Precision Medicine in Inflammatory Bowel Disease

Submitted by Gareth Walker to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Medical Studies, June 2019.

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

……

Gareth Walker

If it were not for the great variability among individuals, Medicine might be a Science, not an Art

Sir William Osler, 1892

Abstract

Background: Individual patient variability may explain why despite a growing number of therapeutic options in the treatment of inflammatory bowel disease (IBD) many patients still suffer from disabling disease. Precision medicine aims to address this dilemma by improving the timing and delivery of healthcare for each patient by targeting treatment according to the application of biomarkers. I therefore sought biomarkers that could help deliver two key aspects of precision IBD medicine: accelerating time to diagnosis from the first onset of symptoms and predicting the risk of adverse drug reactions.

Methods: I used prospective observational cohort studies firstly to explore the diagnostic accuracy of faecal calprotectin in distinguishing IBD from functional gut disorder in the primary care setting, and secondly, the factors associated with a delayed IBD diagnosis. Agnostic genome- and exome-wide association methodologies were used to investigate the association between genetic variants and thiopurine-induced myelosuppression in patients of European ancestry.

Results: Faecal calprotectin is a clinically useful biomarker that helps General Practitioners (GPs) distinguish IBD from functional gut disorder in young adults and children. Use of calprotectin was not associated with time to IBD diagnosis, although its uptake in primary care was poor. The greatest component of the total time to diagnosis was the time it took patients to first present to their GP. In the adverse drug reaction study, I discovered a novel association between a variant in *NUDT15* and thiopurine-induced myelosuppression.

Conclusions and Impact: The actionable findings reported in this thesis have led to changes in clinical practice locally and nationally. Our primary care study

of calprotectin has demonstrated how a faecal biomarker can help prioritise outpatient referrals and deliver cost-savings, leading to the adoption of our clinical pathway across several UK sites. The identification of *NUDT15* variants as determinants of thiopurine-induced myelosuppression in European individuals has led to the rapid development of an NHS clinical service from the Exeter molecular genetics laboratory and in due course adoption of the test to the National Genomic Test Directory.

Acknowledgements

I would like to dedicate what follows to my wife Jess, my sons James and Charlie and my parents Pat and Norman. Little did we realise when I started my research fellowship that we would have to sacrifice so much to complete it; I couldn't have done it without you, especially you Jess. I am so very grateful.

My thanks to my supervisors: Tariq Ahmad, Sarah Richardson and Noel Morgan.

I would especially like to thank the following people: Tariq Ahmad for giving me access to his fascinating World of IBD research and for inspiring me to be the clinician I am today and would like to be in the future; James Goodhand for teaching me paper-craft- although I still do not yet have your wizardry way with words, we are getting there! And to Nick Kennedy, for his patience in teaching me computer coding and for introducing me to *his* fascinating World; 'log-World' was my favourite.

I would like to thank Marian Parkinson, Hanlie Oliver, Helen Gardener-Thorpe, and Claire Bewshea and the Exeter IBD Pharmacogenetics Team.

I would like to thank Paul Eggleton and the St.Luke's Biosciences team as well as Dean Naisbitt, Lee Faulkner and the Liverpool laboratory for making me feel so welcome during my time there.

List of contents

List of tables

List of figures

Declarations relating to the candidate's publications and his role in co-authored publications

In reference to 'The University of Exeter Teaching Quality Assurance Manual, Chapter 11-Presentation of theses/dissertations for degrees in the Faculty of Graduate Research: statement of procedures' (published July 2018: accessed May 2019), specifically sub-section 2.2.3, I acknowledge that the thesis that I present includes published material. This guidance can be found at http://as.exeter.ac.uk/academic-policy-standards/tqa-manual/pgr/presentationoftheses/

I herein submit four papers and one book chapter as a part of this PhD thesis. I set out in **Table 1.1-1** (below) where these publications are reported in the thesis, the citation where relevant, my contribution to the publication and the publication status (i.e. published, accepted, submitted or pending submission).

Where appropriate (i.e. for published papers), and in accordance with sub-section 2.2.5 I present papers in their published form. I accept that in order to meet with copyright restrictions before uploading to the University of Exeter Repository (ORE) that the final version of this thesis may need to be redrafted to include the pre-publication copy of each of the papers in Microsoft Word format. This will ensure that tables and figures are presented *in situ* within the papers and the broader referencing and page numbering is consistent through-out the thesis.

Table 1.2-1. Candidate's contribution to publications

Contribution of Gareth J. Walker (**GJW**) highlighted in bold

Paper	Relevant chapter and section in the thesis	Citation/Title	The candidate's contribution to the publication	Publication status
1	Chapter 3, section 3.3	'Faecal calprotectin effectively excludes inflammatory bowel disease in 789 symptomatic young adults with/without alarm symptoms: a prospective UK primary care cohort study' **Walker, G. J.,** Moore, L., Heerasing, N., Hendy, P., Perry, M. H., McDonald, T. J., Debenham, T., Bethune, R., Bewshea, C., Hyde, C., Heap, G. A., Singh, A., Calvert, C., Kennedy, N. A., Goodhand, J. R. and Ahmad, T. Alimentary Pharmacology & Therapeutics, 47(8), pp. 1103–1116. (2018) doi: 10.1111/apt.14563.	**GJW,** TA, GH, CB, AS, TJM, RB, MHP and TD participated in the conception and design of the study. **GJW** coordinated the study, was the primary author of the paper and first author on the manuscript. **GJW**, LM, NH and PH collected primary care data. **GJW**, AS, LM, NH and PH collected secondary care data. **GJW** was the primary data-analyst and wrote the Stata script used in its analysis. **GJW**, TA, TJM, CC, CH, NAK, JRG, RB, NH, PH, CB, MHP and TA contributed to data analysis or interpretation. **GJW**, JRG, LM and TA drafted the article and all authors revised it critically and approved the final version of the manuscript.	Manuscript published in Alimentary Pharmacology & Therapeutics, 2018

Table 1.1-1 continued…

Table 1.1-1 continued…

Paper	Relevant chapter and section in the thesis	Citation/Title	The candidate's contribution to the publication	Publication status
2	Chapter 3, section 3.4	'Diagnostic accuracy of primary care faecal calprotectin testing in children with suspected inflammatory bowel disease' **Gareth J Walker**, Amanda Thomas, Lucy Moore, Neel Heerasing, Peter Hendy, Mohammed Abdulrahim, Sean Mole, Mandy H Perry, Timothy J McDonald, Claire Bewshea, Neil Chanchlani, Simeng Lin, Jim Hart, Richard K Russell, Tariq Ahmad, James R Goodhand, Nicholas A Kennedy	**GJW,** TA, GH, CB, AS, TJM, RB, MHP and TD participated in the conception and design of the study. **GJW** coordinated the study, was the primary author of the paper and first author on the manuscript. **GJW**, LM, NH and PH collected primary care data and AS, **GJW**, LM, NH, PH, AT, NC, SM, MA and SL collected secondary care data. **GJW** was the primary data-analyst and wrote the R-script used in the analysis. NAK reviewed and amended the R-script. **GJW**, NAK, JRG, AT, NH, PH, CB, MH, SM, MP and TA contributed to data analysis or interpretation. **GJW**, AT, JH, NC, SL, RR, JRG, NAK and TA made further drafts of the article before all authors revised it critically and approved the final version of the manuscript.	Manuscript pending submission

Table 1.1-1 continued…

Table 1.1-1 continued…

Paper	Relevant chapter and section in the thesis	Citation/Title	The candidate's contribution to the publication	Publication status
3	Chapter 3, section 3.5	'A prospective cohort study to identify factors associated with a delay in IBD diagnosis' **Gareth Walker**, Amanda Thomas, Simeng Lin, Neil Chanchlani, Peter Hendy, Neil Heerasing, Lucy Moore, Harry Green, Nick Kennedy, Claire Bewshea, Joe Mays, James Goodhand, Tariq Ahmad.	**GJW**, TA, NH, LM, PH and CB participated in the conception and design of the study **GJW** coordinated the study, was the primary author of the paper and first author on the manuscript. **GJW**, LM, NH and PH collected primary care data and AS, **GJW**, LM, NH, PH, AT, NC, SM, MA and SL collected secondary care data. **GJW** was the primary data-analyst and wrote the R-script used in the analysis. NAK reviewed amended the R-script. **GJW**, TA, NAK and JRG contributed to data analysis or interpretation.	Manuscript pending submission

Table 1.1-1 continued…

CANDIDATE'S CONTRIBUTIONS TO PUBLICATIONS

Paper	Relevant chapter and section in the thesis	Citation/Title	The candidate's contribution to the publication	Publication status
4	Chapter 4, section 4.3	'Association of Genetic Variants in *NUDT15* With Thiopurine-Induced Myelosuppression in Patients with Inflammatory Bowel Disease' **Walker, G. J**., Harrison, J. W., Heap, G. A., Voskuil, M. D., Andersen, V., Anderson, C. A., Ananthakrishnan, A. N., Barrett, J. C., Beaugerie, L., Bewshea, C. M., Cole, A. T., Cummings, F. R., Daly, M. J., Ellul, P., Fedorak, R. N., Festen, E. A. M., Florin, T. H., Gaya, D. R., Halfvarson, J., Hart, A. L., Heerasing, N. M., Hendy, P., Irving, P. M., Jones, S. E., Koskela, J., Lindsay, J. O., Mansfield, J. C., McGovern, D., Parkes, M., Pollok, R. C. G., Ramakrishnan, S., Rampton, D. S., Rivas, M. A., Russell, R. K., Schultz, M., Sebastian, S., Seksik, P., Singh, A., So, K., Sokol, H., Subramaniam, K., Todd, A., Annese, V., Weersma, R. K., Xavier, R., Ward, R., Weedon, M. N., Goodhand, J. R., Kennedy, N. A., Ahmad, T. and IBD Pharmacogenetics Study Group. JAMA, (2019) 321(8), p. 773. doi: 10.1001/jama.2019.0709.	**GJW** coordinated the study, was the primary author of the paper and is joint first author on the manuscript. JWH performed the genomic analyses and is joint first author on the manuscript. **GJW** and NAK had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: TA, **GJW**, GH, CB, KS, AS Acquisition, analysis, or interpretation of data: **GJW**, JWH, GH, MV, JK, MW, NAK Genotyping and exome sequencing: MD. Sanger sequencing: ReW Drafting of the manuscript: **GJW**, JWH, GH, CB, JRG, NAK, TA, DMcG Statistical analysis: **GJW**, JWH, MV, JK, NAK	Manuscript published in JAMA, 2019

Table 1.1-1 continued…

Table 1.1-1 continued …

Book chapter	Relevant chapter and section in the thesis	Citation/Title	The candidate's contribution to the publication	Publication stat
4	Chapter 4, section 4.1	Biomarkers in Inflammatory Bowel Diseases, 978-3-030-11445-9, 430817_1_En, (21). (eds.) N. S. Ding, P. De Cruz. Chapter: 'The utility of genetic biomarkers to reduce adverse drug reactions in IBD' by Gareth Walker and Tariq Ahmad	**GJW** performed the literature search and wrote the first draft of the chapter. TA edited it.	Literature review in published book, Springer, nature, 2019

Key: GJW, Gareth J Walker (the candidate); TA, Tariq Ahmad; AT, Amanda Thomas; LM, Lucy Moore, NH, Neel Heerasing; PH, Peter Hendy; MA, Mohammed Abdulrahim; SM, Sean Mole; MHP, Mandy H Perry; TM, Timothy J McDonald; CB, Claire Bewshea, NC, Neil Chanchlani, JH, Jim Hart; RR, Richard K Russell; TA, Tariq Ahmad; JRG, James R Goodhand; NAK, Nicholas A Kennedy; AS, Abhey Singh; NC, Neil Chanchlani; SL, Simeng Lin; TD, Tom J. Debenham; RB, Rob Bethune, GH, Graham Heap; JWH, James W.Harrison; Mikael D. Voskuil; VA, Vibeka. Andersen; CA, Carl A. Anderson; AA, A. N Ananthakrishnan; JB, Jeff. C.Barrett; Laurent B, L. Beaugerie; ATC, Andrew. T Cole; FC, Fraser. R. Cummings; MJD, Mark.J. Daly; PE, Pierre P. Ellul; RF, Richard N. Fedorak; EAMF, Eleonora M. Festen, TF, Tim H. Florin; DG, Daniel R. Gaya; JH, Jonas Halfvarson, Ailsa L. Hart; PI, Peter M. Irving; SJ, Samual E. Jones, JK, Jukka Koskela; JL, James O. Lindsay; JM, John Mansfield; D.McG, Dermot McGovern, MP, Miles Parkes; RP, Richard Pollok; SR, Sadeesh Ramakrishnan; DR, David S. Rampton; M.A.R, Manuel A. Rivas; MS, Michael Schultz; SS, Shaji Sebastian; PS, Phillipe Seksik; KS, Kenji So; HS, Harry Sokol, H., RS, Ramikrishnan Subramaniam, VA, Vito Annese, RKW, Rinse K. Weersma; RX, R.Xavier; RW, Rebecca Ward; MW, Mike Weedon; LM, Loukas Moutsianos; AS, Aleksej Sazonovs.

The aims and objectives of this thesis

Despite the arrival of a number of new IBD therapies no one treatment leads to sustained remission for even half of treated patients. Precision medicine in the field of IBD has been proposed as a potential solution for this clinical conundrum, and although studies over the last decade have begun to address this goal, knowledge gaps remain.[1]

The four central precepts of precision IBD medicine are:

1. making an early diagnosis
2. disease prognostication
3. predicting drug response and avoiding toxicity
4. treat-to-target with tight control

The aim of this thesis is to investigate how biomarkers may be used to help deliver two of the four key aspects of this precision medicine approach for the treatment of IBD patients: making an early diagnosis of IBD, as soon after the onset of symptoms as possible to maximise the effectiveness of modern therapies and ultimately to modify the natural history of these lifelong disabling diseases; and, predicting adverse drug reactions and minimising drug toxicity.

I will explore these aims through the 10 objectives that I set out below in **Table 1.1-2** below.

Table 1.2-2. Research questions and objectives

Chapter	Research question(s)	Objectives	Methodology
3	What is the diagnostic accuracy of a new calprotectin based-referral pathway in distinguishing functional gut disorder from IBD in the ***adult*** primary care setting? What is the diagnostic accuracy of faecal calprotectin in distinguishing non-IBD from IBD in the ***paediatric*** primary care setting? Do the presence of gastrointestinal alarm symptoms in adults alter the diagnostic accuracy of the test? Does faecal calprotectin alter referral behaviour in paediatric and adult patients?	To ascertain: 1) the diagnostic accuracy of faecal calprotectin in distinguishing functional gut disorder from IBD in adult and paediatric patients in the primary care setting 2) whether faecal calprotectin testing altered primary care referral behaviour 3) whether the presence of gastrointestinal alarm symptoms altered the performance of the test in adults	Prospective observational cohort study to describe the diagnostic accuracy of calprotectin testing to exclude IBD in primary care

Table 1.1-2 continued…

Table 1.1-2 continued…

Chapter	Research question(s)	Objectives	Methodology
3	What is/are the factor/s which influence time to diagnosis in IBD? Does the use of faecal calprotectin reduce time to IBD diagnosis?	To identify: 4) where delays occur in the referral pathway between onset of symptoms through primary and secondary care to diagnosis of IBD 5) the clinical and laboratory factors which influence time to diagnosis in IBD patients 6) if a delayed diagnosis is associated with more hospitalisations, surgeries and a greater requirement for immunosuppressive and biologic therapies in the first year after diagnosis than patients with a timely diagnosis	Retrospective review of all new IBD diagnoses between 2014-2018 at the Royal Devon and Exeter Hospital

Table 1.1-2 continued…

Table 1.1-2 continued…

Chapter	Research question(s)	Objectives	Methodology
4	What genetic factors are associated with thiopurine-induced myelosuppression (TIM) in patients with IBD?	To investigate 7) the association between genetic variants and TIM in European patients with IBD 8) if genetic variants are present, to explore if the frequency of these variants is enriched in those patients with early drug reactions (≤ 8 weeks from start of maximum dose) To define: 9) the clinical phenotype and morbidity related to carriage of a TIM associated genetic variant(s) 10) the clinical usefulness (e.g. sensitivity, specificity, negative and positive predictive values) of genetic testing to identify patients at risk of TIM	Case-control disease matched genetic study of patients who suffered either thiopurine-induced myelosuppression versus thiopurine-tolerant patients utilising genome wide- and exome-wide association methodologies

The structure of this thesis

I present this thesis as five chapters. The first two chapters introduce IBD and precision medicine in IBD.

Chapter 1: Introduction to IBD. Presents a literature review of the latest research on the aetiology, pathophysiology, diagnosis, treatment and complications of IBD. Specifically, it details how seminal genetic and observational studies have informed current thinking in the field. This chapter includes a summary of current treatment paradigms and their limitations, serving as a backdrop for the precision medicine approach described in Chapter 2.

Chapter 2: Introduction to Precision Medicine. Details the evidence supporting the use of a precision medicine approach in the field of IBD with a focus on four specific areas: the importance of making an early diagnosis of IBD; prognostication of disease course; predicting response to drug therapy and avoiding toxicity; and, treating to target with tight control.

In Chapters 3 and 4, I present four papers (**RESEARCH PAPERS I-IV**), each relating to a different component of precision medicine. All of the papers are self-contained i.e. all tables, references and figures are separate to the rest of the thesis and are located either within the manuscript itself or in the manuscript supplement that immediately follows.

In each of the two data chapters (chapters 3 and 4) I set out:

- the background to the chapter;
- the objective(s) of the chapter;
- the publication or work of the chapter;
- how the chapter contributes to addressing the aims of the thesis; and
- the implications for practice of the work that I present

Chapter 3: **The role of faecal calprotectin in the delivery of precision medicine: making an early diagnosis of IBD.** In this chapter I first present two prospective observational cohort studies which describe the real-world experience of introducing faecal calprotectin into primary care to help GPs differentiate patients with functional gut disorder and IBD. These separate papers relate to the use of a National Institute for Health and Care Excellence (NICE) approved faecal biomarker in paediatric and adult patients, respectively.

RESEARCH PAPER I: 'Faecal calprotectin effectively excludes inflammatory bowel disease in 789 symptomatic young adults with/without alarm symptoms: a prospective UK primary care cohort study' *Walker, G. J., Moore, L., Heerasing, N., Hendy, P., Perry, M. H., McDonald, T. J., Debenham, T., Bethune, R., Bewshea, C., Hyde, C., Heap, G. A., Singh, A., Calvert, C., Kennedy, N. A., Goodhand, J. R. and Ahmad, T.*

RESEARCH PAPER II: 'The diagnostic accuracy of primary care faecal calprotectin testing in children with suspected inflammatory bowel disease.' *Walker, G. J., Thomas A., Chanchlani, N., Lin, S., Moore, L.N. Heerasing, N., Hendy, P., Perry, M. H., McDonald, T., Abdulrahim, M., Mole, S., Bewshea, C., Hyde, C., Heap, G. A., Singh,*

A., Calvert, C., Russell, R.K., Hart, J., Goodhand, J. R., Ahmad, T., and Kennedy, N. A.

I then present a retrospective cohort study of the factors associated with a delayed diagnosis in IBD. The overall time to diagnosis is sub-divided into 3 sections: patient delay (i.e. the time from onset of symptoms to first GP presentation); primary care delay (i.e. the time from first GP presentation to GP referral) and secondary care delay (i.e. the time from GP referral to IBD diagnosis). Specifically, I evaluate whether use of primary care faecal calprotectin was associated with a reduction in the time to diagnosis. I then explore whether complications, such as surgery and hospitalisations and, drug use in the first year after diagnosis, were associated with diagnostic delay. Finally, I sought the demographic and clinical factors associated with an emergent presentation prior to IBD diagnosis.

RESEARCH PAPER III: 'A prospective cohort study to identify factors associated with a delay in IBD diagnosis.' *Walker, G. J., Thomas A., Chanchlani, N., Lin, S., Moore, L.N. Heerasing, N., Hendy, P., Perry, M. H., McDonald, T., Abdulrahim, M., Mole, S., Bewshea, C., Singh, A., Kennedy, N. A, Ahmad, T. and Goodhand, J. R.*

Chapter 4: **The role of pharmacogenetics in the delivery of precision medicine: avoiding adverse drug reactions.** This chapter starts with a literature review that has been published as a book chapter relating to the use of biomarkers in the reduction of adverse drug reactions in IBD**.**

BOOK CHAPTER: 'The utility of genetic biomarkers to reduce adverse drug reactions in IBD.' *Walker, G. J and Ahmad, T.* Chapter in **'**Biomarkers in Inflammatory Bowel Diseases' N. S. Ding, P. De Cruz. (eds.) Publisher, Springer Nature (2019).

I then present a retrospective case-control study that aimed to identify novel clinical and genetic biomarkers associated with thiopurine-induced myelosuppression (TIM).

RESEARCH PAPER IV: 'Association of Genetic Variants in *NUDT15* With Thiopurine-Induced Myelosuppression in Patients with Inflammatory Bowel Disease.' ***Walker, G. J.****, Harrison, J. W., Heap, G. A et al.*

Finally, in **Chapter 5** I summarise the findings and discuss the issues and challenges arising from the thesis and look ahead to the future.

Definitions; abbreviations

ADA	Adalimumab
ADAb	Anti-drug antibody
ADR	Adverse drug reaction
ASG	American society gastroenterology
AZA	Azathioprine
BMI	Body mass index
BSG	British society gastroenterology
CD	Crohn's disease
CEUS	Contrast enhanced ultrasound
CPIC	Clinical pharmacogenetics implementation consortium
CRP	C-reactive protein
CV	Co-efficient of variation
DNA	Deoxyribonucleic acid
DRESS	Drug reaction with hypereosinophilia and systemic symptoms
ECCO	European Crohn's Colitis Organisation
EWAS	Exome-Wide Association Study
ELISA	Enzyme-linked immunosorbent assay
EU	European union
GI	Gastrointestinal
GP	General practitioner
GWAS	Genome-wide association study
IBD	Inflammatory bowel disease
IBD-U	Inflammatory bowel disease-unclassified

DEFINITIONS; ABBREVIATIONS

IBS	Irritable bowel syndrome
IFX	Infliximab
LOR	Loss of response
MP	Mercaptopurine
MRE	Magnetic resonance enterography
NICE	National Institute Health and Care Excellence
NK	Natural killer
NPV	Negative predictive value
OGD	Organic gastrointestinal disease
PNR	Primary non-response
PPV	Positive predictive value
RNA	Ribonucleic acid
SBCE	Small bowel capsule endoscopy
SCAR	Severe cutaneous adverse reaction
SCFA	Short chain fatty acids
SJS	Stephens-Johnson syndrome
TDM	Therapeutic drug monitoring
TEN	Toxic epidermal necrolysis
TIM	Thiopurine-induced myelosuppression
TILI	Thiopurine-induced liver injury
TNF	Tumour necrosis factor
TRFIA	Time-resolved fluorescent immunoassay
UC	Ulcerative colitis

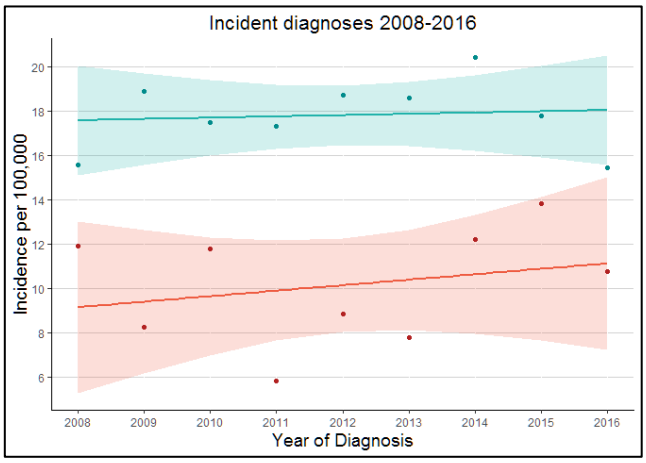
Chapter 1

1 Introduction to IBD

Inflammatory bowel disease (IBD) is the collective term given to describe chronic relapsing-remitting inflammation of the gastrointestinal (GI) tract. The two commonest forms of IBD are Crohn's disease (CD) and ulcerative colitis (UC); the former can affect any part of the gastrointestinal tract whilst UC only affects the large bowel/colon. In approximately 9% of patients the clinical, histological or radiological features do not permit a definitive diagnosis of either CD or UC and such patients are deemed to have 'IBD-unclassified' (IBD-U).[2]

IBD can present at any age although typically a bimodal distribution is described in Western populations, with a younger peak between 15-40 years and a second peak in older adults aged 60-70 years old.[3] Disease prevalence varies with ethnicity and geography and to a lesser extent by social-class and sex.[4] The incidence of IBD is highest in Northern and Western Europe with annual rates as high as 24.3 per 100,000 and 12.7 per 100,000 persons for UC and CD respectively.[5] Although the incidence of IBD is reportedly static in Northern and Western Europe[6], globally the incidence of IBD is increasing.[5] Work from Devon, UK (unpublished) and Lothian, Scotland report a growing prevalence of IBD (0.80% and 0.78%, respectively), despite a static incidence, reflecting the compounding effects of an ageing population and falling rates of disease related mortality (**see Figure 1.1-1**).[7,8]

Figure 1.2-1. Incidence per 100,000 of background population against time for ulcerative colitis (green) and Crohn's disease (red) in Exeter, UK from 2008-2016



The mean incidence of UC was 13.1 (95% CI 12.4 – 13.6) per 100,000 background population per year, and the mean incidence of Crohn's disease was 10.2 (95%CI 8.6 – 11.7) per 100,000 background population per year. No statistically significant change was demonstrated for the period 2008-2016. Incident cases were defined as patients who lived within the Exeter, UK catchment at date of diagnosis. Image used with permission from Dr Ben Hamilton, Exeter IBD Pharmacogenetics Team

Sex differences differ by age group and IBD subtype in Western countries (**Figure 1.1-2**).[9] Recent evidence suggests that female sex hormones may play a role in IBD pathogenesis: in a recent meta-analysis authors concluded that the use of the oral contraceptive pill was associated with an increased risk of IBD[10]. Furthermore, data from a large cohort study found that women with IBD reported changes in symptom severity during times of hormone fluctuation (e.g., menstruation, pregnancy, postpartum, post-menopause).[11]

Figure 1.2-2. Sex-differences in the incidence of Crohn's disease and ulcerative colitis, based on a pooled analysis from Western countries

Sex-Based Differences in Incidence of Inflammatory Bowel Diseases—Pooled Analysis of Population-Based Studies from Western Countries. Figure taken from S. C. Shah et al. in Gastroenterology Volume 155, Issue 4, Pages 1079-1089.e3, October 2018.

1.1.1.1 Disease presentation

CD typically presents with symptoms of abdominal pain, diarrhoea, fatigue and weight loss, although presentation can vary with disease site and severity.[4,12] UC is characterised by symptoms of bloody diarrhoea, faecal urgency and abdominal pain. Although some patients may experience only mild gut inflammation, symptoms can be severe with significant disruption to education, employment and family life.[13–15] For some patients a debilitating disease course ensues, requiring the initialisation of long-term drug therapies and surgery in order to quell inflammation and treat complications. In CD, up to 38% of patients may require surgery in the first 5-10 years after diagnosis.[16,17] Whereas approximately 6-10% of UC patients undergo colectomy within the first 10 years of diagnosis for refractory symptoms.[18,19] After 15 years, the number of surgeries for refractory UC starts to decrease, although the incidence of colorectal cancer in UC rises, with one meta-analysis citing a cumulative probability of cancer of 2% by 10 years, 8% by 20 years, and 18% by 30 years.[19,20] Colorectal cancer in IBD is discussed further later in this introduction.

1.2 Aetiology of IBD

The exact pathogenesis of IBD is yet to be fully elucidated, however, current knowledge of this complex disorder may suggest an inappropriate immune response to antigenic stimulation by the gut microbiota on a background of genetic susceptibility.

1.2.1 Genetic factors

A genetic component to the aetiology of IBD is suggested by observations such as the high concordance rates of first-degree relatives with IBD in family studies[21], the higher heritability of IBD found in monozygotic rather than dizygotic twins[22,23], as well as the

high prevalence of the disease in certain populations such as the Ashkenazi Jews.[24] The risk of IBD is higher in first-degree relatives of a CD proband (overall risk between 2 and 14%) than in UC (overall risk between 7 and 11%), although more distant relatives of patients with CD and UC are also at increased risk.[25] In large European twin studies the concordance rate among monozygotic twins is estimated to be between 20-50%, whilst for dizygotic twins it is lower at approximately 10%.[22,23] These data support a role for genetics in the aetiology of IBD, although shared environmental factors may also contribute to this clustering of disease in families. The importance of environmental factors is highlighted by the fact that only a minority of patients carrying a particular variant will develop IBD, the exception being the development of very early onset IBD in children who carry highly penetrant monogenic variants.[26]

Over the last 20 years a number of different genetic approaches have been employed to unravel IBD genetic loci which now total more than 240.[27] These discoveries have provided novel insights into the pathogenesis of IBD and targets for new therapies.[28] The first IBD susceptibility locus, termed 'IBD1', was identified using linkage analysis.[29] Such agnostic linkage studies investigate how genetic markers along a chromosome co-segregate among members of a single large family with multiple affected individuals. A score is used to quantify the likelihood that a genetic marker is inherited together with the true disease-causing gene; markers in close proximity to such a gene are more likely to be inherited together following meiosis (chromosomal recombination during gamete production) and have a higher score than more distant markers. IBD1 was subsequently mapped to variants within the *NOD2* gene (a.k.a. *CARD15).*[30] NOD2 plays a crucial role in the innate immune response by recognising the muramyl dipeptide (MDP) component of bacterial cell walls and triggering immune

activation and autophagy of dendritic cells through a series of nuclear transcription factors (most notably NF-κB).[31] Further loci, termed IBD2 were identified by linkage studies but mapping studies failed to identify the causal genes reflecting the limited power of such analyses.[32]

1.2.1.1 The GWAS era

In the 21st Century a combination of World-wide collaboration, with successful endeavours such as the Human Genome and HapMap projects, and technological advances, heralded a new tool in the exploration of IBD genetic susceptibility: the hypothesis-free genome-wide association study (GWAS).

The GWAS approach compares the allele frequencies of thousands of single nucleotide polymorphisms between affected cases and unaffected controls in a bid to uncover the association between genetic regions (loci) and phenotypic traits, such as IBD (**see Figure 1.2-1**).

Figure 1.2-1: Diagram to show basic principles of GWAS

Figure illustrates how GWAS utilises the haplotype structure of the human genome: each chromosome consists of multiple haplotypes–regions that are inherited together during meiosis. Within each haplotype, there are typically many SNPs, which are co-inherited within the larger genetic region, and thus, their alleles are inherited non-randomly (i.e. they are in linkage disequilibrium with one and other). This means that it is possible to infer the genotypes at multiple SNPs within the haplotype (shown as grey vertical lines) if the genotype at one or more SNPs is known. GWAS SNPs (shown in black vertical lines) are selected so as to tag each haplotype, but where association is observed, they are unlikely to be the causal variant at the locus (shown as red vertical line). By genotyping SNPs from each haplotype in the genome in disease cases and healthy controls, it is possible to identify SNPs where the allele frequency is significantly different between the cases and controls, and which are associated with the disease. Taken from Verstockt, B., Smith, K.G.C. and Lee, JC. Genome-wide association studies in Crohn's disease: Past, present and future. Clinical & Translational Immunology 2018 [33]

At first, GWAS findings were modest, typically only uncovering up to 10 new genetic associations as a consequence of small sample sizes and thus limited study power. However, in time, a combination of World-wide collaboration (ultimately with sample sizes ~ 50,000 cases and controls) and more affordable genotyping uncovered several crucial insights into the pathophysiology of IBD:

i) ***autophagy related genes (ATG16L1***[34]***, IRGM***[35]***):*** autophagy is a process which recycles cell organelles and cytosolic macromolecules by presentation to lysosomes. This process is important for anti-bacterial host defence against intracellular invading micro-organisms (also known as xenophagy) and resolving endoplasmic reticular stress, a key component of intestinal inflammation and the pathogenesis of CD.

ii) ***defective colonic mucosal barrier in UC:*** the diminished mucosal barrier permits increased microbial adhesion and invasion which is thought to trigger mucosal immune reactions that result in chronic intestinal inflammation.[36,37]

iii) ***a failure to suppress an aberrant immune response:*** disruption of the normal mechanisms by which the body prevents aberrant responses to non-pathogenic commensal gut organisms leads to colitis in animal models.[38] Focus has been on the role of the anti-inflammatory IL-10 cytokine with several other genes implicated in this pathway (*IL10, IL10RB, STAT3 and TYK2*).[39,40]

iv) ***the IL-23/IL-17 axis:*** IL-23 is a cytokine which promotes expansion and maintenance of mucosal effector Th17 cells, which in turn produce pro-inflammatory IL-17. Numerous genetic studies identified variants within or near to genes implicated in Th17 biology (e.g. RORC, IL23R, IL12B, TYK2, JAK2, STAT3, CCR6 and ICOSLG).[41,42] Although the functional consequences of these risk alleles still need further elucidation, this discovery was crucial in the development of CD drugs targeting this system (anti-IL12/23: Ustekinumab, [Janssen Biotech] and anti-IL12: Risankizumab [AbbVie]).

Subsequent genetic meta-analyses increased statistical power even further and enabled the detection of 240 susceptibility loci, each exerting a low effect size.[28] For

the majority of these loci the causal gene and/or causal variants have not been mapped. In some cases, this may be possible using statistical fine mapping and in others it will require a functional characterisation of the downstream effects of each SNP locus.[33] Currently, the CD and UC loci identified to date only account for ~23% and ~16% of disease heritability, respectively, and yet observations of concordance in twin studies informs us that the genetic contribution to IBD aetiology should be much greater; this phenomenon is termed 'missing heritability'. Explanations for this paradox include the presence of gene-gene interactions (epistasis), gene-environmental interactions, as well as deficiencies inherent in the GWAS methodologies and technologies upon which these estimates of missing heritability have been made.[43] With regards this latter explanation, further progress may be made with the advent of widespread whole-exome and whole genome- sequencing.

1.2.1.2 Whole-exome (WES) and whole-genome sequencing (WGS)

The advent of next generation sequencing, which permits the rapid sequencing of large amounts of DNA, has moved genetic studies into a new era once more and enables the detection of rare variants across thousands of individuals. Whole-exome sequencing (WES) characterises nucleotide sequences in the protein coding exomes which make up approximately 1% of our genome. Whereas whole-genome sequencing (WGS) will also detect rare variants in the remaining non-coding intronic regions. Although in reality, due to the difficulty in sequencing technically challenging regions of the genome with current sequencing platforms (i.e. high guanine-cytosine content, large repeat regions, centromeres, telomeres, etc.), whole-genome sequencing still only covers 95% to 98% of the genome.[44] Conceivably these rare variants may have much larger effect sizes than the common variants detected by

GWAS. However, in common with first GWAS studies, the initial forays into the use of WES and WGS in the field of IBD and autoimmune disease pathogenesis have been a little disappointing and yielded few interesting hits.[45,46] Further gains may require collaboration across centres, new methods for combining sequence data-sets and greater computational power.

1.2.2 Environmental factors

That IBD has a higher incidence in developed Western countries when compared with undeveloped and developing nations may be explained by both environmental and genetic factors.[5,47] However, two important epidemiological observations highlight the importance of environmental factors as distinct from genetics in the pathogenesis of IBD: first, the rapid rise in disease incidence in both developed and developing nations cannot be easily explained by genetics; second, the incidence of IBD increases in migrant populations moving from developing countries with a low incidence of IBD to Western counties with higher IBD incidence.[48–50] Environmental changes such urbanisation, diet, lifestyle (smoking and alcohol) and hygiene may underpin these observations.[47] In particular Western diets, characterised by processed foods high in animal fats, protein, carbohydrate and emulsifiers and low in fibre, may be responsible. Changing to a predominantly animal-based diet in humans (composed of meats, eggs, and cheeses) has been shown to rapidly alter host microbiota composition, specifically, increasing the abundance of bile-tolerant microorganisms (*Alistipes, Bilophila, and Bacteroides*) and decreasing the levels of Firmicutes that metabolise dietary plant polysaccharides (*Roseburia, Eubacterium rectale, and Ruminococcus bromii*).[51] In contrast, predominantly plant-based diets (rich in grains, legumes, fruits, and vegetables) did not alter either the microbial diversity within each subject at a given time-point (α-diversity) or the difference between each subjects' baseline and

diet-associated gut microbiota (β-diversity).[51] Such changes in the microbiota, in particular *Faecalibacterium prausnitzii* and *Roseburia intestinalis,* may alter the production of metabolites such as Short Chain Fatty Acids (SCFA's) and bacterial metabolites that are formed as result of anaerobic fermentation of dietary fibre.[51,52] SCFA's such as acetate, propionate and butyrate, are reduced in IBD patients, and are important as a primary energy source for colonic epithelial cells, maintain intestinal homeostasis, strengthen gut barrier function, and may also have immunomodulatory functions.[52,53]

Established IBD treatment options such as faecal diversion[54] and exclusive enteral nutrition[55] have both been shown to alter microbiota composition. In the future, further therapeutic manipulation of the gut microbiome may be achieved by through whole-food exclusion diets[56], pro- and pre-biotics[57] as well as faecal transplantation.[58] However, it remains unclear whether dysbiosis is a cause or a consequence of IBD; future, inception cohorts, such as the RISK[59] and GEM[60] studies, that describe real-time genetic, environmental, and microbial interactions may help address this conundrum.

1.2.3 Epigenetics: Linking genetic and environmental factors

Epigenetics is possibly the missing key that links the effects of the environment, genetic predisposition and intestinal microbiota with IBD pathogenesis. This term was first proposed by Waddington in 2012 to describe how an organism's phenotype may result from an interaction between its genes and environment.[61] Epigenetics is more commonly used now to refer to alterations in gene expression events that occur independently of the primary sequence of Deoxyribonucleic acid (DNA).[62] A few studies have found these events to be inheritable, although this is a somewhat

contentious issue.[63] Such epigenetic mechanisms include DNA methylation, histone modification and micro RNA interference - which may be influenced through lifestyle factors such as diet and smoking.[64–66]

Evidence supporting the importance of epigenetics in the pathogenesis of IBD can be found from both genome- and epigenome-wide methylation association studies. The International IBD Genetics Consortium reported an association between a SNP linked to *DNMT3a*, a gene encoding an enzyme responsible for establishment of DNA methylation, and IBD pathogenesis.[41] Whereas Nimmo *et al* reported 50 differentially methylated sites, including several in important immune response genes (*IL21R, S100A13, FASLG, MAPK13, RIPK3 or PRF1)* which differed in patients with CD as compared with healthy controls.[67] Such epigenetic studies are limited by the heterogeneity of the cell types and tissue investigated (e.g. peripheral blood mononuclear cells [PBMCs] vs. epithelial cells vs. tissue biopsies) as well as the controls which are used (i.e. healthy controls vs. unaffected tissue from the same patient). Each methylome signature is specific for a given cell type, therefore changes in cell proportions in conditions of inflammation may mimic true epigenetic changes and lead to misleading results. Although machine learning algorithms are commonly used to help differentiate signatures from different cell lines, methylome profiles should ideally be studied in sorted cell populations to allow definitive conclusions about epigenetic changes to be made.[68]

1.2.4 Clinical presentation

The clinical manifestations of IBD are dependent on the area of the gut involved. UC commonly presents with bloody diarrhoea, urgency and tenesmus, sometimes associated with abdominal pain. In contrast, CD commonly presents with recurrent

abdominal pain, weight loss and diarrhoea. Children may additionally present with growth retardation and delayed or failed sexual maturation.

Systemic symptoms are commonly reported by IBD patients and include weight loss, fever, sweats, malaise, arthralgia and fatigue. In 10%-20% of cases, patients present with extra-intestinal manifestations including, arthritis, dermatoses, uveitis, or liver disease.[69]

1.3 Diagnosis of IBD

1.3.1 Serological and faecal biomarkers

At diagnosis, current European IBD guidelines recommend that every patient should have a biochemical assessment with full blood count, inflammatory markers (C-reactive protein [CRP]), electrolytes, liver enzymes, and a stool sample for microbiological analysis, including *C. difficile*.[69]

CRP broadly correlates with clinical severity in CD but less so in UC, except in the case of acute severe colitis.[69] However, it is not uncommon for CRP to be normal, even in the context of active disease.[70] Faecal calprotectin, a neutrophil-derived protein, appears to be the most sensitive marker of intestinal inflammation in IBD and its role is described in more detail later in Chapter 3.

Approximately 30% of UC patients and 66% of CD patient receive immunosuppressive therapies in the first 5 years after diagnosis.[17,18] Therefore, it is recommended that patients are screened and vaccinated at diagnosis to prevent severe infections: hepatitis B surface antibody, hepatitis B antigen, hepatitis B core antibody, hepatitis A

IgG, measles serology, varicella serology hepatitis C serology, Epstein Barr serology and HIV serology.[69]

1.3.2 Endoscopy

Ileocolonoscopy with a minimum of two biopsies from the inflamed regions[69] remains the gold-standard diagnostic test in IBD and allows classification of disease based on endoscopic extent, severity of mucosal disease and histological features. Whilst complete colonoscopy, and the use of purgatives may be best avoided in severely active UC, flexible sigmoidoscopy with a phosphate enema is considered safe.

CD is characterised by patchy transmural inflammation anywhere in the gastrointestinal tract and may be defined by age of onset, location, and behaviour. UC is characterised by diffuse mucosal inflammation which is limited to the colon and is classified according to the maximal extent of inflammation observed at colonoscopy. It is more broadly categorised into the following three subgroups: pan-ulcerative colitis; left-sided disease and distal disease/proctitis.[71] The implication of *microscopic* (histological) disease activity in the absence of *macroscopic* inflammation in UC has long been debated. Indeed, Wright and Truelove reported that microscopic inflammation was associated with disease relapse disease back in 1966.[72] More recently, microscopic inflammation in UC was associated with higher corticosteroid use and hospitalisation over a median of 6 years follow up.[73,74] Unsurprisingly, histology is increasingly being used as an endpoint in modern clinical UC trials, although there remains considerable debate about the most appropriate scoring system to use and how to deal with intra-observer variability.

1.3.3 Imaging

Imaging modalities used in the diagnosis of IBD include magnetic resonance enterography (MRE), small bowel capsule endoscopy (SBCE) and small intestinal contrast enhanced ultrasound (CEUS). Imaging is particularly required to detect disease in the small bowel, especially in patients with a clinical suspicion of CD and normal ileocolonoscopy. MRE is now routinely available throughout the UK and uses magnetic resonance imaging (MRI) following distension with an oral contrast agent. It offers several advantages including no radiation exposure, high-contrast resolution, multiplanar ability and dynamic (cine) imaging. Importantly, it can help distinguish between inflammatory, stricturing and penetrating disease as well as both mural and extramural complications; these are key questions for clinicians when deliberating treatment choices.[69] MRI is also the modality of choice to delineate the extent and severity of peri-anal CD.

Small intestinal contrast enhanced ultrasound is not yet widely practiced in the UK, although advocates of this technology cite a lack of radiation, low cost in comparison to MRI and CT, and real-time dynamic assessment, which make it an attractive option for the detection of strictures.[69]

SBCE uses a camera within a small pill which is either swallowed or placed endoscopically into the stomach. It then wirelessly records real-time images from within the small bowel which are viewed by the reporting clinician at a later date. SBCE is a sensitive tool to detect mucosal abnormalities and is comparable to other modalities including CEUS and MRE.[69] Patients with normal MRE and/or CEUS and

an elevated calprotectin should be considered for SBCE, as cross sectional imaging modalities may miss proximal small bowel CD.[75]

1.3.4 Pathology of IBD

The typical histological hallmarks of CD are of granulomas with focal crypt architectural abnormalities, in conjunction with focal or patchy chronic inflammation in untreated adults.[69] The inflammatory changes are defined by the presence of lymphocytes and plasma cells.

In contrast, UC is typified by focal or diffuse basal plasmacytosis which may be present as early as two weeks after symptom onset in nearly a third of patients.[76] Later changes include widespread mucosal and crypt architectural distortion, mucosal atrophy, an irregular or villous mucosal surface and mucin depletion.[76]

IBD-U is used to describe approximately 12% of paediatric and 6% of adult patients where endoscopic appearances and biopsies are inconclusive for making a firm diagnosis of either CD or UC.[77] The histopathology of childhood-onset IBD is distinctly different from adult-onset IBD and in UC may lack the architectural distortion seen with chronic disease.[78] The distinction of infectious colitis from IBD is usually characterised by preserved crypt architecture and acute inflammation, although as eluded to above, these changes are often absent in early IBD.

1.4 Modern treatment paradigms

Treatment paradigms in IBD are changing with the realisation that IBD, particularly Crohn's disease is a progressive disease for a substantial proportion of patients. Treatment plans are increasingly tailored to individual patients according to severity

and prognosis rather than using an empirical one-approach-fits-all strategy. Clinicians are also adopting more ambitious treatment goals for their patients. Clinical remission and clinical response have been replaced by more objective end-points that have prognostic significance such as mucosal healing; a so-called 'treat-to-target' approach. Therapeutic drug monitoring (TDM) is widely used to help guide the management of treatment failure and increasingly is being used proactively in patients in remission to optimise drug levels and reduce the risk of treatment failure.

Currently, in the UK, the most common IBD treatment strategy is based on an accelerated stepwise escalation of pharmacotherapy from corticosteroid to immunosuppressive and then biologic therapies in response to repeated flares or persistently active disease: this is termed the 'accelerated step-up' approach. This 'accelerated step-up' strategy aims to avoid over-treating patients but conceivably exposes some patients to the complications of persistently active disease if progress up the steps is too slow.

In 2013 the AZTEC[79] and RAPID[80] studies cast doubt on the effectiveness of such an accelerated step-up approach. These studies failed to demonstrate that the early initiation of thiopurines improved effectiveness over standard care. However, both studies had a number of limitations including an open label rather than blinded design (RAPID); use of an unselected cohort of newly diagnosed patients with CD, a proportion of which were destined to experience an uncomplicated and mild disease course in any case[16]; and, neither study optimised thiopurine dosing according to drug metabolite levels. There were, however, some positive secondary end-points. The AZTEC study demonstrated that early treatment reduced the occurrence of active

perianal lesions with less need for perianal surgery, and the RAPID study reported that early thiopurine therapy reduced the rate of more severe disease flares.

The seminal TOP-DOWN vs. STEP-UP study demonstrated that early combined immunosuppression with an immunomodulator and an anti-TNF drug ('top-down') was superior to the step-up approach for the treatment of CD patients.[81] The top-down group received standard induction with infliximab [0, 2, and 6 weeks] and then continued with azathioprine monotherapy (as was standard practice at the time additional infliximab infusions were only provided if patients deteriorated clinically). In contrast, the step-up group received two tapering courses of corticosteroids, followed by treatment with azathioprine and then infliximab as required. Steroid-free and surgery-free clinical remission was found to be higher in the top-down group at 14, 26, and 52 weeks, but did not persist beyond a year. The rates of serious adverse events among the two cohorts were reported as no different, although the study was underpowered for this analysis. A follow-up study of 37% (49/133) of participants found that mucosal healing was superior in the top-down group at 2-years [71% vs 30%, P = 0.036].[82]

Furthermore, the SONIC study by Colombel *et al* demonstrated that treatment with both a thiopurine and anti-TNF drug ('combination therapy') in CD patients naïve to both drugs, resulted in higher rates of steroid-free clinical remission and mucosal healing at 26 weeks as compared with azathioprine or infliximab monotherapy.[83] However, given our modern understanding of pharmacokinetics, it is unclear to what extent the addition of a thiopurine offers a therapeutic advantage in and of itself,

beyond a reduction in antibody formation against anti-TNF (so-called 'immunogenicity') which often precipitates a loss of response.[79,80,84]

Despite the findings of SONIC and the TOP-DOWN studies, concerns over both cost and the risks inherent in exposing up to one half of patients who might only ever have suffered a benign disease course to potentially life threatening side effects, have prevented widespread adoption of a top-down approach.[85–89] One possible solution is suggested by the contemporary open-label randomised REACT study[90]: Khana *et al* report that the initiation of combination therapy earlier in the treatment algorithm (within 12 weeks if the disease remained active after corticosteroid treatment) was more effective at reducing major adverse events (IBD-related surgery, hospital admissions, and serious disease-related complications) than a conventional step-wise approach. However, the surprising omission of mucosal healing as a primary end-point led to much criticism and has prompted the follow-up REACT II study.

Other therapeutic options in the modern management of IBD patients include the use of exclusive enteral nutrition (EEN), not only in paediatric cohorts[55] but increasingly in adults as well.[91] The role of surgery should also not be dismissed. Often seen as a consequence of a failure of medical management, timely surgery can in fact be a valuable tool in the treatment of IBD, allowing long-term drug free remission in some patients.[92,93] As discussed further in Chapter 2, the dilemma for clinicians is selecting the right strategy for the right patient at the right time. The solution to this problem may lie in the application of a combination of biomarkers; a central tenet of precision medicine.

1.5 Complications and comorbidities

1.5.1 Strictures

Strictures occur as a consequence of intestinal fibrosis, an exaggerated accumulation of collagen-rich extracellular matrix deposited by myofibroblasts which are themselves activated by inflammatory conditions.[94] In CD, fibrosis appears to take place in two layers: first, in the submucosa there is smooth muscle fibrosis and hyperplasia; and, second, in the muscularis propria there is thickening and hyperplasia.[95]

Strictures are present in approximately 13-21% of CD patients at diagnosis, and approximately 15-25% of patients 5 years after diagnosis.[17,96] However, given that many patients with stricturing CD are asymptomatic, the true number of patients with strictures and fistulae at diagnosis may be as high as 40%.[97] Although classically associated with CD, strictures also complicate UC in approximately 3-6% of patients.[98,99]

Inflammation may persist in the absence of symptoms and in some patients this leads to a destructive progressive evolution from fibrostenotic stricturing to penetrating lesions, such as fistulae and abscesses (**see Figure 1.4-1**).[100]

Figure 1.5-1. Progression of digestive damage and inflammatory activity in a theoretical patient with CD.

Progression of digestive damage and inflammatory activity in a theoretical patient with CD. Figure taken from Pariente B et al. Development of the Crohn's disease digestive damage score, the Lémann score. Inflamm Bowel Dis. 2011;17(6):1415–1422.[100]

The degree to which medical therapies can reverse a stricture has previously been thought to be dependent on the extent to which the lesion is comprised of active inflammation versus the amount of established, and therefore assumed irreversible, fibrosis. However, this paradigm has recently been questioned by the findings of two studies in mice that demonstrated reversal of established fibrosis through systematic administration of IL-36 antibodies[101] and a topically administered Rho kinase inhibitor.[102] For the time being, the mainstay of treatment for established fibrotic strictures is with endoscopic balloon dilation, surgical stricturoplasty and surgical resection. Endoscopic balloon dilatation is immediately successful in 97% of patients and over a median follow up of 5.8 yrs (IQR 3.0-8.4 yrs). Van Assche *et al* reported that no further dilatations were required in over half (54%) of patients.[103] Ding *et al* found that the likelihood of requiring a further dilatation could be reduced by use of combination therapy (thiopurine and anti-TNF), independent of the length of stricture, duration of CD and Rutgeerts score.[104]

Colonic strictures should raise concerns about the risk of colorectal cancer: the GETAID group published a nationwide retrospective study of 12,000 French patients who underwent surgery for colonic strictures between 1992-2014 and reported that 3.5% (248 patients with CD and 39 patients with UC) were found to have dysplasia or cancer.[105]

1.5.2 Fistulae and abscesses

A fistula is an abnormal opening between organs or other structures in the body. Symptoms of peri-anal discomfort and faecal leakage may be indicative of peri-anal abscesses and fistulae formation in perianal CD. The reported incidence of perianal CD varies widely depending on whether the definition includes the commonplace findings of skin tags and haemorrhoids.[71] The incidence of perianal CD, when defined by the presence of fistulae and abscesses, increases with more distal involvement and is present in approximately 12% of isolated ileal CD, 15% of ileocolonic CD and 41% of colonic CD.[106]

1.5.3 Dysmotility and anorectal dysfunction

It has long been recognised that colonic dysmotility and anorectal dysfunction can complicate long-term UC. These sequelae result from a loss of colonic elasticity and consequently a narrow and stiff colon which may lead to diarrhoea, urgency, tenesmus and incontinence even in the absence of active inflammation.[107] In the bygone era of barium enema investigation, it was not uncommon to see the so-called ‘lead-pipe’ colon, characterised by a loss of haustral folds, shortened length and reduced caliber in patients with long-term disabling UC.

Colonic inflammation may damage and alter the number and function of the nerves, glial cells (which support and insulate neurons) and the interstitial cells of Cajal (which

act as pacemakers and regulators of smooth muscle contraction).[108,109] This suggests that in UC it isn't just the mucosal layer which is targeted by the disease. Furthermore, smooth muscle cells have been shown to be permanently altered in UC, even after the inflammation has subsided.[110]

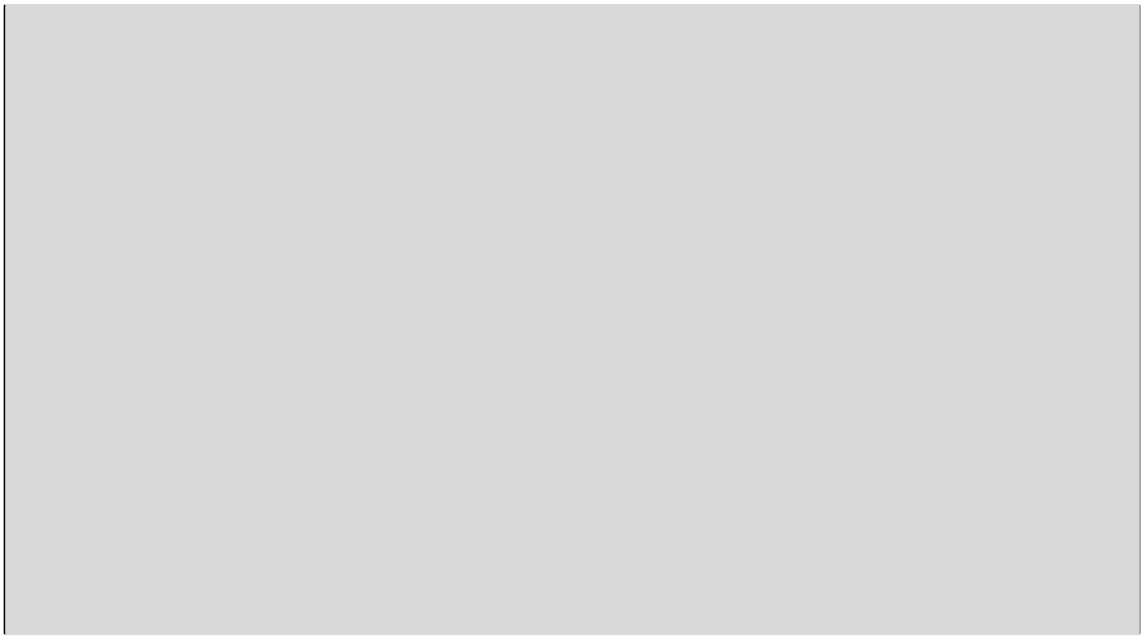
1.5.4 Colorectal cancer

Patients with UC and colonic CD are at increased risk of colorectal cancer and hence national and international guidelines suggest frequent surveillance in all patients apart from those with proctitis.[69,111,112] In a 2001, a meta-analysis based on 116 studies reported a cumulative probability of colorectal cancer in UC of 2% after 10 years, 8% after 20 years, and 18% after 30 years of disease duration.[20] However, this study may have overestimated the risk of colorectal cancer, as many of the studies included were from the pre-surveillance era. In a more recent study of a large cohort of 1356 patients undergoing surveillance at a tertiary Canadian IBD centre, the incidence of colorectal cancer/high grade dysplasia in UC and colonic CD was 19.5/58.5 and 25.1/37.6 per 100,000 patient-years, respectively.[95] The incidence of dysplasia was low prior to 8 years disease duration, both in UC and CD (19.5 and 12.5/100,000 patient-years, respectively). Reassuringly, and in keeping with current European surveillance guidelines, no colorectal cancer was detected prior to 8 years of disease duration.[111]

There are several phenotypic and genetic differences between sporadic and IBD-related colorectal cancer. IBD-related colorectal cancer affects younger individuals, more frequently progresses to adenocarcinoma from flat and non-polypoid dysplasia, has a higher proportion of mucinous and signet ring cell histology and is more likely to occur in one or more sites of the colon at the same time (a.k.a. 'synchronous') as compared with sporadic colorectal carcinoma.[113] The higher incidence of synchronous

cancer is thought to reflect the broader 'field-effect' of widespread inflammation on the colonic mucosa of IBD patients. Whilst the same broad molecular pathogenic mechanisms are seen in both types of colorectal cancer; namely, chromosomal instability and microsatellite instability, the order of the mutations appear to be different in IBD-related and sporadic colon cancer (**see Figure 1.4-2**) [114]

Figure 1.5-2. Comparison of molecular alterations in sporadic colon cancer and colitis-associated colon cancer.



Mut, mutation. Diagnosis and management of dysplasia in patients with inflammatory bowel diseases. Gastroenterology. 2004; 126:1634–1648. Modified from Itzkowitz SH., Harpaz N. by Xie J. and Itkowitz AH. Cancer in inflammatory bowel disease. World J Gastroenterol. 2008; 14(3): 378–389.

Dysplastic lesions in IBD are categorised either as endoscopically 'visible' or 'invisible'. With respect to colorectal cancer surveillance, multiple (≥ 33 biopsies) throughout the colon used to be advocated in order to detect about 90% of invisible lesions. However, both international[115] and national guidelines[111,116] now strongly recommend utilising chromoendoscopy (dye spray) with targeted biopsies as the standard surveillance practice. With the advent of high-definition white-light endoscopy and chromoendoscopy, 'invisible' lesions now only account for about 10%

of dysplastic lesions.[115] Endoscopically visible lesions are described as either polypoid or non-polypoid-like with management determined by the size, morphology, ulceration and border. Often the fibrosis associated with inflammation makes removal of the lesions difficult and requires endoscopic mucosal resection, endoscopic submucosal dissection or hybrid techniques. Colectomy is not necessary if a dysplastic polyp can be entirely removed endoscopically in the absence of dysplasia in the surrounding tissues. Traditionally colectomy was advocated for invisible lesions, however, now with the advent of chromoendoscopy for both low- and high-grade invisible dysplasia repeat surveillance by an experienced endoscopist is recommended and the relative risks of continued intense surveillance versus colectomy are discussed with the patient.[117] While proctocolectomy abolishes the risk of CRC, it does not remove the low risk of anal cancer or cancer of the rectal cuff or ileo-anal pouch.

Chapter 2

2 Introduction to precision medicine in IBD

Precision medicine is a “move away from a *one size fits all* approach to the treatment and care of patients with a particular condition, to one which uses new approaches to better manage patients health and target therapies to achieve the best outcomes in the management of a patient’s disease or predisposition to disease”. This concept is far from new, and some 2500 years ago the Greek physician Hippocrates wrote of the individuality of disease and the necessity of giving ‘different [drugs] to different patients; for the sweet ones do not benefit everyone, nor do the astringent ones, nor are all the patients able to drink the same things’.[118]

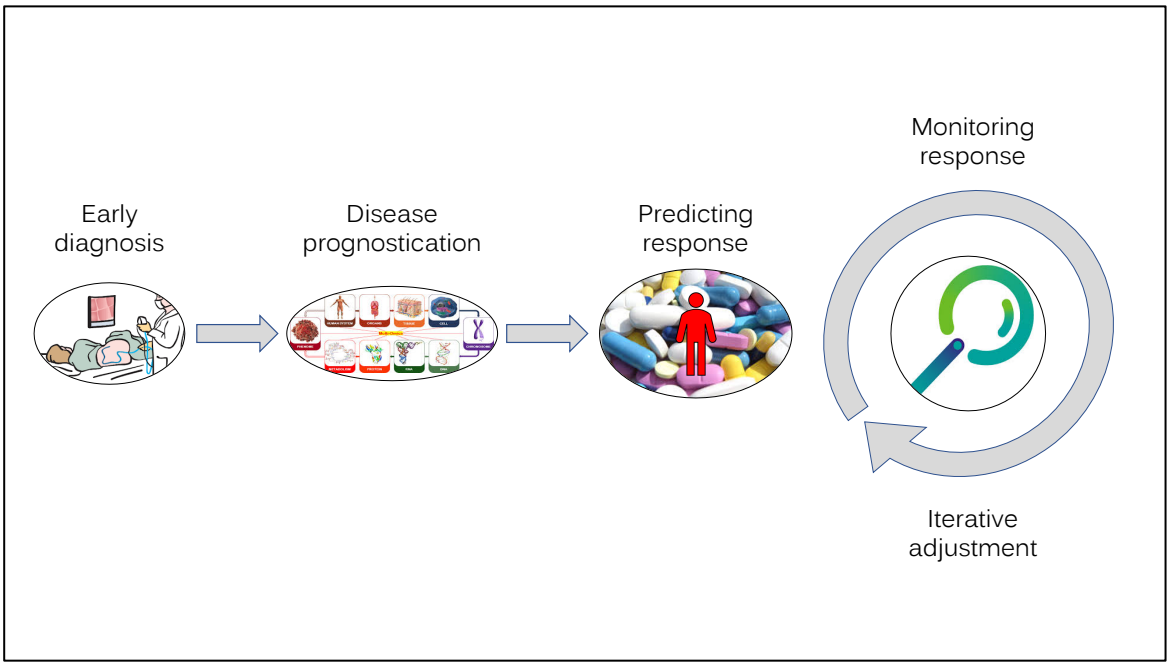
Over the next couple millennia little progress was made or indeed possible beyond characterising responses in patients based on the most rudimental of phenotypic differences, and in 1892, Sir William Osler commented that, ‘If it were not for the great variability among individuals, Medicine might be a Science, not an Art’.[119] However, just over 100 years after Osler made this statement, scientific advances in the field of genetics meant that clinicians could observe inherited differences between individuals which were associated with response to therapies. A new era of precision medicine had arrived in which clinicians sought to exploit new biomarkers, serological, and genetic data to improve patient response and possibly in the future, even prevent disease.

2.1 Precepts of precision IBD medicine

The need for a new approach in the field of IBD is obviously apparent: current treatment paradigms mean that many patients still suffer a complicated disease course with multiple drug side-effects, hospital admissions and IBD-related surgeries.[120,121] The key to harnessing the potential of precision medicine in IBD is to improve the timing and delivery of healthcare by targeting both pharmacological and non-pharmacological treatment according to specific clinical and biological characteristics of individual patients.[122,123] The central precepts of precision IBD medicine that will be summarised in the following introduction are (**see Figure 2.1-1**):

1. making an early diagnosis
2. prognostication of disease course and complications
3. predicting drug response and adverse drug reactions
4. monitoring response to treatment with stringent targets and iterative adjustments

Figure 2.1-1. Schematic showing the components precision medicine in IBD



This precision medicine approach aims to identify IBD patients early in their disease course, when therapies appear most effective and prior to the development of complications.[124] Physicians will be able to identify those patients who are likely to suffer a complicated disease course and target them with early more aggressive pharmacotherapies. Conversely, patients destined to follow a benign disease course could be spared the risks of such therapies. Clinicians will select the right treatment or combination of treatments based on the pathophysiological mechanisms driving a particular individual's disease. The right treatment will not only vary between patients who have the same disease but also within an individual patient as they get older and their body changes. Additionally, this will not only facilitate escalation of treatment, but safe and appropriate de-escalation in order to minimise the risk of disease relapse and reduce long-term drug side-effects. Deeper pharmacokinetic and pharmacodynamic understanding will enable personalised therapeutic drug monitoring and the fine adjustment of drug dosing and intervals in order to meet stringent treatment targets. Arguably, in an era defined by financial constraints, such an approach within the NHS should also have the equalitarian ambition to make the best use of available resources.

2.2 Precept #1: The importance of making an early diagnosis

The concept of disease modification through early treatment has revolutionised rheumatology and dermatology practice. In IBD, evidence is now accumulating that early diagnosis allows early intervention in a window of opportunity, prior to irreversible bowel damage, when therapies are more effective.

Post hoc analyses of several individual and pooled IBD studies stratified by disease duration at time of enrolment suggest a therapeutic treatment window in which treatment responses were better in CD patients treated early in their disease course.[125–129] For example in a sub-analysis of the SONIC data, the benefit of combination therapy in achieving a stringent composite outcome of clinical remission, mucosal healing and CRP normalisation, was most apparent in patients whose treatment was instigated within 18 months of diagnosis (early disease cohort: IFX combination therapy = 65%; IFX monotherapy= 25%; AZA monotherapy = 10%, [*P* = 0.010]; late disease cohort: IFX combination therapy = 44%; IFX monotherapy = 26%; AZA monotherapy = 14% [*P* = 0.069]).

Unfortunately, there are a paucity of prospective data supporting the early initiation of IBD therapies. As afore mentioned in Chapter 1, the RAPID[80] and AZTEC[79] studies failed to demonstrate an advantage to the early initiation of thiopurine monotherapy, however, to date no study has done the same for anti-TNF mono- or combination-therapy in adults. In contrast in a paediatric CD cohort, the RISK study reported higher rates of one-year steroid-free remission, and reduced progression to year-three internal fistulating complications in patients who received anti-TNF within three months of diagnosis.[59]

The immunological mechanisms underlying the benefits of aggressive early treatment are largely unknown, although differences in the peripheral but not mucosal IL-17/Th1 response[130] as well as a diminished T-cell response to Th1 cytokines in human cell studies[131], and a blunted response to CCR9/CCL25 blockade in treating murine colitis[132] have all been described as differing in late and early disease.

Whilst early combination therapy may offer patients the best chance of long-term disease modification[133,134], such an approach remains expensive, even with the advent of biosimilars, and risks exposing patients who might only ever have suffered a benign disease course to potentially life threatening side effects.[85–89] For other patients, an early surgical intervention is a better option than medical therapy and offers long-term drug free clinical remission.[92,93] Central to these decisions is the concept of disease prognostication; allowing clinicians to predict which patients are likely to suffer disabling sequelae in order that they may apply the correct therapeutic strategy to each individual.

2.3 Precept #2: Prognostication of disease course and complications

Despite extensive work investigating clinical, genetic and serological markers of IBD susceptibility there are no routinely used tools that reliably predict disease course at the point of diagnosis in IBD. Such an advance would not only allow precision treatment strategies but would also revolutionise clinical study design which may have been flawed by disease outcome confounders in the past. Terms such as ‘complicated’ and ‘disabling’ disease are used interchangeably, but invariably refer to patients that require hospitalisation, surgery, prolonged steroid exposure and cancer. It is not only necessary to identify those patients at high risk of disabling disease, but also those patients at low risk of disease progression, to inform discussion of the risks and benefits of treatment options.[1] In future, a combination of genetic clinical and serological markers nay be used to predict disease course and response to treatment. Furthermore, clinicians may come to revise the current sub-phenotypes of IBD (CD, UC, IBD-U) in favour of new descriptors that better reflect the heterogeneity of the disease and the diverse outcomes that are seen in everyday practice. In the following

section I will summarise progress in the field of predicting disease course and behaviour.

2.3.1 Clinical criteria

Simple clinical features have long been used to predict the likelihood of a complicated disease course. In CD, this phenotype has been associated with clinical factors such as an initial need for steroids, age below 40 years old at time of diagnosis and the presence of perianal disease at diagnosis.[135] Although scoring systems using these clinical biomarkers have been replicated and found to be sensitive predictors of disabling disease in IBD patients Worldwide[136], they lack specificity and are present in many patients with benign disease who are therefore at risk of being overtreated. Other predictors include extensive small bowel involvement, severe upper GI disease, early stricturing or penetrating disease and smoking.[137] In contrast, patients with colonic CD rarely require resective surgery, remaining free of complications for many years.[121] Patients with childhood-onset CD are more likely to have severe disease that requires immunosuppressant treatment, although stricturing, penetrating complications and surgery don't appear more common.[138] Whilst many genetic factors have been implicated in the pathogenesis of IBD, patients with a strong family history of CD seem to have no worse a prognosis or disease course when compared with sporadic cases.[139]

Environmental factors may also predict disease course. Following Crohn's disease-related surgery, smoking is associated with a doubling of the risk of disease recurrence and need for further surgery: indeed this can be used to stratify patients who are most appropriately started on prophylactic immunosuppressive treatment following ileal resection.[140]

In UC, approximately 80-90% of patients suffer a relapsing disease course, with disease activity and extent in the first couple years predicting subsequent disease activity over the next few years.[141,142] Patients with more extensive and aggressive disease have a higher risk of relapse, need for surgery and risk of developing colon cancer.[137] Extensive colitis, with disease proximal to the splenic flexure, at presentation is the most reliable independent predictor of colectomy within 10 years.[141,143,144,145] Smoking cessation increases the severity of UC and the risk of being admitted into hospital admission for rescue medical therapy.[146] Unlike CD, UC is a fairly dynamic disease with 27%–54% of patients with initial proctitis or left-sided disease showing proximal extension during the course of their disease.[110] Indeed, studies from the UK[147] and Denmark[141] both suggest that this cohort of patients with proximal extension of disease have a higher rate of colectomy as compared with patients with extensive disease from the outset. Young age at diagnosis[141,148] and the presence extra-intestinal manifestations[141] have both been associated with higher risk of proximal extension.

Following colectomy and ileal pouch-anal anastomosis, some UC patients develop complications such as chronic pouchitis and a Crohn's Disease-like phenotype. These complications are increased in the presence of enteropathic arthropathy, primary sclerosing cholangitis (PSC) and a longer duration of time to ileostomy closure.[149]

2.3.2 Faecal biomarkers

2.3.2.1 Predicting relapse after drug withdrawal in IBD

Two studies exploring the clinical and biochemical factors associated with disease relapse following infliximab withdrawal in patients in long-term corticosteroid-free remission have reported the value of the biomarker faecal calprotectin. Both STORI[150] and Kennedy *et al*[151] found that that a calprotectin of ≥ 300 µg/g and > 50 µg/g, respectively, increased the odds of relapse 3-fold, independent of factors such as age of disease onset, white blood cell count and CRP. Kennedy *et al* reported that re-treatment with infliximab was effective and well tolerated in 88% of patients who experienced a relapse. These studies suggest that faecal calprotectin can be used with other readily available biomarkers to help clinicians decide whom they might safely de-escalate treatment. Whether patients in long-term remission on combination therapy should have their thiopurine or anti-TNF, or both withdrawn, is unknown and the subject of the SPARE study (a prospective randomised controlled trial comparing infliximab and immunomodulatory combination therapy vs. immunomodulator monotherapy and infliximab monotherapy in CD).[152]

2.3.2.2 Predicting relapse in IBD

Costa *et al*[153] report that the median calprotectin in CD patients who relapsed and those who maintained remission was no different among 28 patients in clinical remission and followed-up with serial biochemical and faecal tests over a year: 220 µg/g [95%CI 22 - 419 µg/g] vs. 221 µg/g [95%CI 53 - 388 µg/g], respectively; P = 0.395. In contrast, the same study in 41 UC patients, found that the median calprotectin was higher in those who suffered a relapse than those who didn't: 221

µg/g [95%CI 86 - 355.2 µg/g] vs. 67 µg/g [95%CI 15 - 119 µg/g], respectively $P < 0.001$. The proportional multivariate Cox hazard regression model showed a 2-fold and 14-fold increase in the relapse risk in those patients with CD and UC, respectively, who had a faecal calprotectin concentration > 150 µg/g, independent of other confounding variables. Interestingly in UC, De Vos *et al* report a rise in calprotectin above 300 µg/g may precede a flare by up to 3 months, and that two such consecutive calprotectin measurements with a 1-month interval was the best predictor for this with a 62% sensitivity and 100% specificity.[154]

2.3.2.3 Predicting relapse after ileocaecal resection in CD

The current gold-standard of post-ileocaecal resection evaluation is to review the pre-anastomotic, anastomotic and neo-terminal ileal mucosa at 6 months after surgery and to escalate treatment according to the severity of inflammation as defined by the Rutgeerts score.[155,156] Faecal calprotectin is a good surrogate marker of endoscopic disease recurrence as defined by the Rutgeerts score.[157] In a recent systematic review and meta-analysis of 9 studies by Tham *et al* the authors report that the optimal diagnostic accuracy for determining endoscopic recurrence was obtained using a calprotectin threshold of 150 µg/g. This resulted in a pooled sensitivity of 70% [95%CI 59 – 81%] and specificity 69% [95% CI 61 – 77%] with an area under the receiver operating curve of 0.73.[158] However, given the clinical repercussions of re-instigating long-term immunosuppressive medications, it is unlikely that a 30% false negative rate will mean that calprotectin will replace endoscopic evaluation for this purpose.

2.3.3 Serological Antibodies

Subsets of IBD patients may have an abnormal immune responses to various microbial antigens, which can be used not only to diagnose IBD, and distinguish CD

from UC[159], but also help predict the course of disease.[160–162] Anti-Saccharomyces cerevisiae antibodies (ASCA), Escherichia coli outer-membrane porin C (OmpC), anti-CD related bacterial sequence 12 (anti-I2) and CBir1 flagellin (anti-CBir1) have been associated with early CD onset, a fibrostenosing and penetrating disease course and the need for early small bowel surgery.[163–166] Combining these serological antibodies with age of IBD onset, as well as environmental factors such as smoking can be used to ascertain useful predictions for risk of future surgery in CD.[167] In the population-based IBSEN cohort, ASCA-positive patients aged less than 20 years old who had been diagnosed with penetrating disease and had been initially treated with systemic steroids had a probability of CD-related surgery of 97%.[167]

2.3.4 Genetic factors

2.3.4.1 Genetic factors for disease prognostication

Compared to clinical parameters or serologic markers, genetic markers are more appealing for risk stratification as they are present long before the onset of disease, stable over time and unaffected by disease flares.[168] However, in contrast to the 240 loci found to be associated with susceptibility to IBD, only a few genetic markers have been shown to predict its course.[169,170] Patients carrying a *NOD2* genetic variant were previously thought to be more likely to require earlier resection surgery and experience a higher rate of post-operative recurrence than patients who don't share this allele.[171–175] However, contemporary studies subsequently showed that this association was entirely driven by the association between *NOD2* and ileal disease, which is more commonly treated with surgery.[27]

In the first study to explore prognostic as distinct from disease susceptibility genes, Lee *et al* reported a candidate gene study that identified a non-coding SNP in FOXO3 which was associated with a milder course of CD.[176] In subsequent functional experiments the authors found that the FOXO3-driven pathway abrogated inflammatory responses in monocytes via TGFβ1 and led to reduced TNF-α and IL-6 production in carriers of the mild CD-associated allele.[176]

Gene expression profiling studies illustrate the dynamic function of genes *in vivo* by simultaneously measuring the activity of thousands of genes to create a global picture of cellular function. This design was used by Lee *et al* to further define an expression signature within peripheral blood CD8 T-cells isolated from patients with active untreated UC and CD which stratified all patients into two clinically indistinguishable subgroups, called 'IBD1' and 'IBD2'.[177] Prospective follow-up demonstrated that those in the IBD1 subgroup had a high incidence of treatment-refractory, relapsing disease, while those in the IBD2 subgroup typically achieved stable remission on minimal immunosuppression. These markers were independent of biomarkers for inflammation and superior to clinical and serological factors. To aid translation of this work Lee *et al* have detected a similar gene signature detectable in whole blood samples. This work has formed the basis for PROFILE, a prospective UKCRN study to stratify patients based on this prognostic biomarker to either step-up or top-down therapies; the results are eagerly awaited and may transform practice. In children, similar gene expression signatures that predict disease course have also been discovered.[59]

2.3.4.2 Genetic factors for cancer prediction

Many of the genetic alterations responsible for sporadic colorectal cancer such chromosomal instability, microsatellite instability and CpG island hypermethylation also occur in IBD-related colorectal cancer.[178] Genetic biomarkers may help assess the future risk of colorectal cancer in UC patients; a microarray study found 40 genes that were differently expressed between 10 UC patients diagnosed with cancer and 43 UC patients without cancer.[179] Included in this list of genes was *LRP5* and *LRP6* (low-density lipoprotein receptor–related protein), which promote cancer cell proliferation, tumorigenesis and are considered candidate oncogenes.[179] Studies reporting differential methylation of genes associated with carcinogenesis (e.g. *CDKN2a/p16INK4A, CDKN2a/p14ARF, CDH1, MLH1, HPP1* and *MYOD1)* in the colonic mucosa of UC patients with dysplasia and/or carcinoma as compared with the quiescent mucosa from the same patients may provide an insight into the role of unchecked inflammation in the pathway to IBD-related colorectal cancer.[68,180,181] Interestingly, changes in gene expression[182] as well as abnormal number of chromosomes in a cell (aneuploidy)[183] and loss of p53 heterozygosity[184] occur in the normal looking rectal mucosa of patients with IBD-related colorectal cancer elsewhere in the colon; these changes may act as a biomarker in patients at high risk of dysplasia/cancer and thus could be incorporated into future personalised surveillance algorithms.

2.3.5 Combining genetic, serological and clinical factors

The paediatric RISK CD inception cohort enrolled over 1000 children at diagnosis from 28 sites and sought to define factors associated with a complicated disabling disease course within the first 5 years after diagnosis.[59,185] A validated model was developed

which integrated clinical and serological biomarkers. Those patients of older age-of-onset, African American ethnicity, and ASCA IgA and CBir1 seropositivity were shown to be at higher risk of stricturing and fistulating disease.

2.4 Precept #3: Predicting drug response and adverse drug reactions

Rather than arriving at the best therapy by chance or following multiple failed trials of alternative therapies, one of the cornerstones of precision medicine is to select the drug or combinations of drugs with the greatest effectiveness and the least toxicity. Therapeutic options are rapidly expanding and represent the major cost of IBD healthcare. As a consequence, optimal selection/use of therapies is now an urgent priority. In the following sections exploring prediction of response to drugs used in the treatment of IBD, I will primarily focus on biologic drugs and in particular anti-tumour necrosis factor (anti-TNF) therapies, as arguably these are the most effective therapies for IBD.

2.4.1.1 Predicting response to anti-TNF

TNF-α is potent pro-inflammatory acute phase response cytokine produced mainly by macrophages but also by lymphocytes, natural killer (NK) cells and neutrophils. Anti-TNF treatment has been one of the mainstays of the treatment of IBD for more than two decades, although treatment failure is common: 40% of patients fail to respond to induction therapy (primary non-response, PNR), 46% of patients suffer secondary loss-of-response in the first year of treatment (LOR), and approximately 10% suffer an adverse drug reaction that curtails treatment.[84] Variability in response to anti-TNF therapies, may be a consequence of pharmacokinetic and pharmacodynamic factors, both of which may be influenced by genetics.

A number of clinical factors have been reported to be associated with primary non-response to anti-TNF therapy including body mass index (BMI), longer disease duration, older age at diagnosis, previous surgery, and absence of mucosal lesions.[186–189] However, these factors are neither sensitive nor specific enough to be clinically useful.

Multiple small case control studies have investigated the genetic determinants of response and non-response to anti-TNF drugs, both in IBD and in a range of other inflammatory conditions.[190–192] To date, results have been inconsistent with no clinically useful marker identified. In the largest hypothesis free study to date, GWAS methodologies were used to explore PNR among 474 patients with IBD [359 CD, 99 UC, 16 IBD-U] of European ancestry.[193] A genetic risk score was constructed using 11 IBD susceptibility loci associated with PNR with a *P* value < 0.05 and 4 novel variants associated with PNR with a more stringent *P* value of < $1x10^{-4}$. Like many previous reports in this field this study was limited by a retrospective design and lack of therapeutic drug monitoring (TDM) data (to distinguish pharmacokinetic from pharmacodynamic treatment failure).

Pre-treatment gene expression from mucosal biopsies may help to determine future response to anti-TNF and could attractively be performed at the time of diagnosis. In a study of UC patients by Arijs *et al* expression profiles of 5 genes separated non-responders from responders with a sensitivity of 95% and 85% specificity.[194] However, like many similar studies responders and non-responders were stratified by expression levels *after* drug administration. Therefore, it is likely that these biomarkers

simply represent another proxy measure of inflammation, when what is really needed is a biomarker that separates patients *before* drug exposure which predicts response to anti-TNF prior to induction. In addition, gene expression profiling is challenged by its inherently unstable nature and sensitivity to external factors such as environmental factors and drugs.

Measuring tissue cytokine levels targeted by drugs may seem like a more obvious correlate of future treatment success. Atreya *et al* used FITC labelled adalimumab, which was applied directly to the mucosa at the time of pre-treatment colonoscopy using a spray catheter, and real-time confocal laser endomicroscopy to yield specific signals for cells expressing mucosal-TNF (mTNF).[195] After *in vivo* imaging the patients were then treated with adalimumab and clinical response evaluated at week 12. The authors found a significant correlation in the mean number of mTNF+ cells per confocal image and clinical response. Importantly the high and low mTNF groups did not differ in terms of histological activity or serum CRP. Translation of this promising study has partly been delayed by the legislation surrounding the manufacture of monoclonal antibodies which must meet the same level of European Medicine Agency (EMA) quality control as any other drug.

There are many causes of secondary loss of response, and not all are due to active disease (e.g. fibrostenotic strictures, bile salt malabsorption, functional gut disorder overlap etc.). Immunogenicity and anti-drug antibody (ADAb) formation is the most studied underlying mechanism and leads not only to treatment failure but also infusion reactions. In a recent meta-analysis of 13 studies that evaluated 1378 patients with IBD treated with IFX, the pooled relative risk of secondary loss of response in patients

with ADAb was 3.2 (95%CI 2.0-4.9; *P* < 0.001), when compared to patients without ADAb.[196] The ability to identify patients at risk of immunogenicity prior to treatment might allow targeted use of immunomodulatory therapies or alternative treatments to be used. It is possible that some patients are primed to develop immunogenicity through environmental exposures: in a small exploratory single centre retrospective study from Denmark the presence of high titres of pre-treatment cross reacting murine antibodies targeting the Fab portion of IFX, were associated with treatment failure at 12 months.[197]

The Exeter PANTS study[198] found that among 955 biologic naïve CD patients treated with infliximab and 655 treated with adalimumab the only factor independently associated with primary non-response was low anti-TNF drug levels at week 14; the optimal week 14 drug concentrations associated with remission at both week 14 and week 54 were 7 mg/L for infliximab and 12 mg/L for adalimumab. The proportion of patients who developed immunogenicity (anti-drug antibodies-ADAb) was 63% (95%CI 59 - 63%) for infliximab and 29% (95%CI 24-33%) for adalimumab. In unpublished work, we also report that the Human Leukocyte Antigen (HLA) allele, HLA-DQA1*05, carried by approximately 40% of Europeans, significantly increased the rate of immunogenicity (hazard ratio [HR] 1.90 [95%CI 1.60-2.25]; P = 5.9 $x10^{-13}$). This finding was confirmed in a replication cohort (HR 2.00 [95%CI 1.35 to 2.98]; P = 6.60 $x10^{-4}$) and was consistent for patients treated with adalimumab (HR 1.89 [95% CI, 1.32-2.70] and infliximab (HR 1.92 [95%CI, 1.57-2.33], and for patients treated with anti-TNF therapy alone (HR 1.75 [95% CI, 1.37-2.22] or in combination with an immunomodulator (HR 2.01 [95%CI, 1.57-2.58].

2.4.1.2 Predicting response to anti-integrin therapy

Etrolizumab is a humanised monoclonal antibody that selectively binds the β7 subunit of the heterodimeric integrins α4β7 and αEβ7. In a post-hoc analysis, high as compared with low αE (ITGAE) gene expression at baseline colonic biopsy predicted clinical remission at 10 weeks.

2.5 Precept #4: Monitoring response to treatment

The 'treat-to-target' strategy is based on the frequent assessment of disease activity, using objective markers of inflammation and subsequently adjusting therapy accordingly to reach the pre-established target.[200] The 2015 STRIDE guidelines suggest that the ideal target in both UC and CD should be a composite of clinical and endoscopic remission with faecal calprotectin and blood CRP only used as adjuncts.[201] It is possible that histological remission, that is, healing at the cellular/microscopic level, may be added in the future, although evidence is still being gathered for this endpoint.[202]

'Tight-control' is the use of regular (suggested at least every 3 months) monitoring of patient symptoms and biomarkers to direct treatment escalation. Data from the multicenter open-label CALM study showed that escalation of treatment based on both clinical symptoms and biomarkers (faecal calprotectin and CRP) was superior to a conventional symptom-based approach.[203] Crucially, the study protocol meant that patients in the tight-control arm who were in symptomatic remission but had either a raised CRP and/or faecal calprotectin had their treatment escalated. Escalation in both arms consisted in the sequential use of adalimumab every other week, adalimumab weekly, and finally adalimumab weekly plus azathioprine. At week 48, patients in the

tight control arm had better clinical and endoscopic outcomes than in the conventional treatment arm. These data suggest that tight-control may prevent, or at least delay, disease progression. In the long-term follow up of CALM patients, when patients were stratified by outcomes at 1 year into (1) clinical remission (Crohn's disease activity index, CDAI <150), (2) endoscopic remission (Crohn's disease endoscopic index of severity, CDEIS <4 with no deep ulcerations); and, (3) deep remission (CDAI <150, CDEIS <4 with no deep ulcerations, and no steroids for ≥ 8 weeks) both endoscopic remission and deep remission, but not clinical remission were significantly associated with lower risk of major adverse outcomes (new internal fistula/abscesses, strictures, perianal fistula/abscesses, CD hospitalisations, or CD surgeries). Thus, the authors concluded that the early CD patients who achieve endoscopic or deep remission after 1 year of intensive treatment are less likely to have disease progression over a median of 3 years' follow-up.[204]

Chapter 3

3 The role of faecal calprotectin in the delivery of precision IBD medicine: making an early diagnosis

3.1 Background to the chapter

Calprotectin is an abundant calcium and zinc binding protein with antimicrobial and anti-proliferative activity found in the cytosol of neutrophils and to a lesser extent monocytes and reactive macrophages.[205] It can be measured in numerous fluids including serum and faeces, although for discriminating IBD from healthy controls, faecal is superior to serum calprotectin.[206] Raised calprotectin levels detected in stool reflect cellular damage and apoptosis and therefore correlate with levels of gastrointestinal inflammation.[207] This enables its use as a biomarker for a number of purposes in gastroenterology including: differentiation of inflammatory and functional disease in patients presenting with lower GI symptoms; evaluation of disease activity; and, assessment of treatment response in patients with a prior IBD diagnosis. In this regard, faecal calprotectin has been shown to outperform older inflammatory biomarkers such as FBC, ESR and CRP, both in distinguishing IBD from functional gut disorder, and as an adjunct in the assessment of disease activity in patients with known IBD.[208–210]

3.1.1 Measurement of faecal calprotectin

Calprotectin levels are higher when time between bowel movements is longer; hence morning stool samples are recommended in order to measure it at its nadir. Calprotectin is stable in faeces stored at room temperature for up to 3 days, thereafter, levels fall by approximately 28% between days 3-7 days, which may be of clinical relevance at lower calprotectin thresholds in the 100-200μg/g range that is most commonly used to influence investigation and treatment decisions.[211] Further sample variability may be introduced by the method of faecal extraction; in comparison to manual weighing, commercial extraction devices may under-recover calprotectin by 8-28%.[212] Once extracted into buffer, calprotectin levels are stable for ~2.5 months at −20°C.[213]

Several faecal calprotectin tests are available to the NHS in England, including[214]:

i. fully quantitative laboratory-based tests (mostly ELISA based platforms)
ii. fully quantitative rapid tests (Immunoassays)
iii. semi-quantitative point−of−care tests (POCTS) (Immunochromatographic rapid test)

For each type of test there are several methods of performing the assay and multiple manufacturers. The most commonly used methods of calprotectin testing include:

3.1.1.1 Enzyme Linked Immunosorbent Assay (ELISA)

An antibody against calprotectin is attached to a microtiter plate by the manufacturer. When the patient faecal sample is added to the plate, calprotectin within the sample is bound by the antibody. The plate is washed to remove remaining sample and a detection antibody to calprotectin which is linked to an enzyme is then added. When

an enzyme substrate is then added the product of this reaction is proportional to the amount of calprotectin present and can be detected by colorimetry or fluorimetry (**see Figure 3.1-1**).[214] Whitehead *et al* demonstrated that different manufacturers of calprotectin ELISA assays differ in their performance and precision profiles.[212] In this study of three commonly used two-step ELISA assays (EK-CAL, by Bühlmann Laboratories AG; PhiCal by Immunodiagnostik AG and Calprest by Eurospital) the authors found that mean intra-assay imprecision (as measured by percentage coefficient variation [%CV]) was 6.4%, 10.0% and 10.1%, respectively. The inter-assay %CV ranges were 5.3–8.2%, 7.0–8.9% and 7.1–8.2%, respectively.[212] A general laboratory rule of thumb is that the intra-and inter-assay %CV should be <10% and <15%, respectively, and therefore all three assays tested met this criteria.

Figure 3.1-1. Examples of different enzyme-linked immunosorbent immunoassays (ELISA's)

Direct, enzyme labelled primary antibody 'directly' binds to the antigen (Ag) that is immobilised to the plate surface. The enzyme then reacts with substrate to produce measured visible signal proportional to concentration of Ag. Indirect, both primary and secondary antibody used with enzyme bound to second antibody. Sandwich, instead of binding the Ag to the plate, it is the capture antibody which is bound to the plate. The Ag binds to this antibody, which is then bound by the primary antibody. Thirdly, the secondary antibody bound to enzyme binds the primary antibody. Image taken from *https://www.bosterbio.com/protocol-and-troubleshooting/elisa-principle*

3.1.1.2 Time-resolved fluorescent immunoassay (TRFIA)

The TRFIA is similar to an ELISA, with a capture antibody which has been adsorbed onto the surface of a microtiter plate, and steps involving adding sample, washing and using a detection antibody. During standard fluorometric detection, excitation and

emission both occur at the same time, which can lead to self-quenching when the two overlap. In contrast, TRFIA relies on the use of very specific fluorescent molecules, called lanthanide chelate labels, which allow detection of the emitted light to take place after excitation has occurred with fluorescence measured during a defined time window. The most commonly used lanthanide chelate label is the Europium ion (Eu3+). A further advantage to a TRFIA is that biological samples, which often have their own autofluorescence, are washed away, thus reducing background noise. These technical differences mean that TFRIA's are more sensitive, have a greater measurement range – as there is no need to further dilute samples, and are ~25% cheaper, as compared with ELISA's.[215,216]

3.1.1.3 Point-of-care semi-quantitative/ quantitative tests

Most semi-quantitative and quantitative methods are based on a chromatographic immunoassay involving lateral flow of sample and reagents along a membrane strip encased within a plastic palette which contains a sample well and an elongated window for result viewing (**see Figure 3.1-2**). The expected position of the lines developed during the test are usually imprinted on the plastic at the side of the window. The test lines contain anti-calprotectin antibodies and the control line contains anti-immunoglobulin antibodies, both of which have been dried onto the membrane strip. When the liquid sample is added to the sample well the labelled antibodies bind to calprotectin in the sample and the complex migrates along the membrane by capillary action. The calprotectin/labelled antibody complexes are bound to the test line by the immobilised antibodies to a different part of the calprotectin molecule. The unbound labelled antibody moves on to be bound by the immobilised immunoglobulin antibodies in the control line. A visible developed control line is essential to show that

the test has run properly. Where there is more than one test-line they indicate different concentrations of calprotectin present. The results are available after 10-15 minutes and can be quantitative or semi-quantitative; the latter show the results as ranges or as a traffic light rating scale. Lateral flow technology is ideal for home use and may, if proven reliable and robust, herald a new era in disease monitoring in IBD with electronic patient smart phone links to clinician databases. [157,217]

Figure 3.1-2. Schematic of lateral flow assay

Labelled antibody-calprotectin complexes migrate along the membrane by capillary action and are bound by the primary antibody to a different part of the calprotectin molecule. Taken from https://www.cd-diatest.com/[218]

3.1.2 Applications of calprotectin in gastroenterology

The clinical validity of calprotectin is specific to the stated clinical application, which is principally related to the test setting and thereby disease prevalence and pre-test probability.[219] Calprotectin testing is used in primary, secondary and tertiary care centres, both in paediatric and adult patients, and for a range of purposes; namely, differentiating IBS from IBD, IBD treatment monitoring, predicting relapse following drug withdrawal and stratifying therapy.

3.1.3 Distinguishing IBS and IBD in primary care

The following is a summary of the use of faecal calprotectin in primary care which is the focus of the two papers presented later in this chapter.

3.1.3.1 The clinical need for calprotectin

Gastrointestinal symptoms are reported in 8-10% of all presenting complaints reported in new primary care appointments: whilst the majority of these patients will be diagnosed with functional gut disorder a few will have IBD.[220–223] Distinguishing between functional and organic diagnoses based on symptoms alone can be notoriously difficult for GPs and secondary care specialists alike.[214] Whilst blood tests such as haemoglobin, albumin and CRP are specific for the presence of organic intestinal disease they lack sensitivity, and therefore risk missing IBD.[70,224,225]

Several studies have shown prior to the introduction of faecal calprotectin in the primary care setting, that the conventional GP assessment strategy which included history taking, physical examination and serological biomarkers had a high sensitivity, but low specificity for the diagnosis of IBD.[214,226,227] This resulted in a low number of missed IBD cases at the expense of referring many disease-free patients to secondary care.

Endoscopic examination and histological analysis of biopsies remain the 'gold-standard' investigation for the diagnosis and quantification of inflammatory and other organic pathology of the lower gastrointestinal tract, however, this test can be uncomfortable and has notable risks including bleeding (1 in 400) and bowel perforation (1 in 2500).[228,229] Therefore, calprotectin is an attractive non-invasive cost-

effective alternative to endoscopy, especially in children, where a general anaesthetic in usually performed to facilitate the test. Furthermore, it is an attractive option for GPs when venesection in children might be difficult or distressing.

It is estimated that up to one in four CD patients wait more than two years from the onset of symptoms to a definitive IBD diagnosis being made.[230] Delays in the order of this magnitude are associated with an increase in bowel stenosis and intestinal surgery, even when immunosuppressive and anti-TNF therapy use are taken into account.[231] In childhood IBD, diagnostic delay has additionally been associated with malnutrition and growth failure.[232] In UC, data suggest that preventing the proximal extension of disease and reducing exposure of the colon to unchecked inflammation reduces the risk of colonic dysfunction, colectomy[233], dysplasia and cancer[234], as well as reducing sick leave[235] and improving quality of life.[236]

Diagnostic delay is more often reported in CD than UC, most likely as CD may present exclusively with abdominal pain which might mimic and be incorrectly attributed to a functional gut disorder.[231] Amongst CD patients, ileal disease location[230], age less than 40 years old[230], smoking[231], use of NSAIDs[231] and female sex[231] have all previously been independently associated with diagnostic delay. Whereas in UC, male gender and recent NSAID use were associated with a delayed diagnosis.[230] In paediatric and adolescent CD cohorts, male sex was associated with diagnostic delay.[232]

3.1.3.2 Introduction of NICE guidance in the UK

Calprotectin has been NICE (DG11; 2013) approved since 2013 to help clinicians distinguish IBD from irritable bowel syndrome (IBS) in patients in whom cancer is not suspected (Figures 3-3).[214] Although this guidance extended the use of calprotectin to the primary care setting, by the authors own admission, all of the literature they reviewed related to studies in patients who had already been referred by their GP to secondary care. In such 'referred' cohorts there is a higher pre-test probability (prevalence) of organic disease such as IBD, colorectal cancer and diverticulitis, than in an 'unreferred' cohort. Increasing the prevalence of disease results in higher positive predictive values and lower negative predictive values, and therefore, it might not be appropriate to extrapolate performance from one setting to the other. This clearly demonstrated the urgent need for primary care specific calprotectin studies. Given the importance of pre-test probability of disease on the performance of the calprotectin in the primary care setting, herein I will preferentially cite positive (PPV) and negative predictive values (NPV) in preference to sensitivity and specificity which are less effected by disease prevalence.

Figure 3.1-3. NICE DG11 (2013) Section 1.1: Use of faecal calprotectin in adults

Faecal calprotectin testing is recommended as an option to support clinicians with the differential diagnosis of inflammatory bowel disease (IBD) or irritable bowel syndrome (IBS) in adults with recent onset lower gastrointestinal symptoms for whom specialist assessment is being considered, if:

- *cancer is not suspected, having considered the risk factors (for example, age) described in the NICE guideline on suspected cancer, and;*
- *appropriate quality assurance processes and locally agreed care pathways are in place for the testing.*

3.1.3.3 The importance of cut-off thresholds

The clinical validity of faecal calprotectin is dependent on the cut-off threshold used to distinguish positive and negative tests. Most enzyme-linked immunosorbent assay (ELISA) manufacturers and NICE guidance (DG11; 2013) recommend using a cut-off of 50 µg/g to distinguish a positive result suitable for onward referral and investigation.[237,238] However, there is no nationally accepted cut-off value, partly due to the paucity of primary care specific calprotectin literature, but also the large number of different assays used throughout the UK. The aim of the test, and consequently the threshold employed in these settings is quite different: in primary care, the prevalence of IBD is lower and the objective of the test is to 'rule out' disease; a negative result from a test with a high NPV therefore either rules out disease and provides reassurance, or prompts a 'watchful waiting strategy'.[239] In contrast, in secondary care the role of calprotectin is to 'rule in' IBD: increasing the probability of disease in order to justify invasive and expensive tests such as endoscopy and/or cross-sectional imaging; therefore, a high positive likelihood ratio is preferred.[239] Increasing the calprotectin cut-off threshold increases the specificity of the test and therefore reduces unnecessary secondary care referrals, but this is at the expense of sensitivity, and false negative tests, thus delaying diagnosis of IBD.

3.1.3.4 Primary care specific faecal calprotectin studies

To date, aside from the initial NICE endorsed pilot projects, only two large scale primary care calprotectin studies from Brighton[240] and York[241] have been reported.

Turvill *et al* demonstrated that doubling the calprotectin cut-off threshold from 50 µg/g to 100 µg/g (EK-CAL ELISA, Bühlmann) increased the PPV for identifying IBD from

0.20 to 0.40 with a negligible reduction in NPV from 0.98 to 0.97.[241] This study noted that referral of patients with indeterminate results (first samples in the range 50-100 µg/g) results and ongoing symptoms may be avoided by repeating the test, rather than immediate referral. The probability of IBD in patients with such an indeterminate and then negative test was less than 3%.

In the Brighton cohort of 962 patients who underwent faecal calprotectin testing in the primary care setting, 71% had a negative test (< 50µg/g).[240] 28% (77/276) of the patients testing positive and 3% (17/686) of those patients testing negative were later diagnosed with an organic lower GI disorder such as, colorectal cancer, diverticulitis and IBD. Using a 50µg/g cut-off, the NPV and PPV were 98% and 28%, respectively. Increasing the calprotectin threshold to 150 µg/g reduced the NPV by 1% whilst increasing the PPV to 71%. The authors estimated that by doubling the recommended threshold to 100µg/g they would reduce colonoscopy and flexible sigmoidoscopy bookings by 10% at the cost of four missed cases of inflammatory bowel disease. Interestingly, 38% of the patients tested were outside the age criteria specified in the pathway (18-45 yrs old), suggesting that GPs 'gate-keep' a significant proportion of older patients with lower gastrointestinal symptoms from secondary care referral.

3.1.3.5 Financial Savings

NICE endorsed pilot projects of faecal calprotectin in primary care were completed in Stafford & Surrounds CCG; Yorkshire and Humber ASHN; Northumberland CCG and Durham Dales CCG.[242] Although the projects used a mix point of care tests and different cut-off thresholds the estimated reduction in secondary care referrals ranged from 56-86%, which led to an average cost saving of £134,000 (range £29,000 -

£280,000) over a year. Specifically, the Yorkshire and Humber pilot project rolled out a new calprotectin-based referral pathway across 7 CCGs and 198 GP practices in the North of England.[243] Using a threshold of 50 µg/g to determine a positive result, the authors reported has a 40-57% reduction in new hospital outpatients' appointments and a 21-50% reduction in colonoscopies.

3.1.3.6 Use of calprotectin in paediatric patients

Approximately 10% of IBD presents before adulthood, with a median age at diagnosis of 11.5 years old. As in adults, clinical symptoms are a poor discriminant of IBD and non-IBD pathology. Typically, only 25% of children diagnosed with CD present with the usual triad of diarrhoea, weight loss and abdominal pain, and nearly half report no diarrhoea.[244] Calprotectin varies with age and is typically higher in children aged 1-4 yrs old than in adults, although levels in older children and adults are approximately the same.[245] This observation may reflect increased migration of neutrophil granulocytes into the gut lumen in early life along with development of the gut immune system and establishment of a normal bacterial flora.[246]

NICE guidance for use of calprotectin in paediatric primary care patients differs to that in adults (**Figure 3.1-4**): specifically, it should be used as an option to help clinicians distinguish IBD from non-IBD, but importantly, only in referred patients. In this regard, it is really a tool to help secondary care clinicians stratify referrals and increase the productiveness of the first clinical encounter, given that a result should already be present at the time of the appointment.

Figure 3.1-4. NICE DG11 (2013) section 1.2: Use of faecal calprotectin in children

> *Faecal calprotectin testing is recommended as an option to support clinicians with the differential diagnosis of IBD or non-IBD (including IBS) in children with suspected IBD who have been referred for specialist assessment, if: appropriate quality assurance processes and locally agreed care pathways are in place for the testing.*

To my knowledge, no primary care faecal calprotectin studies in children have been published, and all data relates to secondary care.

3.1.3.7 The importance of not missing other organic disease

It is important for primary care practitioners that calprotectin not only detects IBD, but also identifies all treatable organic disease, including high risk adenomas and colorectal cancer. It is reassuring that this biomarker performs well in this regard as well. In an urgently referred cohort (median age 64 yrs [IQR 52-73 yrs]) with new gastrointestinal symptoms Mowat *et al* reported that a threshold of 50 µg/g (EK-CAL ELISA, Bühlmann) had a PPV = 0.51 and NPV = 0.98 for detecting colorectal cancer, high risk adenoma and IBD.[247] Likewise, Turvill *et al* found calprotectin performed well in patients referred with suspected colorectal cancer via the two week wait pathway with a NPV of 98.6% for colorectal cancer and 97.2% when including polyps greater than 10mm in diameter.[248]

3.1.3.8 Limitations of current guidance for clinicians

In addition to the setting in which the test is applied, age, symptoms, family history, and comorbidities also all alter the pre-test probability of disease. The NICE implementation document ‘Faecal Calprotectin in Primary Care as a Decision Diagnostic for Inflammatory Bowel Disease and Irritable Bowel Syndrome’

recommended calprotectin is only used in patients aged less than 50-60 years old[249]; older patients with new lower gastrointestinal symptoms have a higher likelihood of underlying colorectal cancer and should be urgently referred without requiring a calprotectin. Likewise, patients with symptoms meeting the relevant criteria in the latest NICE lower gastro-intestinal cancer referral guidelines (NG12; 2017) should also be referred urgently. NG12 no longer refers to 'red-flag' symptoms, unlike the NICE IBS guidelines (NICE; CG61), and instead advises either 'urgent suspected cancer referral', 'routine referral' or 'consideration of referral' based on symptoms and age. However, patients under 50 years old will often report symptoms which NG12 advises GPs to consider referral (**Figure 3.1-5**).

Figure 3.1-5. NICE NG12 (2017) Section 1.3.3

> *Consider a suspected cancer pathway referral (for an appointment within 2 weeks) for colorectal cancer in adults aged under 50 with rectal bleeding and any of the following unexplained symptoms or findings: abdominal pain; change in bowel habit; weight loss; and, iron-deficiency anaemia. [new 2015]*

Rectal bleeding is common, and reported by 10% of the general population[250] and up to 30% of patients with IBS per year.[251] It is likely therefore to occur concomitantly in patients with symptoms of IBS, which has an estimated UK prevalence of approximately 10%.[221] GPs might look to faecal calprotectin to aid them when deciding if onward referral is appropriate, but its use in patients with rectal bleeding is discouraged.[249] The Brighton study, similarly discouraged use of the test in patients with alarm symptoms, but even so symptoms meeting these criteria were present in 14% (136/962) of cases reviewed retrospectively, illustrating the real-world dilemma facing GPs.[240]

3.2 The objective(s) of the chapter

Early diagnosis is an essential element of IBD precision medicine. It has been proposed that primary care use of faecal calprotectin may help distinguish functional gut disorder from IBD and allow earlier diagnosis, but knowledge of how this test performs in this setting is limited. Implementation requires knowledge of the clinical validity of the test in the real-World primary care setting, both in adults and children. This includes exploration of the calprotectin cut-off threshold in order to deliver on the purpose of the test: to distinguish functional gut disorder from IBD.

- **Objective 1:** Assess the diagnostic accuracy of faecal calprotectin in distinguishing functional gut disorder from IBD in adult and non-IBD from IBD in paediatric patients in the primary care setting

- **Objective 2:** Assess the optimal calprotectin cut-off threshold for the purpose of minimising referrals of patients later diagnosed with IBS whilst also not missing IBD

- **Objective 3:** Assess whether faecal calprotectin testing altered primary care referral behaviour

The NICE cancer (NG12) and calprotectin (DG11) guidelines do not reconcile whether clinicians should use calprotectin to guide referral in patients that report GI-alarm symptoms in whom cancer is not suspected by their GP.

- **Objective 4:** Assess whether the presence of GI-alarm symptoms altered the performance of the test in adults

There are a paucity of data relating to factors that influence time to diagnosis in UK patients with IBD. In particular, it is unknown if calprotectin helps triage patients to urgent review or straight-to-test endoscopy.

- **Objective 5:** Ascertain where delays occur in the referral pathway between onset of symptoms through primary and secondary care to diagnosis of IBD

- **Objective 6** Explore the clinical and laboratory factors which influence time to diagnosis in IBD patients, and specifically whether faecal calprotectin reduces time to diagnosis

- **Objective 7:** Assess if a delayed diagnosis is associated with a more complicated disease course in the first year after diagnosis

RESEARCH PAPER I

‘Faecal calprotectin effectively excludes inflammatory bowel disease in 789 symptomatic young adults with/without alarm symptoms: a prospective UK primary care cohort study’

PUBLISHED: Alimentary Pharmacology & Therapeutics, 47(8), pp. 1103–1116. (2018) doi: 10.1111/apt.14563

Walker, G. J., Moore, L., Heerasing, N., Hendy, P., Perry, M. H., McDonald, T. J., Debenham, T., Bethune, R., Bewshea, C., Hyde, C., Heap, G. A., Singh, A., Calvert, C., Kennedy, N. A., Goodhand, J. R. and Ahmad, T.

3.3.1 Summary

Background: Primary care faecal calprotectin testing distinguishes inflammatory bowel disease (IBD) from functional gut disorder in young patients presenting with abdominal symptoms: however, previous evaluations have excluded patients with alarm symptoms.

Aims: We sought to evaluate the diagnostic accuracy of calprotectin to distinguish IBD from functional gut disorder in young adults in whom general practitioners (GPs) suspected IBD; including, patients reporting gastrointestinal alarm symptoms. We hypothesised that calprotectin would reduce secondary care referrals and healthcare costs.

Methods: We undertook a prospective cohort study of 789 young adults (18-46 years old) presenting with gastrointestinal symptoms to 49 local general practices that had undergone calprotectin testing (1053 tests: between Jan 2014 and May 2016) because of suspected IBD. We considered calprotectin levels of ≥100μg/g positive. Primary and secondary care records over 12 months' from the point of calprotectin testing were used as the reference standard.

Results: Overall, 39% (308/789) patients reported gastrointestinal alarm symptoms and 6% (50/789) tested patients were diagnosed with IBD. The positive and negative predictive values of calprotectin testing for distinguishing IBD from functional gut disorder in patients with gastrointestinal alarm symptoms were 50% [95% confidence interval 36-64%] and 98% [96-100%]: and in patients without gastrointestinal alarm symptoms were 27% [16-41%] and 99% [98-100%], respectively. We estimate savings of 279 referrals and £160 per patient.

Conclusions: Calprotectin testing of young adults with suspected IBD in primary care accurately distinguishes IBD from functional gut disorder, even in patients with

gastrointestinal alarm symptoms and reduces secondary care referrals and diagnostic healthcare costs.

3.3.2 Background

Gastrointestinal symptoms constitute 8 to 10% of all presenting complaints reported in new primary care appointments[220,221]: the majority of young adults will be diagnosed with a functional gut disorder; a minority, however, will have inflammatory bowel disease (IBD) and even fewer colorectal cancer.[222,223,252]

Faecal calprotectin (calprotectin) is a stool biomarker that reliably distinguishes IBD and other organic intestinal diseases from functional gut disorder, but its use is only recommended in patients in whom colorectal cancer is not suspected.[214] Many young patients with functional gut disorder will report incidental symptoms, including anorectal bleeding, that together satisfy referral guidelines for suspected colorectal cancer.[251,253] Without the application of clinical acumen a significant proportion of young patients risk being prioritised inappropriately along suspected cancer pathways[253] obviating the potential benefits of calprotectin assessment.

The National Institute for Clinical Excellence (NICE) has endorsed calprotectin based-referral pathways for use in primary care because they estimate that calprotectin increases diagnostic yields of IBD and reduces unnecessary secondary care costs for the investigation and management of functional gut disorder.[214,243] Previous calprotectin studies in primary care setting have largely excluded patients with gastrointestinal alarm symptoms and therefore it is unknown what effect inclusion of these patients would have had on the diagnostic accuracy of the test and subsequent secondary care referrals.[240,241]

3.3.2.1 Objectives

We aimed to assess:

1. the diagnostic accuracy of a new calprotectin based-referral pathway in distinguishing functional gut disorder from IBD in young adults including those with gastrointestinal alarm symptoms.
2. whether calprotectin testing altered referral behaviour in young patients, both with and without gastrointestinal alarm symptoms.

We hypothesised that a primary care calprotectin referral pathway would:

- distinguish IBD and organic intestinal disease from functional gut disorder regardless of gastrointestinal alarm symptoms with clinically useful positive and negative predictive values.
- reduce referrals to secondary care services and subsequent healthcare costs.

3.3.3 Methods

3.3.3.1 Study design

We designed a prospective observational cohort study to describe the diagnostic accuracy of calprotectin testing to exclude IBD in primary care.

3.3.3.2 Clinical setting

The Royal Devon & Exeter (RD&E) NHS Trust is a tertiary referral centre for IBD in the South West of England. We serve the Eastern locality of the Northern, Eastern and Western Devon Clinical Commissioning group comprising 49 primary care practices that serve 378,000 people.[254] Patients with gastrointestinal symptoms are

referred to either gastroenterology or surgical services at the RD&E. A working group comprising representation from primary and secondary care devised a new calprotectin referral pathway in January 2014 based on the 2013 NICE guidance.[214] Patients tested between January 2014 and May 2016 were followed-up for at least 12 months and diagnoses were captured from both primary and secondary care records.

3.3.3.3 Participants

All adult patients aged between 18-46 years old referred on the calprotectin pathway for investigation of gastrointestinal symptoms were included. Exclusion criteria were a previous diagnosis of IBD, suspicion of colorectal cancer, and use of NSAIDS/aspirin within the previous six weeks. The cohort represents a convenience series as calprotectin was not mandated in all patients meeting eligibility criteria and use of the test was at the discretion of General Practitioners (GPs).

3.3.3.4 New East Devon faecal calprotectin referral pathway

We disseminated information about the new calprotectin pathway to GPs through a series of educational meetings. In accordance with NICE guidelines[214], GPs were asked to use calprotectin in patients presenting with lower gastrointestinal symptoms whom they suspected but were uncertain had IBD. Purpose-designed request forms were embedded within routine electronic primary care pathology requesting systems (**Supplementary Appendix 3.3-2**). Incomplete request forms were rejected by the blood sciences laboratory and prompts sent to aid completion. Test results, including defined thresholds, and recommended actions were returned to GPs within 10 days.

3.3.3.5 Variables and data acquisition

Our purpose-designed request forms to captured patient demographic data, presenting symptoms, family history of IBD and colorectal or ovarian cancer. These data were used to determine whether patients met NICE lower gastrointestinal cancer referral guidelines ('Suspected cancer: recognition and referral'; NG12)[253] (**Supplementary Appendix 3.3-1**). Patients satisfying these criteria were deemed to have 'gastrointestinal alarm symptoms'.

GPs were asked the following hypothetical referral question to assess expected referral behaviour: "If calprotectin was not available would you have referred this patient to secondary care?" ["Yes"; "No"; "Unsure"]. GPs were also prompted to send blood tests for full blood count (FBC), ferritin, C-reactive protein (CRP) and coeliac serology (Tissue TransGlutaminase [TTG]). Pathology databases were accessed to capture the above data. Where a laboratory test was reported as greater or less than a threshold, for statistical purposes it was assigned to one more or less than the threshold respectively.

Health-care utilisation data in the year after calprotectin testing including: outpatient clinic referrals (gastroenterology, colorectal and upper gastrointestinal surgeons, dieticians and private clinics); diagnostic imaging (ultrasound [USS], computerised tomography [CT] and magnetic resonance imaging [MRI]) and endoscopy (colonoscopy, flexible sigmoidoscopy and gastroscopy) were recorded from electronic secondary care databases.

3.3.3.6 Faecal calprotectin (index test)

Samples were analysed using a quantitative monoclonal enzyme-linked immunosorbent assay (ELISA, K6927, Immundiagnostik, Bensheim, Germany) on a Dynex DS2 analyser (Werfen, Cheshire, UK). Inter-run coefficient of variation (CV) for the patient pool was 10.1% at 60µg/g. Calprotectin levels were reported in the range 6 to 2100µg/g, with results lower than this reported as <6µg/g and results above the upper analytical limits not diluted and re-assayed but reported as >2100µg/g.

Based on calprotectin manufacturers and NICE recommendations the pre-specified calprotectin cut-offs were: calprotectin ≥100µg/g = positive, calprotectin 50-99µg/g = intermediate and calprotectin <50µg/g = negative. GPs were asked to send repeat stool samples from patients with intermediate results and second samples considered positive if calprotectin ≥50µg/g. We advised referral of patients with a positive calprotectin and GP management for negative tests; although GPs were also able to refer patients whom they felt required specialist review based on their clinical assessment. Regrettably, repeat tests were rarely undertaken for intermediate calprotectin results (50-99µg/g) and excluding these "valid inconclusive" data[255] as per our *a priori* analysis plan, would have removed critical data in the calprotectin cut-off zone, where the test is likely to perform worst and consequently over-estimate its performance. In view of this, we adjusted our analysis and calprotectin test result status was determined by the first calprotectin test only, with values <100µg/g deemed negative, and those ≥100µg/g, positive.

3.3.3.7 Reference standard

The diagnosis of IBD and other organic intestinal disease usually requires ileocolonoscopy with or without gastroscopy and small bowel imaging. This burden of investigation is impractical and inappropriate for the diagnosis of functional gut disorder; therefore, and in common with previous published literature[240], we recorded diagnoses after a 12-month period of follow-up to allow sufficient time for organic pathology to evolve and any missed cases of IBD and organic intestinal disease to be correctly diagnosed.

3.3.3.8 Definition of diagnostic outcomes

Diagnoses were recorded firstly as per the responsible clinician, and then assigned to one of three groups for the final analysis: functional gut disorder; organic intestinal disease and inflammatory bowel disease (**Supplementary Figure 3.3-1**). Clinicians were not blinded to the index test results. The diagnosis of IBD was based on clinical, radiological and histopathological findings.[256] A diagnosis of functional gut disorder was assigned to patients based on a composite of normal lower gastrointestinal endoscopy and cross-sectional imaging with CT or MRI if available, and an absence of organic intestinal disease after at least 12 months' follow-up from the time of the index test, if not. Patients not referred to secondary care were followed-up by a primary care researcher who visited every practice to record the GP diagnosis using the patient's electronic and paper-based Lloyd George notes. Patients with inflammatory lower gastrointestinal lesions insufficient to record a diagnosis of IBD were recorded as having non-specific inflammation, and were grouped as having organic intestinal disease, but not IBD.

Where a definitive diagnosis was not recorded by either a GP or secondary care consultant, relevant data were reviewed independently by two consultant gastroenterologists and a consensus reached (NH, PH). Where data were deemed insufficient to reach a diagnosis an outcome of "lost to follow-up" was recorded and excluded. Patients who were diagnosed with non-enteric diseases (e.g. gynaecological diagnoses, respiratory tract infections, thyroid disease) are reported but not included in the final analyses.

3.3.3.9 Sample size calculation

Because this study was designed as a service evaluation *a priori* power calculations were not undertaken. Rather we decided to allow our new pathway to bed-in and then assessed its utility over two years.

3.3.3.10 Statistical Methods

All analyses were two tailed and *P*-values <0.05 were considered significant. Summary statistics are reported based on normality: descriptive statistics are reported as mean (SEM) and median [IQR]. We included patients with missing clinical data in analyses for which they had data, and specified the denominator for each variable.

Chi-squared analyses were used to compare expected and observed referrals to determine whether calprotectin and alarm symptoms influenced referral behaviour. We calculated observed healthcare utilisation costs based on the 2017 National Tariff[257] in patients referred to gastrointestinal outpatients and/or endoscopy and/or diagnostic imaging. We used the observed proportions of patients investigated to

estimate the expected secondary care healthcare utilisation costs saved by calprotectin testing in patients subsequently not referred to secondary care.

We performed univariable analyses using chi-squared analyses for categorical data, Student's t-test for continuous normally distributed variables and Mann-Whitney U tests for nonparametric data to identify baseline clinical variables and biomarkers predictive of a diagnosis of IBD and organic intestinal disease. Factors that predicted these diagnoses were entered into a stepwise forward multivariable logistic regression model.

Receiver operator characteristic curves and area under the curve (AUC) analyses were undertaken to determine clinical validity of calprotectin as a continuous variable to diagnose IBD and organic intestinal disease: Youden's formula was used to determine the optimal cut-off.[258] Sensitivity and specificity analyses were undertaken to calculate positive (PPV) and negative (NPV) predictive values at our pre-specified cut-off and then at various calprotectin cut-off thresholds to optimise its use in diagnosing IBD and organic intestinal disease. These analyses were performed in patients with and without gastrointestinal alarm symptoms. All statistical analyses were undertaken in Stata (v14.2. StataCorp. College Station, TX USA).

3.3.3.11 Ethical consideration and patient involvement

This project was endorsed by the Local Medical Council, Devon Clinical Commissioning Group, primary and secondary care Caldicott guardians and the Southwest Academic Health Sciences Network (SWAHSN). Patients were not involved in the conception or design of this study and in accordance with UK Health

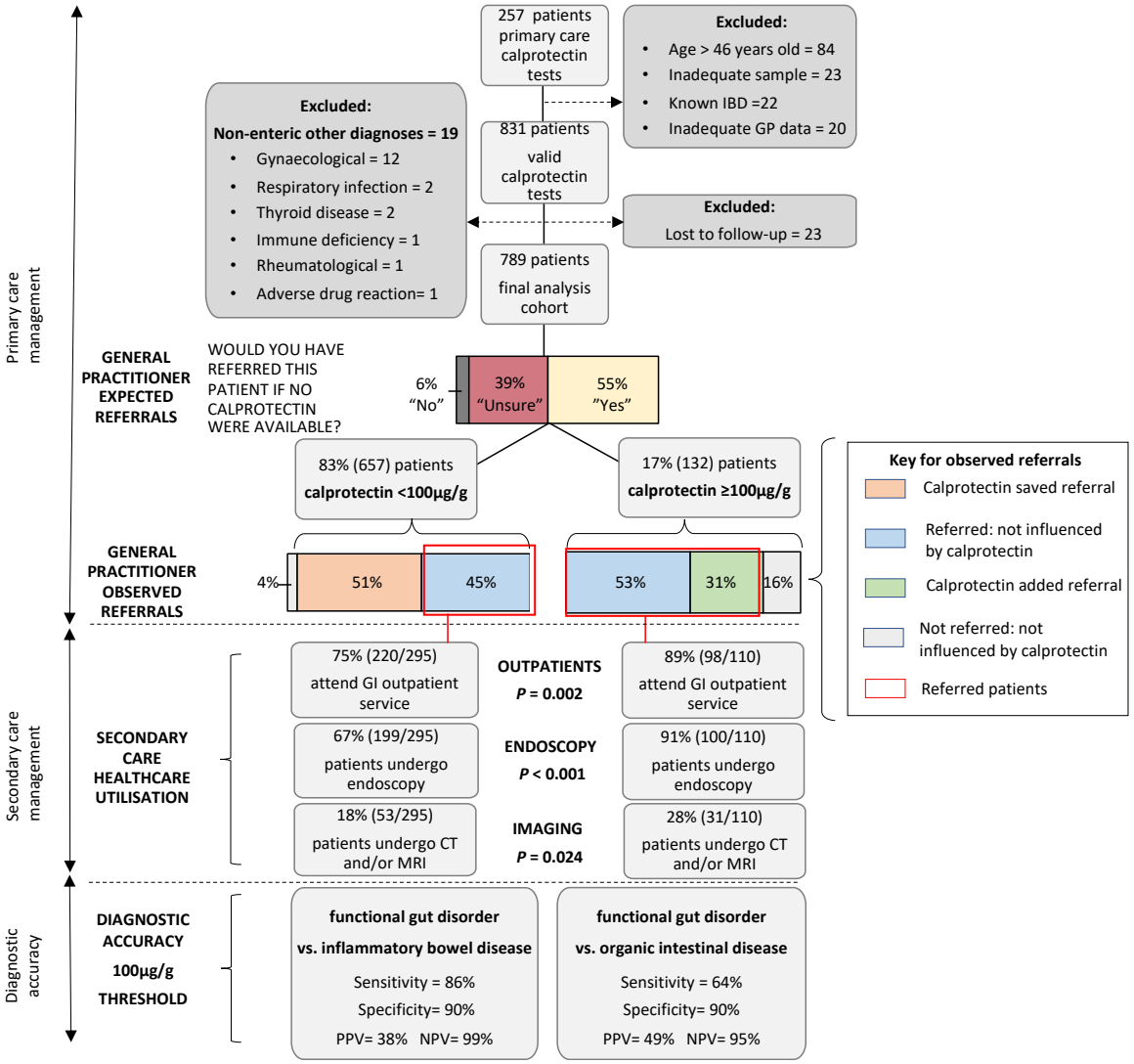
Research Authority guidelines we did not require formal ethical approval.[259] Results will be disseminated to patients through posters, educational sessions at GP practices and lay summaries.

3.3.4 Results

3.3.4.1 Study overview

We report data in the order displayed in **Figure 3.3-1**; 1053 calprotectin samples were received from 980 primary care patients aged 18 to 46 yrs between January 2014 and May 2016. 149 patients were excluded from the final analysis: age ≥46 years (n=84), inadequate faecal sample (n=23), known IBD (n=22), inadequate GP request form data (n=20). Of the remaining 831 adult patients with valid samples, 23 patients were lost to follow-up (calprotectin <100μg/g [n=16], calprotectin ≥100μg/g [n=7]), and a further 19 patients were diagnosed with non-enteric diseases. 789 patients were included in the final analysis. A family history of IBD was reported in 15% (112/757) and of colorectal cancer or ovarian cancer in 11% (84/754). Gastro-intestinal alarm symptoms were reported by 39% (311/789) of all patients: 38% (302/789) rectal bleeding and one or more of abdominal pain/ change in bowel habit/ weight loss/ iron-deficiency anaemia in patients under 50 years old; 2% (16/789) abdominal pain and weight loss in patients over 40 years old. Positive calprotectin tests (≥100μg/g) were returned in 17% (132/789) patients.

Figure 3.3-1: Flow diagram showing derivation of the cohort, faecal calprotectin result and diagnosis of functional gut disorder, inflammatory bowel disease and organic intestinal disease



CT, computerised tomography; MRI, magnetic resonance imaging; NPV, negative predictive value, PPV, positive predictive values; GI, gastrointestinal

3.3.4.2 Primary care management

GPs were asked to report their referral intentions before the calprotectin result was available to them: *"Would you have referred this patient if no calprotectin test were available?*" Over half (55%, 409/739) of GP responses stated that they intended to refer their patient had calprotectin testing not been available, a further 39% (287/739) were unsure if they would have referred, and 6% (43/739) stated that they would not

have referred. Regardless of initial referral intentions, calprotectin testing significantly altered referral behaviour (**Table 3.3-1**). Overall, a negative calprotectin saved 317 referrals; whereas, a positive calprotectin added 38 referrals, and thus 279 referrals were saved overall. Patients without gastrointestinal alarm symptoms were less likely to be referred to secondary care gastrointestinal services than patients with such symptoms (48% [230/478] vs. 56% [175/311], respectively, *P* = 0.025). Likewise, patients with a calprotectin <100µg/g were also less likely to be referred by their GP than calprotectin positive patients (45% [295/657] vs. 83% [110/132], respectively, *P* < 0.001).

Table 3.3-1: Table comparing expected and observed general practitioner referral behaviour by calprotectin result

Expected referral behaviour (pre-calprotectin test result)[a]	**Calprotectin test result (µg/g)**	**Observed referral behaviour (post-calprotectin test result)**		**P value**
		No referral made	**Referral made**	
GP not intending to refer the patient if calprotectin testing were unavailable = 6% (43/739)	< 100	69% (25/36)	31% (11/36)	**0.011**
	≥ 100	14% (1/7)	86% (6/7)	
GP unsure whether to refer the patient if calprotectin testing were unavailable = 39% (287/739)	< 100	70% (170/244)	30% (74/244)	**<0.001**
	≥ 100	26% (11/43)	74% (32/43)	
GP did intend to refer the patient if calprotectin testing were unavailable = 55% (409/739)	< 100	44% (147/336)	56% (189/336)	**<0.001**
	≥ 100	11% (8/73)	89% (65/73)	

*** *P* Value represents chi-squared or Fisher's exact test as appropriate.**

Total saved referrals using 100µg/g calprotectin threshold = (170 + 147) – (32 + 6) = 279; Additional saved referrals if referral pathway strictly enforced = (11 + 74 + 189) – (1 + 11 + 8) = 254; Total saved referrals if referral pathway strictly enforced = 254 + 279 = 533

	Calprotectin saved referral
	Referred: not influenced by calprotectin
	Calprotectin added referral
	Not referred: not influenced by calprotectin

3.3.4.3 Diagnostic accuracy

Primary outcome diagnoses: The incidence of IBD, organic intestinal disease (including IBD) and functional gut disorder was 6% (50/789), 13% (100/789), 87% (689/789) respectively (**Table 3.3-2**).

Table 3.3-2: Frequency of diagnoses contained within each of three primary analysis groups

Inflammatory bowel disease	**% (n)**	**Functional gut disorder**	**% (n)**	**Organic intestinal disease**	**% (n)**
Ulcerative colitis	52 (26/50)	Functional gut disorder/Irritable bowel syndrome	91 (625/689)	Inflammatory bowel disease	50 (50/100)
Crohn's disease	38 (19/50)	Haemorrhoids	4 (25/689)	Upper gastrointestinal disorders	16 (16/100)
IBD-unclassified	10 (5/50)	Symptoms resolved	2 (12/689)	Infective gastrointestinal condition	14 (14/100)
		Eating disorder or chronic pain disorder	1 (9/689)	Microscopic colitis	5 (5/100)
		Polyp/s: adenoma <1cm	1 (7/689)	Non-specific inflammation	4 (4/100)
		Anal fissure	1 (6/689)	Appendicitis	3 (3/100)
		Diverticular disease	1 (5/689)	Bile acid malabsorption	2 (2/100)
				Coeliac disease	2 (2/100)
				Polyp/s: adenoma ≥ 1cm	2 (2/100)
				Rectocoele	1 (1/100)
				Intermittent small bowel obstruction	1 (1/100)
Total	**50**		**689**		**100**

3.3.4.4 Clinical variables and biomarkers to distinguish IBD from functional gut disorder

Symptoms: Patients subsequently diagnosed with IBD reported significantly more stools per day, less abdominal pain and had a shorter duration of symptoms prior to calprotectin testing than patients with functional gut disorder **(Table 3.3-3)**. There was no difference in the proportion of patients later diagnosed with either IBD or functional gut disorder who initially reported diarrhoea (defined as Bristol stool score >4 and/or >3 stools per day) (88% [44/50] vs. 81% [560/689]) respectively, *P* = 0.235). Gastrointestinal alarm symptoms were frequently observed in both groups but more commonly in patients diagnosed with IBD than functional gut disorder, (64% [32/50] vs. 38% [260/689], *P* <0.001 respectively).

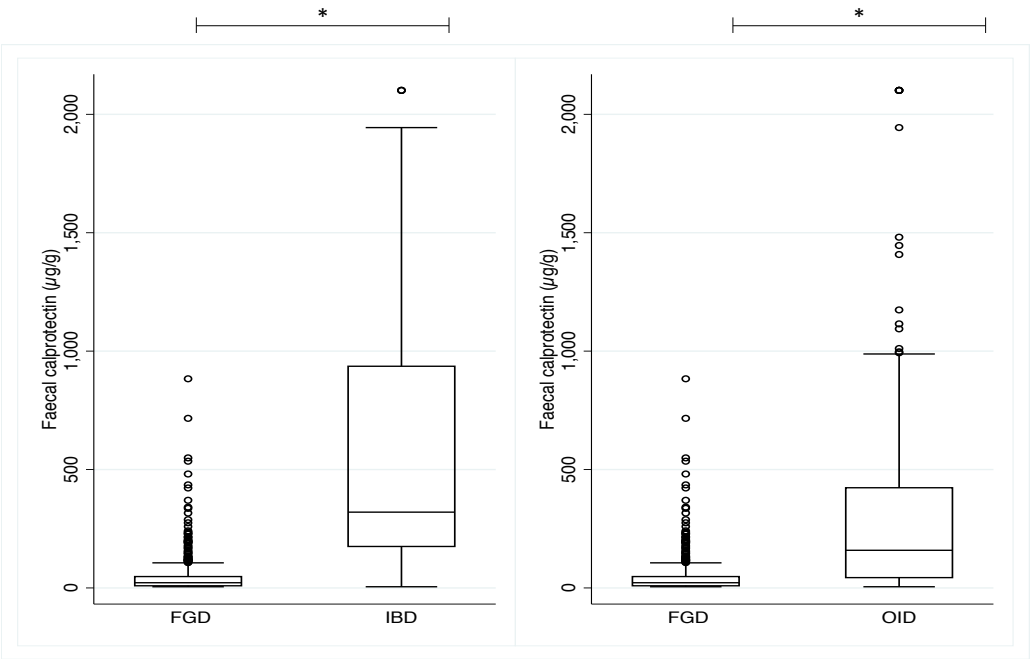
Table 3.3-3:Comparison of clinical variables and biomarkers in patients diagnosed with FGD, IBD and OID

	Functional gut disorder (n=689)	Inflammatory bowel disease (n=50)	IBD vs. Functional gut disorder *P* value †	Organic intestinal disease ‡ (n=100)	Organic intestinal disease vs Functional gut disorder *P* †
Demographics					
Female	60% (416/689)	52% (26/50)	0.243	54% (54/100)	0.225
Age (years)	30.0 [23.2-36.5]	29.1 [23.2-33.9]	0.514	30.6 [24.8-38.0]	0.156
Family history colorectal/ovarian cancer	11% (71/658)	8% (4/48)	0.594	14% (13/96)	0.423
Family history IBD	14% (95/660)	24% (12/49)	0.057	18% (17/97)	0.417
Symptoms					
Abdominal pain	85% (580/680)	74% (37/50)	0.033	79% (78/99)	0.095
Change in frequency	82% (547/667)	86% (43/50)	0.476	81% (79/98)	0.738
Change in appearance	80% (509/636)	83% (40/48)	0.579	85% (81/95)	0.228
Defaecation improves pain	42% (270/636)	54% (25/46)	0.116	52% (48/92)	0.079
Rectal bleeding	38% (259/677)	65% (32/49)	<0.001	51% (50/98)	**0.016**
Gastrointestinal alarm symptoms*	38% (260/689)	64% (32/50)	<0.001	51% (51/100)	**0.011**
Nocturnal symptoms	25% (167/664)	35% (17/49)	0.141	32% (31/96)	0.136
Unintentional weight loss	17% (115/674)	18% (9/49)	0.815	24% (23/97)	0.110
Symptom duration (months) *n=765*	6 [3-12]	4 [2-8]	0.001	4 [2-10]	**<0.001**
Number of stools per day *n=756*	3 [2-5]	4 [3-6]	0.048	3 [2-5]	0.582
Blood/Stool Markers					
CRP (mg/L) *n=673*	1 [<1-4]	2 [1-10]	0.001	2 [1-7]	**<0.001**
B12 (ng/L) *n=216*	334 [260-481]	346.5 [252-423]	0.811	394 [256-490]	0.590
Albumin (g/L) *n=566*	48 [45-49]	46 [43-48]	0.002	46 [44-48]	**0.001**
Ferritin (µg/L) *n=486*	65.5 [34-126]	69 [28-100]	0.233	61 [30-114.5]	0.373
Folate (µg/L) *n=216*	8.5 [6.4-12.5]	5.8 [4.9-8.0]	0.004	6.3 [5.4-9.9]	**0.018**
Haemoglobin (g/L) *n=705*	139 [131-149]	139 [129-150]	0.437	139.5 [132-149]	0.756
Platelet count (x10^9/L) *n=705*	225 [189-266]	246 [209-295]	0.008	239 [204-293]	**0.011**
White cell count (x10^9/L) *n=705*	6.5 [5.4-8]	7.3 [5.9-8.5]	0.030	7.2 [5.6-8.6]	**0.050**
Calprotectin (µg/g) *n=789*	22 [9-48]	320 [175-936]	<0.001	159 [44-423]	**<0.001**

***See Supplementary Appendix S6 for gastrointestinal alarm criteria (NICE; NG12); † P value represents Mann-Whitney U, chi-squared or Fisher's exact test as appropriate; ‡ Organic intestinal disease category**

Faecal calprotectin: Calprotectin was higher in patients diagnosed with IBD than functional gut disorder (**Figure 3.3-2**). 86% of patients subsequently diagnosed with IBD had a calprotectin ≥100µg/g compared to 10% of patients with functional gut disorder. Amongst patients with a normal CRP (≤5mg/L), median calprotectin was higher in patients with IBD than functional gut disorder (241µg/g [IQR 116-453] vs. 21µg/g [IQR 9-46], *P* <0.001, respectively).

Figure 3.3-2: Box plot showing difference in faecal calprotectin between patients with a) functional gut disorder and inflammatory bowel disease b) functional gut disorder and organic intestinal disease * P < 0.001



FGD, functional gut disorder; IBD, inflammatory bowel disease; OID, organic intestinal disease

Blood markers: Patients diagnosed with IBD had significantly higher CRP levels, total white cell and platelet counts, but lower albumin and folate levels than patients diagnosed with functional gut disorder (**Table 3.3-3**). CRP was abnormal (>5mg/L) in 33% patients with IBD (15% [4/26] ulcerative colitis; 65% [11/17] Crohn's disease; 20% [1/5] IBD-unclassified) compared to 16% of patients with functional gut disorder (*P* = 0.002).

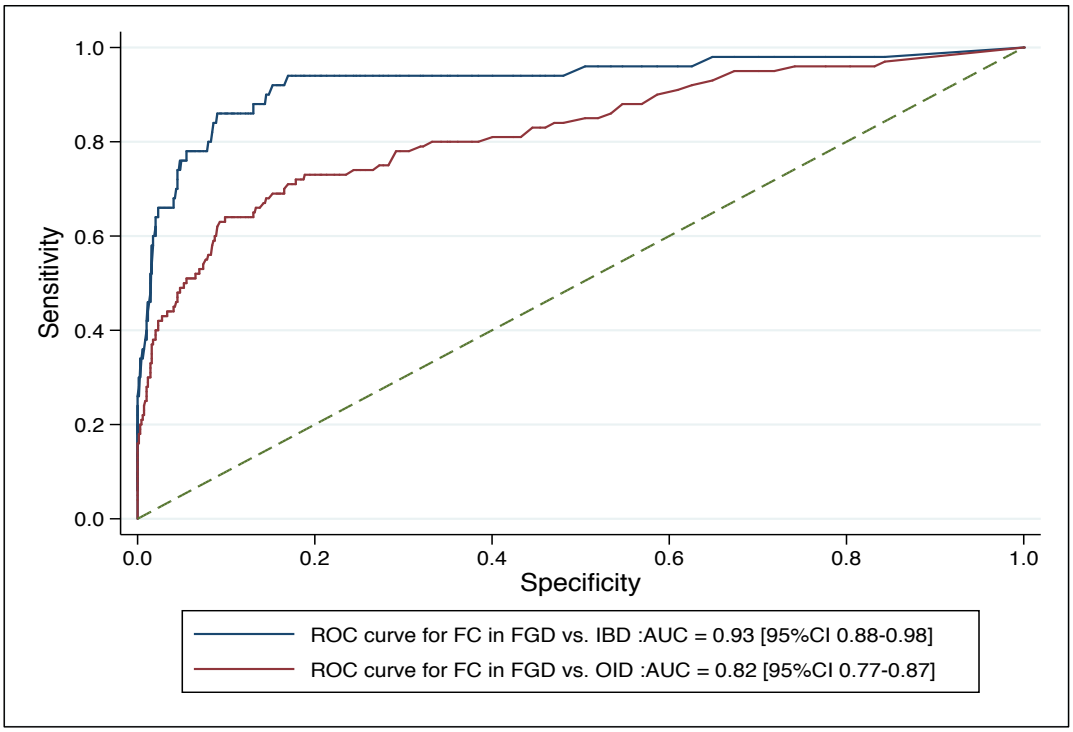
Multivariable logistic regression: The above significant clinical biomarkers were included in our multivariable logistic regression model. Co-linearity was demonstrated between rectal bleeding and gastrointestinal alarm symptoms and we used the latter more inclusive term. In our model, calprotectin ≥100µg/g increased the odds of IBD 54-fold (OR 53.9, 95%CI 23.2-125.2, *P* <0.001), independent of the presence of gastrointestinal alarm symptoms, which increased the odds 3-fold (OR 2.6, 95%CI 1.3-5.2*, P* = 0.008).

3.3.4.5 Diagnostic accuracy of calprotectin in distinguishing IBD from functional gut disorder

Sensitivity and specificity analyses: Using our calprotectin threshold of 100µg/g, the sensitivity for distinguishing IBD vs. functional gut disorder was 86.0%, the specificity was 90.1%, the positive predictive value (PPV) was 38.7%, the negative predictive value (NPV) 98.9%, with an overall accuracy of 89.9%.

Receiver operator characteristic (ROC) analyses: Using calprotectin as a continuous variable in ROC analyses revealed an area under curve (AUC) for distinguishing IBD from functional gut disorder of 0.93 [95%CI 0.88-0.98] **(Figure 3.3-3)**. We estimated the optimum calprotectin threshold as 107 µg/g using Youden's formula.

Figure 3.3-3: Receiver operating characteristic curves for faecal calprotectin as predictors of inflammatory bowel disease or organic intestinal disease vs. functional gut disorder. 2x2 tables for diagnosis of inflammatory bowel disease or organic intestinal disease vs. functional gut disorder.



		Disease Status		Total
		IBD	FGD	
FC (µg/g)	<100	7	621	628
	≥100	43	68	111
Total		50	689	739

		Disease Status		Total
		OID	FGD	
FC (µg/g)	<100	36	621	657
	≥100	64	68	132
Total		100	689	789

AUC, area under the curve; CI, confidence interval

False negative IBD cases: Seven (14%) patients diagnosed with IBD had a false negative calprotectin test (median calprotectin 65µg/g [IQR 14-75]). Four of the seven patients had an intermediate first calprotectin but were not re-tested. These seven patients had a longer time to diagnosis (149 [IQR 133-240] days vs. 55 [IQR 29-69] days; *P* = 0.043) and lower serum CRP levels (<1mg/L [IQR <1-2]

vs. 2mg/L [IQR 1-11]; *P* = 0.050) than true positive cases (**Supplementary Table 3.3-1).**

3.3.4.6 Diagnostic accuracy of calprotectin in distinguishing organic intestinal disease from functional gut disorder

The sensitivity for distinguishing organic intestinal disease vs. functional gut disorder using a threshold of 100µg/g was 64.0%, the specificity was 90.1%, the PPV was 48.5%, the NPV 94.5%, with an overall accuracy of 86.8%. The ROC AUC for organic intestinal disease vs. functional gut disorder was 0.82 [95%CI 0.77-0.87] with an estimated optimal cut-off of 62µg/g.

3.3.4.7 Optimisation of calprotectin thresholds for diagnosis of IBD and organic intestinal disease

Raising the calprotectin threshold increases the PPV for both IBD and organic intestinal disease with a negligible reduction in NPV for IBD and a modest reduction for organic intestinal disease. Raising the calprotectin threshold from the manufacturers recommended limit (50µg/g) to the level used in our analysis (100µg/g) approximately doubles the post-test probability of IBD (**Table 3.3-4**).

3.3.4.8 Effect of gastrointestinal alarm symptoms on distinguishing IBD and organic intestinal disease from functional gut disorder

Comparing the performance of calprotectin to distinguish IBD from functional gut disorder in patients with and without gastrointestinal alarm symptoms, demonstrated that the presence of such symptoms nearly doubled the PPV at lower calprotectin thresholds (50µg/g & 100µg/g), with a more modest effect at higher thresholds (150µg/g, 200µg/g and 150µg/g). The converse was seen with

NPV; at lower calprotectin thresholds the performance of the test was very similar in patients with and without alarm symptoms. However, at higher thresholds, a more modest reduction in NPV was seen in patients with alarm symptoms compared to those patients not reporting such symptoms. Similar trends, albeit with a more modest increase PPV, were seen in the performance of calprotectin to distinguish functional gut disorder from organic intestinal disease in patients with and without alarm symptoms (**Table 3.3-4)**.

Table 3.3-4: Effect of gastrointestinal alarm symptoms on diagnostic accuracy of faecal calprotectin at different thresholds for the diagnosis of inflammatory bowel disease and organic intestinal disease

Calprotectin threshold	Sensitivity %(95% CI)	Specificity %(95% CI)	PPV %(95% CI)	NPV %(95% CI)
inflammatory bowel disease vs. functional gut disorder				
≥ 50 µg/g	100 (82-100)	75 (71-79)	14 (0-22)	100 (99-100)
≥ 50 µg/g	91 (75-98)	77 (71-82)	32 (23-43)	99 (96-100)
≥ 100 µg/g	83 (59-96)	91 (88-93)	27 (16-41)	99 (98-100)
≥ 100 µg/g	88 (71-97)	89 (85-93)	50 (36-64)	98 (96-100)
≥ 150 µg/g	78 (52-94)	95 (93-97)	40 (24-58)	99 (98-100)
≥ 150 µg/g	78 (60-91)	93 (89-96)	58 (42-73)	97 (94-99)
≥ 200 µg/g	72 (47-90)	98 (96-99)	57 (35-77)	99 (97-100)
≥ 200 µg/g	63 (44-79)	95 (91-97)	59 (41-75)	95 (92-98)
≥ 250 µg/g	67 (41-87)	99 (97-100)	71 (44-90)	99 (97-100)
≥ 250 µg/g	56 (38-74)	97 (94-98)	67(46-84)	95(91-97)
organic intestinal disease vs. functional gut disorder				
≥ 50 µg/g	69 (55-82)	75 (71-79)	24 (17-32)	96 (93-97)
≥ 50 µg/g	78 (65-89)	77 (71-82)	40 (30-50)	95 (91-97)
≥ 100 µg/g	55 (40-69)	91 (88-93)	40 (28-53)	95 (92-97)
≥ 100 µg/g	73 (58-84)	89 (85-93)	57 (44-69)	94 (91-97)
≥ 150 µg/g	43 (29-58)	95 (93-97)	50 (34-66)	94 (91-96)
≥ 150 µg/g	59 (44-72)	93 (89-96)	63 (47-76)	92 (88-95)
≥ 200 µg/g	39 (25-54)	98 (96-99)	66 (46-82)	93 (91-95)
≥ 200 µg/g	49 (35-63)	95 (91-97)	64 (47-79)	90 (86-94)
≥ 250 µg/g	33 (20-48)	99 (97-100)	76 (53-92)	93 (90-95)
≥ 250 µg/g	43 (29-58)	97 (94-98)	71 (52-86)	90 (85-93)

PPV, positive predictive value; NPV negative predictive value; CI, confidence interval

Values in black represent diagnostic accuracy of faecal calprotectin in patients without gastrointestinal alarm symptoms (n=447 inflammatory bowel disease vs. functional gut disorder; n=478 organic intestinal disease vs. functional gut disorder); values in red represent the diagnostic accuracy of calprotectin in patients with gastrointestinal alarm symptoms (n=292 inflammatory bowel disease vs. functional gut disorder; n=311 organic intestinal disease vs. functional gut disorder). Black boxes represent data at 100µg/g threshold

3.3.4.9 Secondary care diagnostics utilisation

In referred patients, significantly fewer patients with a negative calprotectin underwent outpatient review (75% [220/295] vs. 89% [98/110], *P* = 0.002), endoscopic evaluation (67% [199/295] vs. 91% [100/110], *P* <0.001) and

diagnostic imaging (18% [53/295] vs. 28% [31/110], *P* = 0.024) than did positive calprotectin patients (**Table 3.3-5**).

Table 3.3-5: Influence of faecal calprotectin on primary care referrals to gastrointestinal services and secondary care investigation of referred patients

	Calprotectin <100µg/g	Calprotectin ≥100µg/g	Total	*P* Value†
Primary care referral of patients to secondary care gastrointestinal services				
Referred to GI services	*45% (295/657)*	*83% (110/132)*	*51% (405/789)*	***<0.001***
Secondary care investigation of patients referred to secondary care gastrointestinal services				
Reviewed in outpatient clinic 75% (220/295)*		*89% (98/110)*	*79% (318/405)*	***0.002***
Gastroenterology	56% (124/220)	89% (87/98)	66% (211/318)	**<0.001**
Colorectal surgeons	28% (61/220)	5% (5/98)	21% (66/318)	**<0.001**
Dieticians	8% (18/220)	1% (1/98)	6% (19/318)	**0.010**
Private clinic	4% (8/220)	2% (2/98)	3% (10/318)	0.729
Did Not Attend (DNA)	2% (5/220)	3% (3/98)	3% (8/318)	0.706
Advice and guidance	2% (4/220)	0% (0/98)	1% (4/318)	0.316
Endoscopic assessment	*67% (199/295)*	*91% (100/110)*	*74% (299/405)*	***<0.001***
Colonoscopy	50% (100/199)	57% (57/100)	53% (157/299)	0.270
Flexible sigmoidoscopy	16% (32/199)	10% (10/100)	14% (42/299)	0.153
Gastroscopy (OGD)	13% (25/199)	4% (4/100)	10% (29/299)	**0.021**
OGD + lower GI endoscopy	21% (42/199)	29% (29/100)	23% (71/299)	0.130
Endoscopy referral pathway				
Straight-to-test	53% (105/199)	56% (56/100)	54% (161/299)	0.596
Outpatient clinic prior-to-test	47% (94/199)	44% (44/100)	46% (138/299)	0.596
Radiological assessment	18% (53/295)	28% (31/110)	21% (84/405)	**0.024**
CT abdomen and pelvis	47% (25/53)	16% (5/31)	36% (30/84)	**0.005**
MRI abdomen and pelvis	53% (28/53)	84% (26/31)	64% (54/84)	**0.005**

CT, computerised tomography; MRI, magnetic resonance imaging; GI, gastrointestinal

*** represents first service seen**

† *P* value represents chi-squared or Fisher's exact test as appropriate

Costings analysis: Using the aforementioned assumptions pertaining to GP intended (expected) and actual (observed) referral behaviour (**Table 3.3-1**), we

estimate that 279 referrals were saved by the primary care calprotectin pathway. This equated to 212 saved outpatient appointments and 228 endoscopies (32 flexible sigmoidoscopies, 120 colonoscopies, 22 gastroscopies and 54 combined upper and lower gastrointestinal endoscopies) and 64 cross-sectional diagnostic imaging tests. To the point of diagnosis, we estimated that the pathway delivered savings of £52,355 per year (£160 per patient). Additionally, had we enforced the referral pathway such that only patients with a calprotectin ≥100μg/g were referred to secondary care services, this would have saved a further 254 referrals; doubling the total savings to £106,469 per year (£326 per patient) (**Supplementary Appendices 3.3-3 to 3.3-5**).

3.3.5 Discussion

3.3.5.1 Key Results

In this real-world primary care cohort of young patients, including for the first-time a large subset of patients with gastrointestinal alarm symptoms, we have shown that a calprotectin ≥100μg/g distinguishes functional gut disorder from IBD (PPV 39%; NPV 99%) and other organic intestinal diseases (PPV 49%; NPV 95%) with clinically useful positive and negative predictive values. For both diagnostic categories at the 100μg/g threshold, the negative predictive value remained high, and was nearly identical for patients reporting and not reporting gastrointestinal alarm symptoms, supporting the extension of calprotectin testing to this group. Both calprotectin and the presence of gastrointestinal alarm symptoms influenced referral behaviour and subsequent onward investigations: patients with a negative calprotectin and those without alarm symptoms were less likely to be

referred to gastrointestinal services, although in those who were, fewer underwent lower gastrointestinal endoscopy.

3.3.5.2 Interpretation

Based on the results of our multivariable model, faecal calprotectin and gastrointestinal alarm symptoms independently predicted the diagnosis of IBD. Therefore, one might consider two strategies combining the two: (i) investigating patients with either gastrointestinal alarm symptoms or calprotectin ≥100μg/g; or (ii) only investigating patients with both features (**Supplementary Table 3.3-2**). The first strategy would reduce the number of missed cases of IBD (sensitivity increased from 86% to 94%), but the PPV (14%) is poor. However, the high NPV (99%) means that this strategy could be used to rule out IBD in patients who met neither of these criteria. The second strategy could potentially reduce the number of referrals with functional gut disorder (specificity increases to 96%) but the sensitivity falls (56%) and consequently many cases of IBD may be missed. By using a calprotectin ≥100μg/g, regardless of gastrointestinal alarm symptoms, we missed 7 false negative cases of IBD who perhaps as a result incurred a diagnostic delay. Four had an intermediate calprotectin result and in accordance with our pathway should have undergone a repeat test but did not. These patients had a less severe phenotype than cases with calprotectin ≥100μg/g. All of the Crohn's disease patients (4/4) with a false negative calprotectin had ileocaecal disease (L1): a location previously associated with a diminished diagnostic calprotectin performance.[260,261] Additionally, all of the missed ulcerative colitis cases (3/3) had proctitis (E1), which is likely to return lower calprotectin results than more extensive disease. These findings are similar to those reported in the Brighton study (14% mis-rate at 100μg/g)[240] and taken together demonstrate the

need to closely monitor adherence to the pathway and clinical review of patients with refractory lower gastrointestinal symptoms.

Reassuringly for GPs, using our *a priori* threshold calprotectin also adequately distinguishes functional gut disorder from other treatable organic intestinal diseases, and even in patients with gastrointestinal alarm symptoms there were no missed cases of colorectal cancer and only 1 missed case of a large (13mm) adenoma. Further improvements in PPV by raising the cut-off above 100μg/g are offset by increased missed cases of organic intestinal disease. Overall, based on this data and work from others we advocate a threshold for referral of 100μg/g.[240,241,248]

3.3.5.3 Strengths and Limitations

The strengths of our study lie in the prospective design, meticulous follow-up of all the primary care records of non-referred patients and the cohort size with a large subset with gastrointestinal alarm symptoms. We acknowledge, however, some important limitations.

Firstly, there is selection bias due to inter-user variability whereby patients with gastrointestinal alarm symptoms suspected of having cancer were excluded; therefore, the clinical judgment of GPs is fundamental to the successful application of calprotectin in this setting and our data are only representative of patients deemed unsuitable for urgent cancer referral by their GP.

Secondly, the uptake of calprotectin testing in primary care was relatively poor with only 26% (50/192) of patients aged 18 to 46 diagnosed with IBD during the

time-period of the study referred by participating practices via the pathway. Therefore, patients diagnosed with IBD in our cohort may have a milder phenotype than patients diagnosed outside of the pathway and a spectrum bias may exist. Despite our larger catchment population, we received only 36 calprotectin samples/month compared with 57 and 43/month in the Brighton and York studies, respectively.[240,241] Explanations may include the cessation of GP educational sessions soon after study commencement and that we did not prompt calprotectin assessment before accepting referrals in eligible patients. In line with this, there was poor adherence to our pre-specified pathway for intermediate test results, and we are therefore unable to comment on the value of retesting in this group. Because of our convenience sampling, we also acknowledge that we cannot report to what extent our data reflect the total eligible population presenting to their GP with lower gastrointestinal symptoms.

Thirdly, about three quarters of patients with a negative calprotectin, and one third of patients with a positive calprotectin later diagnosed with functional gut disorder did not undergo a gold standard lower gastrointestinal endoscopy, leading to an inevitable partial verification bias that may have overestimated the accuracy of calprotectin. We took various measures, however, to limit the effect of this bias. Like others we used a 12-month follow-up period to allow for IBD to evolve[240], but for the first-time we captured all data across both primary and secondary care. Furthermore, the capture of primary care data in un-referred patients, should have avoided an erroneous diagnosis of functional gut disorder being assigned to patients who may have been diagnosed with IBD and organic intestinal disease in private clinics or in other hospitals.

3.3.5.4 Generalisability

We acknowledge that our data may have limited external validity to healthcare systems where referrals are made direct to specialists and not regulated by GP 'gatekeepers'. However, based on data with two other primary care calprotectin studies in patients of a similar age range in Brighton[240] and York[241] (albeit noting that patients with gastrointestinal alarm symptoms were discouraged from these other two cohorts), it is reassuring that there was just one reported case of colorectal cancer in 2055 (0.05%) patients who underwent the test in this setting. This is not only due to the low prevalence of colorectal cancer in this age group[222,252], but also the clinical acumen of primary care physicians, meaning that calprotectin can safely be applied in young patients with gastrointestinal alarm symptoms deemed unsuitable for urgent referral using current national cancer guidelines by their GP (NICE, NG12)[253]. Existing studies of the performance of calprotectin for distinguishing organic from non-organic intestinal diseases, that mostly excluded patients with gastrointestinal alarm symptoms, report negative predictive values of 98 to 99%, and positive predictive values 14 to 40%; the wide variation in the latter reflecting the impact that age and presenting symptoms have on the prevalence of disease.[240,241,262]

Despite a much higher number of patients with gastrointestinal alarm symptoms in our cohort and a different manufacturer of our ELISA assay, we report a similar prevalence of IBD (6.3%) and organic intestinal disease (excluding upper gastrointestinal disease; 11%), as well as similar diagnostic accuracy to both the Brighton and York cohorts. Given these similarities, it is possible that either GPs under reported gastrointestinal alarm symptoms in the previous two cohorts or

that a marginal increase in disease prevalence results but that the effect size is too small to be detected with the given sample sizes. In contrast to the original 2008 version of the NICE IBS guidelines (CG61), the latest 2017 version, in keeping with the 2015 NICE cancer guidelines (NG12), no longer refer to "red flag" symptoms.[253,263] The presence of some symptoms, such as weight loss and abdominal pain in patients over 40 years old (NG12; Section 1.3.1)[253], are advised to lead to an urgent referral, whilst other symptom combinations should prompt consideration of an urgent referral. However, if all patients under 50 years old with rectal bleeding and abdominal pain or change in bowel habit (NG12; section 1.3.3)[253], are excluded from using calprotectin pathways (DG11, 2013; section 1.1)[214], then rectal bleed pathways may be overwhelmed, more urgent cases delayed and calprotectin will be withheld from a large number of patients who may benefit from it.

3.3.5.5 Implications for future practice

In the light of our findings, we will continue to allow GPs to use the test in patients with gastrointestinal alarm symptoms and will make the following revisions to our calprotectin pathway: a single 100µg/g calprotectin cut-off; eight-week safety-net review of patients in non-referred calprotectin negative patients; closer oversight by a lead clinician; and sending patients with a calprotectin ≥250µg/g straight-to-test, as over two thirds (68% [30/44]) will have IBD once infective conditions have been excluded. Indeed, the presence of alarm symptoms, which raise the pre-test probability of IBD, may be crucial to the successful application of calprotectin diagnostic pathways in primary care; without which the PPV of calprotectin may too low to be clinically useful in identifying IBD.[262] Further research is required to

establish how calprotectin will integrate with faecal immunochemical testing (FIT), in particular in older age-groups than included here.[247,248,264,265]

Our service evaluation demonstrates that faecal calprotectin is a clinically useful primary care test to distinguish IBD and organic intestinal disease as a whole from functional gut disorder in patients aged less than 46. However, simply introducing the pathway is not sufficient to either maximise gains, or guarantee its success. To do so requires consistent interpretation of calprotectin results, both in primary and secondary care, with buy-in from all relevant stakeholders, oversight by a responsible consultant, on-going GP educational and feedback sessions as well as careful adjustment of pathways and thresholds based on audit data.

3.3.6 Acknowledgements

We would like to thank all primary care practices who helped us to collect the clinical information necessary for this study. We are very grateful for Dr James Turvill's sage advice and guidance on our manuscript. We would also like to thank Dr Siobhan Ahmad, Dr Lisa Gibbons, Dr Joe Mays and Dr Tom Debenham for piloting the data collection forms; Dr Mark Sanford-Wood (Devon LMC) for his crucial support; and Hanlie Olivier, Marian Parkinson and Helen Gardner-Thorpe for their on-going administrative support. Immundiagnostik supplied ELISA assays through Biohit (UK distributor).

3.3.7 Statement of Interests

Authors declaration of personal interests: GJW has consulted for AbbVie and received honoraria from Falk for unrelated topics. GAH reports non-financial

support from AbbVie, outside the submitted work; and that he is now an employee of AbbVie and owns stock in the company. CC received honoraria from Norgine and non-financial travel support from AbbVie for unrelated topics. NAK has consulted for Falk and received honoraria from Falk, Allergan, Pharmacosmos and Takeda for unrelated topics. JRG received honoraria from Falk, Abbvie and Shield therapeutics for unrelated topics. TA has received unrestricted research grants, advisory board fees, speaker honorariums and support to attend international meetings from AbbVie, Merck, Janssen, Takeda, Ferring, Tillotts, Ferring, Pfizer, NAPP, Celltrion, Hospira for unrelated topics, no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Declaration of funding interests: GJW was supported by research grants from Crohn's and Colitis UK and forCrohns, LM was supported by a research grant from Tillotts and the South West Academic Health Sciences Network (SWAHSN). TJM is an NIHR senior clinical lecturer. MHP and JRG are supported by the Royal Devon and Exeter Externally Funded Research scheme (EFR). CH is supported by the National Institute for Health Research (NIHR) Collaboration for Leadership in Applied Health Research and Care South West Peninsula. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health. The funding organisations had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

3.3.8 Authorship

Author contributions: Author contributions: TA, GJW, GAH, CB, AS, TJM, MHP, and TD participated in the conception, design and coordination of the study. GJW, LM, NH and PH collected primary care data and AS, GJW, LM, NH and PH collected secondary care data. NH and PH performed adjudication. TA, GJW, TJM, CC, CH, NAK, JRG, RB, NH, PH, CB, MP and TA contributed to data analysis or interpretation. GJW, JRG, LM, TA drafted the article and all authors revised it critically and approved the final version of the manuscript.

Guarantor of the article: Tariq Ahmad

3.3.9 References

A list of references can be found at the end of this thesis.

RESEARCH PAPER I

SUPPLEMENT

Supplementary Table 3.3-1: Disease severity in false negative and true positive IBD cases

Characteristic		False Negatives/Missed IBD cases (n=7)*	True Positives/Correctly identified IBD cases (n=43)*	*P* Value‡
Age	Age (years) median [IQR]	29.6 [IQR 23.6-32.9]	29.0 [IQR 22.9-34.9]	0.900
Sex	Female	43 (3/7)	51 (22/43)	1.000
Smoking	Current	29 (2/7)	12 (5/43)	**0.036**
	Ex	29 (2/7)	30 (13/43)	
	Never	14(1/7)	54(23/43)	
	Unknown	29 (2/7)	5 (2/43)	
IBD	CD	57 (4/7)	35 (15/43)	0.488
	IBD-U	0 (0/7)	12 (5/43)	
	UC	43 (3/7)	54 (23/43)	
Montreal Classification CD	A2: 17-40 years	100 (4/4)	100 (15/15)	1.000
	A3: >40 years	0 (0/4)	0(0)	
	L1: Ileal	100 (4/4)	53 (8/15)	0.398
	L2: Colonic	0 (0/4)	27 (4/15)	
	L3: Ileocolonic	0 (0/4)	20 (3/15)	
	+ L4: Upper GI	0 (0/4)	0 (0/15)	1.000
	B1: Inflammatory	100 (4/4)	87 (13/15)	1.000
	B2: Stricturing	0 (0/4)	13.3(2/15)	
	B3: Penetrating	0 (0/4)	0 (0/15)	
	+ p: Perianal	0 (0/4)	0 (0/4)	1.000
Montreal Classification UC	E1: proctitis	100 (3/3)	36 (10/28)	0.200
	E2: left-sided	0 (0/3)	43 (12/28)	
	E3: pan-colitis	0 (0/3)	21 (6/28)	
Blood markers at diagnosis	CRP (mg/L)	<1 [<1-2]	2 [1-11]	**0.050**
	B12 (ng/L)	328 [230-425]	347 [254-421]	0.874
	Albumin (g/L)	48 [47-49]	46 [42.5-47]	0.078
	Ferritin (µg/L)	46 [41-73]	69 [27-107]	0.732
	Folate (µg/L)	5.3 [4.9-5.7]	6.1 [4.8-8.2]	0.525
	Haemoglobin (g/L)	150 [141-154]	138 [127-149]	0.073
	Platelet count ($x10^9$/L)	227 [208-245]	251 [210-305]	0.458
	White cell count ($x10^9$/L)	7.2 [5.4-8.2]	7.3 [5.9-8.6]	0.685
Faecal calprotectin	Faecal calprotectin (µg/g)	65 [14-75]	364 [234-997]	**<0.001**
Time to diagnosis (FC to diagnostic test)	Time (days)	133 [149-240]	55 [29-71]	**0.043**

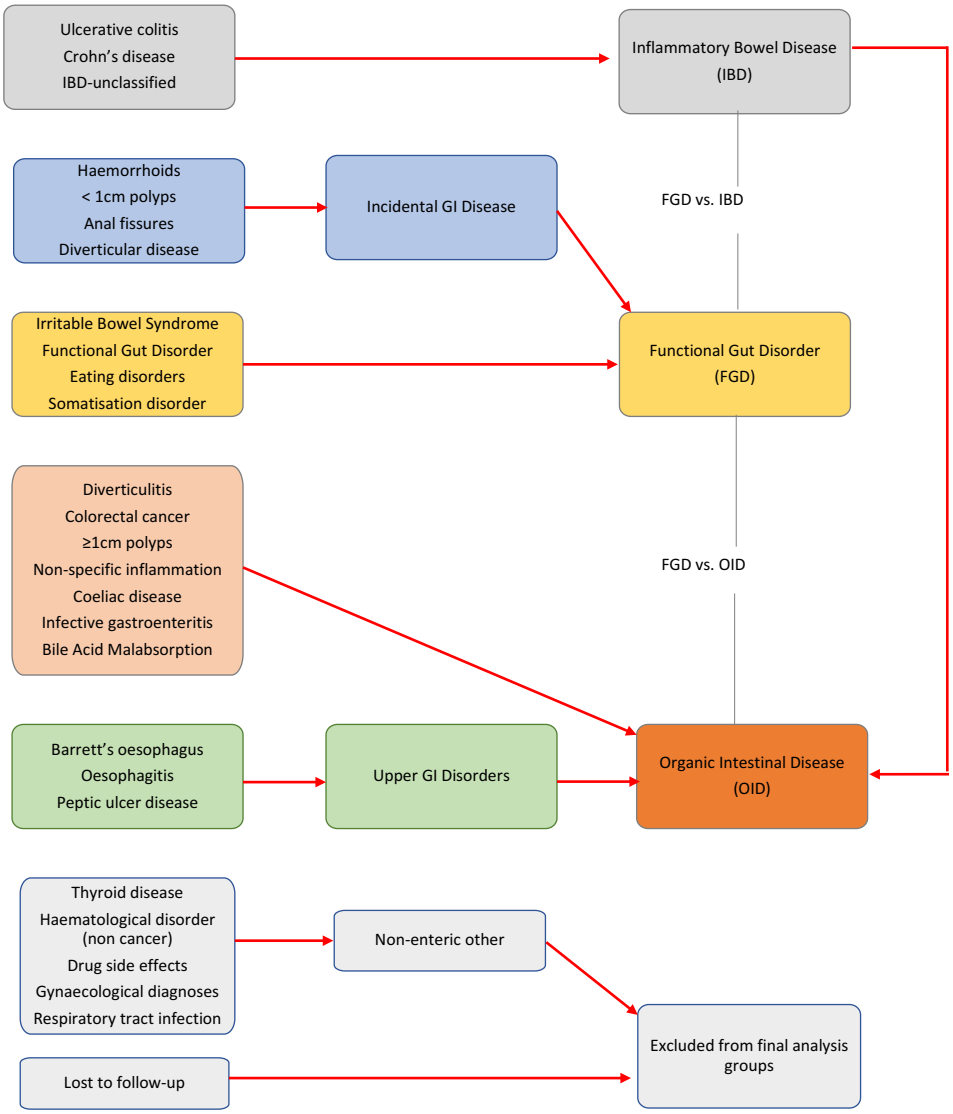
*** values represent % (n) or median [IQR] ‡ P value represents chi-squared, Fisher's exact, Mann-Whitney U test as appropriate. CD, Crohn's disease; UC, ulcerative colitis; IBD-U, IBD-unclassified; GI, gastrointestinal; FC, faecal calprotectin; CRP, C-reactive protein.**

Supplementary Table 3.3-2: Referral strategies for IBD using model from multiple logistic regression

Strategy	Sensitivity %(95%CI)	Specificity %(95%CI)	PPV %(95%CI)	NPV %(95%CI)	Interpretation of strategy
GI alarm symptoms only	64 (49-77)	62 (59-66)	11 (8-15)	96 (94-98)	Too many referrals with functional gut disorder
FC ≥ 100µg/g only	86 (73-94)	90(88-92)	39 (30-49)	99 (98-100)	Optimal combination of PPV and NPV
GI alarm symptoms or **FC ≥ 100µg/g**	94 (84-99)	56 (53-60)	14 (10-18)	99 (98-100)	Too many referrals with functional gut disorder
GI alarm symptoms and **FC ≥ 100µg/g**	56 (41-70)	96 (94-97)	50 (36-64)	97 (95-98)	Too many missed cases of IBD

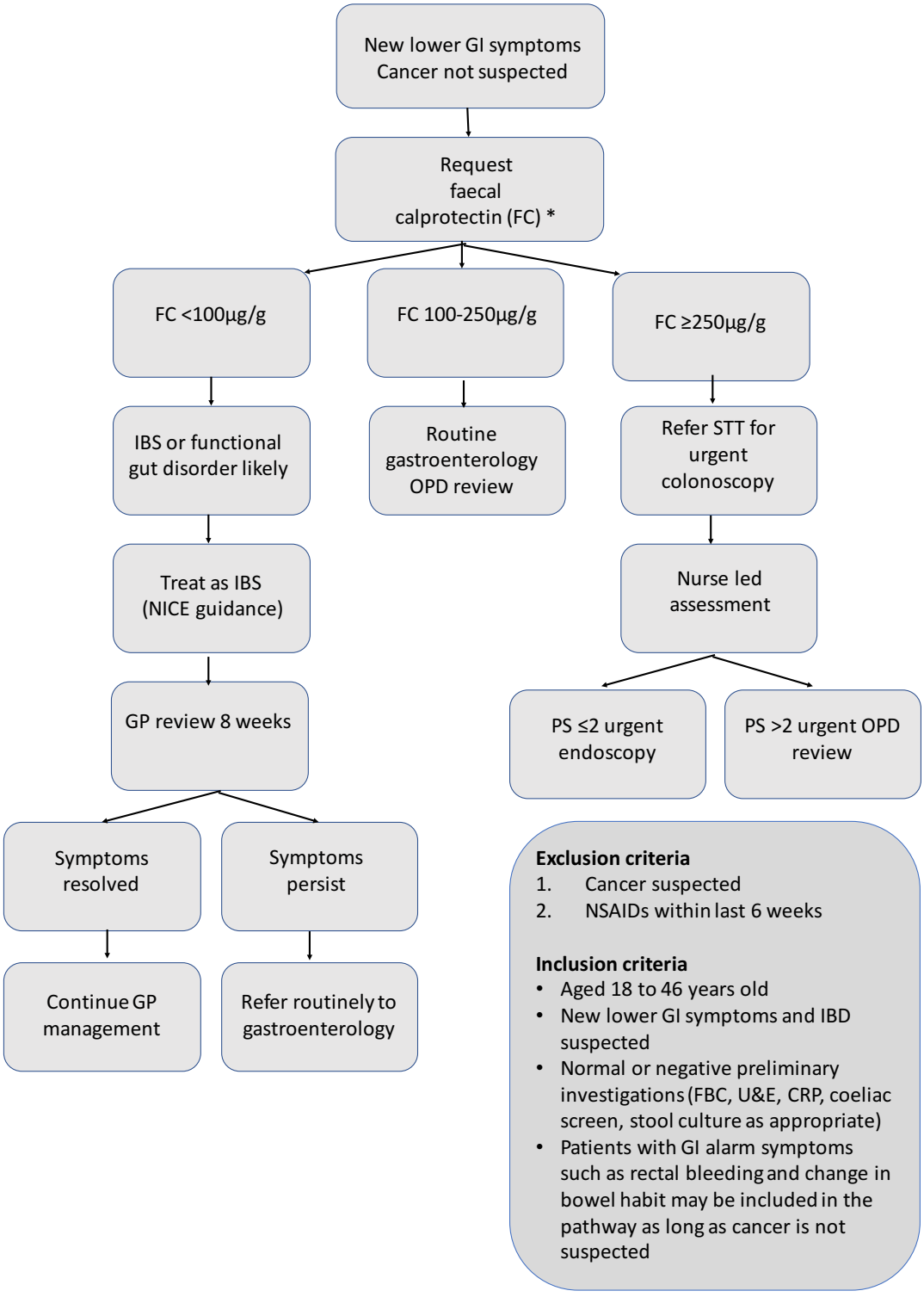
PPV, positive predictive value; NPV negative predictive value; CI, confidence interval; GI, gastrointestinal; FC, faecal calprotectin

Supplementary Table 3.3-3: Image depicting how each diagnosis was collapsed into one of the three primary diagnostic outcome groups, or excluded



GI, gastrointestinal; FGD, functional gut disorder; IBD, inflammatory bowel disease; OID, organic intestinal disease (includes IBD)

Supplementary Table 3.3-4: Proposed guidelines for the use of faecal calprotectin (FC) in the management of patients presenting with lower gastrointestinal symptoms.



IBS, Irritable Bowel Syndrome; IBD, Inflammatory Bowel Disease; NICE, National Institute for Health and Care Excellence; PS, World Health Organisation (WHO) performance status; NSAIDs, Non-steroidal anti-inflammatory drugs; GP, general practitioner; FBC, full blood count; U&E, urea and electrolytes; CRP, C-reactive protein.

Supplementary Appendix 3.3-1: NICE referral guidelines for lower gastrointestinal tract cancers: NG12, 2015[253]

Section 1.3.1 Refer adults using a suspected cancer pathway referral (for an appointment within 2 weeks) for colorectal cancer if:

- they are aged 40 and over with unexplained weight loss and abdominal pain or
- they are aged 50 and over with unexplained rectal bleeding or
- they are aged 60 and over with:
- iron-deficiency anaemia or
- changes in their bowel habit, or
- tests show occult blood in their faeces. **[new 2015]**

Section 1.3.2 Consider a suspected cancer pathway referral (for an appointment within 2 weeks) for colorectal cancer in adults with a rectal or abdominal mass. **[new 2015]**

Section 1.3.3 Consider a suspected cancer pathway referral (for an appointment within 2 weeks) for colorectal cancer in adults aged under 50 with rectal bleeding and any of the following unexplained symptoms or findings:

- abdominal pain
- change in bowel habit
- weight loss
- iron-deficiency anaemia. **[new 2015]**

Supplementary Appendix 3.3-2: Faecal calprotectin request form for primary care

EXETER PATHOLOGY SERVICES
DEPARTMENT OF BLOOD SCIENCES
REQUESTS ENQUIRIES (01392 40)2934

Faecal Calprotectin (FC) Request Form for Primary Care

Affix Label Here

NHS Number / / Date of Birth Gender ○ M ○ F

Surname (Block CAPITALS please) First name(s) (Block CAPITALS please)

Doctor's Laboratory Code Practice Laboratory Code Sample date For EXTRA copy reports, state doctor & location

When to use Faecal Calprotectin in Primary Care:

Suspected Inflammatory Bowel disease (IBD) in patient aged 45 or below
If you suspect a patient age ≤45 might have IBD then perform FC as part of your usual work up/investigations.

- Positive: FC≥100 µg/g faeces, referral to Gastroenterology recommended.
- Negative: FC<50 µg/g faeces, IBD unlikely- consider primary care IBS management.
- Indeterminate: FC 50-99 µg/g faeces, if symptoms persist re-test FC. If on re-test FC ≥50 µg/g faeces referral to Gastroenterology is recommended.

If FC is normal, but CRP raised without another obvious explanation, then consider referral for suspected IBD

If you remain clinically concerned about a patient despite a negative FC then there is no need to repeat the test. You may refer in the normal way but please state in your referral letter what features are concerning you and prompting the referral.

Secondary care referral:

- Please title your referral letter "SUSPECTED IBD" and send to RD&E gastroenterology only. All patients will be seen in gastroenterology outpatients within 3 weeks.
- For more urgent opinions please Fax 01392 402810 or call one of the gastroenterologists

IMPORTANT - STOOL SAMPLES MUST ARRIVE IN THE LABORATORY WITHIN 24 HOURS OF COLLECTION

Criteria for Sample Analysis:

*** Please note, samples will only be processed when the following questions are marked "Yes" ***

Is patient age ≤45 years?	Yes ○	No ○
Is IBD suspected or possible?	Yes ○	No ○
Is there a low suspicion of colorectal cancer?	Yes ○	No ○
Do you confirm it has been >6 weeks since any NSAID, including aspirin?	Yes ○	No ○

Impact of FC in Primary Care:

If FC was not available would you have referred this patient to secondary care?	Yes ○	No ○	Unsure ○
Are you planning to refer this patient to secondary care even if FC is normal?	Yes ○	No ○	Unsure ○

Please also request:
a) FBC, Ferritin, CRP if not done in the past 6 weeks
b) TTG if not previously done

Clinical Information

Duration of Symptoms (months):		
Abdominal Pain:	Yes ○	No ○
Pain improves with defecation:	Yes ○	No ○
Change in stool frequency:	Yes ○	No ○
24 hour stool frequency (number of times):		
Change in stool appearance/consistency:	Yes ○	No ○
Stool consistency (see Bristol Stool Chart on reverse):		
Rectal bleeding:	Yes ○	No ○
Unintentional weight loss:	Yes ○	No ○
Nocturnal symptoms:	Yes ○	No ○
Family history of IBD:	Yes ○	No ○
Family history of bowel or ovarian cancer:	Yes ○	No ○
Alcohol (units per week):		

Version 1.1

Supplementary Appendix 3.3-3: Estimated current savings

Estimated current savings using 100µg/g threshold (279 referrals saved)

Resource saved (% based on secondary care healthcare utilisation in referred patients)	Number of referrals saved to each resource (assuming 279 fewer referrals)	Secondary Care Service (% based on secondary care healthcare utilisation in referred patients)	Resources saved if 279 referrals (n/£*)	Total savings (£)
Outpatient clinic only (18.1%)	50.5	Surgical outpatient clinic (25.8%)	13 £1,859.00	£8,815
		Gastroenterology outpatient clinic (74.2%)	37 £6,956.00	
Endoscopy only (24.1%)	67.2	Flexible sigmoidoscopy (12.5%)	8 £2,728.00	£29,553
		Colonoscopy (48.9%)	33 £14,322.00	
		Gastroscopy (13.6%)	9 £3,280.50	
		Combined upper and lower endoscopy (25.0%)	17 £9,222.50	
Endoscopy and outpatient clinic (57.8%)	161.3	Flexible sigmoidoscopy (14.7%)	24 £8,184.00	£99,319
		Colonoscopy (54.0%)	87 £37,758.00	
		Gastroscopy (8.1%)	13 £4,738.50	
		Combined upper and lower endoscopy (23.2%)	37 £20,072.50	
		Surgical outpatient clinic (25.8%)	42 £6,006.00	
		Gastroenterology outpatient clinic (74.2%)	120 £22,560.00	
Ultrasound (12.0%)	33.5	£6,195	£1,591.25	£6,195
Computerised Tomography (4.2%)	11.7	_	£1,269.45	
Magnetic Resonance Imaging (6.8%)	19.0	_	£3,334.50	
			TOTAL	**£143,882**
			Cost FC testing in 789 adult patients (£22/test)	**£17,358**
			TOTAL SAVING (29 months)	**£126,524**
			TOTAL SAVING (per year)	**£52,355**
			Per patient	**£160**

***using 2017-18 National Tariff[257]**

Supplementary Appendix 3.3-4: Estimated potential savings

Estimated additional savings if pathway strictly enforced at 100µg/g threshold (additional 254 referrals saved; total 533)

Resource saved (% based on secondary care healthcare utilisation in referred patients)	Number of referrals saved to each resource (assuming 533 fewer referrals)	Secondary Care Service (% based on secondary care healthcare utilisation in referred patients)	Resources saved if 533 referrals (n*/£)	Total savings (£)
Outpatient clinic only (18.1%)	96.5	Surgical outpatient clinic (25.8%)	25	£17,111
			£3,575.00	
		Gastroenterology outpatient clinic (74.2%)	72	
			£13,536.00	
Endoscopy only (24.1%)	128.5	Flexible sigmoidoscopy (12.5%)	16	£56,355
			£5,456.00	
		Colonoscopy (48.9%)	63	
			£27,342.00	
		Gastroscopy (13.6%)	17	
			£6,196.50	
		Combined upper and lower endoscopy (25.0%)	32	
			£17,360.00	
Endoscopy and OPD (57.8%)	308.1	Flexible sigmoidoscopy (14.7%)	45	£189,368
			£15,345.00	
		Colonoscopy (54.0%)	166	
			£72,044.00	
		Gastroscopy (8.1%)	25	
			£9,112.50	
		Combined upper and lower endoscopy (23.2%)	71	
			£38,517.50	
		Surgical outpatient clinic (25.8%)	79	
			£11,297.00	
		Gastroenterology outpatient clinic (74.2%)	229	
			£43,052.00	
Ultrasound (12.0%)	64.0	£11,824	£3,040.00	£11,824
Computerised Tomography (4.2%)	22.4	–	£2,430.40	
Magnetic Resonance Imaging (6.8%)	36.2	–	£6,353.10	
			TOTAL	**£274,657**
			Cost calprotectin testing in 789 adult patients (£22/test)	**£17,358**
			TOTAL SAVINGS (29 months)	**£257,299**
			TOTAL SAVINGS per year	**£106,469**
			TOTAL SAVINGS per patient	**£326**

***using 2017-18 National Tariff[257]**

Supplementary Appendix 3.3-5: 2017-18 National tariffs used for costings analysis[257]

Item	Cost (£)	Average Cost (£)
Diagnostic Colonoscopy with Biopsy, ≥ 19 years	465	434.0
Diagnostic Colonoscopy, ≥ 19 years	403	
Diagnostic Flexible Sigmoidoscopy, ≥ 19 years	310	341.0
Diagnostic Flexible Sigmoidoscopy with Biopsy, ≥ 19 years	372	
Combined Upper and Lower GI Tract Diagnostic Endoscopic Procedures	496	542.5
Combined Upper and Lower Gastrointestinal Tract Diagnostic Endoscopic Procedures with Biopsy, 19 years and over	589	
Diagnostic Endoscopic Upper GI Tract Procedures, ≥ 19 years and over	341	364.5
Diagnostic Endoscopic Upper GI Tract Procedures + Biopsy, ≥ 19 years	388	
Colorectal OPD (first appointment)	143	-
Gastroenterology OPD (first appointment)	188	-
Ultrasound Scan with duration of less than 20 minutes, without Contrast	40	47.5
Ultrasound Scan with duration of less than 20 minutes, with Contrast	48	
Ultrasound Scan with duration of 20 minutes and over, without Contrast	48	
Ultrasound Scan with duration of 20 minutes and over, with Contrast	54	
MRI Scan of Two or Three Areas, without Contrast (plus reporting)	159	175.5
MRI Scan of Two or Three Areas, with Contrast (plus reporting)	192	
CT Scan of Two Areas, without Contrast (plus reporting)	97	108.5
CT Scan of Two Areas, with Contrast (plus reporting)	120	

RESEARCH PAPER II

'The diagnostic accuracy of primary care faecal calprotectin testing in children with suspected inflammatory bowel disease.'

SUBMITTED FOR PUBLICATION: UNDER PEER REVIEW

Gareth J Walker, Amanda Thomas, Lucy Moore, Neel Heerasing, Peter Hendy, Mohammed Abdulrahim, Sean Mole, Mandy H Perry, Timothy J McDonald, Claire Bewshea, Neil Chanchlani, Simeng Lin, Jim Hart, Richard K Russell, Tariq Ahmad, James R Goodhand, Nicholas A Kennedy

3.4.1 Abstract

Objective: To determine the diagnostic accuracy of calprotectin to diagnose IBD in children in whom general practitioners (GPs) suspected IBD.

Design: Prospective observational cohort study of a new calprotectin-based primary care referral pathway.

Setting: 49 GP practices and gastroenterology secondary care services at the Royal Devon and Exeter NHS Foundation Trust in the South-West of England, UK.

Patients: 142 children aged between 4-18 years referred on the pathway between January 2014 and August 2017 for investigation of gastrointestinal symptoms were included.

Interventions: Primary-care-driven faecal calprotectin testing. Primary and secondary care records over 12 months from the point of calprotectin testing were used as the reference standard.

Main outcome measures: Diagnostic accuracy of calprotectin testing to detect IBD.

Results: 8% (11/142) tested patients were diagnosed with IBD. Using our pre-specified cut-off of 100 µg/g, calprotectin had a diagnostic accuracy of 93% (95% CI 87-97%) with a sensitivity for distinguishing IBD from non-IBD of 100% (95% CI 72-100%), a specificity of 92% (95% CI 86-96%), a positive predictive value of 52% (95% CI 30-74%) and a negative predictive value of 100% (95% CI 97-100%). Calprotectin testing had no effect on the time to diagnosis, but a negative test saved referrals in 39% (40/102) and was associated with fewer diagnostic tests in secondary care.

Conclusions: Calprotectin testing of children with suspected IBD in primary care accurately distinguishes IBD from a functional gut disorder, reduces secondary care referrals and associated diagnostic healthcare costs.

3.4.2 Background

Most children who present to their general practitioner (GP) with gastrointestinal symptoms are diagnosed with a self-limiting infection or functional gut disorder that can be managed in primary care [266–268]; a minority, however, will have inflammatory bowel disease (IBD).

Because of the practical constraints of venepuncture, in particular in young children, primary care physicians often rely on presenting symptoms alone to formulate a differential diagnosis. The presence of 'red-flag' symptoms including weight loss, rectal bleeding, and a family history of IBD should prompt secondary care referral; however, these symptoms are common and have poor discriminative power.[227,269,270] Where C-reactive protein (CRP) is available, its diagnostic accuracy for IBD is limited by moderate sensitivity, particularly for diagnosing terminal ileal Crohn's disease and left-sided ulcerative colitis.[224,225] Faecal calprotectin is a stool biomarker that distinguishes paediatric IBD and other organic intestinal disease from functional gut disorders.[219,271] We, like others, have shown that in adults with suspected IBD, calprotectin can be used by GPs to risk stratify patient referrals and permit timely diagnoses.[240,241,272,273] Despite the benefits of being non-invasive, there is a paucity of data exploring the real-world use of faecal calprotectin testing in paediatric primary care referral pathways.[270]

We hypothesised that calprotectin testing would distinguish IBD from non-IBD with clinically useful positive and negative predictive values, save secondary care referrals and reduce the time to diagnosis from GP presentation.

3.4.2.1 Objectives

We aimed to:

i) compare the diagnostic accuracy of primary care calprotectin testing in children to distinguish IBD from non-IBD diagnoses with 'red-flag' symptoms and other biomarkers of inflammation including CRP

ii) define whether calprotectin testing alters primary care referral behaviour, reduces the time to diagnosis from presentation to GP and influences the use of secondary care investigations

iii) compare the phenotype and time to diagnosis of patients referred on and off our primary care calprotectin pathway

3.4.3 Methods

3.4.3.1 Design & clinical setting

We designed a prospective observational cohort study to describe the diagnostic accuracy of calprotectin in children with suspected IBD in primary care.

The Royal Devon & Exeter (RD&E) NHS Trust provides paediatric secondary care services to the Eastern locality of the Northern, Eastern and Western Devon Clinical Commissioning group and serves a local population of 378,000 people, of whom 75,000 are under 18 years old.

3.4.3.2 Patients

Children aged between 4-18 years referred on the calprotectin pathway between January 2014 and August 2017 for investigation of gastrointestinal symptoms were included and followed-up for at least 12 months. Exclusion criteria were a previous diagnosis of IBD, suspicion of cancer and use of non-steroidal anti-inflammatory drugs (NSAIDs) within the previous six weeks. This was a convenience sampling series: the use of calprotectin was not mandated in all patients meeting our eligibility criteria; rather, the test was used at the discretion of the treating GP.

3.4.3.3 Variables and data acquisition

We used a purpose-designed request form that captured patient demographic data, presenting symptoms and family history of IBD, colorectal or ovarian cancer at the point of calprotectin testing (**Supplemental Appendix 3.4-1**). General Practitioners (GPs) were prompted to send blood tests for full blood count (FBC), ferritin, C-reactive protein (CRP) and coeliac serology (tissue TransGlutaminase

[tTG]). Incomplete request forms were rejected by the blood sciences laboratory, and prompts sent to aid completion. Patients with one or more of rectal bleeding, unintentional weight loss, a family history of IBD, and anaemia (defined according to the age adjusted World Health Organization criteria[274]), were deemed to have 'red-flag' symptoms. GPs were also asked the following hypothetical referral question to assess expected referral behaviour: 'Would you have referred this patient if calprotectin had been unavailable?' ['Yes'; 'No'; 'Unsure']

Using electronic secondary care databases, we recorded health-care utilisation data in the year after calprotectin testing including: outpatient clinic referrals (paediatrics, gastroenterology, colorectal surgeons, upper gastrointestinal surgeons, dieticians and private clinics); diagnostic imaging (ultrasound [USS], computerised tomography [CT] and magnetic resonance imaging [MRI]); and endoscopy (colonoscopy, flexible sigmoidoscopy and gastroscopy).

We compared baseline demographics, IBD phenotype according to the Paris classification[275], presenting symptoms, emergency department presentations, faecal calprotectin, inflammatory biomarkers, and time to diagnosis between patients with IBD referred using our new pathway and those referred directly without calprotectin testing.

3.4.3.4 Reference standard

The diagnosis of IBD was made on clinical, radiological and histopathological findings in line with the Porto criteria. [276] This burden of investigation is impractical and inappropriate for the diagnosis of a functional gut disorder, particularly in

children. In common with previous published literature[267], absence of IBD was defined as no endoscopic, histopathological and radiological evidence of IBD, or no IBD diagnosis after a 12-month period of follow-up. We mandated this period of follow-up to allow sufficient time for organic pathology to evolve and any missed cases of IBD to be correctly diagnosed. The primary care notes of children not referred to secondary care were reviewed to see whether there had been any further contact with a healthcare practitioner.

3.4.3.5 Faecal calprotectin (index test)

Samples were analysed in accordance with our previously described methods.[272] In short, a quantitative ELISA (Immundiagnostik, Bensheim, Germany) reported levels in the range 6-2100 μg/g. Results ≥ 100 μg/g were deemed positive. Calprotectin results and recommended actions were returned to GPs within 10 days. Primary care management was suggested for those with a negative test, although GPs were encouraged to refer patients whom they felt required specialist review based on their clinical assessment.

3.4.3.6 Statistical methods

Because this study was designed as a service evaluation, *a priori* power calculations were not undertaken. Rather we decided to allow our new pathway to become established and then assessed its utility over three years. All analyses were two tailed and *P*-values < 0.05 were considered significant and were conducted in R 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). We included patients with missing clinical data in analyses for which they had data and specified the denominator for each variable.

We used chi-squared analyses to compare expected and observed GP referrals to determine whether calprotectin influenced GP referral behaviour. We used Fisher's exact tests for categorical data and Mann-Whitney U tests for continuous data to identify baseline clinical variables and biomarkers associated with a diagnosis of IBD and to identify phenotypic differences, including the time to diagnosis between children diagnosed with IBD referred on and off our new pathway.

Receiver operator characteristic curves and area under the curve (AUC) analyses were undertaken to determine clinical validity of calprotectin as a continuous variable to diagnose IBD and organic intestinal disease; Youden's formula was used to determine the optimal cut-off. [258] We calculated sensitivity, specificity, positive (PPV) and negative (NPV) predictive values at our pre-specified cut-off and then at various calprotectin cut-off thresholds to optimise its use in diagnosing IBD. We used stepwise forward multivariable logistic regression models to compare diagnostic strategies using calprotectin and CRP with 'red-flag' symptoms.

3.4.3.7 Ethical consideration and patient involvement

This project was endorsed by the Local Medical Council, Devon Clinical Commissioning Group, primary- and secondary-care Caldicott guardians and the Southwest Academic Health Sciences Network (SWAHSN). Patients were not involved in the conception or design of this study, and in accordance with UK Health Research Authority guidelines, we did not require formal ethical approval.[259]

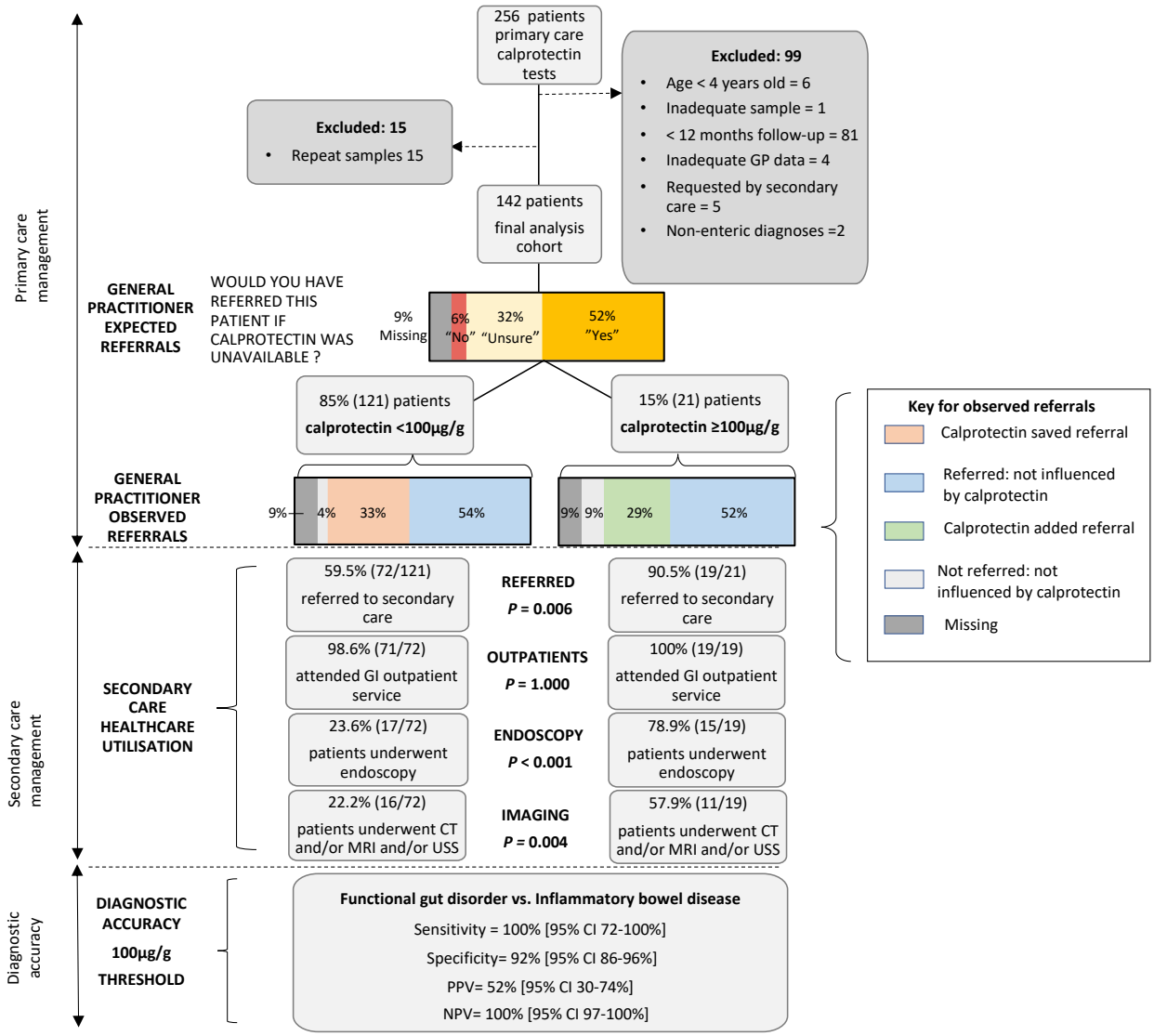
3.4.4 Results

3.4.4.1 Study overview

We report data according to the sequence of events on our pathway as outlined in **Figure 3.4-1**. The laboratory received 256 calprotectin samples from 241 children between January 2014 and May 2016. Overall, 99 children were excluded from the final analysis for the following reasons: less than 12 months' follow-up (n=81), age < 4years (n=6), inadequate GP data or faecal sample (n=5), requested in secondary care (n=5) and non-enteric diagnosis (n=2: Rett's syndrome and pyelonephritis).

142 patients were included in the final analysis: 49% (69/142) were female with a median [range] age of 15.0 [5.9-17.9] yrs. At least one 'red-flag' criterion was present in 49% (72/142) of all patients. 21% (29/136) children had a family history of IBD. Overall, 7% (14/142) tested patients were not referred to secondary care and had no further primary care record entries. For the purposes of our study they were coded as non-IBD after a median follow-up 1.6 years [IQR 1.3-2.1].

Figure 3.4-1: Flow diagram showing derivation of the cohort, faecal calprotectin and diagnosis of functional gut disorder



Flow diagram showing derivation of the cohort, faecal calprotectin result and diagnosis of functional gut disorders and Inflammatory Bowel Disease (IBD). CT, computerised tomography; MRI, magnetic resonance imaging; NPV, negative predictive value, PPV, positive predictive values; GP, general practitioner; GI, gastrointestinal. Note, 13 patients missing data for GP expected referral behaviour.

3.4.4.2 Primary care referrals

Approximately half (52%, 74/142) of GPs indicated that they intended to refer their patient had calprotectin testing not been available; 32% (46/142) were unsure if they would have referred; and 6% (9/142) stated that they would not have referred (**Table 3.4-1**). On multivariable logistic regression, the final decision to refer was independently associated with pre-calprotectin intention to refer (OR

= 3.2 [95% CI 1.5-7.0] for 'yes' vs. 'unsure' or 'no', p=0.003) and faecal calprotectin ≥ 100 µg/g (OR = 6.5 [95% CI 1.7-43.1, *P* = 0.018]).

Table 3.4-1. Table comparing expected and observed GP behaviour by calprotectin result

Expected referral behaviour (pre-calprotectin test result)[a]	**Calprotectin test result (µg/g)**	**Observed referral behaviour (post-calprotectin test result)**	
		No referral made	Referral made
GP not intending to refer the patient if calprotectin testing were unavailable = 6% (9/142)	< 100	62% (5/8)	38% (3/8)
	≥ 100	0% (0/1)	100% (1/1)
GP unsure whether to refer the patient if calprotectin testing were unavailable = 32% (46/142)	< 100	54% (21/39)	46% (18/39)
	≥ 100	29% (2/7)	71% (5/7)
GP did intend to refer the patient if calprotectin testing were unavailable = 52% (74/142)	< 100	30% (19/63)	70% (44/63)
	≥ 100	0% (0/11)	100% (11/11)
Missing GP response 9% (13/142)	< 100	36% (4/11)	64% (7/11)
	≥ 100	0% (0/2)	100% (2/2)

Key

	Calprotectin saved referral
	Referred: not influenced by calprotectin
	Calprotectin added referral
	Not referred: not influenced by calprotectin

3.4.4.3 Diagnostic accuracy

8% (11/142) patients were diagnosed with IBD (8 Crohn's disease, 1 UC, 2 IBD-U). The most frequent non-IBD (n = 131) diagnoses were: IBS/functional gut disorder 82%; anal fissure/haemorrhoids 4%; infective gastroenteritis 4% and coeliac disease 1% (**Supplemental Table 3.4-1**).

3.4.4.4 Clinical and inflammatory biomarkers to distinguish IBD and non-IBD

Symptoms: Patients diagnosed with IBD more commonly reported diarrhoea (Bristol stool ≥ type 6) (P = 0.010), a change in stool appearance (P = 0.017) and change in stool frequency (P = 0.036) than patients with non-IBD (**Table 3.4-2**). At the time of GP referral, 55% (6/11) of patients diagnosed with IBD and 51% (66/131) of patients subsequently diagnosed with non-IBD (P = 1.0) met one or more of the 'red-flag' criteria.

Table 3.4-2. Comparison of clinical variables and biomarkers in patients diagnosed with inflammatory bowel disease and non-IBD

Variable	N[f]	IBD n = 11	Non-IBD n = 131	*P* value[e]
Demographics				
Age	142	14.9 (14.2 - 15.9)	15.0 (11.8 - 16.5)	0.497
Gender	142	45% (5/11)	49% (64/131)	1
Family history IBD	136	20% (2/10)	21.4% (27/126)	1
Symptoms				
Duration of symptoms (months)	139	3.0 (2.0 - 5.5)	6.0 (2.8 - 12.0)	0.08
Abdominal pain	140	73% (8/11)	90% (116/129)	0.114
Pain improves on defaecation	128	22% (2/9)	36% (43/119)	0.492
Number of stools in 24 hours	137	4.0 (3.2 - 4.0)	3.0 (1.0 - 4.5)	0.226
Diarrhoea (Bristol stool ≥ 6)	97	78% (7/9)	31.8% (28/88)	**0.010**
Change in stool frequency	134	100% (11/11)	70.7% (87/123)	**0.036**
Change in stool appearance	130	100% (11/11)	65.5% (78/119)	**0.017**
Rectal bleeding	139	18% (2/11)	20.3% (26/128)	1
Unintentional weight loss	140	18% (2/11)	18.6% (24/129)	1
Nocturnal symptoms	137	45% (5/11)	26.2% (33/126)	0.178
Blood Biomarkers: Continuous				
Haemoglobin (g/L)	119	128 (116 - 132)	136 (129 - 147)	**0.006**
White Blood Cell count (x 10^9/L)	119	9.2 (8.0 - 10.1)	6.2 (5.0 - 7.8)	**<0.001**
Platelet count (x 10^9/L)	119	305 (250 - 380)	248 (220 - 296)	**0.039**
CRP (mg/L)	111	17 (6 - 25)	<1 (<1 - 2)	**<0.001**
Ferritin (µg/L)	65	30 (15 - 52)	38 (29 - 55)	0.38
Albumin (g/L)	89	44 (38 - 49)	48 (47 - 51)	**0.007**
B12 (ng/L)	29	628 (273 - 700)	452 (289 - 721)	0.878
Folate (µg/L)	29	7.2 (6.0 - 10.2)	11.0 (7.6 - 12.8)	0.185
Blood Biomarkers: Binary				
Anaemia [a]	119	36% (4/11)	7% (8/108)	0.014
Raised CRP (> 5 mg/L)	111	73% (8/11)	5% (5/100)	**<0.001**
Raised platelets (> 400 µg/L)	119	27% (3/11)	3% (3/108)	**0.010**
Low ferritin [b]	63	86% (6/7)	20% (11/56)	**0.001**
Raised ferritin [c]	119	0% (0/11)	5% (5/108)	1
Low B12 (< 180 ng/L)	29	0% (0/7)	5% (1/22)	1
Stool Biomarker				
Faecal calprotectin (µg/g)	142	1140 (264 - 1489)	22 (10 - 48)	**<0.001**
Red-flag criteria [d]				
Red-flag criteria present	142	55% (6/11)	51% (66/129)	1.0

% (numerator/denominator); median (interquartile range)

IBD, inflammatory bowel disease; CRP, C-reactive protein

[a] Anaemia threshold: 4-12 years < 115 g/L; males 4-18 < 130 g/L; females 4-18 < 120 g/L

[b] Low ferritin threshold: If CRP > 5 mg/L, then ferritin threshold <100 µg/L; if CRP ≤ 5 mg/L, then ferritin threshold 13 µg/L for females and 30 µg/L for males

[c] Raised ferritin threshold: female > 150 µg/L; male > 400 µg/L

[d] Red-flag criteria include one or more of: unintentional weight loss, rectal bleeding, family history of IBD

[e] *P* values represent Mann-Whitney U, chi-squared or Fisher's exact test as appropriate

[f] denominator denoted by column N

Calprotectin: Calprotectin levels were significantly greater in the IBD than the non-IBD group (**Supplemental Figure 3.4-1**). Using our pre-specified cut-off of 100 µg/g, calprotectin had a diagnostic accuracy of 93% (95% CI 87-97%) with a sensitivity for distinguishing IBD from non-IBD of 100% (95% CI 72-100%), a specificity of 92% (95% CI 86-96%), a positive predictive value of 52% (95% CI 30-74%) and a negative predictive value of 100% (95% CI 97-100%).

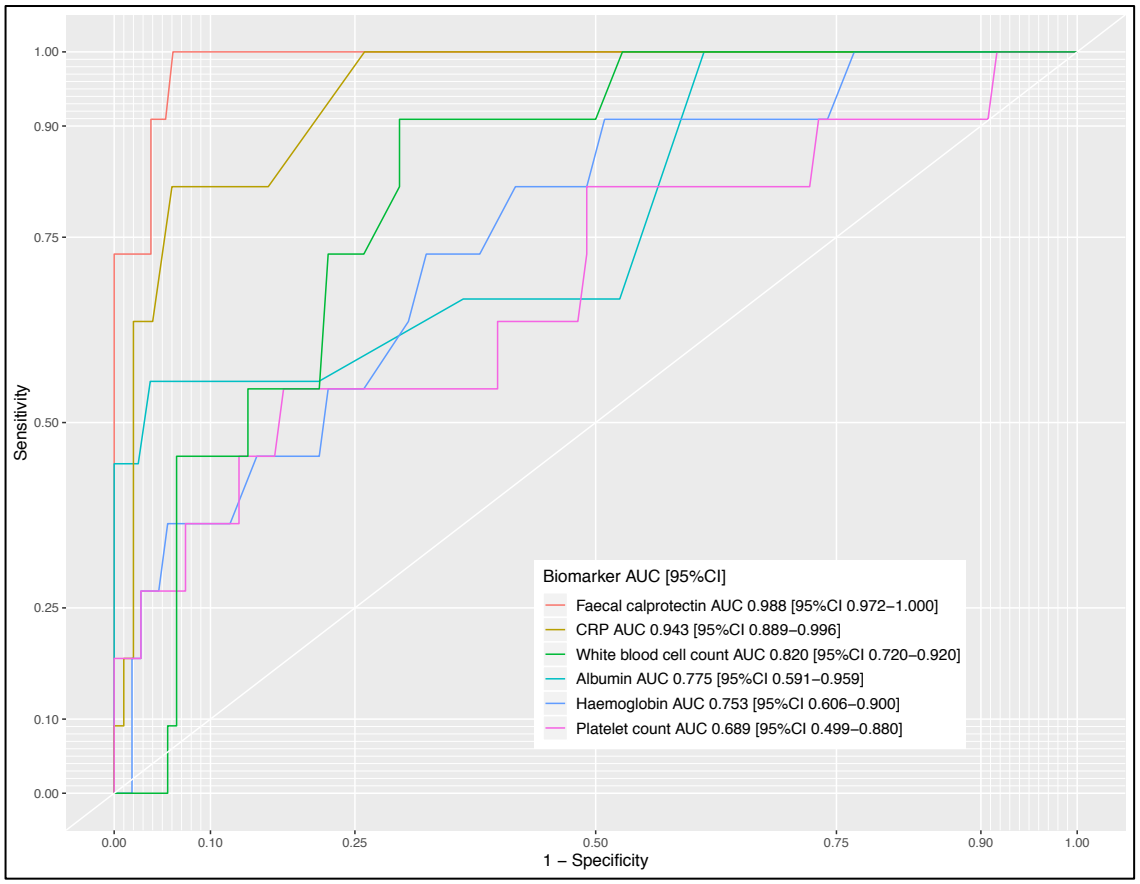
Increasing the threshold separating positive and negative calprotectin results from 50 µg/g, as recommended by the manufacturers, to 100 µg/g doubled the positive predictive value (PPV) of the test with no reduction in negative predictive value (NPV) (**Supplemental Table 3.4-2**). Further increases likewise increased the PPV but at the expense of a reduction in the NPV and a reduction in sensitivity due to missed IBD cases.

13 patients had a false positive calprotectin, 11 of whom were referred by their GP to secondary care. Of the two patients not referred to secondary care, one (calprotectin = 125 µg/g) was given an inferred diagnosis of non-IBD after 1.9 years' follow-up and the other (calprotectin = 102 µg/g) a diagnosis of gastroenteritis by their GP. Of the 11 referred patients, 9/11 underwent imaging investigations (1 CT, 1 ultrasound and 9 MRI small bowel) and all 11 children

underwent upper and lower GI endoscopy with no other organic treatable diagnoses made.

Calprotectin versus other biomarkers: Faecal calprotectin had a significantly better AUC when compared with albumin (P = 0.016), haemoglobin (P = 0.001), white blood cell count (P < 0.001) and platelets (P = 0.001) but not CRP (P = 0.144). Using Youden's method to maximize the difference between the true positive and false positive rate over all possible cut-off values, the optimal cut-off threshold for distinguishing IBD from non-IBD was 112 µg/g (**Figure 3.4-2)**.

Figure 3.4-2. Receiver operating characteristic curves for faecal calprotectin and blood biomarkers as predictors of inflammatory bowel disease



Receiver operating characteristic curves for faecal calprotectin and blood biomarkers as predictors of Inflammatory Bowel Disease (IBD) or Non-IBD. AUC, area under the curve; CI, confidence interval; CRP, C-reactive protein.

27% (3/11) of patients diagnosed with IBD had a normal CRP (≤ 5 mg/L); in all three cases the calprotectin was ≥ 100 µg/g (in two patients the blood and stool tests were performed on the same day, in the third, the calprotectin was taken 13 days after the CRP) (**Supplemental Table 3.4-3**).

3.4.4.5 Strategies for predicting an IBD diagnosis

Red-flags alone: Using a referral strategy based on the presence of red-flag symptoms alone was not predictive of IBD (logistic regression with 'red-flag' as univariable: P = 0.83, AIC = 81.0). The AUC for the receiver operator curve for the red-flag model was 0.517.

Red-flags and abnormal CRP (> 5 mg/L): Adding CRP to this model, showed that irrespective of the presence of red-flag symptoms, an abnormal CRP (> 5mg/L) increased the odds of IBD 50-fold (95% CI 11.1 to 294.4, $P = 4.8 \times 10^{-6}$) with a signifcant increase in the ROC AUC to 0.855 (P = 0.002).

Red-flags and calprotectin: We were unable to include abnormal calprotectin (≥ 100 µg/g) as a binary covariate as it had a perfect sensitivity for IBD. However, adding this biomarker as a continuous covariate (after log transformation) demonstrated that independently of red-flag symptoms, for every 10-fold increase in calprotectin, the risk of IBD increased 408-fold (95% CI 32.5 to 37564), $P = 3.9 \times 10^{-4}$, AUC 0.98).

3.4.4.6 Secondary care investigations

Patients with a negative calprotectin were referred to secondary care less frequently than the positive calprotectin cohort (60% [72/121] vs 91% [19/21] respectively, P = 0.006), they also underwent fewer endoscopic investigations

(14% [17/121] vs 71% [15/21] respectively, *P* < 0.001) and underwent less imaging (13% [16/121] vs 52% [11/21] respectively, *P* < 0.001) (**Table 3.4-3**)

Table 3.4-3: Influence of faecal calprotectin on primary care referrals to secondary care services and secondary care investigation of referred patients

Variable	Calprotectin < 100 µg/g n = 121	Calprotectin ≥ 100 µg/g n = 21	*P* Value
Primary care referral of patients to secondary care			
Referred to GI services	72/121 (60%)	19/21 (90%)	**0.006**
Emergency presentation to medical or surgical assessment unit[a]	4/121 (3%)	3/21 (14%)	**0.066**
Secondary care investigation of referred patients referred			
Reviewed in outpatients	71/121 (59%)	19/21 (90%)	**0.006**
Gastroenterology	11/71 (15%)	4/18 (22%)	0.676
Paediatrics	56/71 (79%)	14/18 (78%)	0.676
Surgeons	4/71 (6%)	0/18 (0%)	0.676
Radiological investigations			
One or more radiological investigations	16/121 (13%)	11/21 (52%)	**<0.001**
X-Ray	3/121 (2%)	4/21 (19%)	**0.009**
CT	0/121 (0%)	1/21 (5%)	0.148
MRI	7/121 (6%)	10/21 (48%)	**<0.001**
USS	11/121 (9%)	2/21 (10%)	1
Endoscopic Investigations			
One or more endoscopic procedures	17/121 (14%)	15/21 (71%)	**<0.001**
Flexible sigmoidoscopy	2/121 (2%)	2/21 (10%)	0.104
Colonoscopy	9/121 (7%)	13/21 (62%)	**<0.001**
Gastroscopy	11/121 (9%)	12/21 (57%)	**<0.001**

CT, computerised tomography; MRI, magnetic resonance imaging; GI, gastrointestinal

[a] includes GP referrals straight to medical and surgical assessment units as well as patients self-presenting to accident and emergency and admitted to the medical and surgical assessment units

3.4.4.7 IBD phenotype diagnosed on and off the calprotectin pathway

Between January 2014 and May 2016, a total of 37 children were diagnosed with IBD, of whom 75% (26/37) were diagnosed without primary care faecal calprotectin testing. Of the 26 children who did not submit a primary care calprotectin test, 19 had a secondary care calprotectin measured prior to diagnosis. In all 19 instances the faecal calprotectin result was ≥100 µg/g (median 615 µg/g, IQR 411-1930 µg/g, range 124->2100 µg/g). Patients diagnosed without use of a primary care calprotectin test reported more rectal bleeding (76% [19/26] vs. 18% [2/11], P = 0.002) but rates of unintentional weight loss (P = 0.067) and a family history of IBD (P = 1.0) were similar. Despite the differences in red-flag symptoms, the IBD Montreal phenotype, number of emergency hospital attendances (P = 0.67), serological biomarkers, including CRP and faecal calprotectin were not significantly different between the two cohorts (**Supplemental Table 3.4-4 and Supplemental Figure 3.4-2**). There was no difference in the median [IQR] time to diagnosis between patients who underwent primary care calprotectin testing and those who went straight to secondary care (53 [32-56] days vs 79.5 [49.2-189] days respectively, P = 0.11).

3.4.5 Discussion

3.4.5.1 Key results & implementation

Based on the results of our predictive modelling, stratifying paediatric referrals using red-flag symptoms alone is futile. Calprotectin, however, is a useful biomarker: the high negative predictive value allows exclusion of IBD and over half of patients with a raised calprotectin were subsequently diagnosed with UC or Crohn's disease despite the lower pre-test probability in primary care[227,277] than in referred cohorts.[271,278] Although, CRP and calprotectin both discriminated

IBD from non-IBD, calprotectin was more sensitive, detecting IBD in 3 cases where CRP was within the normal range (≤ 5 mg/L). Calprotectin has the added benefit of being non-invasive and therefore widely applicable even in young children in primary care, where bloodletting is not easily undertaken. In referred patients with a negative calprotectin, 13% underwent radiological and 14% endoscopic investigation. In all instances these tests were normal suggesting that secondary care clinicians can use this biomarker to reduce unnecessary investigations. Implementation of a primary calprotectin based-pathway is likely to reduce the risk incurred by unnecessary endoscopic procedures, particularly in children that need to undergo endoscopy using a general anaesthetic.[279–281]

3.4.5.2 Strengths and limitations

The strengths of our study lay in the prospective design and meticulous follow-up of all primary care records of non-referred patients. We acknowledge, however, some important limitations. Firstly, the uptake of calprotectin testing in primary care was relatively poor with only approximately one third (31%, 11/36) children diagnosed with IBD during the time-period of the study referred by participating practices via the pathway. We were unable to demonstrate a disease spectrum bias in the non-tested compared with calprotectin-tested patients, supporting our recommendations for faecal calprotectin in all primary care patients with possible IBD not meeting criteria for emergent referral. However, it is interesting to observe that three-quarters of children with IBD who did, and one-fifth of children who didn't undergo a primary care calprotectin test reported rectal bleeding: this illustrates that GPs still use this red-flag symptom to determine direct referral and secondly the likely inherent limitation of conflating bloody diarrhoea and fresh rectal bleeding together as one

entity. Secondly, 91% (110/121) of patients with a negative calprotectin, and 29% (6/21) of patients with a positive calprotectin later diagnosed with non-IBD did not undergo a gold standard lower gastrointestinal endoscopy, leading to an inevitable partial verification bias that may have overestimated the accuracy of calprotectin. We took various measures to limit the effect of this bias, including using a 12-month follow-up period to allow for IBD to evolve and capturing data across primary and secondary care. For non-referred patients this should have avoided an erroneous diagnosis of functional gut disorder being assigned to patients who may have been diagnosed with IBD in other hospitals. Thirdly, despite a relatively small sample size we did detect IBD and report and no false negatives unlike previous studies [227,270].

3.4.5.3 Generalisability

It is unlikely that our proposed calprotectin cut-off is applicable in children age 0-4 years, as normal values of faecal calprotectin are higher than children aged > 4 years . [282–284] Despite this, calprotectin remains a useful test in the very young, with careful interpretation of the result. We acknowledge too that our data comes from a single secondary care centre in South-west England, UK. In this regard, nearly all of our patients were diagnosed with IBD within 100 days (median 63 days) and calprotectin did not reduce the time to diagnosis of IBD. Contemporary UK data is lacking, but our time to diagnosis is shorter than previously reported in a UK multicentre population based study of 739 new IBD diagnoses diagnosed between 1998-1999, where median time to diagnosis was 5 months. [28] Whether our findings are applicable to other centres with less capacity is therefore unknown. It is likely, however, that the phenotype of paediatric IBD is similar to

that presenting elsewhere in the UK [285] and that calprotectin is likely to be useful when stratifying referrals.

3.4.5.4 What is already known on this topic

Distinguishing paediatric IBD from non-IBD in patients presenting to their GP for the first time with lower GI symptoms can be notoriously difficult. Currently GPs use a combination of 'red-flag' symptoms such as rectal bleeding, as well as blood biomarkers, including CRP, to guide onward specialist referral. Faecal calprotectin is routinely used in the secondary care setting to rule out IBD, but there is a paucity of UK data exploring its real-world use in paediatric primary care referral pathways.

3.4.5.5 What this study adds

In this real-world application in children in primary care, we have shown that a calprotectin ≥ 100 µg/g discriminates accurately IBD from non-IBD and out-performs 'red-flag' symptoms and/or CRP. Calprotectin testing did not influence the time to diagnosis of IBD, but saved outpatient referrals and a negative test reduced secondary care investigations.

3.4.5.6 Supplementary Methods

New East Devon faecal calprotectin referral pathway

We disseminated information about the new calprotectin pathway to GPs through a series of educational meetings. In accordance with NICE guidelines[214], GPs were asked to use calprotectin in patients presenting with lower gastrointestinal symptoms whom they suspected, but were uncertain, had IBD. Purpose-designed request forms were embedded within routine electronic primary care pathology requesting systems (Supplementary Appendix 5.4-1). Incomplete

request forms were rejected by the blood sciences laboratory and prompts sent to aid completion. Test results, including defined thresholds, and recommended actions were returned to GPs within 10 days.

Definition of diagnostic outcomes

Diagnoses were recorded firstly as per the responsible clinician, and then grouped as either inflammatory bowel disease (IBD) or non-IBD; the latter encompassing all enteric conditions other than IBD which may have led to the initial GP presentation. Clinicians were not blinded to the index test results. The diagnosis of IBD was based on clinical, radiological and histopathological findings.[256] A non-IBD diagnosis was assigned to patients based on a composite of lower gastrointestinal endoscopy and cross-sectional imaging with CT or MRI if available, and an absence of IBD after at least 12 months' follow-up from the time of the index test, if not. Patients not referred to secondary care were followed-up by requesting diagnoses from the responsible primary care clinicians. Patients who were diagnosed with non-enteric diseases (e.g. urological diagnoses, gynaecological diagnoses, respiratory tract infections, thyroid disease) are reported but not included in the final analysis.

RESEARCH PAPER II

SUPPLEMENT

Supplementary Appendix 3.4-1: Case Report Form

EXETER PATHOLOGY SERVICES
DEPARTMENT OF BLOOD SCIENCES
REQUESTS ENQUIRIES (01392 40)2934

Faecal Calprotectin (FC) Request Form for Primary Care

Affix Label Here

NHS Number / / Date of Birth Gender ○ M ○ F

Surname (Block CAPITALS please) First name(s) (Block CAPITALS please)

Doctor's Laboratory Code Practice Laboratory Code Sample date For EXTRA copy reports, state doctor & location

When to use Faecal Calprotectin in Primary Care:

Suspected Inflammatory Bowel disease (IBD) in patient aged 45 or below
If you suspect a patient age ≤45 might have IBD then perform FC as part of your usual work up/investigations.

- Positive: FC≥100 µg/g faeces, referral to Gastroenterology recommended.
- Negative: FC<50 µg/g faeces, IBD unlikely- consider primary care IBS management.
- Indeterminate: FC 50-99 µg/g faeces, if symptoms persist re-test FC. If on re-test FC ≥50 µg/g faeces referral to Gastroenterology is recommended.

If FC is normal, but CRP raised without another obvious explanation, then consider referral for suspected IBD

If you remain clinically concerned about a patient despite a negative FC then there is no need to repeat the test. You may refer in the normal way but please state in your referral letter what features are concerning you and prompting the referral.

Secondary care referral:

- Please title your referral letter "SUSPECTED IBD" and send to RD&E gastroenterology only. All patients will be seen in gastroenterology outpatients within 3 weeks.
- For more urgent opinions please Fax 01392 402810 or call one of the gastroenterologists

IMPORTANT - STOOL SAMPLES MUST ARRIVE IN THE LABORATORY WITHIN 24 HOURS OF COLLECTION

Criteria for Sample Analysis:

*** Please note, samples will only be processed when the following questions are marked "Yes" ***

Is patient age ≤45 years?	Yes ○	No ○
Is IBD suspected or possible?	Yes ○	No ○
Is there a low suspicion of colorectal cancer?	Yes ○	No ○
Do you confirm it has been >6 weeks since any NSAID, including aspirin?	Yes ○	No ○

Impact of FC in Primary Care:

If FC was not available would you have referred this patient to secondary care?	Yes ○	No ○	Unsure ○
Are you planning to refer this patient to secondary care even if FC is normal?	Yes ○	No ○	Unsure ○

Please also request:
a) FBC, Ferritin, CRP if not done in the past 6 weeks
b) TTG if not previously done

Clinical Information

Duration of Symptoms (months):		
Abdominal Pain:	Yes ○	No ○
Pain improves with defecation:	Yes ○	No ○
Change in stool frequency:	Yes ○	No ○
24 hour stool frequency (number of times):		
Change in stool appearance/consistency:	Yes ○	No ○
Stool consistency (see Bristol Stool Chart on reverse):		
Rectal bleeding:	Yes ○	No ○
Unintentional weight loss:	Yes ○	No ○
Nocturnal symptoms:	Yes ○	No ○
Family history of IBD:	Yes ○	No ○
Family history of bowel or ovarian cancer:	Yes ○	No ○
Alcohol (units per week):		

Version 1.1

Supplementary Table 3.4-1: Summary of diagnostic frequencies in IBD and non-IBD patients

IBD diagnoses (n= 11)		**Non-IBD diagnoses (n = 131)**	
Diagnosis	**Frequency (%)**	**Diagnosis**	**Frequency (%)**
Crohn's disease	8/11 (73%)	Functional gut disorder/ Irritable bowel syndrome	107/131 (82%)
IBD-U	2/11 (18%)	Eating disorder/Chronic pain/Anxiety-related	6/131 (5%)
Ulcerative colitis	1/11 (9%)	Anal fissure/Haemorrhoids	5/131 (4%)
		Infective gastroenteritis	5/131 (4%)
		Symptoms resolved	5/131 (4%)
		Coeliac disease	1/131 (1%)
		Gastritis	1/131 (1%)
		Chronic appendicitis	1/131 (1%)

Supplementary Table 3.4-2: Effect of altering faecal calprotectin thresholds defining positive and negative test results on diagnostic performance for distinguishing IBD and non-IBD

FC threshold (µg/g)	Sens	Sens CI	Spec	Spec.CI	PPV	PPV.CI	NPV	NPV.CI
20	1.00	(0.68 - 1.00)	0.46	(0.37 - 0.55)	0.13	(0.07 - 0.23)	1.00	(0.93 - 1.00)
50	1.00	(0.68 - 1.00)	0.76	(0.67 - 0.82)	0.26	(0.14 - 0.41)	1.00	(0.95 - 1.00)
70	1.00	(0.68 - 1.00)	0.85	(0.77 - 0.90)	0.35	(0.20 - 0.55)	1.00	(0.96 - 1.00)
100	1.00	(0.68 - 1.00)	0.92	(0.86 - 0.96)	0.52	(0.30 - 0.74)	1.00	(0.96 - 1.00)
150	0.73	(0.39 - 0.93)	0.97	(0.92 - 0.99)	0.67	(0.35 - 0.89)	0.98	(0.93 - 0.99)
200	0.73	(0.39 - 0.93)	0.97	(0.92 - 0.99)	0.67	(0.35 - 0.89)	0.98	(0.93 - 0.99)
250	0.73	(0.39 - 0.93)	0.98	(0.94 - 1.00)	0.80	(0.44 - 0.96)	0.98	(0.93 - 0.99)
300	0.73	(0.39 - 0.93)	0.99	(0.95 - 1.00)	0.89	(0.51 - 0.99)	0.98	(0.93 - 0.99)

FC, faecal calprotectin; Sens, sensitivity; Spec, specificity; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval

Supplementary Table 3.4-3. IBD phenotype in patients diagnosed in and outside primary care calprotectin pathway

Primary Care Calprotectin Pathway	Sex	Age at diagnosis (years)	IBD subtype	Index Lower GI Endoscopy Result	Paris classification CD	Paris classification UC	FC result (µg/g)	CRP (mg/L)	Family history IBD	Rectal bleeding	Weight loss
No	F	12.1	CD	Mild left-sided Crohn's disease		A1bL3B1	>2100	2	No	Yes	No
No	F	13.8	CD	Severe ileocolonic Crohn’s disease		A1bL3B1	>2100	133	Yes	Yes	Yes
No	F	18.1	CD	Mild ileocolonic inflammation		A2L3B1		1	No	No	No
No	M	7.3	IBDU	Mild patchy colonic inflammation	E3		>2100		No	Yes	Yes
No	F	8.9	CD	Severe ileocolonic Crohn’s disease		A1aL3B1	440	110		Yes	Yes
No	F	16.0	UC	Severe recto-sigmoiditis	E1			<1	No	Yes	Yes
No	M	6.3	UC	Moderate pan-colitis	E3		>2100	27		Yes	Yes
No	M	9.8	UC	Mild-moderate pan-colitis	E3		>2100	<1	No	Yes	Yes
No	F	14.4	IBDU	Severe pan-colitis	E3		544	23		Yes	
No	M	15.2	IBDU	Moderate-severe left sided colitis	E2			<1	No	Yes	No
No	M	17.2	UC	Moderate active proctitis	E1			<1		Yes	
No	M	16.9	IBDU	Mild patchy left-sided inflammation worse	E3			<1	No	No	Yes

				on right side, rectal sparing							
No	M	15.8	CD	Moderate patchy colitis and ileitis		A1bL3B1	671	12	Yes	No	No
No	M	16.9	CD	Moderate ileocolonic Crohn's disease		A1bL3B1	586	12	Yes	No	No
No	M	11.1	UC	Moderate pan-colitis	E3		1633	4	No	Yes	Yes
No	F	16.2	CD	Moderate ileitis		A1bL1B1	615	47	No	No	No
No	M	6.3	UC	Severe proctitis	E1		279	<1	No	Yes	No
No	M	12.6	CD	Moderate Crohn's colitis		A1bL3B1	382	17	No	Yes	Yes
No	M	15.1	IBDU	Moderate segmental colitis	E2		866		No	Yes	No
No	F	14.3	UC	Moderate pan-colitis	E3			9	Yes	Yes	
No	M	7.7	CD	Ileocolonic Crohn’s disease		A1aL3B1		61		Yes	Yes
No	M	15.6	UC	Moderate pan-colitis	E3		124	5	No	Yes	Yes
No	M	16.4	IBDU	Moderate right-sided colitis	E3		135	3			
No	F	10.2	CD	Moderate right-sided pan-colitis		A1bL3B1	516	19		Yes	Yes
No	M	13.7	CD	Moderate colonic Crohn's disease		A1bL2B1	125	<1	Yes	No	No
No	M	15.0	IBDU	Mild pan-colitis	E3		1758	<1	No	Yes	No
Yes	F	15.6	CD	Severe ileocolonic Crohn’s disease		A1bL3B1	1223	16		No	No
Yes	M	17.9	UC	Severe proctitis	E1		119	17	No	Yes	No
Yes	F	14.5	CD	Mild ileal Crohn’s disease		A1bL1B1	2101	6	Yes	No	No

Yes	M	14.4	CD	Moderate Ileocolonic Crohn's disease		A1bL3B2	548	25	No	No	Yes
Yes	M	13.6	CD	Moderate Crohn’s colitis		A1bL2B3	2085	25	No	No	Yes
Yes	F	15.0	CD	Moderate pan-colitis and ileitis		A1bL3B1	1373	19	No	No	No
Yes	M	15.7	CD	Severe rectosigmoid Crohn's disease		A1bL2B1	396	65	No	No	No
Yes	M	16.3	IBDU	Mild caecal inflammation	E3		1605	5	No	Yes	No
Yes	F	17.9	CD	Mild ileal Crohn's disease		A1bL1B1	130	98	No	No	No
Yes	F	14.2	IBDU	Moderate patchy pan-colitis and ileitis with rectal sparing	E3		1140	2	Yes	No	No
Yes	M	13.7	CD	Patchy mild Ileal Crohn's disease		A1bL1B1	132	2	No	No	No

CD, Crohn’s disease; UC, ulcerative colitis; FC: Faecal calprotectin; IBDU, inflammatory bowel disease unclassified; M, male; F, female; E1, proctitis; E2, left sided; E3 pancolitis; L1, ileal +/- limited caecal; L2, colonic; L3 ileocolonic; B1, inflammatory; B2, stricturing; B3, penetrating

Supplementary Table 3.4-4. Comparison of phenotype between patients diagnosed on and outside of primary care faecal calprotectin pathway

Variable		All IBD patients diagnosed 2014:2017 n = 37	Primary care calprotectin n = 11	Direct to secondary care n = 26	*P* value [b]
Demographic and IBD-phenotype					
Sex	Female	14/37 (38%)	5/11 (45%)	9/26 (35%)	0.713
Family history of IBD		7/29 (24.1%)	2/10 (20.0%)	5/19 (26%)	1
IBD subtype	CD	19/37 (51.4%)	8/11 (72.7%)	11/26 (42.3%)	0.255
	IBDU	9/37 (24.3%)	2/11 (18.2%)	7/26 (26.9%)	
	UC	9/37 (24.3%)	1/11 (9.1%)	8/26 (30.8%)	
UC extent	E1	4/18 (22.2%)	1/3 (33.3%)	3/15 (20.0%)	1
	E2	2/18 (11.1%)	0/3 (0.0%)	2/15 (13.3%)	
	E3	12/18 (66.7%)	2/3 (66.7%)	10/15 (66.7%)	
CD age	A1a	2/19 (10.5%)	0/8 (0.0%)	2/11 (18.2%)	0.274
	A1b	16/19 (84.2%)	8/8 (100.0%)	8/11 (72.7%)	
	A2	1/19 (5.2%)	0/8 (0.0%)	1/11 (9.1%)	
CD location	L1	4/19 (21.1%)	3/8 (37.5%)	1/11 (9.1%)	0.149
	L2	3/19 (15.8%)	2/8 (25.0%)	1/11 (9.1%)	
	L3	12/19 (63.2%)	3/8 (37.5%)	9/11 (81.8%)	
CD behaviour	B1	17/19 (89.5%)	6/8 (75.0%)	11/11 (100.0%)	0.164
	B2	1/19 (5.3%)	1/8 (12.5%)	0/11 (0.0%)	
	B3	1/19 (5.3%)	1/8 (12.5%)	0/11 (0.0%)	
Emergency hospital presentation		8/37 (21.6%)	5/26 (19.2%)	3/11 (27.3%)	0.672
Symptoms prior to diagnosis					
Duration symptoms (months)		3.5 (2.0 - 5.2)	3.0 (2.0 - 5.5)	4.0 (1.5 - 5.0)	0.691
Abdominal pain		25/35 (71.4%)	8/11 (72.7%)	17/24 (70.8%)	1
Change in stool frequency		30/35 (85.7%)	11/11 (100.0%)	19/24 (79.2%)	0.157
Number of stools per day		4.0 (2.0 - 4.0)	4.0 (3.2 - 4.0)	2.5 (1.9 - 4.2)	0.434
Bristol stool score (1-7)		6.0 (5.5 - 6.0)	6.0 (6.0 - 6.0)	6.0 (5.4 - 6.0)	0.962
Rectal bleeding		21/36 (58.3%)	2/11 (18.2%)	19/25 (76.0%)	**0.002**
Unintentional weight loss		14/33 (42.4%)	2/11 (18.2%)	12/22 (54.5%)	0.067
Red-flag symptoms[a]		29/36 (80.6%)	6/11 (54.5%)	23/25 (92.0%)	**0.018**
Blood tests					
Haemoglobin (g/L)		126.0 (112.0 - 131.0)	128.0 (115.5 - 132.5)	124.5 (111.0 - 130.8)	0.666

White blood cell count ($x10^9$/L)	9.2 (7.6 - 10.2)	9.2 (8.0 - 10.1)	9.2 (7.3 - 10.9)	0.881
Platelets ($x10^9$/L)	322.0 (251.0 - 405.0)	305.0 (249.5 - 380.0)	339.0 (265.2 - 413.8)	0.682
CRP (mg/L)	9.0 (1.5 - 24.0)	17.0 (5.5 - 25.0)	4.5 (0.5 - 20.0)	0.116
Raised CRP (>5mg/L)	19/35 (54.3%)	8/11 (72.7%)	11/24 (45.8%)	0.167
Ferritin	34.0 (15.0 - 60.0)	30.0 (15.0 - 52.0)	34.5 (18.2 - 63.5)	0.649
Albumin	44.0 (39.5 - 47.5)	44.0 (38.0 - 49.0)	44.5 (40.2 - 47.0)	0.97
B12	628.0 (343.0 - 931.5)	628.0 (272.5 - 699.5)	656.5 (446.0 - 977.0)	0.278
Folate	9.6 (6.7 - 14.7)	7.2 (6.0 - 10.2)	11.2 (8.8 - 17.2)	0.067
Faecal tests				
Faecal calprotectin	643.0 (385.5 - 1726.8)	1140.0 (264.0 - 1489.0)	615.0 (411.0 - 1929.5)	0.713
Raised calprotectin (≥ 100µg/g)	30/30 (100.0%)	11/11 (100.0%)	19/19 (100.0%)	1
Time to diagnosis				
From symptom onset to first GP presentation (months)	3.5 (2.0 - 5.2)	3.0 (2.0 - 5.5)	4.0 (1.5 - 5.0)	0.691
From first GP presentation to GP referral (days)	22.0 (8.8 - 60.8)	32.0 (14.0 - 32.8)	18.5 (9.2 - 76.5)	0.912
From GP referral to diagnosis (days)	41.5 (20.5 - 63.0)	21.0 (15.5 - 47.5)	45.5 (38.0 - 76.8)	0.15
Total time to diagnosis (days)	63.0 (41.5 - 101.5)	53.0 (32.0 - 56.0)	79.5 (49.2 - 189.0)	0.111

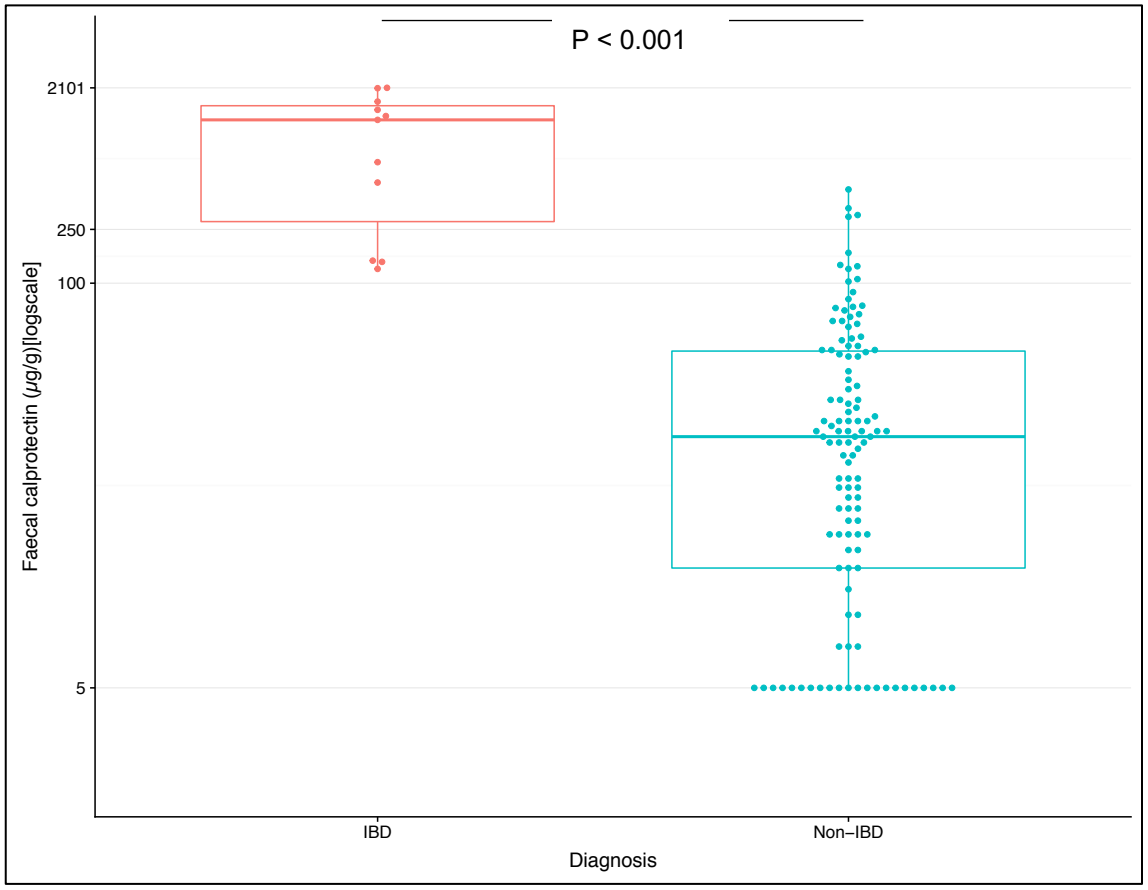
% (numerator/denominator); median (interquartile range)

IBD, inflammatory bowel disease; CRP, C-reactive protein

[a] Red-flag criteria include one or more of: unintentional weight loss, rectal bleeding; family history of IBD

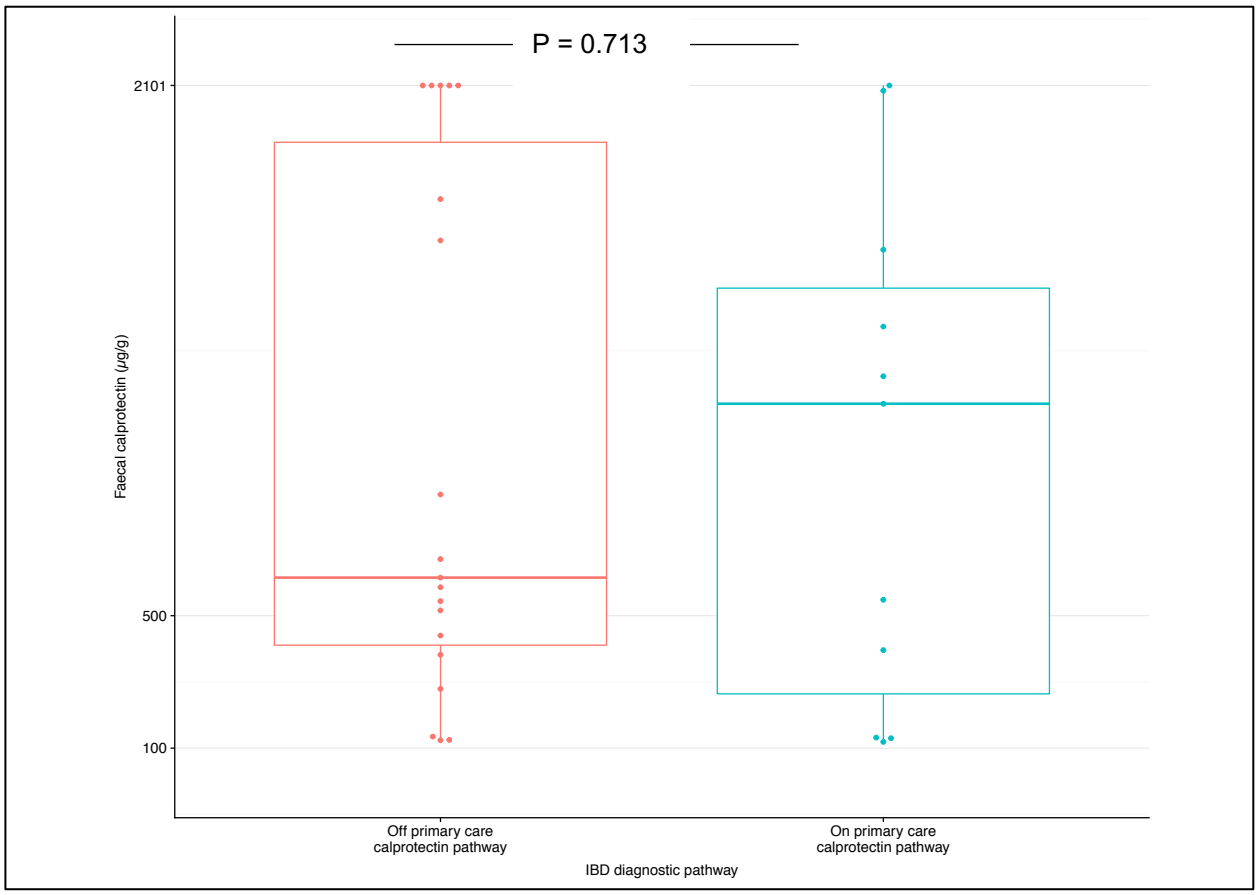
[b] *P* values represent Mann-Whitney U for primary vs. direct

Supplementary Figure 3.4-1:Box plot showing difference in faecal calprotectin between patients with Inflammatory Bowel Disease (IBD) and Non-IBD



Box plot showing difference in faecal calprotectin between patients with Inflammatory Bowel Disease (IBD) and non-IBD.

Supplementary Figure 3.4-2. Faecal calprotectin among IBD patients diagnosed on and off primary care calprotectin pathway



Box plot showing the faecal calprotectin in Inflammatory Bowel Disease (IBD) patients diagnosed on and off the primary care calprotectin pathway

RESEARCH PAPER III

‘A prospective cohort study to identify factors associated with a delay in IBD diagnosis.’

PENDING SUBMITION FOR PUBLICATION

Gareth Walker, Amanda Thomas, Simeng Lin, Neil Chanchlani, Peter Hendy, Neil Heerasing, Lucy Moore, Harry Green, Nick Kennedy, Claire Bewshea, Joe Mays, James Goodhand, Tariq Ahmad.

3.6.1 Abstract

Background: Delay in the diagnosis of inflammatory bowel disease (IBD) is common and contemporary UK studies exploring this issue are lacking.

Objective: To determine the factors associated with, and the consequences of, a prolonged time to diagnosis in IBD.

Design: Prospective observational cohort study

Setting: 49 GP practices and gastroenterology secondary care services at the Royal Devon and Exeter NHS Foundation Trust, UK.

Patients: 304 adults with a new IBD diagnosis made between January 2014 - December 2017.

Main outcome measures: Multivariable logistic regression of demographic, disease and biomarkers associated with a prolonged (>75th centile) time to: (a) *patient* presentation (b) *GP* referral (c) *secondary care* diagnosis. Factors associated with complicated disease course (hospitalisation and/or surgery and/or biologic treatment) in the year after diagnosis.

Results: Of 304 eligible patients: female 48%; median [IQR, range] age-at-diagnosis 36 [27-53, 18-91] yrs; ulcerative colitis 64%; emergent presentation 20%. Median [IQR] diagnosis sub-intervals: (a) *patient* = 2 [1-5] months; (b) *GP* = 0.3 [0-1] months; (c) *secondary care* = 1 [0.5-2] months. 50% of patients diagnosed within 4-months and 11% within 2-years of symptom onset: diagnostic delay more common in Crohn's disease than ulcerative colitis (P < 0.001). Patients who presented emergently (P < 0.001) but not those with a delayed overall time to diagnosis (P = 0.35) more likely to have a complicated disease course in first year after diagnosis.

Conclusion: Time to patient presentation is the biggest component of time to IBD diagnosis supporting the need to raise public awareness of seeking medical attention

for new lower GI symptoms. Emergent presentation is common and associated with a complicated disease course, unlike a delayed time to diagnosis.

3.6.2 Introduction

Delay in the diagnosis of inflammatory bowel disease (IBD) and in particular Crohn's disease, is common. Overall, about one-third of patients report symptoms for more than a year before a diagnosis of IBD is made.[18,230,286–288] Timely diagnosis of IBD is important because earlier use of biologic therapies leads to mucosal healin[125,127,128], a reduction in hospitalisations and surgeries[289,290], and improvements in quality of life.[235]

Contemporary studies of the time to IBD diagnosis from the United Kingdom (UK), where healthcare is free at the point-of-access and general practitioners (GPs) act as gatekeepers to secondary care services, are lacking.[286] Most patients who present to primary care with gastrointestinal symptoms will have a functional gut disorder and only a minority will have IBD.[220,221,223] Distinguishing between irritable bowel syndrome (IBS) and IBD, even in the presence of red-flag symptoms is difficult, and misdiagnosis is common.[251,291] Faecal calprotectin is a National Institute for Health and Care Excellence (NICE) endorsed biomarker that helps physicians rule out IBD, and is increasingly being used in primary care.[214,241] However, few studies have assessed whether the use of calprotectin in primary care helps avoid a delay to the diagnosis of IBD.

3.6.2.1 Objectives

We aimed to define the clinical and laboratory factors associated with a delay to diagnosis of IBD. We hypothesised that primary care calprotectin testing would reduce the time to diagnosis and that diagnostic delay would be associated with a more complicated disease course.

3.6.3 Methods

3.6.3.1 Study design and clinical setting

We designed a prospective observational cohort study to identify factors associated with a prolonged time to diagnosis of IBD. The Royal Devon & Exeter (RD&E) NHS Foundation Trust provides secondary care services to a locality that comprises 49 GP practices covering a population of 378,000 people in the South West of England, UK. GPs refer patients with gastrointestinal symptoms to either gastroenterology or surgical services.[292]

We introduced a new calprotectin-based primary care referral pathway for patients with suspected IBD in 2014.[272] In brief, calprotectin testing was encouraged, but not mandated, to stratify referrals of patients reporting new gastrointestinal symptoms, to secondary care. We report data related to the time to diagnosis of IBD made between January 2014 and December 2017.

3.6.3.2 Inclusion & exclusion criteria

Each patient was diagnosed with IBD by their gastroenterologist using standard endoscopic, histological and/or radiological criteria. Patients who first presented to their GP with gastrointestinal symptoms after January 2014 were eligible for inclusion. Patients aged less than 18 years old and those diagnosed with IBD at other centres were excluded.

3.6.3.3 Primary outcome

Time to diagnosis was defined as the time from onset of patient symptoms to diagnosis of IBD by endoscopy and/or imaging. We divided this into three subintervals: the time from symptom onset to first GP presentation; the time from first GP presentation to GP referral; and the time from GP referral to IBD diagnosis. Delay was defined as a time to diagnosis greater than the upper quartile and was calculated for each sub-interval: hereafter referred to as patient, primary and secondary care delays.

3.6.3.4 Variables and data acquisition

Identification of patients with IBD

We searched our IBD database for new cases of IBD diagnosed after 2014. Checks were made, using free-text searches of both our endoscopy (Unisoft Medical Systems, Enfield, UK) and histology databases (Swift Integrated Healthcare Solutions DXC Technology Company, Virginia, US) to identify cases not registered in our database. Data was extracted from primary and secondary care records into a purpose designed electronic database in REDCap (Vanderbilt University Medical Centre, Tennessee, US).

Patient delay variables

We recorded demographic data (age at IBD diagnosis, sex, ethnicity, family history of colorectal or ovarian cancer, family history of IBD, home postcode at diagnosis and smoking history), presenting symptoms at the time of first GP presentation and IBD phenotype according to the Montreal Classification.[71] Patient income deciles were estimated using data from the English Indices of Deprivation 2015 and patients' postcodes.[293]

Primary care delay variables

Blood (full blood count, urea and electrolytes, liver function tests, C-reactive protein [CRP], tissue transglutaminase, vitamin B12, folate and ferritin) and faecal biomarker tests (faecal calprotectin, microscopy culture and sensitivity) within 28 days of GP referral were captured using secondary care electronic pathology records.

Secondary care delay variables

Electronic records and patient notes were used to capture the priority category assigned to each referral to secondary care (routine or two-week wait) and whether the patient was referred by their general practitioner or self-presented to secondary care emergency services. In our Trust, GP referrals are vetted and triaged by gastroenterologists or surgeons to either outpatient clinic review or straight to diagnostic test. The following dates prior to diagnosis were recorded: first outpatient appointment, emergency hospital presentation (accident and emergency, medical assessment unit and/or surgical assessment unit), index endoscopy and cross-sectional imaging (computerised tomography/magnetic resonance imaging). Secondary care work-force capacity was estimated through three proxy measures: firstly, by year of diagnosis; secondly, by ascertaining if any given patient's IBD diagnosis occurred within one week either side of a national UK holiday; and thirdly, after developing a continuous variable (adjusted outpatient capacity), which reflected outpatient gastroenterology capacity between the date of referral and diagnosis (see methods in the Supplement).

Treatment and complications in the first year after diagnosis

We captured the following data within the first year of diagnosis: the number of and indication for IBD-related surgeries; IBD-related hospitalisations; and treatment (5-aminosalicyates, corticosteroids, immunomodulatory therapies [methotrexate, thiopurine or ciclosporin], biologics [infliximab, adalimumab, vedolizumab and ustekinumab] and exclusive enteral nutrition). Patients were deemed to have complicated disease if they had an IBD-related hospital admission and/or IBD-related surgery and/or biologic therapy in the first year after their diagnosis.

3.6.3.5 Statistical methods

Because this study was designed as a service evaluation *a priori* power calculations were not undertaken: we decided to allow our new calprotectin pathway to become established and then assessed its usefulness over three years.

All analyses were conducted in R 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). Analyses were two tailed, and p-values <0.05 were considered significant. We excluded patients from individual analyses where relevant data were missing and specified the denominator for each variable. Continuous variables are reported as median [interquartile range - IQR] and compared with either Mann Whitney U or Kruskal-Wallis tests. Categorical variables are summarised as frequencies (%) and compared with Fisher's exact test. Univariable analyses were undertaken to identify factors associated with patient-, primary care-, and secondary care delay. We also sought factors associated with emergency department presentations and being triaged straight-to-test by secondary care specialists. We used stepwise forward and backwards multivariable logistic regression models, using Akaike Information Criteria

(AIC) scores to compare models and avoid over-fitting, to identify factors independently associated with delays in diagnosis. We only included significant univariable factors ($P < 0.05$) with more than 100 observations in our models. Continuous data were transformed to a binary categorical variable. Checks were made for multi-collinearity using a variance inflation factor (VIF) score of more than 10. Collinear variables that added least to our regression models were removed. Results are presented as odds ratio (OR) and 95% confidence interval (95% CI).

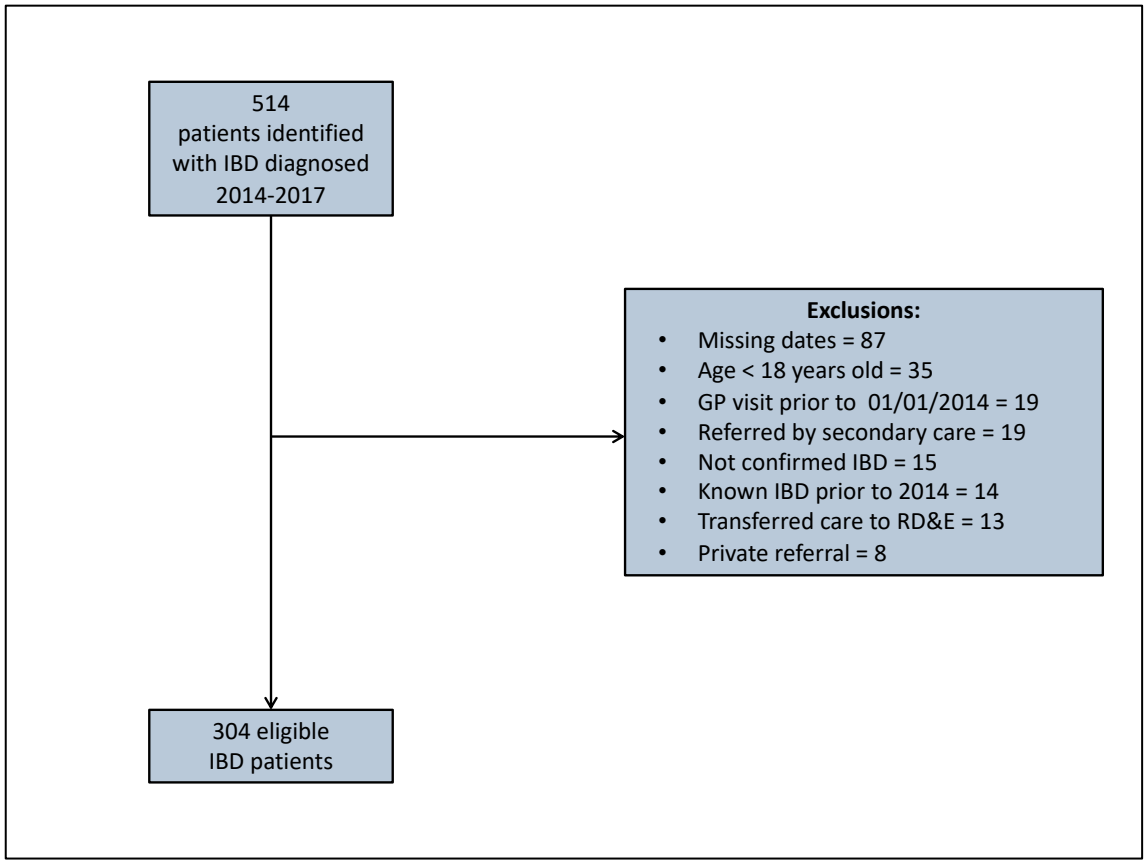
3.6.3.6 Ethical consideration

This quality improvement project was endorsed by primary and secondary care Caldicott guardians, the Southwest Academic Health Sciences Network (SWAHSN), the Local Medical Council, and the Devon Clinical Commissioning Group. Patients were not involved in the conception or design of this study. In accordance with UK Health Research Authority guidelines we did not require formal ethical approval.[294]

3.6.4 Results

We report our data according to the flow diagram in Figure 1: 514 adult patients were identified as diagnosed with IBD between January 2014 and December 2017. We excluded 87 patients because we were unable to define the onset of symptoms, 40 patients who were not-referred from primary care, and 15 subjects where we unable to verify the diagnosis of IBD. Baseline demographics are shown in **Supplemental Tables 3.5-1 and 3.5-2.**

Figure 3.6-1. Flow diagram



Missing dates, missing one or more dates required to calculate three sub-intervals; IBD, inflammatory bowel disease; GP, general practitioner; not confirmed IBD, patients diagnosed with possible or refuted diagnosis of IBD; RD&E, Royal Devon and Exeter Hospital

3.6.4.1 Time to diagnosis

The median time to diagnosis from onset of symptoms was 4.3 months [IQR 2.2 to 10.7 months]. Over half (60%) of patients were diagnosed within 6 months, 79% within 12 months and 92% within 24 months of the onset of symptoms. The greatest contributor to the overall delay was the time it took patients to present to their GP, with a median duration of 2.1 months [IQR 0.9–5.1 months]. In comparison, the median time to secondary care referral from the GP was 0.3 months [IQR 0.0–0.9 months] and the median time from GP referral to diagnosis 1.1 months [IQR 0.5–2.1 months] ($P < 0.001$). Patients with Crohn's disease had a longer overall time to diagnosis: UC,

3.3 months [IQR 1.9–7.3 months], IBD-U, 3.9 months [IQR 2.0–7.2 months] and Crohn's disease, 7.6 months [IQR 3.1–15.0 months]; P < 0.001) (**see Table 3.5-1, Figure 3.5-2 and Supplemental Tables 3.5-1 and 3.5-2**)

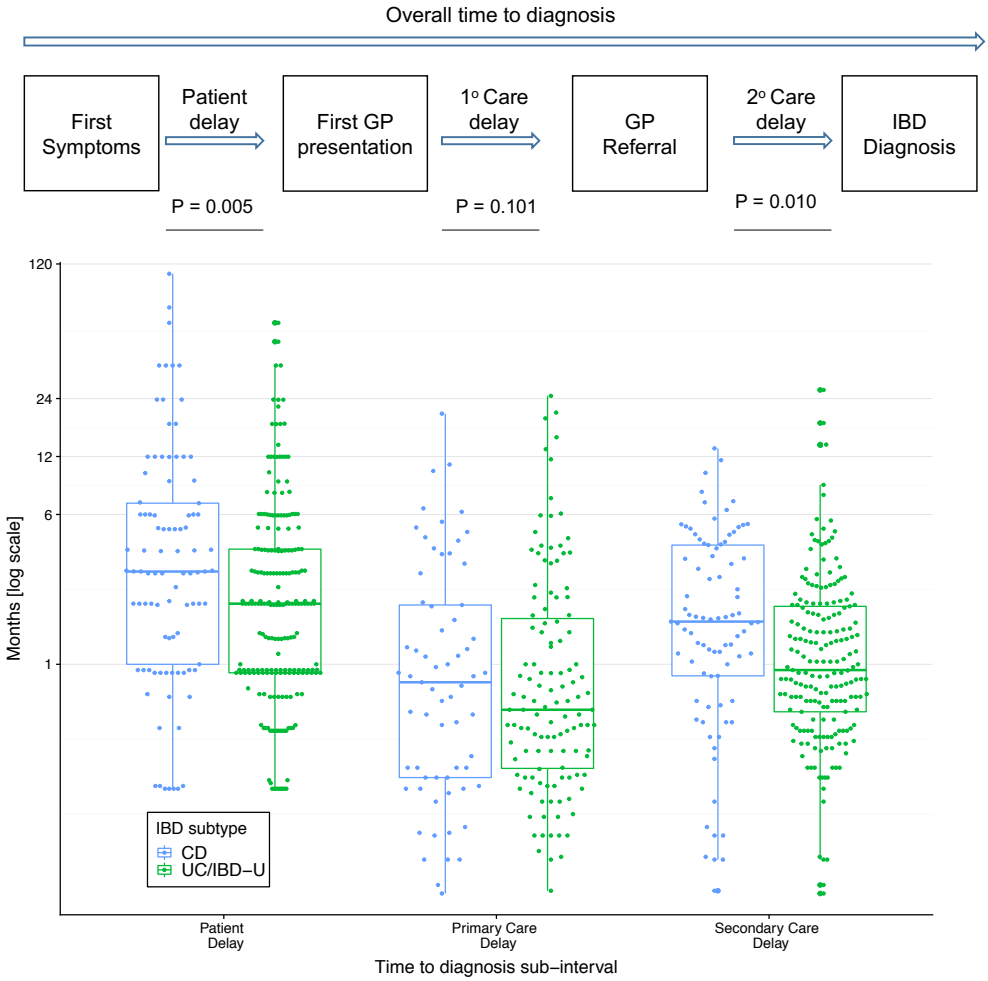
Table 3.6-1. Time to IBD diagnosis by IBD-subtype

Subintervals	**Crohn's disease *n* = 94**	**IBD-unclassified *n* = 15**	**Ulcerative colitis *n* = 195**	**P value[a]**
Patient delay: time from first symptoms to first GP presentation (months) [median, IQR, range]	3, 1-7, 0-107	2, 1-5, 0-12	2, 1-4, 0-59	**0.017**
Primary care delay: time between first GP presentation and GP referral (months) [median , IQR, range]	0, 0-1, 0-20	0, 0-1, 0-4	0, 0-1, 0-25	0.26
Secondary care delay: time between GP referral and secondary care diagnosis (months) [median , IQR, range]	2, 1-4, 0-13	1, 1-1, 0-4	1, 1-2, 0-27	**0.027**
Overall time to diagnosis: time from first symptoms to secondary care diagnosis (months) [median , IQR, range]	8, 3-15, 0-112	4, 2 - 7, 0-16	3, 2-7, 0-65	**<0.001**

IQR, interquartile range; IBD, inflammatory bowel disease

[a] P value represents Kruskal-Wallis test

Figure 3.6-2. Box plot of intervals constituting time to diagnosis among all 304 patients by IBD subtype



CD, Crohn's disease; UC, ulcerative colitis; IBD-U, IBD-unclassified

3.6.4.2 Factors associated with emergent IBD diagnoses

Approximately one-fifth (19%, 58/304) of all new IBD diagnoses from 2014-2018 were made following an emergency presentation to hospital: 86% (50/58) patients were referred to hospital by their GP whilst 14% (8/58) patients self-presented to the A&E department.

Demographic and disease factors associated with either an emergent or a non-emergent IBD diagnosis are presented in **Supplemental Table 3.5-3**. Age under 30

years old at IBD diagnosis (OR 2.42, 95%CI 1.35 - 4.34), duration of symptoms less than 6 weeks (OR 4.97, 95%CI 2.73 - 9.29), abdominal pain (OR 3.15, 95%CI 1.64 - 6.51), unintentional weight loss (OR 4.10, 95%CI 1.89 – 8.94), anaemia (OR 7.18, 95%CI 2.70 - 20.51), raised platelet count (OR 6.30, 95%CI 2.20 - 18.79) and raised white blood cell count (OR 6.00, 95%CI 1.84 – 20.64) were associated with an increased odds of an initial presentation of IBD as an emergency.

In the final multivariable model, duration of symptoms less than 6 weeks and anaemia increased the odds of an emergent presentation 8-fold (OR = 8.26, 95%CI 1.77 – 50.75) and 19-fold (OR 19.01, 95%CI 3.76 – 160.48), respectively (**see Table 3.5-2**).

A diagnosis of IBD following an emergency hospital admission was associated with greater use of corticosteroids ($P < 0.001$), biologics ($P < 0.001$) and exclusive enteral nutrition ($P < 0.001$) than non-emergent diagnoses. In contrast the use of 5-ASAs was reduced ($P < 0.001$) and the use of immunomodulators was no different in those with an emergent rather than non-emergent diagnosis ($P = 0.308$). Patients with an emergent diagnosis experienced more IBD-related hospitalisations ($P < 0.001$) after the index presentation and surgeries ($P < 0.001$).

Figure 3.6-3. Boxplot of time to diagnosis among all IBD patients by emergent and non-emergent diagnosis

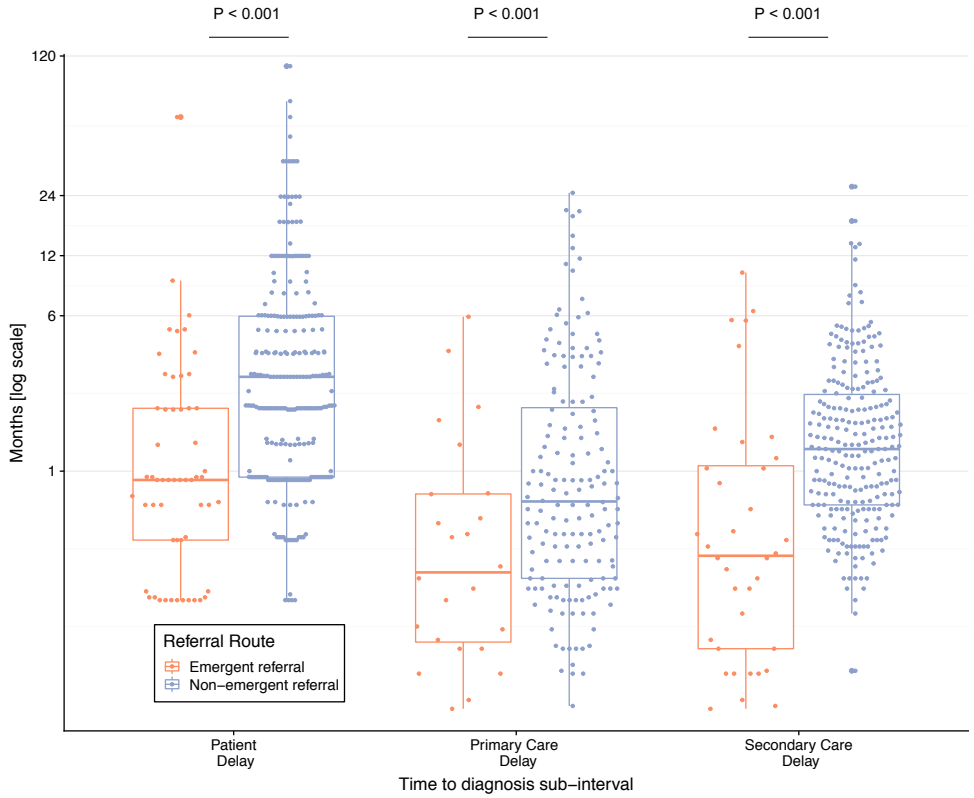


Table 3.6-2. Uni- and multi-variable analysis of factors associated with an emergent diagnosis of IBD

Emergent diagnosis of IBD variables [a]	**Univariable analysis**			**Multivariable analyses**		
	P value	**OR**	**OR 95%CI**	**P value**	**OR**	**OR 95%CI**
Patient factors						
Aged < 30 yrs at IBD diagnosis	0.003	2.42	1.35 - 4.34			
Patient symptoms						
Duration symptoms < 6 wks	2.5×10^{-7}	4.97	2.73 - 9.29	0.012	8.26	1.77 - 50.75
Abdominal pain	0.001	3.15	1.64 - 6.51			
Weight loss	3.4×10^{-4}	4.10	1.89 - 8.94			
Blood biomarkers						
Presence of anaemia[b]	1.2×10^{-4}	7.18	2.70 - 20.51	0.001	19.01	3.76 - 160.48
Raised platelet count	6.9×10^{-4}	6.30	2.20 - 18.79			
Raised white blood cell count	0.003	6.00	1.84 - 20.64			

OR, odds ratio; CI, confidence interval; IBD, inflammatory bowel disease

[a] Only factors with n >100 and P < 0.05 shown. See Supplemental Table 3.5-3 for complete UVA.

[b] Anaemia threshold (World Health Organisation definition) defined as haemoglobin concentration < 120g/L for females and < 130g/L for males

[c] Raised platelets defined as platelet count > 400 x10^9/L

[d] Raised white blood cell count defined as > 10.6 x10^9/L for females and > 11.0 x10^9/L for males

3.6.4.3 Factors associated with patient-delay in presentation to primary care

The median [IQR] patient delay was 2.1 [0.9-5.1] months. Demographic and disease factors associated with either timely or delayed patient presentation to their GP are presented in **Supplemental Table 3.5-4.** Reporting abdominal pain (OR 2.47, 95%CI 1.40 to 4.51), altered bowel habit to diarrhoea (OR 2.81, 95%CI 1.15 to 8.44), unintentional weight loss (OR 2.37, 95%CI 1.19 to 4.66), and a higher estimated higher household income (OR 1.20, 95%CI 1.24 to 5.34) all increased the odds of a delayed patient presentation (**see Table 3.5-3**).

In the final multivariable model, the odds of a delayed patient presentation were increased 2-fold by the presence of abdominal pain (OR 2.11, 95%CI 1.01 to 4.64), 1.3-fold for every increase in estimated income decile (OR 1.27, 95%CI 1.07 to 1.53), and 3-fold by the presence of unintentional weight loss (OR 2.57, 95%CI 1.21 to 5.50), and decreased 2-fold by the presence of rectal bleeding (OR 0.45, 95%CI 0.22 to 0.91).

Table 3.6-3. Uni- and multi-variable analysis of factors associated with a prolonged time (> 5.1 months) from onset of symptoms to first GP presentation

Patient delay variables [a]	Univariable analysis			Multivariable analyses		
	P value	OR	OR 95% CI	P value	OR	OR 95% CI
Patient Factors						
Income decile [b]	0.008	1.2	1.24 - 5.34	0.008	1.27	1.07 to 1.53
Patient Symptoms						
Rectal bleeding	0.001	0.39	0.22 - 0.67	0.025	0.45	0.22 to 0.91
Abdominal pain	0.002	2.47	1.40 - 4.51	0.054	2.11	1.01 to 4.64
Unintentional weight loss	0.013	2.37	1.19 - 4.66	0.014	2.57	1.21 to 5.50
Altered bowel habit-diarrhoea [c]	0.038	2.81	1.15 - 8.44	-	-	-

OR, odds ratio; CI, confidence interval; IBD, inflammatory bowel disease

[a] Only factors with n >100 and P < 0.05 shown

[b] Income estimated using patient postcode (see methods)

[c] Reference = constipation/no change in bowel habit

3.6.4.4 Factors associated with primary care delay

The median [IQR] primary care delay was 0.3 [0-0.9] months. Demographic and disease factors associated with either timely or a delayed patient presentation to their GP are presented in **Supplemental Table 3.5-5.** Only duration of symptoms less than 6 weeks and patient age at diagnosis were significant in both the uni- and multi-variable analysis and therefore only the latter is described (**see Table 3.5-4**). In the multivariable analysis, for every 10-year increase in age at IBD diagnosis the odds of delay were reduced by approximately a third (OR 0.96, 95%CI 0.94 to 0.98). Whereas a shorter than 6-week symptom duration prior to GP presentation reduced the odds of a primary care delay by approximately 80% (equivalent to 5-fold reduction in odds) (OR 0.18, 95%CI 0.08 to 0.36).

Table 3.6-4. Primary Care Delay: Uni- and multi-variable factors associated with a prolonged time (> 0.9 months) from first patient presentation to GP referral

Primary care delay variables [a]	Univariable analysis			Multivariable analyses		
	P value	OR	OR 95% CI	P value	OR	OR 95% CI
Patient Factors						
Age at IBD diagnosis	2.8×10^{-4}	0.97	0.95 - 0.98	2.5×10^{-4}	0.96	0.94 - 0.98
Patient Symptoms						
Duration symptoms < 6 weeks	9.4×10^{-6}	0.19	0.08 - 0.37	7.4×10^{-6}	0.18	0.08 - 0.36

OR, odds ratio; CI, confidence interval; IBD, inflammatory bowel disease

[a] Only factors with n >100 and P < 0.05 shown

3.6.4.5 Factors associated with secondary care delay

From the point of GP referral, 63%(191/304) of IBD patients were reviewed within 4 weeks by a hospital specialist. The median [IQR] secondary care delay was 1.1 [0.5-2.1] months. Demographic and disease factors associated with either timely or a delayed secondary care as shown in **Supplemental Table 3.5-6**. Male sex (OR 0.49, 95%CI 0.28 to 0.83), family history of IBD (OR 0.23, 95%CI 0.05 to 0.66), duration of symptoms < 6 weeks (OR 0.21, 95%CI 0.10 to 0.42), urgent GP referral (OR 0.14, 95%CI 0.06 to 0.30) and being triaged straight-to-test (OR 0.11, 95%CI 0.06 to 0.21) were associated with a reduction in the odds of a prolonged secondary care delay (see Table 5).

In the final model, adjusted by workforce capacity, male sex (OR 0.37, 95%CI 0.13 to 1.02), duration of patient symptoms lasting less than 6 weeks prior to first GP presentation (OR 0.14, 95%CI 0.03 to 0.51), urgent GP referral (OR 0.12, 95%CI 0.04 to 0.35) and being triaged straight-to-test (OR 0.08, 95%CI 0.02 to 0.25) were associated with a reduction in the odds of secondary care delay.

Table 3.6-5. Secondary Care Delay: Univariable and multivariable factors associated with a prolonged time (>2.1 months) from GP referral to diagnosis

Secondary care delay variables [a]	Univariable analysis			Multivariable analyses		
	P value	OR	OR 95%CI	P value	OR	OR 95%CI
Patient factors						
Male sex	0.008	0.49	0.28 - 0.83	0.058	0.37	0.13 to 1.02
Family history IBD	0.017	0.23	0.05 - 0.66	-	-	-
Patient symptoms						
Duration symptoms < 6 weeks	2.4×10^{-5}	0.21	0.10 - 0.42	0.007	0.14	0.03 to 0.51
Change in bowel habit-diarrhoea	0.030	0.46	0.23 - 0.94	-	-	-
Primary Care Factors						
Urgent GP referral [b]	1.6×10^{-6}	0.14	0.06 - 0.30	1.4×10^{-4}	0.12	0.04 - 0.35
Secondary Care Factors						
Straight-to-test	1.1×10^{-11}	0.11	0.06 - 0.21	4.9×10^{-5}	0.08	0.02 - 0.25
Workforce capacity [c]	0.418	1	1.00 - 1.00	0.009	1.01	1.00 - 1.01

OR, odds ratio; CI, confidence interval; IBD, inflammatory bowel disease; straight-to-test, patients referred by primary care are triaged to go directly to undergo a diagnostic test without being seen an outpatient clinic first; GP, general practitioner

[a] Only factors with n >100 and P < 0.05 shown

[b] Reference = routine referral

[c] See supplementary methods, *a priori* this was included in the multivariable analysis

3.6.4.6 Disease course in patients with a delay in diagnosis

The median time [IQR] to an overall IBD diagnosis was 4.3 [2.2 to 10.7] months. IBD-related complications (surgery and hospitalisation) and therapies within the first year of diagnosis among patients with either timely or a delayed overall time to diagnosis are shown in **Supplemental Table 3.5-7**. Patients with a delayed time to diagnosis were no more likely to receive corticosteroids (P = 0.427), immunosuppressives (P = 0.105), aminosalicylates (P = 0.101), biologics (P = 1), exclusive enteral nutrition (P = 0.761), experience more IBD-related hospitalisations (P = 0.149) or undergo more surgeries (P = 0.415) than patients with a timely diagnosis.

In a post-hoc sensitivity analysis having removed the emergently diagnosed patients, there was an association with delayed diagnosis (>2 years from symptom onset) and higher IBD-related hospital admissions (P = 0.038) and steroid use (P = 0.043), but not IBD-related surgeries (P = 0.356), immunosuppressives (P = 0.117) or biologics (P = 0.302) in the first year after diagnosis (**see Supplemental Table 3.5-8**).

3.6.4.7 Faecal calprotectin

Primary care calprotectin was performed in 11% (53/304) of all IBD patients prior to their first point of contact with secondary care (**see Supplemental Table 3.5-9 and Supplemental Figure 3.5-1**). Of the 5 patients (2 male, 3 female) with a false negative calprotectin (< 100µg/g), 4 were diagnosed with ulcerative colitis, all of whom had E1:proctitis (P = 0.021), and 1 with Crohn's disease (A2: Age <40 yrs; L1:ileal; B1:inflammatory); 3 had rectal bleeding and none had either a family history of IBD, unintentional weight loss or an elevated CRP (>5 mg/L). Neither primary- nor secondary-care delay were increased following a false negative calprotectin: P = 0.761 and P = 0.429, respectively. Nor was a positive calprotectin (≥ 100 µg/g) associated with a more rapid (< 25th centile) GP referral or secondary care diagnosis.

3.6.5 Discussion

3.6.5.1 Key results

In this study one-fifth of patients diagnosed with IBD presented emergently, half of patients were diagnosed within 4 months of symptom onset and one-tenth of patients suffered symptoms for more than 2 years before diagnosis. The time taken for patients to present to primary care was the major factor contributing to overall diagnostic delay. Uptake of primary care calprotectin testing was low and had no effect on time to diagnosis. 'Urgent' GP referral and directing patients straight-to-test reduced secondary care delay independently of temporal changes in workforce capacity. Patients who presented emergently and those whose diagnosis was made after 2 years were more likely to have a complicated disease course in the year after diagnosis.

3.6.5.2 Interpretation

The overall time to diagnosis reported here is similar to recent reports from Swiss[25], American[26] and Italian[27] cohorts, but longer than reports from other European countries[5]. The reasons for these differences are likely to be complex and related to local healthcare pathways, in particular the involvement of primary care physicians in the decision to refer, rather than self-referral to a gastroenterologist. We, like others, report a longer time to diagnosis in patients with Crohn's disease compared with either ulcerative colitis or IBD-unclassified[25–27]; an observation possibly explained by the presence of rectal bleeding, which is not only reported more commonly in patients with ulcerative colitis but also associated with timely GP consultation.

The median sub-interval between symptom onset and primary care presentation was 7-times longer than the time it took GPs to refer patients and twice as long as the time it took secondary care services to diagnose referred patients. We have replicated previous findings that rectal bleeding and lower GI symptoms are associated with a shorter time to presentation.[26] It is unclear why we found a seemingly paradoxical association with increased patient delay with weight loss and abdominal pain. Perhaps the latter reflects the widespread public assumption, especially in younger patients that abdominal pain is likely to be due to a functional gut disorder.[2,28] Arguably, as secondary care clinicians we are unable to influence patient or primary care delay.

The uptake of faecal calprotectin testing, limited in clinical practice to patients under the age of 46 years, was low, and we were under-powered to answer whether calprotectin influences time to diagnosis. However, we report that referrals triaged straight-to-test were associated with a reduced time to diagnosis, and in common with previous work, that the wider use of faecal calprotectin and/or faecal immunohistochemical testing (FIT) to triage patients straight-to-test may reduce the time to diagnosis.[29,30]

To our knowledge, we are the first adult UK study to report the frequent GP-directed emergent referral of suspected IBD through medical and surgical assessment units. This emergent cohort had a higher inflammatory burden with a more extensive and complicated phenotype at presentation. Consequently, this cohort were more likely to experience IBD-related surgery, IBD-related hospital admission and receive treatment with immunosuppressive and biologic therapies in the year after diagnosis.

3.6.5.3 Limitations/Strengths

Our study has several strengths: first, to our knowledge this is the largest contemporary adult UK study to identify factors associated with delays at each of the constituent stages that make-up 'overall time to IBD diagnosis'. Second, we captured primary care data using GP records in order to accurately establish the date of first GP presentation, reducing recall bias and inaccuracies. Further work across this primary and secondary care interface should be encouraged and is to the advantage of all stakeholders, but particularly patients. Second, we adjusted our findings using a novel proxy for workforce capacity, a strong confounder that undermines many other previous similar studies.

However, we also note some limitations: although we electronically interrogated hospital pathology records, enabling the assimilation of millions of data-points, the exclusion of both blood and faecal tests prior to 28 days of GP referral and also variables with less than 100 observations, may have limited our power to detect association with these parameters. Second, it is unclear whether the date of symptom onset reflects an underlying inflammatory or functional gut disorder[36], although this limitation is shared by all similar studies. Third, in common with most UK primary care calprotectin pathways[37,38], we discourage use of this biomarker in older patients who constituted one third of our inception cohort. However, limiting our analysis to younger calprotectin-eligible patients would have been to the detriment of the objectives and generalisability of this study.

3.6.5.4 Generalisability

Whilst we accept that time to diagnosis depends on healthcare provision in a locality, our principle findings are likely to be generalisable to most hospitals in the UK. Pressures on NHS services are increasing, and we believe that observations such as the GP referral of one-fifth of patients as emergency admission, secondary care triaging of over half of newly diagnosed patients straight-to-test and our failure to review a third of suspected IBD referrals within the 4-week recommended timeframe reflect this.[39,40] In this regard our performance is in line with over a quarter of UK gastroenterology services surveyed in the 2014 UK IBD audit.[41] Even centres who met this target in 2014 might have struggled to meet it more recently as reflected by 2018 NHS performance indicators.[42] We note a few possible differences between our local secondary care service and others nationally, and, indeed internationally, which may aid interpretation of our findings: first, in comparison with other larger UK cities, the Royal Devon and Exeter Hospital serves an ethnically homogenous white population and the time to presentation and utilisation of healthcare services in other populations may differ.[43] Second, in comparison with national data we report a relatively low endoscopy waiting time for diagnostic flexible sigmoidoscopy and colonoscopy:10 weeks.[295,296] In comparison, in April 2019, the proportion of NHS patients waiting longer than 6 weeks for a diagnostic colonoscopy or flexible sigmoidoscopy was 10.9% and 7.4%, respectively.[44] Third, we report relatively high use of biologics with 34% of Crohn's disease and 9% of ulcerative colitis/IBD-unclassified patients receiving such treatment in the first year from diagnosis. In comparison, the EPI-IBD inception cohort, which comprised data from 22 European countries between 2010-2015, reported that approximately 16% of Crohn's and 4% of ulcerative colitis patients received biologic treatment in the year after diagnosis.[4,34] As these therapies have

been shown to reduce hospitalisation and complications, other hospitals with lower use of such therapies may report different outcomes.

3.6.5.5 For the future

Diagnostic delay can be considered to have patient-, primary care- and secondary care- related components. Our data lend strong support to the need for raising awareness about the importance of seeking medical attention for new lower GI symptoms among members of the general public. Targeting adolescents and young adults seems to be particularly important given the more complicated disease in this cohort. Prompt patient-GP consultation is not only important for the early diagnosis of IBD, but more widely for the diagnosis of colorectal cancer which is rising most rapidly in patients aged 20-29 yrs old.[45] Application of faecal biomarkers in the 'low risk but not no risk' group (NICE; NG12[46]) may help stratify appropriate onward GP referral.[47–49] Furthermore, more widespread early use of calprotectin and blood tests, where diagnostic uncertainty exists, in primary care patients not meeting acute severe IBD parameters may save money and aid appropriate triaging to secondary care services.[29,50]

RESEARCH PAPER III

SUPPLEMENT

3.6.6.1 Faecal Calprotectin Pathway

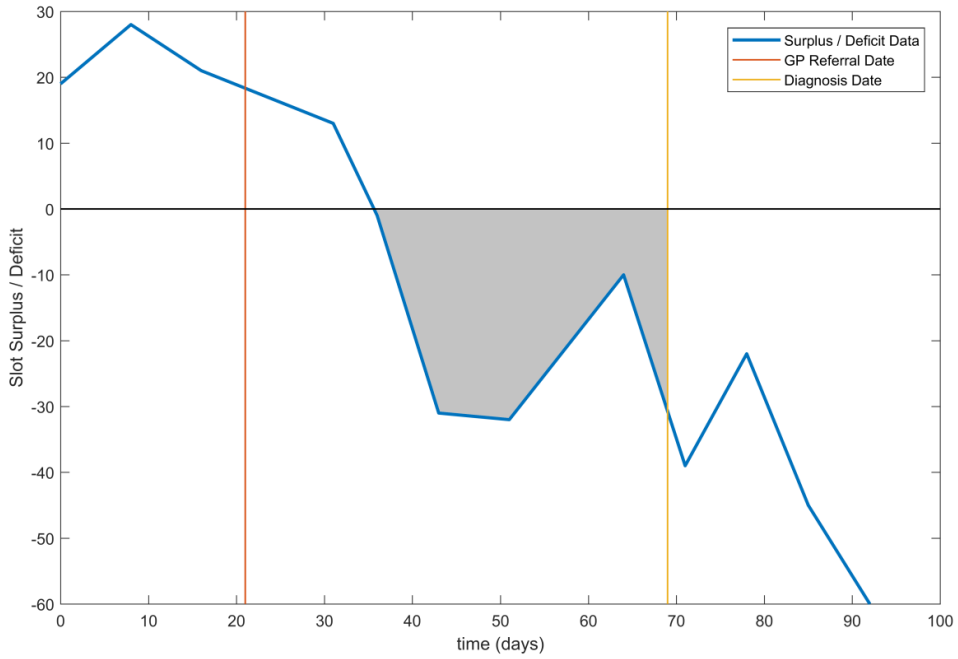
We introduced primary care calprotectin in January 2014 based on 2013 NICE (DG11)[18] guidance to all 49 local and encouraged GPs to use this test in all patients with lower GI symptoms aged under 46 years old where they suspected, but were not confident in a diagnosis of IBD. Calprotectin use was therefore not mandated prior to GP referral. Although we have now modified our calprotectin pathway based on recent data[21], the following thresholds were used during the time of data collection: calprotectin ≥100 µg/g = positive, calprotectin 50-99 µg/g = intermediate and calprotectin <50 µg/g = negative. GPs were asked to send repeat stool samples from patients with intermediate results and second samples considered positive if calprotectin ≥50 µg/g. We advised referral of adult patients with a positive calprotectin and GP management for negative tests; although GPs were also able to refer patients whom they felt required specialist review based on their clinical assessment.

3.6.6.2 Calculation of 'workforce capacity' variable

Data pertaining to the weekly surplus or deficit of outpatient and endoscopy appointments at our centre between 2014 and 2018 were obtained. These data provided either a positive (reflecting a surplus of outpatient clinic and endoscopy slots relative to referrals for each week) or negative integer (reflecting deficit of outpatient clinic and endoscopy slots relative to referrals for each week). A new variable representing shortage in available appointments for each patient between their time of referral and diagnosis was calculated from the area under a curve of appointment surplus / deficit. The area was approximated using the trapezium rule for numerical integration, with endpoints (time of referral and diagnosis) linearly interpolated using neighbouring points. Only area below the x axis was considered; a surplus of

outpatient appointments reflected by areas above this line (y>0) were deemed clinically irrelevant as any referral could have been instantly offered a slot, regardless of whether for example 1 or 10 slots were available. The area was then divided by the time between referral and diagnosis. The variable, called the **adjusted workforce capacity** therefore represents the average deficit each day in that time period. This is illustrated for a hypothetical patient in Supplementary M Fig 1 below.

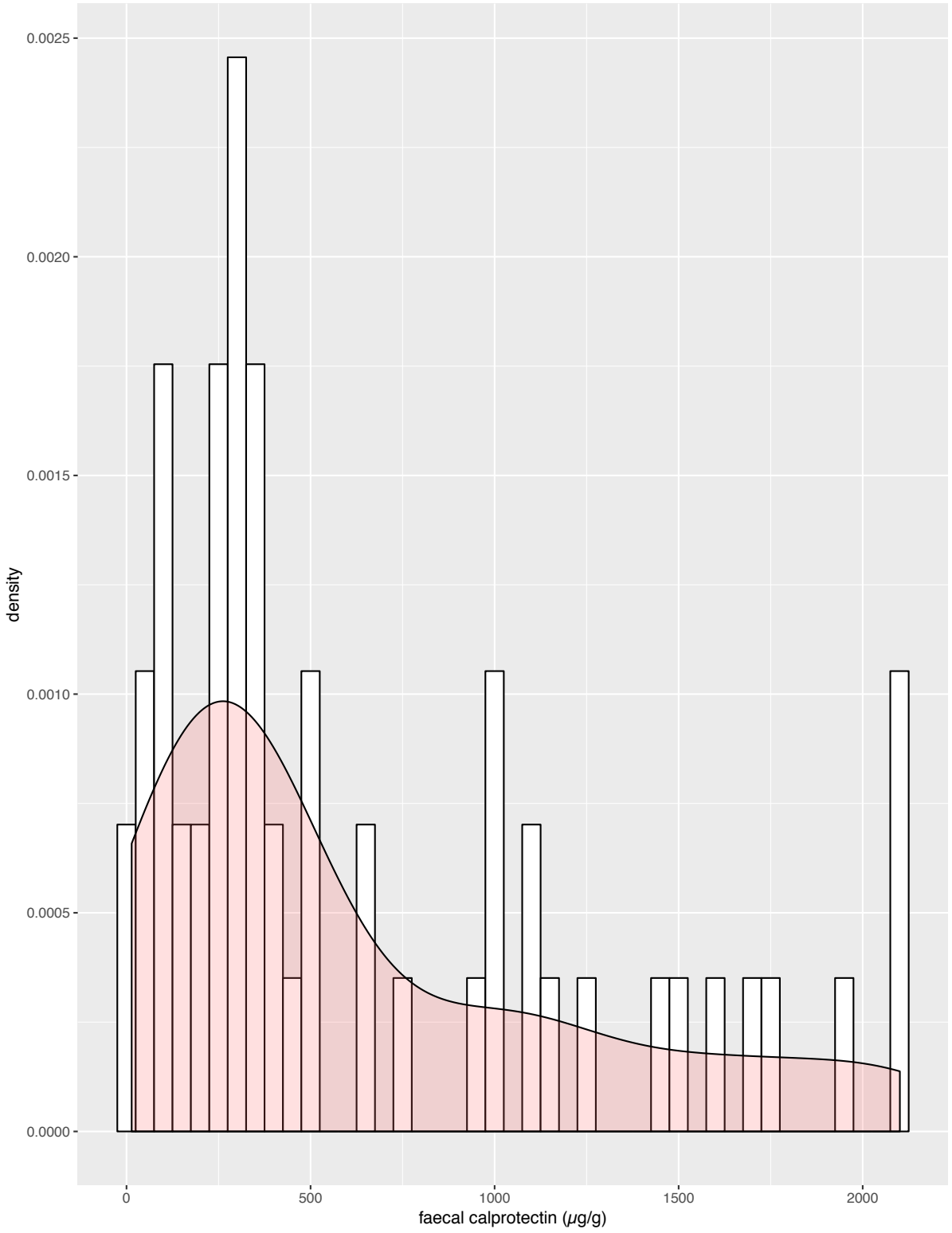
Supplementary M.Fig 1: Hypothetical workforce capacity variable assessment



Patient was referred on day 21 (21st January 2014) and diagnosed on day 69 (10th of March). The plot shows the surplus / deficit (blue) with date of referral (red) and date of diagnosis (yellow) annotated. The area used is shaded area in grey, and divided by the time between referral and diagnosis to give the 'adjusted workforce capacity variable'

Supplemental Figures

Supplementary Table 3.6-1. Calprotectin results among all IBD patients



Supplemental Tables

Supplementary Table 3.6-2. Overall cohort demographics, delay and complications

Variable	Level	Value
Sex	Female	145/303 (47.9%)
Ethnicity	White	288/304 (94.7%)
Age at IBD diagnosis (years)		36.3 (26.8 - 52.5)
Family history of IBD		38/268 (14.2%)
Family history of bowel/ovarian cancer		20/250 (8.0%)
Income decile		6.0 (5.0 - 8.0)
Smoking status	Current smoker	45/303 (14.9%)
IBD subtype	CD	94/304 (30.9%)
	IBDU	15/304 (4.9%)
	UC	195/304 (64.1%)
UC extent	E1: proctitis	88/204 (43.1%)
	E2: left-sided	64/204 (31.4%)
	E3: total	52/204 (25.5%)
CD location	L1: ileal	46/95 (48.4%)
	L2: colonic	20/95 (21.1%)
	L3: ileocolonic	29/95 (30.5%)
CD behaviour	B1: inflammatory	74/93 (79.6%)
	B2: stricturing	10/93 (10.8%)
	B3: penetrating	9/93 (9.7%)
Perianal CD		8/94 (8.5%)
Patient delay (months)		2.1 (0.9 - 5.1)
Primary care delay (months)		0.3 (0.0 - 0.9)
Secondary care delay (months)		1.1 (0.5 - 2.1)
Overall time to diagnosis (months)		4.3 (2.2 - 10.7)
IBD-related hospitalisation	One or more	65/295 (22.0%)
IBD-related surgery	One or more	4/279 (1.4%)
Treatment in the first year after diagnosis	Corticosteroids	159/295 (53.9%)
	Immunomodulator	83/295 (28.1%)
	Aminosalicylate	180/295 (61.0%)
	Biologic	50/295 (16.9%)

CD, Crohn's disease; UC, ulcerative colitis; IBD, inflammatory bowel disease

Supplementary Table 3.6-3. Comparing demographics, symptoms at initial GP presentation, blood and stool tests, primary care factors, secondary care factors and complications among IBD subtypes

Variable	N	Level	Crohn's disease n = 94	IBD-unclassified n = 15	Ulcerative colitis n = 195	P value
Demographics						
Sex	303	Female	50/94 (53.2%)	3/15 (20.0%)	92/194 (47.4%)	0.055
Ethnicity	304	White	91/94 (96.8%)	14/15 (93.3%)	183/195 (93.8%)	0.458
Age at IBD diagnosis	304	Years	32.1 (24.4 - 45.4)	42.4 (37.7 - 52.1)	37.9 (27.7 - 54.1)	0.053
Family history of IBD	268		8/83 (9.6%)	7/14 (50.0%)	23/171 (13.5%)	**0.002**
Income decile	291		7.0 (5.0 - 8.0)	7.0 (5.0 - 7.5)	6.0 (5.0 - 8.0)	0.692
Smoking status	303	Ex-smoker	17/94 (18.1%)	5/15 (33.3%)	57/194 (29.4%)	**<0.001**
		Non-smoker	49/94 (52.1%)	10/15 (66.7%)	120/194(61.9%)	
		Smoker	28/94 (29.8%)	0/15 (0.0%)	17/194 (8.8%)	
Symptoms						
Duration symptoms	304	Months	3.0 (1.0 - 6.8)	2.0 (1.1 - 4.5)	2.0 (1.0 - 4.0)	**0.015**
Rectal bleeding	298		36/90 (40.0%)	9/14 (64.3%)	169/194 (87.1%)	**<0.001**
Abdominal pain	300		74/93 (79.6%)	8/14 (57.1%)	95/193 (49.2%)	**<0.001**
Weight loss	226		29/70 (41.4%)	4/10 (40.0%)	19/146 (13.0%)	**<0.001**
Change appearance	291		65/90 (72.2%)	10/15 (66.7%)	161/186 (86.6%)	**0.004**
Pain improves on defaecation	69		8/27 (29.6%)	2/4 (50.0%)	10/38 (26.3%)	0.637
Number of stools 24 hrs	141		4.0 (3.0 - 6.0)	8.0 (5.5 - 10.0)	4.0 (3.0 - 7.0)	**0.077**
Change stool frequency	296		74/89 (83.1%)	13/15 (86.7%)	167/192 (87.0%)	0.677
Nocturnal symptoms	163		14/49 (28.6%)	2/10 (20.0%)	19/104 (18.3%)	0.385
Change bowel habit	280	diarrhoea	67/83 (80.7%)	11/13 (84.6%)	159/184 (86.4%)	0.458
Blood and stool tests						
Anaemia	102	see footer	20/37 (54.1%)	0/4 (0.0%)	13/61 (21.3%)	**0.001**
Raised CRP	94	> 5mg/L	29/36 (80.6%)	2/5 (40.0%)	21/53 (39.6%)	**<0.001**
Raised platelets	100	> 400 $x10^9$/L	12/36 (33.3%)	0/4 (0.0%)	8/60 (13.3%)	0.055

Supplemental Table 3.5-3 continued…

…continued Supplemental Table 3.5-2

	N	Level	Crohn's disease n = 94	IBD-unclassified n = 15	Ulcerative colitis n = 195	P value
Raised ferritin	49	see footer	2/14 (14.3%)	1/2 (50.0%)	0/33 (0.0%)	**0.012**
Low ferritin	49	see footer	1/14 (7.1%)	0/2 (0.0%)	11/33 (33.3%)	0.151
Raised WBC	102	see footer	6/37 (16.2%)	1/4 (25.0%)	7/61 (11.5%)	0.419
Low B12	18	< 180ng/L	1/6 (16.7%)	0/1 (0.0%)	1/11 (9.1%)	1
Low folate	18	< 3.6µg/L	3/6 (50.0%)	0/1 (0.0%)	0/11 (0.0%)	**0.043**
Low ferritin	49	see footer	1/14 (7.1%)	0/2 (0.0%)	11/33 (33.3%)	0.151
Low albumin	74	< 30g/L	2/26 (7.7%)	1/5 (20.0%)	2/43 (4.7%)	0.306
Primary Care Factors						
Urgent GP referral	148	urgent	24/46 (52.2%)	5/6 (83.3%)	48/96 (50.0%)	0.314
Secondary Care Factors						
Secondary care team triaging GP referral	244	surgeons	29/65 (44.6%)	5/12 (41.7%)	103/167 (61.7%)	**0.036**
Straight-to-test	301		45/92(48.9%)	10/15(66.7%)	123/194(63.4%)	0.055
Time to diagnosis						
Patient delay	304		3.0 (0.9 - 6.7)	2.1 (1.0 - 4.5)	2.1 (0.9 - 3.9)	**0.017**
Primary care delay	296		0.3 (0.0 - 1.2)	0.3 (0.0 - 0.7)	0.2 (0.0 - 0.8)	0.26
Secondary care delay	296		1.6 (0.6 - 3.7)	0.7 (0.5 - 1.3)	0.9 (0.5 - 2.0)	**0.027**
Overall time to diagnosis	304		7.6 (3.1 - 15.0)	3.9 (2.0 - 7.2)	3.3 (1.9 - 7.3)	**<0.001**
Complications						
IBD-related hospitalisation	295		34/93 (36.6%)	3/14 (21.4%)	28/188 (14.9%)	**<0.001**
IBD-related surgeries	293		12/92 (13.0%)	1/14 (7.1%)	5/187 (2.7%)	**0.003**
Treatment in the first year after diagnosis	295	Steroids	68/93 (73.1%)	10/14 (71.4%)	81/188 (43.1%)	**<0.001**
		IS	53/93 (57.0%)	5/14 (35.7%)	25/188 (13.3%)	**<0.001**
		5ASA	8/93 (8.6%)	6/14 (42.9%)	166/188 (88.3%)	**<0.001**
		Biologic	32/93 (34.4%)	2/14 (14.3%)	16/188 (8.5%)	**<0.001**
		EEN	14/93 (15.1%)	0/14 (0.0%)	0/188 (0.0%)	**<0.001**

Straight-to-test, patients referred by primary care are triaged to go directly to undergo a diagnostic test without being seen an outpatient clinic first; GP, general practitioner; WBC, white blood cell count; CRP, c-reactive protein IBD, inflammatory bowel disease; EEN, exclusive enteral nutrition; IS, immunosuppressive medication (azathioprine, mercaptopurine, methotrexate or ciclosporin); 5ASA, 5-aminosalicylate; Steroids, corticosteroids). Data displayed as % (n/N) or median (IQR); P value represents Fisher's exact or Mann Whitney U test as appropriate. Anaemia threshold (WHO definition) as haemoglobin concentration < 120g/L for females and < 130g/L males. Raised WBC defined as > 10.6 x10^9/L for females and > 11.0 x10^9/L for males. Raised ferritin defined as > 150ng/mL for females and > 400ng/mL for males. Low ferritin defined as < 15ng/mL for females and < 30ng/mL for males. Income decile estimated using patient postcode (see methods)

Supplementary Table 3.6-4. Demographics, presenting symptoms, biomarkers and delay intervals among non-emergently and emergently diagnosed IBD patients

Variable	N	Level	Non-emergent n = 246	Emergent n = 58	P value
Demographics					
Sex	303	Female	118/246 (48.0%)	27/57 (47.4%)	1
Ethnicity	304	White	232/246 (94.3%)	56/58 (96.6%)	0.745
Age at IBD diagnosis	304	Years	38.1 (28.3 - 54.0)	30.0 (22.5 - 43.5)	**0.005**
Age < 30 yrs at IBD diagnosis	304		72/246 (29.3%)	29/58 (50.0%)	**0.003**
Family history of IBD	268		30/218 (13.8%)	8/50 (16.0%)	0.657
Family history of colorectal- /ovarian cancer	250		17/200 (8.5%)	3/50 (6.0%)	0.773
Income decile	291		7.0 (5.0 - 8.0)	6.0 (5.0 - 8.0)	0.436
Smoking status	303	Smoker	36/245 (14.7%)	9/58 (15.5%)	0.839
Symptoms					
Duration symptoms < 6 weeks	304		68/246 (27.6%)	38/58 (65.5%)	**<0.001**
Rectal bleeding	298	Yes	179/243 (73.7%)	35/55 (63.6%)	0.139
Abdominal pain	300	Yes	132/243 (54.3%)	45/57 (78.9%)	**<0.001**
Weight loss	226	Yes	36/193 (18.7%)	16/33 (48.5%)	**<0.001**
Change appearance	291	Yes	193/238 (81.1%)	43/53 (81.1%)	1
Pain improves on defaecation	69	Yes	17/63 (27.0%)	3/6 (50.0%)	0.346
Number of stools in 24 hours	141		4.0 (3.0 - 7.0)	5.0 (1.5 - 12.0)	0.824
Change stool frequency	296	Yes	211/243 (86.8%)	43/53 (81.1%)	0.282
Nocturnal symptoms	163	Yes	29/148 (19.6%)	6/15 (40.0%)	0.094
Change bowel habit	280	Diarrhoea	193/226 (85.4%)	44/54 (81.5%)	0.528
Blood and stool tests					
Anaemia	102	see footer	17/78 (21.8%)	16/24 (66.7%)	**<0.001**
Raised CRP	94	> 5mg/L	32/74 (43.2%)	20/20 (100.0%)	**<0.001**
Raised platelets	100	> 400 $x10^9$ /L	9/76 (11.8%)	11/24 (45.8%)	**<0.001**
Raised ferritin	49	see footer	2/46 (4.3%)	1/3 (33.3%)	0.176
Low ferritin	49	see footer	10/46 (21.7%)	2/3 (66.7%)	0.144
Raised WBC	102	see footer	6/78 (7.7%)	8/24 (33.3%)	0.004
Low B12	18	< 180ng/L	2/17 (11.8%)	0/1 (0.0%)	1
Low folate	18	< 3.6µg/L	3/17 (17.6%)	0/1 (0.0%)	1
Low albumin	74	< 30g/L	2/56 (3.6%)	3/18 (16.7%)	0.089
Faecal calprotectin	53	µg/g	338.0 (223.0 - 1031.8)	2100.0 (2100.0 - 2100.0)	0.133
Raised faecal calprotectin	53	> 100µg/g	47/52 (90.4%)	1/1 (100.0%)	1

Supplemental Table 3.5-3 continued…

…continued Supplemental Table 3.5-3

Variable	N	Level	Non-emergent n = 246	Emergent n = 58	P value
Disease subtype					
IBD subtype	304	CD	64/246 (26.0%)	30/58 (51.7%)	**< 0.001**
		IBD-U	12/246 (4.9%)	3/58 (5.2%)	
		UC	170/246 (69.1%)	25/58 (43.1%)	
UC extent	204	E1: proctitis	86/177 (48.6%)	2/27 (7.4%)	**< 0.001**
		E2: left-sided	49/177 (27.7%)	15/27 (55.6%)	
		E3: total	42/177 (23.7%)	10/27 (37.0%)	
CD location	95	L1: ileal	32/65 (49.2%)	14/30 (46.7%)	0.616
		L2: colonic	12/65 (18.5%)	8/30 (26.7%)	
		L3: ileocolonic	21/65 (32.3%)	8/30 (26.7%)	
CD behaviour	93	B1:inflammatory	54/64 (84.4%)	20/29 (69.0%)	0.072
		B2: stricturing	7/64 (10.9%)	3/29 (10.3%)	
		B3: penetrating	3/64 (4.7%)	6/29 (20.7%)	
Perianal CD	94	Yes	7/64 (10.9%)	1/30 (3.3%)	0.429
Year of IBD diagnosis					
Year of IBD diagnosis	304	2014	58/246 (23.6%)	19/58 (32.8%)	0.197
	304	2015	82/246 (33.3%)	20/58 (34.5%)	
	304	2016	78/246 (31.7%)	11/58 (19.0%)	
	304	2017	28/246 (11.4%)	8/58 (13.8%)	
Time sub-intervals					
Patient delay	304		3.0 (0.9 - 6.0)	0.9 (0.3 - 2.1)	**<0.001**
Primary care delay	296		0.3 (0.0 - 1.0)	0.0 (0.0 - 0.3)	**<0.001**
Secondary care delay	296		1.3 (0.7 - 2.4)	0.2 (0.0 - 0.6)	**<0.001**
Overall time to diagnosis	304		5.5 (2.8 - 11.9)	1.5 (0.8 - 3.6)	**<0.001**
Complications					
IBD-related hospitalisation	295	One or more	21/243 (8.6%)	44/52 (84.6%)	**<0.001**
IBD-related surgeries	279	One or more	6/242 (2.5%)	12/51 (23.5%)	**<0.001**
Treatment in the first year after diagnosis	295	Steroids	118/243 (48.6%)	41/52 (78.8%)	**<0.001**
		IS	65/243 (26.7%)	18/52 (34.6%)	0.308
		5ASA	162/243 (66.7%)	18/52 (34.6%)	**<0.001**
		Biologic	32/243 (13.2%)	18/52 (34.6%)	**<0.001**
		EEN	239/243 (1.6%)	42/52 (19.2%)	**<0.001**
Complicated disease in the first year after diagnosis[a]	304		46/246 (18.7%)	45/58 (77.6%)	**<0.001**

Data displayed as % (n/N) or median (IQR); P value represents Fisher's exact or Mann Whitney U test as appropriate. IBD, inflammatory bowel disease; WBC, white blood cell count; CRP, c-reactive protein; EEN, exclusive enteral nutrition; IS, immunosuppressive medication (azathioprine, mercaptopurine, methotrexate or ciclosporin); 5ASA, 5-aminosalicylate; Steroids, corticosteroids). Data displayed as % (n/N) or median (IQR); P value represents Fisher's exact or Mann Whitney U test as appropriate. Anaemia threshold (WHO definition) as haemoglobin concentration < 120g/L for females and < 130g/L males. Raised WBC defined as > 10.6 x10^9/L for

females and > 11.0 x10^9/L for males. Raised ferritin defined as > 150ng/mL for females and > 400ng/mL for males. Low ferritin defined as < 15ng/mL for females and < 30ng/mL for males

[a] complicated disease defined as either IBD-related hospitalisation or IBD-related surgeries or received biologic drug treatment in the first year of diagnosis

Supplementary Table 3.6-5. Patient delay: Patient factors and symptoms associated with a prolonged time (> 5.1 months) from onset of symptoms to first GP presentation

Patient Delay Variable	N	Level	Timely patient presentation n = 230	Delayed patient presentation n = 74	P value
Demographics					
Sex	303	Male	52.8% (121/229)	50.0% (37/74)	0.690
Ethnicity	304	White	95.2% (219/230)	93.2% (69/74)	0.551
Age at IBD diagnosis	304	Years	37.0 (27.4 - 52.5)	34.3 (25.6 - 46.5)	0.412
Family history of IBD	268		14.9% (30/202)	12.1% (8/66)	0.687
Family history of bowel/ ovarian cancer	250		6.4% (12/187)	12.7% (8/63)	0.176
Income decile[a]	291		6.0 (5.0 - 8.0)	7.0 (6.0 - 8.0)	**0.009**
Income in upper quartile (highest earning)	291		14.2% (31/219)	22.2% (16/72)	0.138
Smoking status[b]	303	Current smoker	13.5% (31/230)	19.2% (14/73)	0.258
Symptoms					
Rectal bleeding	298		76.9% (173/225)	56.2% (41/73)	**0.001**
Abdominal pain	300		54.0% (122/226)	74.3% (55/74)	**0.003**
Unintentional weight loss	226		19.1% (33/173)	35.8% (19/53)	**0.015**
Change appearance	291		80.3% (175/218)	83.6% (61/73)	0.607
Pain improves on defaecation	69		31.4% (16/51)	22.2% (4/18)	0.556
Number of stools in 24 hours	141		4.0 (3.0 - 6.5)	6.0 (3.0 - 8.2)	0.100
Change stool frequency	296		84.8% (189/223)	89.0% (65/73)	0.442
Nocturnal symptoms	163		21.3% (26/122)	22.0% (9/41)	1.000
Change in bowel habit	280	Diarrhoea	82.0% (173/211)	92.8% (64/69)	0.034

Data displayed as % (n/N) or median (IQR); P value represents Fisher's exact or Mann Whitney U test as appropriate. IBD, inflammatory bowel disease

[a] Income decile estimated using patient postcode (see methods)

[b] Reference = ex-smokers and non-smokers

Supplementary Table 3.6-6. Primary Care Delay: Factors associated with a prolonged time (> 0.9 months) from first patient presentation to GP referral

Primary Care Delay Variable	N	Level	Timely GP referral n = 222	Delayed GP Referral n = 74	P value
Demographics					
Sex	295	Male	55.2% (122/221)	44.6% (33/74)	0.139
Ethnicity	296	White ethnicity	95.5% (212/222)	93.2% (69/74)	0.540
Age at diagnosis of IBD	296	Years	41.4 (27.4 - 55.6)	31.7 (24.7 - 37.8)	**< 0.001**
Family history of IBD	260		13.8% (26/189)	16.9% (12/71)	0.556
Family history of bowel/ovarian cancer	242		8.8% (15/170)	6.9% (5/72)	0.800
Income decile [a]	283		6.0 (5.0 - 8.0)	7.0 (5.0 - 8.0)	0.057
Smoking status	295	Current smoker	14.9% (33/222)	15.1% (11/73)	1.000
Symptoms					
Duration symptoms < 6 wks	296		42.8% (95/222)	12.2% (9/74)	**< 0.001**
Rectal bleeding	290		74.3% (162/218)	65.3% (47/72)	0.172
Abdominal pain	292		57.5% (126/219)	61.6% (45/73)	0.585
Unintentional weight loss	221		23.1% (39/169)	23.1% (12/52)	1.000
Change appearance	283		81.0% (171/211)	81.9% (59/72)	1.000
Pain improves on defaecation	69		29.8% (14/47)	27.3% (6/22)	1.000
Number of stools in 24 hours	140		4.0 (3.0 - 7.0)	5.0 (3.0 - 8.5)	0.675
Change stool frequency	288		84.7% (182/215)	89.0% (65/73)	0.440
Nocturnal symptoms	161		19.2% (24/125)	30.6% (11/36)	0.170
Change bowel habit	272	Diarrhoea	84.7% (171/202)	84.3% (59/70)	1.000
Bloods and faecal tests					
Anaemia	99	see footer	27.5% (19/69)	36.7% (11/30)	0.476
Raised CRP	91	> 5mg/L	51.6% (32/62)	58.6% (17/29)	0.653
Raised platelets	97	> 400 x10^9/L	19.1% (13/68)	20.7% (6/29)	1.000
Raised ferritin	49	see footer	6.5% (2/31)	5.6% (1/18)	1.000
Low ferritin	49	see footer	16.1% (5/31)	38.9% (7/18)	0.094
Raised WBC	99	see footer	15.9% (11/69)	6.7% (2/30)	0.333
Low B12	18	< 180ng/L	0.0% (0/12)	33.3% (2/6)	0.098
Low folate	18	< 3.6µg/L	25.0% (3/12)	0.0% (0/6)	0.515
Low albumin	71	< 30g/L	10.0% (5/50)	0.0% (0/21)	0.312
Faecal calprotectin (µg/g)	53		348.0 (193.5 - 1056.0)	343.0 (245.0 - 1073.2)	0.986
Raised faecal calprotectin	53	> 100µg/g	87.1% (27/31)	95.5% (21/22)	0.389

Data displayed as % (n/N) or median (IQR); P value represents Fisher's exact or Mann Whitney U test as appropriate. IBD, inflammatory bowel disease; WBC, white blood cell count; CRP, c-reactive protein. Anaemia threshold (WHO definition) as haemoglobin concentration < 120g/L for females and < 130g/L males. Raised WBC defined as > 10.6 x10^9/L for females and > 11.0

x10^9/L for males. Raised ferritin defined as > 150ng/mL for females and > 400ng/mL for males. Low ferritin defined as < 15ng/mL for females and < 30ng/mL for males

[a] Income decile estimated using patient postcode (see methods)

Supplementary Table 3.6-7. Secondary Care Delay: Factors associated with a prolonged time (> 2.1 months) from GP referral to IBD diagnosis

Variable	N	Level	Timely secondary care diagnosis n = 222	Delayed secondary care diagnosis n = 74	P value
Demographics					
Sex	295	Male	57.0% (126/221)	39.2% (29/74)	**0.010**
Ethnicity	296	White ethnicity	94.6% (210/222)	95.9% (71/74)	0.769
Age at IBD diagnosis	296	Years	38.0 (27.8 - 53.1)	32.1 (25.0 - 50.8)	0.150
Family history of IBD	260		17.9% (35/196)	4.7% (3/64)	**0.008**
Family history of bowel/ovarian cancer	242		7.2% (13/181)	11.5% (7/61)	0.291
Income decile	283		7.0 (5.0 - 8.0)	6.0 (5.0 - 8.0)	0.502
Smoking status	295	Current smoker	13.1% (29/221)	20.3% (15/74)	0.137
Symptoms					
Duration symptoms	296	Months	2.0 (1.0 - 4.0)	4.0 (2.0 - 12.0)	**0.001**
Duration symptoms < 6 weeks	296		42.3% (94/222)	13.5% (10/74)	**0.001**
Rectal bleeding	290		73.8% (163/221)	66.7% (46/69)	0.283
Abdominal pain	292		57.8% (126/218)	60.8% (45/74)	0.684
Weight loss	221		23.8% (40/168)	20.8% (11/53)	0.712
Change appearance	283		84.1% (180/214)	72.5% (50/69)	0.050
Pain improves on defaecation	69		31.2% (15/48)	23.8% (5/21)	0.580
Number of stools in 24 hours	140		4.0 (3.0 - 7.0)	5.0 (3.0 - 6.8)	0.924
Change stool frequency	288		87.2% (190/218)	81.4% (57/70)	0.242
Nocturnal symptoms	161		19.2% (23/120)	29.3% (12/41)	0.192
Change bowel habit	272	Diarrhoea	87.3% (179/205)	76.1% (51/67)	**0.033**
Bloods and faecal tests					
Anaemia	99	see footer	33.3% (24/72)	22.2% (6/27)	0.334
Raised CRP	91	> 5mg/L	60.3% (41/68)	34.8% (8/23)	0.052
Raised platelets	97	> 400 $x10^9$/L	23.9% (17/71)	7.7% (2/26)	0.089
Raised ferritin	49	see footer	0.0% (0/37)	25.0% (3/12)	**0.012**
Low ferritin	49	see footer	32.4% (12/37)	0.0% (0/12)	0.024
Raised WBC	99	see footer	18.1% (13/72)	0.0% (0/27)	**0.017**
Low B12	18	< 180ng/L	14.3% (2/14)	0.0% (0/4)	1.000
Low folate	18	< 3.6µg/L	21.4% (3/14)	0.0% (0/4)	1.000

Supplemental Table 3.5-6 continued…

…continued Supplemental Table 3.5-6

Variable	N	Level	Timely secondary care diagnosis n =222	Delayed secondary care diagnosis n = 74	P value
Low albumin	71	< 30g/L	9.3% (5/54)	0.0% (0/17)	0.328
Faecal calprotectin (µg/g)	53		375.5 (241.5 - 1129.8)	328.0 (114.0 - 435.0)	0.336
Raised faecal calprotectin	53	> 100µg/g	92.5% (37/40)	84.6% (11/13)	0.586
Primary Care Factors					
Urgent GP referral	148		66.3% (67/101)	21.3% (10/47)	**< 0.001**
Secondary Care Factors					
Year of IBD diagnosis	296	2014	26.1% (58/222)	21.6% (16/74)	0.672
		2015	33.8% (75/222)	35.1% (26/74)	
		2016	29.7% (66/222)	28.4% (21/74)	
		2017	10.4% (23/222)	14.9% (11/74)	
Referral triaging team [c]	244	Surgeons	52.8% (93/176)	64.7% (44/68)	0.114
Straight-to-test	294		71.0% (157/221)	21.9% (16/73)	**< 0.001**
Diagnosis within 7 days of bank holiday[a]	296		23.0% (51/222)	20.3% (15/74)	0.747
Workforce capacity variable [b]	293		104.7 (30.0 - 217.1)	151.8 (60.5 - 211.4)	0.202

Straight-to-test, patients referred by primary care are triaged to go directly to undergo a diagnostic test without being seen an outpatient clinic first; GP, general practitioner; WBC, white blood cell count; CRP, c-reactive protein. Data displayed as % (n/N) or median (IQR); P value represents Fisher's exact or Mann Whitney U test as appropriate. Anaemia threshold (WHO definition) as haemoglobin concentration < 120g/L for females and < 130g/L males. Raised WBC defined as > 10.6 x10^9/L for females and > 11.0 x10^9/L for males. Raised ferritin defined as > 150ng/mL for females and > 400ng/mL for males. Low ferritin defined as < 15ng/mL for females and < 30ng/mL for males

[a] Bank holidays for England and Wales

[b] See supplementary methods

[c] Reference = gastroenterology

[d] Income decile estimated using patient postcode (see methods)

Supplementary Table 3.6-8. Complications in the first year after diagnosis in patients with a delayed (> 10.7 months) and timely overall time to IBD diagnosis

Overall time to diagnosis	N	Level	Timely diagnosis n = 228	Delayed diagnosis n = 76	P value
Complications					
IBD-related hospitalisation	295	one or more	24.2% (53/219)	15.8% (12/76)	0.149
IBD-related surgeries	293	One or more	5.5% (12/218)	8.0% (6/75)	0.415
Treatment in the first year after diagnosis	295	Steroids	52.5% (115/219)	57.9% (44/76)	0.427
	295	IS	25.6% (56/219)	35.5% (27/76)	0.105
	295	5ASA	63.9% (140/219)	52.6% (40/76)	0.101
	295	Biologic	16.4% (36/219)	18.4% (14/76)	0.724
	295	EEN	4.6% (10/219)	5.3% (4/76)	0.761
Complicated disease course in first year after diagnosis[a]	304		29.8% (68/228)	30.3% (23/76)	1.000

IBD, inflammatory bowel disease; EEN, exclusive enteral nutrition; IS, immunosuppressive medication (azathioprine, mercaptopurine, methotrexate or ciclosporin); 5ASA, 5-aminosalicylate; Steroids, corticosteroids)

[a] composite outcome for patients who had either IBD-related hospitalisation or IBD-related surgeries or received biologic drug treatment in the first year of diagnosis

Supplementary Table 3.6-9. Complications in the first year after diagnosis in patients with a timely (< 1 year) and delayed (> 2 years) time to IBD diagnosis from symptom onset to diagnosis among ONLY NON-EMERGENTLY diagnosed IBD patients

Overall time to diagnosis	**n**	**Level**	**Very timely diagnosis (< 1 yr) n = 185**	**Markedly delayed diagnosis (> 2 yrs) n = 22**	**P value**
Complications					
IBD-related hospitalisation	204	One or more	7.7% (14/182)	22.7% (5/22)	**0.038**
IBD-related surgeries	199	One or more	1.6% (3/182)	4.8% (1/21)	0.356
Treatment in the first year after diagnosis	204	Steroids	44.5% (81/182)	68.2% (15/22)	**0.043**
	204	IS	23.6% (43/182)	40.9% (9/22)	0.117
	204	5ASA	71.4% (130/182)	59.1% (13/22)	0.230
	204	Biologic	11.0% (20/182)	18.2% (4/22)	0.302
	204	EEN	1.6% (3/182)	4.5% (1/22)	0.369
Complicated disease course in first year after diagnosis[a]	207	see footer	15.7% (29/185)	36.4% (8/22)	**0.034**

Data displayed as % (n/N) or median (IQR). IBD, inflammatory bowel disease; EEN, exclusive enteral nutrition; IS, immunosuppressive medication (azathioprine, mercaptopurine, methotrexate or ciclosporin); 5ASA, 5-aminosalicylate; Steroids, corticosteroids)

[a] composite outcome for patients who had either IBD-related hospitalisation or IBD-related surgeries or received biologic drug treatment in the first year of diagnosis

Supplementary Table 3.6-10. Demographic, patient symptom, biomarkers, time to diagnosis and complications in patients undergoing primary care faecal calprotectin prior to diagnosis of IBD

Variable	n	Level	Negative (<100µg/g) Calprotectin n = 5	Positive (≥100µg/g) Calprotectin n = 48	P value
Demographics					
Sex	53	Male	2 (40.0%)	26 (54.2%)	0.658
Ethnicity	53	White ethnicity	5 (100.0%)	47 (97.9%)	1
Age at IBD diagnosis	53		26.2 (24.1 - 30.5)	28.5 (23.4 - 33.8)	0.927
Family history of IBD	53	Yes	0 (0.0%)	11 (22.9%)	0.571
Family history of colorectal- /ovarian cancer	51	Yes	1 (20.0%)	3 (6.5%)	0.347
Income decile	53		5.0 (4.0 - 7.0)	6.5 (4.0 - 8.0)	0.281
Smoking status	52	Smoker	0 (0.0%)	6 (12.8%)	1
Symptoms					
Duration symptoms < 6 wks	53	Yes	3 (60.0%)	32 (66.7%)	1
Rectal bleeding	53	Yes	4 (80.0%)	34 (70.8%)	1
Abdominal pain	49	Yes	0 (0.0%)	11 (25.0%)	0.574
Weight loss	53	Yes	4 (80.0%)	43 (89.6%)	0.465
Change appearance	26	Yes	1 (33.3%)	9 (39.1%)	1
Pain improves on defaecation	36		3.0 (2.5 - 4.2)	4.0 (3.0 - 7.0)	0.42
Number of stools in 24 hours	53	Yes	4 (80.0%)	45 (93.8%)	0.336
Change stool frequency	39	Yes	0 (0.0%)	11 (30.6%)	0.545
Nocturnal symptoms	53	Yes	3 (60.0%)	32 (66.7%)	1
Change bowel habit	45	diarrhoea	4 (80.0%)	40 (100.0%)	0.111
Blood and stool tests					
Anaemia	31	see footer	0 (0.0%)	7 (24.1%)	1
Raised CRP	30	> 5mg/L	0 (0.0%)	12 (42.9%)	0.503
Raised platelets	31	> 400 x10^9/L	0 (0.0%)	4 (13.8%)	1
Raised ferritin	16	see footer	0 (0.0%)	1 (6.7%)	1
Low ferritin	16	see footer	0 (0.0%)	4 (26.7%)	1
Raised WBC	31	see footer	0 (0.0%)	3 (10.3%)	1
Low B12	5	< 180ng/L	1 (100.0%)	4 (100.0%)	NA
Low folate	5	< 3.6µg/L	1 (100.0%)	4 (100.0%)	NA
Low albumin	21	< 30g/L	0 (0.0%)	1 (5.3%)	1

Supplemental Table 3.5-10 continued …

…Supplemental Table 3.5-10 continued

Variable	N	Level	Negative Calprotectin n = 5	Positive Calprotectin n = 48	P value
Disease subtype					
IBD subtype	53	CD	1 (20.0%)	16 (33.3%)	1
		UC/IBDU	4 (80.0%)	32 (66.7%)	
UC extent	35	E1: proctitis	4 (100.0%)	8 (25.8%)	**0.021**
		E2: left-sided	0 (0.0%)	17 (54.8%)	
		E3: total	0 (0.0%)	6 (19.4%)	
CD location	17	L1: ileal	1 (100.0%)	5 (31.2%)	1
		L2: colonic	0 (0.0%)	6 (37.5%)	
		L3: ileocolonic	0 (0.0%)	5 (31.2%)	
CD behaviour	17	B1:inflammatory	1 (100.0%)	14 (87.5%)	1
		B2: stricturing	0 (0.0%)	2 (12.5%)	
		B3: penetrating	0 (0.0%)	0 (0.0%)	
Perianal CD	17		0 (0.0%)	2 (12.5%)	1
Primary Care Factors					
Urgent GP referral	46		2 (40.0%)	26 (63.4%)	0.365
Secondary Care Factors					
Secondary care team triaging GP referral	49	Surgeons	2 (40.0%)	8 (18.2%)	0.267
Straight-to-test	53		1 (20.0%)	23 (47.9%)	0.362
Time to diagnosis					
Patient delay	53	Months	0.9 (0.9 - 3.9)	3.9 (2.1 - 6.5)	0.161
Primary care delay	53	Months	0.7 (0.5 - 0.8)	0.9 (0.5 - 2.4)	0.761
Secondary care delay	53	Months	1.5 (1.5 - 2.9)	1.3 (0.9 - 2.0)	0.429
Overall time to diagnosis	53	Months	3.9 (3.2 - 5.1)	7.5 (3.9 - 12.0)	0.224
Delayed GP referral	53		1/5 (20.0%)	21 (43.8%)	0.389
Delayed secondary care diagnosis	52		1 (20.0%)	14 (29.2%)	1
Patient in first quartile of time to GP referral	52	Rapid GP referral	0 (0.0%)	0 (0.0%)	NA
Patient in first quartile of time to secondary care diagnosis	52	Rapid secondary care diagnosis	0 (0.0%)	2 (4.2%)	1
Complications					
IBD-related hospitalisation	53	One or more	1 (20.0%)	4 (8.3%)	0.403
IBD-related surgeries	53	One or more	0 (0.0%)	1 (2.1%)	1
Treatment in the first year after diagnosis	52	steroids	2 (40.0%)	29 (60.4%)	0.638
		IS	1 (20.0%)	16 (33.3%)	1
		5ASA	4 (80.0%)	30 (62.5%)	0.643
		Biologic	0 (0.0%)	8 (16.7%)	1
		EEN	1 (20.0%)	2 (4.2%)	0.262

Straight-to-test, patients referred by primary care are triaged to go directly to undergo a diagnostic test without being seen an outpatient clinic first; GP, general practitioner; WBC, white blood cell count; CRP, c-reactive protein IBD, inflammatory bowel disease; EEN, exclusive enteral nutrition; IS, immunosuppressive medication (azathioprine, mercaptopurine, methotrexate or ciclosporin); 5ASA, 5-aminosalicylate; Steroids, corticosteroids). P value represents Fisher's exact or Mann Whitney U test as appropriate. Anaemia threshold (WHO definition) as haemoglobin concentration < 120g/L for females and < 130g/L males. Raised WBC defined as > 10.6 x10^9/L for females and > 11.0 x10^9/L for males. Raised ferritin defined as >

150ng/mL for females & > 400ng/mL for males. Low ferritin defined as < 15ng/mL for females and < 30ng/mL for males. Income decile estimated using patient postcode (see methods)

3.7 Discussion

3.7.1 How the chapter addresses the aims and objectives of the thesis

In the following section I will assess whether each of the objectives were successfully addressed by the three calprotectin studies:

Objective 1: Assess the diagnostic accuracy of faecal calprotectin in distinguishing functional gut disorder from IBD in adult and paediatric patients in the primary care setting

In adults, the positive and negative predictive values of calprotectin distinguishing IBD from functional gut disorder using 100µg/g cut-off were 39% and 99%, respectively; the diagnostic accuracy was 90%. In children, the positive and negative predictive values for distinguishing IBD from non-IBD using the same 100 µg/g threshold were 52% and 100%, respectively; the diagnostic accuracy was 93%. By increasing the cut-off threshold from 50µg/g to 100µg/g I noted an approximate doubling of the positive predictive value in both adults and children, with little or no decrease in negative predictive value. 14% (7/43) of adult, but none (0/11) of the paediatric IBD patients had a false negative test result at this threshold.

These data suggest that faecal calprotectin is a useful biomarker to aid GPs in distinguishing IBD from IBS in adults, and IBD from non-IBD in children. In particular, the high negative predictive values (NPV) in both cohorts should empower clinicians to confidently exclude IBD after a negative test. It is, however, worthwhile noting that raising the threshold from the original NICE and manufacturer recommended limit of

50µg/g to 100µg/g will lead to some missed diagnoses of IBD, as I report a 14% false negative rate.[214,238] Reassuringly for the clinician, if not the patient who experiences unchecked inflammatory disease, these false negative cases tend to be of milder IBD phenotype, where inflammatory burden is lower, and complications are less frequent. This observation, however, highlights the importance of GP follow-up of patients with negative tests to ensure that symptoms have resolved or improved with treatment; this is common practice in primary care and is referred to as 'safety-netting'.[297]

Inherent with the exploration of an optimal calprotectin threshold is the trade-off between sensitivity and specificity which is best illustrated by the receiver operator curve (ROC). ROC curves compare sensitivity versus specificity across a range of values for the ability to predict a dichotomous outcome, whereas the area under the ROC curve (AUC) is another measure of test performance.[298] In adults the AUC for distinguishing functional gut disorder from IBD was 0.93 (95%CI 0.88-0.98). In children, the AUC for distinguishing IBD from non-IBD was 0.99 (95%CI 0.97-1.0).

If the aim of calprotectin were solely to facilitate the early diagnosis of IBD, one might argue that the calprotectin cut-off threshold should be reduced from our recommended 100µg/g so as to maximise the sensitivity of the test and thus avoid any missed cases of IBD. However, this would increase the number of false positive tests requiring referral and investigation. Currently endoscopy and gastroenterology departments throughout the NHS are struggling to meet the UK Department of Health targets for the timely investigation of patients due to the increasing demands of a growing and aged population.[295,296,299] Reducing the FC threshold may increase the number of referrals and paradoxically negatively impact the diagnosis of IBD, cancer and other

organic pathology. An equitable balance needs to be found, and this may vary from centre to centre. Therefore, I advocate maximising sensitivity and specificity using Youden's method, which estimated the optimal threshold to be 107µg/g and 112µg/g, in adults and children, respectively.

Objective 2: To assess whether faecal calprotectin testing alters primary care referral behaviour

---

In adults, I estimated that the calprotectin pathway saved 279 referrals over a 17-month period: 196 referrals saved per year. In children, 41 referrals were estimated to have been saved over the same period: 29 referrals saved per year. However, I found that nearly half of adults (45%, 195/657) and nearly two thirds (60%, 72/121) of children who returned a negative calprotectin (≤ 100µg/g) were subsequently referred to secondary care gastroenterology services. Of these calprotectin negative referrals two-thirds of adults and one in seven children underwent either endoscopic or cross-sectional radiological imaging. If the adult calprotectin pathway had been rigidly adhered to, or perhaps enforced, then a further 254 outpatient appointments might have been saved. Whilst neither study was set up as cost-effectiveness analyses, it was estimated that in adults the pathway resulted in savings of £160 per patient (£52,355 per year) to the point of diagnosis. If complete adherence were achieved, the estimated savings would have doubled to £326 per patient - £106,469 saved per year.

In adults, these data clearly show that calprotectin altered GP referral behaviour, however, a large number of calprotectin negative patients were still referred. I believe that this reflects two underlying issues: in some patients, GPs are confident of a

diagnosis of functional gut disorder but have either exhausted treatment options or believe that further patient reassurance would be helpful; in other patients, GPs question the validity of the calprotectin result and refer regardless. This second point may reflect the diagnostic uncertainty created by the presence of alarm symptoms, which have traditionally been used by GPs to determine the urgency of referral. Such symptoms were present in 39% of the adult cohort. Interestingly, as noted by others, these alarm symptoms were also reported by 64% of adult patients later diagnosed with a functional gut disorder, demonstrating their modest value as a predictor of disease.[251,300] This creates an interesting dilemma; in order to maximise the impact of faecal calprotectin in primary care, I advocate pathway oversight, such that test negative referrals are either returned to GP-led care or directed to dietetic therapy.[241,301] This is a difficult scenario, as such a policy over-rides the clinical acumen of responsible primary care clinician who has deemed the patient appropriate for referral. Solutions to this impasse require the following: first, a good relationship between primary and secondary care physicians that empowers GPs to discuss individual patients, thus enabling investigation in exceptional cases; second, concomitant use of other biomarkers, as the likelihood of organic pathology is further diminished in the presence of a normal haemoglobin, platelet count and CRP[302]; and, third, a refractory IBS referral pathway for patients in whom first and second line IBS managements have failed.

In children, the effect of calprotectin on secondary care referrals was less marked; this is consistent with NICE guidance (DG11; 2013[214]) which recommends that the test is only used as, ‘an option to support children suspected of IBD who have already been referred for specialist assessment’. However, I observed that only 84% (16/19) of

patients with a positive- and 86% (95/1010) with a negative-calprotectin test were referred by their GP. This is an interesting example of how the predicted use of a biomarker cannot be assumed when rolled out in the real-World, and furthermore, reflects how GPs quickly adapt new technology into established patterns of practice. In paediatric patients I still advocate secondary care referral of all patients with ongoing symptoms regardless of the calprotectin result, and that calprotectin is used to guide secondary care prioritisation of clinical review and the need for endoscopic investigation.

Objective 3: To ascertain whether the presence of gastrointestinal alarm symptoms altered the performance of the test

---

In adult patients, GI alarm symptoms were reported by 39% of patients. The presence of these symptoms nearly doubled the PPV at lower calprotectin thresholds (50-100µg/g) with a more modest effect above 100µg/g. For NPV the opposite was seen, at lower thresholds the performance of the tests was very similar in patients with and without alarm symptoms; however, at higher thresholds there was a notable but still modest increase in PPV seen patients with alarm symptoms.

In paediatric patients, GI alarm symptoms were present in half of the cohort, although their impact on the diagnostic accuracy of calprotectin was less clear. At thresholds below 70µg/g the presence of alarm symptoms marginally improved the PPV of calprotectin when compared to the performance in patients without such symptoms. However, at thresholds above 70µg/g the PPV was lower in the presence rather than absence of alarm symptoms. This may reflect the difficulty in eliciting such symptoms in a paediatric population.

Using multivariable logistic regression, I demonstrated that calprotectin increased the odds of an IBD diagnosis independently of GI alarm symptoms: in paediatric patients (calprotectin could not be used as binary variable as it was a perfect predictor of disease), for every 10-fold increase in calprotectin level, the odds of IBD increased 408-fold (95% CI 32.5 to 37564, $P = 3.9 \times 10^{-4}$); whereas, in adult patients a positive calprotectin (≥100µg/g) increased the odds of IBD 54-fold (95% CI 23.2-125.2, P < 0.001).

In the adult cohort, I also looked at diagnostic strategies combining calprotectin with GI-alarm symptoms: in adults, the use of calprotectin **or** GI alarm symptoms to determine referral resulted in a notable fall in the PPV when compared with calprotectin alone (PPV 39% and 14%, respectively). Whereas, calprotectin **and** GI alarm symptoms raised the PPV when compared with calprotectin alone (PPV 39% and 50%, respectively) but unfortunately also reduced the sensitivity, thus missing an unacceptable number of IBD cases (Sens 86% and 56%, respectively).

Objective 4: To ascertain where delays occur in the referral pathway between onset of symptoms through primary and secondary care to diagnosis of IBD

In adults, half of patients were diagnosed within 4 months of symptom onset and one-tenth of patients suffered symptoms for more than 2 years before diagnosis. The greatest component of the time to diagnosis was the time taken for patients to present to their GP with symptoms. The median sub-interval between symptom onset and primary care presentation was 7-times longer than the time it took GPs to refer patients

and twice as long as the time it took secondary care services to diagnose referred patients.

Arguably, these data suggest that the most effective way of reducing the delay in IBD diagnosis is to focus on timely patient presentation. This might be achieved through greater public awareness of gastrointestinal symptoms particularly if severe or persistent.[230] The recent UK news coverage of the publication by Vuik *et al* reporting the increasing incidence of colorectal cancer in young patients is a great example of how this can be achieved.[303]

Objective 5: To explore the clinical and laboratory factors which influence time to diagnosis in IBD patients, and specifically whether faecal calprotectin reduces time to diagnosis

---

In adults, the time it took patients to present to their GP (patient delay) was increased by the presence of abdominal pain, higher estimated household income and unintentional weight loss, but reduced by the presence of rectal bleeding. Ileal Crohn's disease may present with isolated abdominal pain which may incorrectly be attributed by patients (and clinicians), as functional rather than inflammatory in aetiology. The factors underlying the association of patient delay and unintentional weight loss are more difficult to explain. I believe the most likely explanation for this observation is an ascertainment bias, whereby this information was elicited more frequently by clinicians reviewing patients with long-standing rather than shorter duration symptoms. This seems more plausible than a situation in which multiple patients with unintentional weight loss actively avoid healthcare consultation. Socioeconomic status has previously been linked disparities in the delivery and effectiveness of healthcare for

patients with IBD.[304] However, a recent French study by Nahon *et al* failed to identify an association between socioeconomic status and diagnostic delay.[287] Interestingly, we found that a higher estimated household income was associated with an increased time to GP presentation. However, again I feel that this result may be confounded: in a relatively small city such as Exeter, households with the lowest estimated income are geographically co-located and served by high performing GP practices. A combination of low waiting times for clinic appointments and highly trained GPs may have led to shorter delay to referral for their patients. The reduction in time to presentation with rectal bleeding has previously been reported several other cohorts; clearly such symptoms lead to health-seeking in most, if not all, patients.[230,287,305,306]

The time-taken for GPs to refer patients with suspected IBD following a first consultation was very short in nearly all patients (median [IQR] = 1.1 months [0.5 - 2.1]). I report that a shorter time to referral (primary care delay) was associated with increasing age at presentation and shorter duration of symptoms prior to presentation. These observations likely reflect the higher incidence of IBS as compared with IBD, longer duration symptoms being more likely to be of a benign/non-sinister aetiology and the poor discriminatory diagnostic performance of clinical symptoms and demographics for distinguishing organic from non-organic lower GI disease.[16,221,291,307]

I also report that independent of estimated workforce capacity, a timely secondary care diagnosis was associated with male sex, shorter duration of symptoms, urgent GP referral and triage of patients straight-to-test. This last finding is particularly interesting and reflects a common pathway used in the NHS to speed up diagnosis and reduce workload by directing patients straight-to-endoscopy or imaging. This

initiative was originally driven by NHS England's National Cancer Programme[308] and has been demonstrated to shorten time to diagnosis of colorectal cancer in the UK.[309,310]

Locally, primary care calprotectin testing was rolled-out to patients < 46 yrs in 2014, but initial uptake in the 4-years following was relatively poor: it was used in only one-quarter of eligible adults, and one-third of children prior to IBD diagnosis between 2014 and 2017. However, calprotectin is unnecessary in cases of acute severe colitis, who should instead be directed towards urgent diagnostic services or emergent care. Similarly, a negative calprotectin test is not a prerequisite to confirm a diagnosis of IBS; indeed, widespread use of the test in this way would inadvertently result in significantly more false positives, and thus negate many of the tests benefits. Zhang *et al* used a decision analytic model to demonstrate that the time to diagnosis for patients with IBD could be reduced by 40 days (95% CI 16 to 65) using calprotectin compared with standard practice.[311] I found no such association with use of calprotectin and time to diagnosis in either paediatric or adult cohorts, although both analyses were underpowered due to low uptake of the test.

Objective 6: To investigate whether a delayed diagnosis is associated with a more complicated disease course in the first year

---

In adult IBD patients, I found no association between diagnostic delay and IBD-related hospital admission, IBD-related surgery or advanced therapeutic strategies, such as use of immunomodulators and biologics in the first year after diagnosis. This remained the case even when I compared complications in rapid- (< 12 months) and very delayed- (>2 years) patients. In keeping with other European data[17,230,312], I report that

approximately 20% of our adult Crohn's patients had stricturing (B2) and fistulating (B3) disease at presentation. This finding reinforces the need to better identify patients at risk of developing IBD and to streamline their diagnosis in order to commence early disease-modifying therapies. The lack of an association between time to diagnosis and complications is explained by the inclusion of emergently diagnosed patients; this cohort have a higher inflammatory burden, are more likely to experience a complicated disease course and yet have shorter time to diagnosis. When emergently diagnosed patients were removed and the analysis repeated in markedly (> 2 yrs to diagnosis) and rapidly diagnosed (<12 months to diagnosis) patients, I found that diagnostic delay *was* associated with both IBD-related hospitalisation and the need for corticosteroids in the first year after diagnosis, thus supporting my hypothesis that inclusion of the emergent cohort biased the original analysis towards the null.

3.7.2 The implications for practice

In light of these data we have redesigned our adult primary care faecal calprotectin pathway for the North, East and West Devon catchment area which covers a population of approximately 378,000 people (see Appendix A). Advanced plans are also in place to extend this pathway to Torbay, which covers an additional population of 286,000 people.

This new pathway uses a single calprotectin cut-off of 100µg/g, a direct-to-test option in patients with a calprotectin of ≥ 250µg/g, as over two thirds (68%) of patients with this level of calprotectin are later diagnosed with IBD and an 8-week GP safety-net review of patient symptoms. It is hoped that the time to diagnosis of IBD will be reduced by the widespread adoption of the test by GPs at patients' first presentation.

In patients who present with symptoms of severe colitis (> 6 bloody stools per day and pulse > 90 beats per minute or temperature >37.8℃) there is no need to perform calprotectin prior to referral.[313] Such patients can be emergently directed to secondary care services.

We have also appointed a 'clinical lead' to oversee the new pathway. Both Turvill *et al* and the National NHS Business Authority recommend initiation of a 'clinician champion' responsible for, 'organising support, delivering guidance on the correct use of the pathway and leading educational sessions for local stakeholders'.[241,314] Additionally, I think it is also important for the clinical lead to have oversight of secondary care endoscopy usage: locally, in 2015-16 (1-year after primary care- and 8-years after secondary care- calprotectin was introduced) only 20% of routine

referrals for diagnostic colonoscopy/flexible sigmoidoscopy in patients < 46 yrs had a faecal calprotectin test performed prior to their endoscopy (unpublished data). I feel that the appointment of a clinical lead was probably the main determinant of the notable difference in the pathway adherence between the York and Exeter studies: 90% and 60%, respectively.[241,272,315]

The predicted cost savings from the initiation of our new pathway have been used to pay for a ring-fenced primary care dietician to deliver and oversee specialist interventions, such as the FODMAP diet, in patients who have failed first-line IBS therapies. The low–fermentable oligo-, di-, and monosaccharide and polyol (FODMAP) diet is a two-phased intervention, with strict reduction of all slowly absorbed or indigestible short-chain carbohydrates (i.e. FODMAPs) followed by reintroduction of specific FODMAPs according to tolerance. In one case series evaluating this dietary intervention, 63% of patients reported satisfactory control of their IBS after specialist dietetic input with 74% reporting improved quality of life.[301] This provides GPs with an extremely effective second-line option for the treatment of refractory IBS and helps to provide equality of services for both patients with IBS and IBD.

In 2017, NICE issued guidance (DG30; 2017) supporting use of a new faecal occult blood test (FOBT), called the faecal immunohistochemical test (FIT), to replace the older and less sensitive guaiac assay.[316] This guidance recommended that FIT is used to aid referral decisions in patients with lower GI symptoms that did not meet 2-week wait criteria. In 2019, the qFIT project was rolled out across Southwest England by the

Cancer Alliance: GPs were asked to consider its use in the following 'low risk but not no risk' patients:[317]

- ≥ 50 years old with unexplained abdominal pain or weight loss
- 50 to 60 years old with changes in bowel habit or iron-deficiency anaemia
- ≥ 60-year-old with anaemia without iron deficiency

The qFIT age cut-off dovetails nicely with our own Devon calprotectin pathway, such that calprotectin and FIT may be used in patients under- and older- than 50 yrs old, respectively. FIT performs very similarly to calprotectin in distinguishing IBD from IBS and is reportedly cheaper and superior to faecal calprotectin for detecting cancer and high risk adenoma.[247,264,318] Only time will tell it replaces calprotectin entirely in the future.

Chapter 4

4 The role of pharmacogenomics in the delivery of precision medicine: avoiding adverse drug reactions in IBD

4.1 Background to the chapter

This work was published as a book chapter in Biomarkers in Inflammatory Bowel Diseases, N. S. Ding, P. De Cruz (eds.) Springer (2019). This particular chapter was written by Walker G and edited by Ahmad T.

4.1.1 Clinical significance of ADRs in IBD

An adverse drug reaction (ADR) is defined as an appreciably harmful or unpleasant reaction resulting from the use of a medicinal product; adverse effects usually predict hazard from future administration and warrant prevention, or specific treatment, or alteration of the dosage regimen, or withdrawal of the product.[319] ADRs result in morbidity and mortality as well as placing a significant financial burden on health-care resources. In Europe (EU) and North America, they are responsible for between 3.5-6.5% of all hospital admissions[320–322], with a further 10% of ADRs occurring during the subsequent hospital stay.[320] ADRs are thought to contribute to the deaths of approximately 197,000 EU, and 159,000 US citizens annually, which places ADRs as one of the top 10 causes of death.[2,4,6] It is estimated that EU countries spend 15-20%

of their healthcare budgets dealing with the consequences of ADRs and in the UK alone this exceeds £500 million per year. [4,6]

4.1.2 Types of ADRs

Traditionally ADRs have been classified as Type A and Type B. Type A reactions (80% of ADRs) are predictable through the known pharmacological mode of action of the drug and are often a consequence of an exaggerated on-target effect. They have a strong dose relationship such that a dose reduction will usually lead to resolution of sequelae (e.g. oral iron and gastrointestinal side effects). Type B reactions (20% of ADRs) are off-target interactions, often associated with a high mortality and require drug cessation. These ADRs were previously thought to be idiopathic and independent of drug dose, however, recent studies have shown that they actually have a complex dose relationship[324], and many are predictable through knowledge of the underlying immunological and genetic aetiology (e.g. thiopurine-induced pancreatitis[325]). They are often referred to as 'allergic' or 'hypersensitivity' reactions because they involve complex interactions of multiple components of the host's adaptive immune system including IgE antibodies, drug-specific T-cells and immune complexes.[326–328] Indeed, the same drug may activate many different arms of the immune system via different pathways.[196] A common theme to many hypersensitivity ADRs is the activation of T-lymphocytes, which occurs in some cases exclusively in patients with a specific human leukocyte antigen (HLA) type. Candidate gene studies as well as hypothesis-free genome wide association studies (GWAS), have shown a number of HLA associations with Type B ADRs, for example, abacavir hypersensitivity [HLA-B*57:01][329], carbamazepine hypersensitivity in Caucasians and Japanese [HLA-A*31:01][330,331] and carbamazepine-induced Stevens-Johnson syndrome in Han Chinese [HLA-B*15:02][332]. However, these studies have shown that the carriage of specific HLA

genotype is often not sufficient, nor indeed necessary, to cause an ADR in patients exposed to a particular drug. This suggests that other factors such as regulatory T-cells (T-reg), the cytokine milieu and danger signals caused by tissue damage also may also contribute to the development of ADRs.[333]

4.1.3 Pharmacogenetic biomarkers of ADRs

Predictive biomarkers allow the identification of individuals who are more likely to respond to a particular therapy. This response could be a symptomatic benefit, improved survival, or an ADR. Predictive biomarkers of ADRs may direct drug-avoidance, dose-reduction or enhanced monitoring in at risk individuals. Pharmacogenetic biomarkers are particularly attractive for the purpose of predicting ADRs as they are present at diagnosis and are unaffected by disease phenotype, disease activity and other drug therapies. Pharmacogenetic biomarker discovery has been made possible by the increasing availability of reliable, cheap high throughput genotyping and sequencing platforms. This has led to a rapid expansion in the number of publications reporting pharmacogenetic associations, although very few have reached clinical practice. The first step towards implementation is independent replication and many claimed biomarkers have fallen at this first hurdle. In this review we highlight the most promising examples of pharmacogenetic associations for drugs used in patients with inflammatory bowel disease (IBD).

4.1.4 Biomarker discovery

The essential requirements of an ADR pharmacogenetic biomarker discovery study include strict phenotype definitions, a robust assessment of causality and an adequate sample size. Rare idiosyncratic drug reactions are notoriously difficult to characterise due to the small number of cases available to individual researchers. Therefore,

nationwide and global collaboration is essential to build cohorts of sufficient size for hypothesis-free genome wide pharmacogenetic studies. Recent efforts of the UK IBD pharmacogenetics network[334], the international IBD genetics consortium[335] and the serious adverse events consortium (iSAEC)[336] have demonstrated that collaboration can successfully deliver sufficient patient numbers to adequately power such studies. Strict phenotype definitions allow the inclusion of a homogenous population and work by the Phenotype Standardisation Project[337] has been instrumental in the effort to address this issue. In clinical practice it is often difficult to be certain that an ADR has been caused by the drug of interest. Adjudication is an essential part of ADR pharmacogenetic studies; this process maximises the likelihood that symptoms experienced by recruited patients are due to the drug rather than other unrelated causes. Case adjudication is typically carried out by an independent panel of clinicians using a validated adjudication pathway e.g. the Liverpool Causality Pathway (**see Figure 4.4-1**).[338] High quality cases demonstrate a clear temporal relationship with drug administration, no other identifiable risk factors for the ADR, including the concomitant use of other drugs recognised as causing a similar ADR, and resolution of the ADR on drug withdrawal. A positive rechallenge with a second ADR developing after re-exposure to the same drug provides even stronger evidence of causality. Cases which successfully pass through this adjudication process are sent for genotyping using hypothesis-free array and/or exome sequencing or whole genome sequencing methodologies. Replication of positive findings in an independent cohort is crucial. Particular attention should be paid to minimising population stratification in the analysis of data, especially when cases and controls are recruited from populations of differing ethnic backgrounds. This confounding factor could lead researchers to assume an association with an ADR is present, when in fact this variant is simply more

commonly found in patients of one particular ethnicity who are over or under represented in either cases or controls.

Figure 4.1-1: Causality assessment tool

Adapted version of the Liverpool Adverse Drug Reaction Causality Assessment Tool used in the adjudication process. Adapted from Gallagher et al. (Gallagher, R.M. et al. Development and inter-rater reliability of the Liverpool adverse drug reaction causality assessment tool. PLoS One, e28096, 2011). [338]

4.1.5 Overview of gene-drug adverse drug reaction biomarkers in IBD

4.1.5.1 Adverse reactions to thiopurine drugs, azathioprine and mercaptopurine

The thiopurines (mercaptopurine and its prodrug azathioprine) are commonly used in patients with IBD to maintain corticosteroid-free remission, prevent postoperative recurrence and reduce the risk of immunogenicity associated with biologic therapy. 59% of CD and 33% of UC patients receive thiopurine therapy within the first 5 years of diagnosis.[339] Despite this widespread use, up to 40-50% of European IBD patients have to discontinue therapy, most commonly (~15%) because of the development of

one or more ADRs.[340,341] Thiopurine-induced ADRs include: pancreatitis (4-7% prevalence)[342,343]; liver injury (3-10%)[343–345]; myelosuppression (7%)[346]; GI side effects (1-6%)[347,348] and a flu-like hypersensitivity reaction (8-12%).[24,31] Over recent years there has been significant progress in our understanding of thiopurine metabolism (reviewed in González-Lama and Gisbert, 2015[351]) and the mechanisms underlying ADRs.

4.1.5.2 *Thiopurine-induced myelosuppression (TIM)*

TIM may occur at any time during thiopurine treatment and whilst most patients are asymptomatic, serious opportunistic infections may occur, especially if neutrophils fall ≤ $1.0x10^9$/L, with an estimated mortality of 1%.[340,341] In the 1980's, Weinshilboum and others recognised that TPMT activity in white Europeans followed an autosomal co-dominant mode of inheritance with a trimodal distribution.[352,353] Approximately 89% of individuals possess high TPMT activity levels, 11% intermediate activity and 0.3% low activity.[354] This phenotypic observation correlates with genetic variation in the thiopurine S methyltransferase (*TPMT*) gene, with variant alleles resulting in decreased TPMT enzyme activity and higher production of the active 6 thioguanine nucleotides (6TGNs), which predispose patients to bone marrow suppression.[352,353] Pre-treatment phenotyping (usually measurement of TPMT activity in red blood cells) or genotyping of *TPMT* is recommended by the European Medicine Agency (EMA)[355] and U.S Food and Drug Administration (FDA)[356] and routinely carried out prior to initiation of treatment to identify patients at risk of over-production of 6TGNs and therefore TIM: in those with reduced TPMT activity, thiopurines are used in reduced dose or avoided altogether.[357] However, *TPMT* variants are only found in 25% of TIM cases in European populations, suggesting the presence of other genetic and

environmental determinants.[357,358] In contrast, variant *TPMT* haplotypes are rare in East Asian patients; a population where TIM is particularly prevalent.[359–361] Recently, Yang *et al* identified a common variant in *NUDT15* associated with myelosuppression in East Asians.[362] The exact mechanism of action of NUDT15 is still being elucidated, however, it is thought to catalyse the hydrolysis of nucleoside triphosphates. Patients with defective *NUDT15* variants therefore have excessive levels of thiopurine active metabolites (thioguanosine triphosphate [TGTP] and DNA-incorporated thioguanine [DNA-TG]) and increased host toxicity.[363]

4.1.5.3 Thiopurine-induced pancreatitis (TIP):

Thiopurine-induced pancreatitis is a well-recognised, idiosyncratic, dose-independent ADR with an incidence of approximately 4-7% in patients with IBD.[342,343] This ADR most commonly occurs within the first month after commencement of therapy, and re-challenge with either AZA or MP usually leads to recurrence of symptoms. Most episodes of acute pancreatitis are mild and resolve after the discontinuation of the drug, although more severe cases can occur (with local and systemic complications of pancreatitis, including death).[364] The pathogenesis of thiopurine-induced pancreatitis is unknown. We previously reported the first large scale clinical and genetic analyses of thiopurine-induced pancreatitis and identified an association with a common variant (rs2647087) in the Class II HLA region which tags HLA-DRB1*07:01.[325] In our study we estimated that the odds of developing pancreatitis amongst variant carriers was increased 2.5 times for heterozygous and 5 times for homozygote patients. This finding has recently been replicated in a cohort of 373 azathioprine exposed patients from Canada.[365] In this cohort, which included 13 patients with a history of azathioprine pancreatitis, the risk was highly predictable and

genotype dependent: 0.5% for wild type (A/A), 4.3% (OR = 4, 95% CI 1-36, *P* = 0.044) for heterozygous (A/C), and 14.6% (OR = 16, 95% CI 4-145, *P* = 0.0001) for homozygous variant (C/C) patients. Data from our UK study suggests that for every 1000 patients tested, 77 risk allele homozygotes will be identified, and these individuals will have a 17% risk of pancreatitis. If azathioprine/mercaptoprine are subsequently avoided in all homozygote risk allele individuals (and we believe most clinicians would consider this reasonable), this equates to an overall number needed to genotype of 76 patients to prevent one case of pancreatitis.

4.1.5.4 Thiopurine-induced liver injury (TILI):

TILI most commonly leads to an asymptomatic hepatocellular liver injury characterised by elevated transaminases (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) within the first 12 weeks of treatment, or soon after dose escalation.[366] This hepatocellular liver injury generally resolves after dose reduction or drug cessation.[343,367] Less commonly, approximately 1 in 1000 treated patients, thiopurines cause a cholestatic liver injury in association with symptoms of jaundice, fatigue and itching.[367] This ADR often is typically seen between 2-12 months after starting treatment and resolves after drug cessation, although some persistent cases have been described.[367] Finally, after long-term therapy thiopurines rarely lead to chronic liver injury with symptoms and signs of portal hypertension. Histologically such cases demonstrate nodular regenerative hyperplasia, sinusoidal dilatation, central congestion and injury to sinusoidal endothelial cells suggestive of veno-occlusive disease.[367–369]

The enzyme, thiopurine S-methyltransferase (TPMT*)* inactivates thiopurines to methylated metabolites, reducing the production of the active 6-thioguanine nucleotides (6TGN). High TPMT enzyme activity may result in a greater 6-methylmercapopurine (6MMP) production, which has been associated with liver toxicity.[370,371] In such cases of thiopurine hypermethylation, the use of adjunctive allopurinol (a xanthine oxidase inhibitor) has proven effective in shunting thiopurine metabolites towards active 6TGN's without increasing 6MMP levels.[372] However, TILI may still occur in the absence of elevated 6MMP and 6TGN levels.[373] To date, there have been no hypothesis-free genome wide association (GWAS) approaches employed to investigate the genetic basis of drug-induced liver injury. However, data from our study of over 200 patients with thiopurine induced-liver injury using GWAS and whole exome sequencing methodologies will be published shortly.

4.1.5.5 Thiopurine-induced hypersensitivity reactions (THR):

Thiopurine hypersensitivity reactions are dose independent and occur in 8-12% of patients treated with azathioprine and mercaptopurine.[342,349,350] Most hypersensitivity reactions are mild, presenting with a flu-like illness within the first four weeks of therapy and resolve rapidly on drug withdrawal. Symptoms and signs of mild hypersensitivity reactions are poorly defined in the literature but include fever, myalgia, arthralgia, headache and fatigue often leading to drug cessation. These symptoms can be associated with an acute inflammatory response, supported by a rise in serum markers e.g. CRP, mimicking active IBD. The mechanism of thiopurine hypersensitivity is unknown. It has been proposed that the imidazole component of azathioprine may be responsible by binding to endogenous proteins resulting in hapten formation and immune activation. This might explain why a small proportion of patients who develop

flu-like illness in response to azathioprine therapy are subsequently able to tolerate mercaptopurine.[374] However, this theory must be challenged as there is no evidence to suggest that the syndrome is more common with azathioprine than mercaptopurine and a number of patients experience identical reactions to mercaptopurine rechallenge. This hypersensitivity syndrome does not appear to be associated with *TMPT* genotype and is not dose related, suggesting an idiosyncratic mechanism.[375] An association with flu-like hypersensitivity to thiopurines and an exonic variant in *ITPA* has been described in a case-control candidate gene study, however, this finding has not been replicated.[376] Our preliminary data from a genome-wide association study suggests the presence of a genetic determinant in the class II HLA region. Further work is underway to replicate this finding prior to publication.

4.1.5.6 Mesalazine-induced nephrotoxicity

5-Aminosalicylates (5-ASAs) are the most frequently prescribed class of drug to induce and maintain remission in patients with mild to moderately active ulcerative colitis. The use of these agents in maintenance therapy over decade's means that long-term toxicity is an important consideration. Mesalazine-induced nephrotoxicity is rare (incidence of approximately at 0.17 cases per 100 patients per year[377]) but the consequences may be serious including the development of end stage renal failure and the need for renal replacement therapy. As a consequence, regular monitoring of renal function for the duration of mesalazine treatment is advised by European Crohn's and Colitis Organisation (ECCO), British National Formulary (BNF) and American Gastroenterology Society (AGA).[111,112,378] Data from our previous work has shown that 5-ASA-induced nephrotoxicity may present at any age and is characterised histologically by chronic tubulointerstitial nephritis.[379] In our case control study, median

time to renal injury was three years, following which only 30% of our cohort fully recovered renal function, with 10% requiring permanent renal replacement therapy. A genome wide association demonstrated association within the HLA region although this failed to reach genome wide significance (OR = 2, 95%CI 2–3, $P = 1\times10^{-7}$). Limiting the association analyses to the biopsy positive cases significantly strengthened the HLA association signal despite the smaller number of cases, with an odds ratio 3.1, and a genome-wide significant P-value ($P = 4x10^{-9}$). The high frequency of this single nucleotide polymorphism (SNP) and the low frequency of the adverse event limits its clinical utility and we therefore cannot recommend its use in guiding treatment choice or monitoring intervals.

4.1.5.7 Sulphasalazine-induced agranulocytosis

Sulphasalzine consists of a sulphonamide antibiotic (sulphapyridine) linked via an azo bond to 5-aminosalicylic acid (5-ASA). It is rarely used in IBD, aside for maintenance treatment of UC patients suffering from IBD associated arthropathy, having largely been replaced by 5-ASAs which have a comparatively better side effect profile. Sulphasalazine reaches the colon mostly unchanged and is split by gut bacteria at the azo linkage, releasing 5-ASA and sulphapyridine.[380] Whilst the systemic absorption of 5-ASA is limited, a positive correlation exists between serum sulphapyridine concentration and both therapeutic efficacy and toxicity.[380] The more severe adverse drug reactions include, agranulocytosis, liver injury, Stevens-Johnson syndrome (SJS) and Toxic Epidermal Necrolysis (TEN). Plasma levels of sulphapyridine are influenced by common polymorphisms in genes that encode N-acetyl transferase 2 (*NAT2*) and ATP-binding cassette protein G2 (ABCG2).[381] Allelic variation at the *NAT2* gene locus determines whether individuals are fast or slow acetylators[382] with fast

acetylators having lower plasma concentrations.[380,383] Prevalence of the slow acetylator phenotype shows marked ethnic variation: 40-70% Caucasians and African-Americans, 10-20% Japanese, >80% Egyptians and certain Jewish populations.[384–386] However, to date, studies involving low patient numbers have mostly failed to detect a relationship between *NAT2* acetylator status and drug toxicity[381,387] and pre-treatment genotyping of *NAT2* or phenotyping of acetylator status is not carried out in clinical practice.

4.1.5.8 Allopurinol-induced severe cutaneous adverse reactions (SCAR)

Allopurinol a commonly prescribed medication for gout and hyperuricemia, and is increasingly used alongside thiopurines in order to reduce thiopurine toxicity or increase efficacy in hypermethylators.[388,389] Up to 0.4% of patients treated with allopurinol suffer severe cutaneous adverse reaction (SCAR) with a mortality rate up to 25% including drug reaction with eosinophilia and systemic symptoms (DRESS), SJS, or TEN.[390] Allopurinol induced SCAR is strongly associated with HLA-B*58:01 carriage (OR = 165 when compared to allopurinol-tolerant controls).[391] This allele is rare in Europeans with a 1% carriage rate, but common in Asians, including the Han-Chinese in whom the PPV of this association is 2% and NPV 100%.[392] The clinical utility of pre-treatment genetic testing for HLA-B*5801 has been demonstrated in a non-randomised trial design using historical data as control.[393] Given the high negative predictive value of the allele, especially in patients of Asian descent (>99%), the clinical and pharmacogenetics implementation consortium (CPIC) states that HLA-B*58:01 testing could significantly reduce the incidence and risk for allopurinol-associated SCAR. [394]

4.1.5.9 Methotrexate-induced mucositis, hepatotoxicity and haematological toxicity

Methotrexate is a commonly used immunosuppressive agent used in the maintenance treatment of IBD. Therapy is frequently limited by side effects including mucositis, hepatotoxicity and haematological toxicity. In a meta-analysis of 14 paediatric oncology candidate gene studies of ADRs methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms were associated with hepatotoxicity and haematological toxicity and mucositis.[362] These authors concluded that 'in children with malignancy, genotyping of the MTHFR C677T polymorphism is expected to be a useful tool in reducing toxicity and improving outcome in personalised MTX therapy.[362] This is not currently advocated by CPIC (evidence level C/D).[395]

4.1.5.10 Calcineurin-induced hypertension and nephrotoxicity

The calcineurin inhibitors include: ciclosporin, which is used as rescue therapy in acute severe ulcerative colitis; and tacrolimus, which is used to induce and maintain remission in patients with ulcerative colitis refractory to systemic corticosteroids.[396] Dosing of ciclosporin and tacrolimus is routinely directed by therapeutic drug level monitoring because of their narrow therapeutic index and significant inter-individual variability in blood concentrations. The calcineurin inhibitors are metabolised by CYP3A5 and genetic variation in this gene contributes to the pharmacokinetic variability of these drugs and the risk of developing hypertension.[397,398] Data from the solid organ and stem cell transplantation literature suggests that CYP3A5 genotype based dosing of tacrolimus may allow target tacrolimus levels to be achieved earlier, although whether this translates to improved efficacy and reduced toxicity is not known.[399] Using this algorithm CYP3A5 extensive (*1/*1) or intermediate (*1/*-) metabolisers are started with 1.5-2 times the standard dose. To date

CYP3A5 genotype directed dosing of calcineurin inhibitors has not be studied in patients with IBD.

4.1.5.11 Anti-TNF induced skin reactions

The use of anti-tumour necrosis factor (anti-TNF) drugs are associated with the development of paradoxical inflammatory skin eruptions in up to 30% of treated patients across all disease indications.[400,401] Skin manifestations may present after many years of anti-TNF treatment and include palmoplantar pustulosis, psoriasis, psoriasiform eczema, eczema and xerosis.[400] Smoking and obesity have been identified as risk factors particularly of palmoplantar psoriasis but these clinical factors are not currently used to stratify patients.[402–405] Initial treatment of skin lesions includes the use of topical steroids in mild-moderate cases (<5% of skin affected), but 10-40% of patients fail to respond necessitating anti-TNF drug withdrawal.[400,403] Switching to an alternative anti-TNF drug does not lead to resolution of skin lesions suggesting a class effect for this ADR.[403] In contrast, switching out-of-class to ustekinumab (an antibody directed against the p40 subunit of IL-12 and IL-23, approved for use in psoriasis and Crohn's disease) has been shown to be effective in the treatment of anti-TNF induced skin lesions refractory to topical steroids.[404,406] Severe skin lesions cause patients with inflammatory bowel disease to discontinue anti-TNF therapy. The mechanism of anti-TNF induced skin lesions is not well understood but recent data suggests that the skin lesions are characterised by infiltration of interferon-γ expressing Th1 lymphocytes and IL-17A/IL-22 expressing Th17 cells, with the severity of skin lesions correlating with the density of Th17 cell infiltrates.[402] It is speculated that the Fc region of anti-TNF antibodies bind to Fc-gamma CD64 (Fc-gamma receptor I (FcγRI)) and CD16/32 (Fc-gamma receptor III/II (FcγRIII/II)) on monocytes and

macrophages triggering secretion of IL-23 which drives Th17 production of IL-17 and IL-22 and the development of skin lesions.[407] A preliminary small candidate gene study has reported association with the rare IL23R variant rs11209026 (p.Arg381Gln) and severe anti-TNF induced psoriasiform skin lesions suggesting it might be possible to identify patients at risk of adverse skin reactions prior to treatment.[402]

4.1.6 Clinical implementation and future clinical use of pharmacogenetic biomarkers of ADRs

The clinical implementation of a genetic association into a pre-treatment test has traditionally demanded a randomised controlled trial (RCT) to assess its clinical utility and cost-effectiveness. However, these studies are costly, require large sample sizes and often fail to deliver consistent actionable results.[408] To hold pharmacogenetic studies up to the same standards designed to assess drug efficacy may be inappropriate and delay translation of research from bench-to-bedside; although clearly an appropriate balance is needed. The greater availability and falling costs of whole genome sequencing, (currently less than US$ 1,500)[409,410], means that the question is increasingly not whether to genotype but how best to utilise existing sequence data, perhaps generated at diagnosis or even at birth.

As our knowledge of gene-drug interactions increases this information needs to be curated, reviewed and translated into actionable prescribing guidelines for clinicians who lack knowledge and confidence of pharmacogenetic testing. This crucial work is being supported by bodies such as Clinical Pharmacogenetics Implementation Consortium (CPIC)[395] and Pharmacogenetics and Pharmacogenomics KnowledgeBase (PharmGKB).[411] There is a need to integrate genetic data into electronic patient systems to help physicians choose and deliver the right drug at the

right dose first time for individual patients. A number of genomic prescribing systems are being developed by academic institutions. These typically employ a web-based portal which displays interactive, patient-specific, pharmacogenomic results in the form of a patient-tailored synopsis including prescribing recommendations and suggested alternative medications. Finally, the turnaround time for these tests needs to be short so that clinicians are able to receive actionable results in a time-frame which doesn't delay the instigation of treatments in the acutely unwell patient.

4.2 The objective(s) of the chapter

The thiopurines are the commonest immunosuppressive drugs used in the maintenance treatment of IBD, although up to one-third of patients have to stop treatment due to drug side-effects, including thiopurine-induced myelosuppression (TIM).[412,413] Pre-emptive dose adjustments based on *TPMT* genotype, and drug avoidance in homozygotes have reduced thiopurine-induced adverse effects without compromising desired immunosuppressive therapeutic effects. Whilst this strategy is fairly commonplace, it only identifies one-quarter of European patients that suffer TIM.[357,414] Recently, retrospective studies in East Asian patients have uncovered association between variants in nudix (nucleoside diphosphate linked moiety X)-type motif 15 (*NUDT15*) and TIM, although their relevance in European patients in unknown.[362,363,415]

Therefore, in this chapter I set the following objectives:

- **Objective 7:** To investigate the association between novel genetic variants and thiopurine-induced myelosuppression in European patients with IBD

- **Objective 8:** If genetic variants are present, to explore if the frequency of these variants were enriched in those patients with early drug reactions (≤ 8 weeks from start of maximum dose)

It is crucial that this TIM cohort is accurately phenotyped in order to describe the morbidity associated with each adverse drug reaction and to uncover any genotype-phenotype associations.

- **Objective 9:** explore the clinical phenotype and morbidity related to carriage of a TIM associated genetic variant(s)

Finally, I aim to explore the clinical validity of any novel genetic variants; that is, the performance of the test including sensitivity, specificity as well as positive and negative predictive values in light of disease prevalence. This may aid translation of any positive findings into clinical practice.

- **Objective 10:** To ascertain the clinical validity (e.g. sensitivity, specificity, negative and positive predictive values) of genetic testing to identify patients at risk of TIM

RESEARCH PAPER IV

‘Association of Genetic Variants in *NUDT15* With Thiopurine-Induced Myelosuppression in Patients with Inflammatory Bowel Disease.’

PUBLISHED: JAMA, (2019) 321(8), p. 773. doi: 10.1001/jama.2019.0709.

Walker, G. J., Harrison, J. W., Heap, G. A et al

4.3.1 Key Points

Question: What genetic variants are associated with thiopurine-induced myelosuppression among patients of European ancestry with inflammatory bowel disease?

Findings: In this case-control study that used whole exome sequence data from 961 thiopurine-exposed patients of European ancestry with inflammatory bowel disease, three coding *NUDT15* variants, including a 6bp in-frame deletion (odds ratio 38.2), were identified that were associated with thiopurine-induced myelosuppression.

Meaning: Among patients of European ancestry with inflammatory bowel disease, variants in *NUDT15* were associated with increased risk of thiopurine-induced myelosuppression.

4.3.2 Abstract

Importance: Thiopurines are commonly prescribed immunosuppressants but their use may be limited by myelosuppression. *TPMT* pharmacogenetic testing identifies only 25% at-risk patients of European ancestry. In East Asians, *NUDT15* variants are associated with thiopurine-induced myelosuppression (TIM).

Objective: To identify genetic variants associated with thiopurine-induced myelosuppression in patients of European ancestry with inflammatory bowel disease (IBD).

Design, Setting, and Participants: Case-control study of 491 patients affected by TIM and 679 thiopurine-tolerant unaffected patients who were recruited from 89 international sites between March 2012 and November 2015. Affected patients were verified by an independent panel of clinicians. Genome-wide (GWAS) and exome-wide association studies (EWAS) of patients of European ancestry were conducted. The replication cohort comprised 73 affected and 840 unaffected patients.

Exposure: Genetic variants associated with TIM.

Main Outcome and Measures: Thiopurine-induced myelosuppression was defined as a decline in absolute white blood cell count to ≤ 2.5×10^9/L or decline in absolute neutrophil cell count to ≤ 1.0×10^9/L leading to thiopurine drug dose reduction or withdrawal.

Results: Among 1077 patients (398 affected and 679 unaffected patients) in the final analysis (median age at IBD diagnosis 31.0 years [IQR 21.2 to 44.1], 540 [50%] women, 602 [56%] Crohn's disease), 919 patients (311 affected and 608 unaffected patients) were included in the GWAS and 961 patients (328 affected and 633 unaffected patients) in the EWAS analyses. The GWAS confirmed association of *TPMT* (chromosome 6, rs11969064) with thiopurine-induced myelosuppression

(30.5% [95/311] affected patients versus 16.4% [100/608] unaffected patients; odds ratio [OR] 2.3, 95% CI 1.7 to 3.1; $P = 5.2\times10^{-9}$). The EWAS demonstrated an association with an in-frame deletion in *NUDT15* (chromosome 13, rs746071566) and thiopurine-induced myelosuppression (5.8% [19/328] affected patients versus 0.2% [1/633] unaffected patients; OR 38.2, 95% CI 5.1 to 286.1; $P = 1.3 \times 10^{-8}$) which was replicated in a different cohort (2.7% [2/73] affected versus 0.2% [2/840] unaffected patients; OR 11.8, 95% CI 1.6 to 85.0; $P = .03$). Carriage of any of three coding *NUDT15* variants, including the in-frame deletion, was associated with an increased risk (OR 27.3, 95% CI 9.3 to 116.7; $P = 1.1\times10^{-7}$) of thiopurine-induced myelosuppression, independent of *TPMT* genotype and thiopurine dose.

Conclusions and Relevance: Among patients of European ancestry with inflammatory bowel disease, variants in *NUDT15* were associated with increased risk of thiopurine-induced myelosuppression. These findings suggest that *NUDT15* genotyping may be considered prior to initiation of thiopurine therapy; however, further study including additional validation in independent cohorts is required.

4.3.3 Introduction

The thiopurines (mercaptopurine and its prodrug azathioprine) are the most commonly used immunosuppressive drugs in the management of patients with inflammatory bowel disease [IBD]. However, approximately15% of patients develop adverse drug reactions that necessitate drug withdrawal.[342,346] Thiopurine-induced myelosuppression (TIM) has a cumulative incidence of 7% and usually occurs within a few weeks of starting the drug.[346] Most patients are asymptomatic, but serious opportunistic infection may occur and there is an estimated mortality of 1%.[346]

The enzyme, thiopurine S-methyltransferase (TPMT) converts thiopurines to methylated metabolites, reducing the production of the active 6-thioguanine nucleotides.[370] Genetic variation in the *TPMT* gene [RefSeqGene NG_012137.2] can result in decreased TPMT enzyme activity and higher production of 6-thioguanine nucleotides, predisposing patients to bone marrow suppression.[346,358,370] Pre-treatment testing of *TPMT* is recommended by the US Food and Drug Administration (FDA) to identify patients at risk of TIM[356]. Among patients with reduced TPMT activity, the drug may be avoided or the dose reduced.[357] However, *TPMT* variants are only found in 25% of TIM affected patients of European ancestry, suggesting the presence of other genetic and environmental determinants.[357,414] Recently, studies in patients of East Asian ancestry[362,415] and other populations[416–420] have identified variants in nudix hydrolase 15 (*NUDT15*; RefSeqGene NG_047021.1) as risk factors for TIM. Although a novel *NUDT15* variant (rs746071566, p.Gly17_Val18del) was described by Moriyama *et al* (2017)[416] in a single paediatric patient with TIM of European ancestry, the association of *NUDT15* genetic variation with TIM in this population has not been fully evaluated.

The primary objective of this study was to investigate the association between genetic variants and TIM in patients of European ancestry with IBD. It was hypothesised that the frequency of these variants would be increased among TIM affected patients and enriched in those with early TIM (≤ 8 weeks from start of maximum dose).[346]

4.3.4 Methods

4.3.4.1 Study Design and Setting

The protocol was approved by the National Research Ethics Committee (11/SW/0222, Exeter pharmacogenetic PRED4 program and STB1, Exeter IBD Genetics cohort, England). All participants provided informed written consent. A retrospective case-control study of the association of genetic variants with TIM was designed as part of the Exeter pharmacogenetic PRED4 program, which aims to investigate the genetic basis of serious adverse drug reactions to drugs commonly used in gastroenterology (www.ibdresearch.co.uk).[325,379] Both genome-wide (GWAS) and exome-wide association (EWAS) platforms were used to investigate common and rare genetic variation, respectively.

4.3.4.2 Study Populations and Case Definition

Thiopurine-induced myelosuppression cases (affected patients) were recruited from 82 UK and 7 international sites between March 2012 and November 2015 and not followed up after their initial research visit. They were identified through: opportunistic clinical encounters; systematic searches of electronic records; recall via the Medicines

and Healthcare Products Regulatory Agency (MHRA) Yellow Card Scheme; and by direct advertising to patients.

Inclusion criteria included all of the following: diagnosis of IBD; history of thiopurine exposure in the seven days prior to onset of TIM; decline in absolute white blood cell count to ≤ 2.5×10^9/L or decline in absolute neutrophil cell count to ≤ 1.0×10^9/L; treating physician concluded that the thiopurine was the likely cause of myelosuppression and the dose was reduced or the drug withdrawn.

Investigators at each site completed a custom-designed case report form (**eAppendix 4.3-1 in the Supplement**), that captured the following data: patient demographics (age, weight, height, ethnicity and smoking history); adverse drug reaction data (thiopurine, thiopurine dose, drug start date, drug stop date, full blood count parameters before, during and after drug exposure and full blood count normal range reference values) and IBD phenotype. Each patient was diagnosed with IBD by their gastroenterologist using endoscopic, histological and/or radiological data and phenotyped using the Montreal classification. This classifies ulcerative colitis extent as: limited to the rectum (E1), distal to the splenic flexure (E2) or proximal to the splenic flexure (E3). For Crohn's disease, patients are categorised by age (years) at disease onset (A1: < 17 years, A2: 17 to 40 years or A3: >40 years); location of disease (L1: ileal, L2: colonic or L3: ileocolonic) and disease behaviour (B1: non-stricturing and non-penetrating, B2: stricturing or B3: penetrating).

Consistent with previous published pharmacogenetics studies,[325,379] all recruited affected patients were reviewed independently by at least four gastroenterologists and

assigned an adjudication category (**eAppendices 4.3-2 to 4.3-4, eFigure 4.3-1 and eMethods in the Supplement**).[338] Only patients assigned as definitely or probably affected by TIM were included in the discovery and replication analyses.

Thiopurine-exposed controls without TIM (unaffected patients) were identified from the Exeter IBD Genetics (IBDGEN) cohort recruited at the Royal Devon & Exeter Hospital, UK (**additional details appear in the eMethods in the Supplement**). In the final analysis, only patients with an absolute white blood cell count ≥ 3.0×10^9/L and an absolute neutrophil cell count ≥ 1.5×10^9/L for the duration of their treatment with a thiopurine were included in the final analyses.

The replication cohort met the identical inclusion criteria and included nonoverlapping patients from the same central study site (Royal Devon and Exeter Hospital) and patients from 4 new sites (Saint-Antoine Hospital in France, University Medical Centre Groningen in the Netherlands, Cedars-Sinai Medical Centre in the United States, and Massachusetts General Hospital in the United States). Patients at these new sites were identified from searches of pre-existing genetics cohorts in April 2017. These sites had started recruitment in 2005 (Massachusetts General Hospital and Cedars-Sinai Medical Centre), 2011 (University Medical centre Groningen), and 2013 (Saint-Antoine Hospital).

4.3.4.3 Genetic Analysis

Details of the genetic data generation and quality control prior to the GWAS and EWAS analyses appear in the eMethods in the Supplement. For the GWAS, 245,185 variants were genotyped using the Illumina Infinium G4L Genome-Wide Association Study

(GWAS) array (Illumina, San Diego, CA, USA). Patients were excluded if they had variants with a call rate of less that 98%, had variants with a minor allele frequency of less than 1% or had variants with a Hardy Weinberg equilibrium (HWE) of $P < 1\times10^{-6}$ in the unaffected patients. Principal component analysis was carried out using Genome-wide Complex Trait Analysis v1.24[421] with data from the 1000 Genomes project.[422] Only data from patients clustering with non-Finnish Europeans (NFE) were included. This process minimised the potential confounding effects of population stratification, which might have resulted in association of variants with TIM when in fact the association was with a specific ethnicity, which was by chance over- or under-represented among affected patients compared with unaffected patients.

We excluded patients of Finnish ancestry due to their unique genetic background, which has occurred as a consequence of the geographical and cultural isolation of this population leading to enrichment of some disease-causing gene variants and losses of others. Other quality control measures included a sex-mismatch check (a method which used X chromosome homozygosity rates to determine sex and identify patients for whom the sex recorded in the case report form/phenotype database did not match the predicted sex based on genetic data) and relatedness-checking"(in which sample and pedigree integrity were both simultaneously examined by reconciling genomic data with self-reported relationships between patients).

After pre-phasing with Eagle2[423], imputation with PBWT[424] was performed into the 1000 Genomes Project Phase 3[422] reference panel using the Wellcome Trust Sanger Imputation Service. Only single nucleotide polymorphisms (SNPs) with a post-

imputation info score of < 0.85 or minor allele frequency < 0.01 were included. After all quality control measures, 6,272,335 variants remained.

For the *EWAS*, exonic regions were sequenced using the Illumina HiSeq platform (150bp paired reads) and reads mapped to the human genome reference sequence (GRCh37) using BWA-MEM.[425] Each sample was sequenced to an average depth of 34×, with ~99% of the targeted regions covered by ≥1×, ~92% covered by ≥10× and ~70% covered by ≥25×. Variants with a Hardy Weinberg equilibrium (HWE) $P < 1\times10^{-6}$ were excluded as were any variants with a genotyping success rate of < 0.98, a read depth of < 10× or with a genotype skew $P < 5\times10^{-9}$ (binomial test). For the post quality control EWAS quantile–quantile plot (**eFigure 4.3-2 in the Supplement**).

4.3.4.4 Statistical Analysis

Phenotype comparisons: Continuous data were summarised using medians and interquartile ranges and compared using Mann-Whitney U tests. The estimate of the median of the difference between affected and unaffected patients and its confidence interval were also calculated, as implemented in R 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). Categorical data were summarised as the number and percentage and compared using Fisher's exact tests.

Primary analyses: Associations for both the GWAS and EWAS were determined using the Fisher exact test implemented in PLINK 1.9. Manhattan plots were generated using R to display negative $\log_{10}$ *P* values at each SNP. A genome-wide significance threshold of $P < 5\times10^{-8}$ was deemed significant. Gene burden testing using PLINK-seq 0.10 and Sequence Kernel Association Tests (SKAT)[426] were used to evaluate if an

association existed between sets of rare variants across individual candidate genes associated with affected patients. Technical validation of variants was carried out using Sanger sequencing (**see eMethods in Supplement**). For the replication cohort, case adjudication, genotype data generation, genetic quality control and analyses were undertaken using the same platforms and methods as the discovery cohort. Replicated variants with a Fisher exact *P* value < .05 were considered significant.

Exploratory analyses: Having found an association with a variant and TIM, the final dataset was examined for any other non-monomorphic variants within this gene annotated as 'missense' or 'loss of function' in the Genome Aggregation Database (gnomAD).[427] Further missense variants were evaluated using *in silico* PROtein Variation Effect ANalyzer (PROVEAN).[428] As the functional significance of this modelling is uncertain, only replicated *NUDT15* variants and those previously described in other thiopurine-induced myelosuppression cohorts[362,415] were used in subsequent genotype-phenotype, multivariable logistic regression, and clinical usefulness analyses.

Combinations of *TPMT* variants on the same chromosome have been reported as haplotypes; these were reconstructed using Eagle2[423] and matched to the Clinical Pharmacogenetics Implementation Consortium (CPIC) definitions.[429] Categorical *TPMT* enzyme activity (i.e. absent; low; normal; high) was measured in red blood cells using radiometric high-performance liquid chromatography (HPLC) as part of routine clinical practice. The relationship between *TPMT* haplotypes and enzyme activity was determined.

Genotype-phenotype interactions were explored using Mann-Whitney U and the Fisher exact tests. All statistical tests were two-sided, and a $P < .05$ was considered significant. No adjustment of the *P*-value was made for multiple comparisons of phenotype data. Weight-adjusted dose (mg/kg) was calculated using the following formula: [mercaptopurine dose (mg)·2.08 / weight (kg)], or for azathioprine: [azathioprine dose (mg) / weight (kg)].

A multivariable logistic regression analysis was undertaken to assess the independent associations of *NUDT15*, *TPMT*, and weight adjusted thiopurine dose with risk of myelosuppression. Time to TIM (stratified by genotype) was analysed using Mann-Whitney U statistics.

The potential for clinical usefulness (sensitivity, specificity, negative and positive predictive value) of genotyping for variants associated with TIM was estimated according to adapted methods by Tonk *et al*[430] and de Graaff *et al*[431] (**see eMethods in Supplement**). These estimates assumed the following: an overall risk of TIM of 7%[346]; either avoidance of drug (reducing risk of TIM to zero) or target dose reduction in those people carrying deleterious variants (reducing risk of TIM to that seen in patients with the reference haplotype/genotype); the non-Finnish European population variant carrier frequency from gnomAD[427] and the odds ratio of TIM for the variant in multivariable logistic regression analysis. Confidence intervals for the number needed to genotype were estimated using 10,000 bootstraps of the case-control cohort and randomly generated estimates of the population *NUDT15* and *TPMT* variant carriage and TIM rates based on sampling from binomial distributions. For *TPMT*, detailed methods can be found in **eMethods in the Supplement**.

The prevalence of *NUDT15* variants in patients of other ancestry was explored using all adjudicated affected patients and population data from gnomAD.[427]

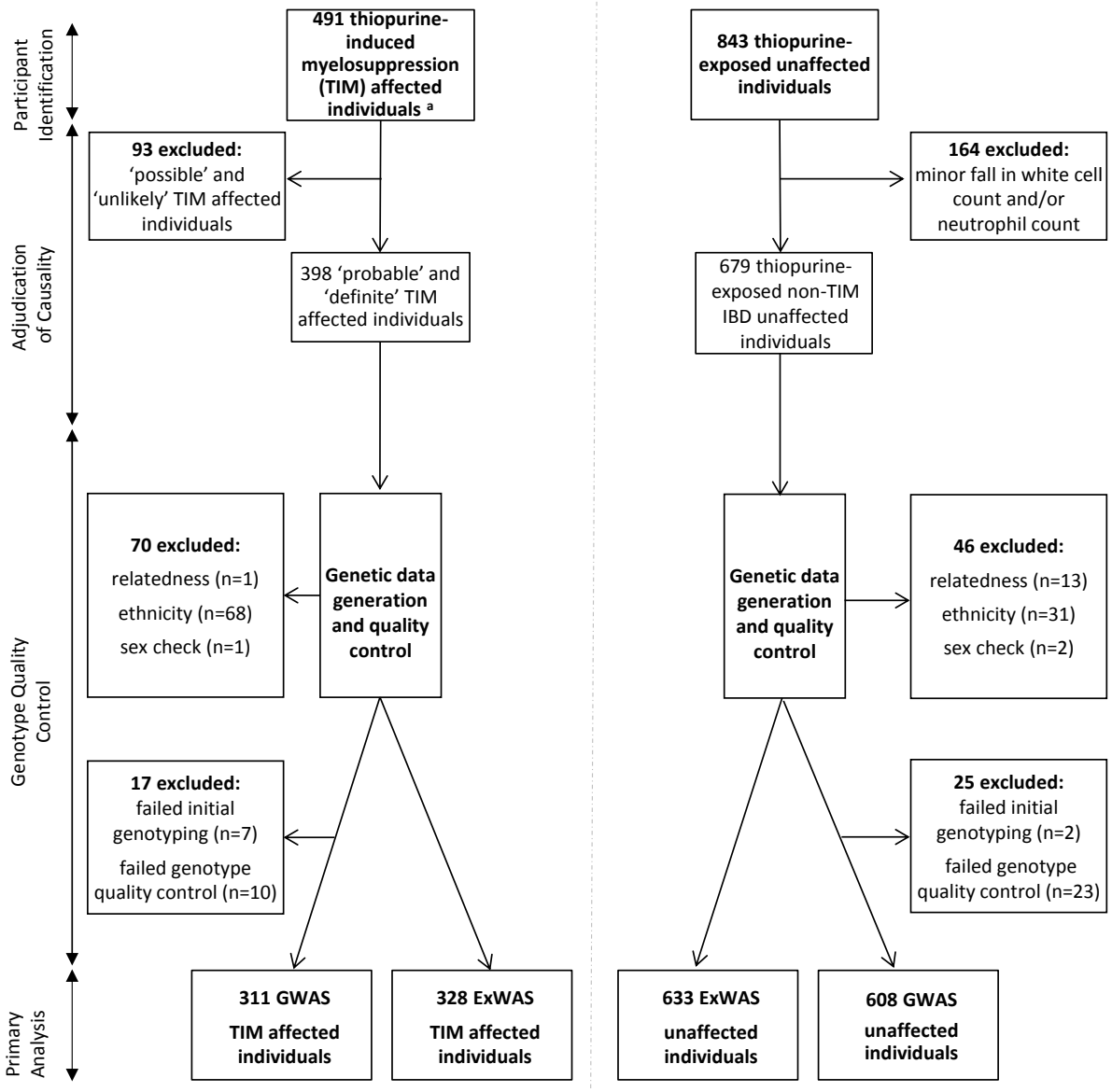
4.3.5 Results

4.3.5.1 Study Overview

Participant flow through the study is shown in **Figure 4.3-1**. 491 patients with IBD and TIM (affected patients) were recruited from 82 UK and 7 international sites between March 2012 and November 2015. One UK centre recruited 843 thiopurine-exposed patients with IBD and no history of myelosuppression (unaffected patients). Following the adjudication process, 1077 patients (398 affected and 679 unaffected patients) entered the final analysis.

After assessment using the genetic quality control measures, 70 affected patients were excluded (68 for ethnicity, 1 for relatedness, and 1 for sex mismatch) and 46 unaffected patients were excluded (31 for ethnicity, 13 for relatedness, and 2 for sex mismatch). In addition, for the GWAS analysis, 17 affected patients were excluded (10 due to failure of quality control genotyping and 7 to failure of genotyping) and 25 unaffected patients were excluded (23 due to failure of quality control genotyping and 2 to failure of genotyping). Thus, 919 patients (311 affected and 608 unaffected patients) were included in the GWAS and 961 patients (328 affected and 633 un-affected patients) were included in the EWAS analysis. Replication was conducted in 73 affected and 840 unaffected patients recruited from 5 international sites.

Figure 4.3-1. Flow diagram and study overview



TIM, Thiopurine-induced myelosuppression; EWAS, Exome Wide Association Study; GWAS, Genome Wide Association Study. Genetic quality control terms: "Sex mismatch"- discrepancy between genetically determined sex and phenotype data; "Relatedness"- exclusion of patients too closely related to each other; "Ethnicity"-exclusion of patients not of non-Finnish European ancestry based on principal component analysis.

4.3.5.2 Phenotype Comparisons

There were no differences in sex when comparing affected and unaffected patients (female 53.0% [211/398] vs 48.5% [329/679], respectively, *P* = .17; **Table 4.3-1**). There were no differences when comparing affected and unaffected patients by type

of IBD diagnosis: Crohn disease (57.8% [230/398] vs 54.8% [372/679], respectively), ulcerative colitis (39.7% [158/398] vs 44.0% [299/679]), and IBD-unclassified (2.5% [10/398] vs 1.2% [8/679], *P* = .12).

Table 4.3-1. IBD and drug exposure phenotype in adjudication affected patients and unaffected patients prior to genomic quality control

Characteristic	Affected patients n = 398 [a]	Unaffected patients n = 679 [a]
Sex		
Female	211 (53.0%)	329 (48.5%)
Male	187 (47.0%)	350 (51.5%)
Diagnosis		
Crohn's disease	230 (57.8%)	372 (54.8%)
IBD-Unclassified	10 (2.5%)	8 (1.2%)
ulcerative colitis	158 (39.7%)	299 (44.0%)
Age at inflammatory bowel disease diagnosis (years)	30.1 (19.3 to 43.1)	31.6 (22.2 to 44.7)
Estimate of difference [95% Confidence Interval]	2.3 [0.4 to 4.2]	
Montreal Crohn's disease classification [b]		
Age at diagnosis [c]		
A1: < 17y	52 (22.7%)	23 (7.7%)
A2: 17-40y	122 (53.3%)	235 (78.3%)
A3: > 40y	55 (24.0%)	42 (14.0%)
Location [c]		
L1: ileal	57 (24.9%)	132 (44.0%)
L2: colonic	74 (32.3%)	79 (26.3%)
L3: ileocolonic	98 (42.8%)	89 (29.7%)
Behaviour [d]		
B1: non-stricturing and non-penetrating	123 (57.7%)	175 (58.9%)
B2: stricturing	62 (29.1%)	82 (27.6%)
B3: penetrating	28 (13.1%)	40 (13.5%)
Montreal ulcerative colitis /Inflammatory Bowel Disease-Unclassified extent [e]		
E1: limited to the rectum	15 (9.4%)	14 (6.0%)
E2: distal to the splenic flexure	73 (45.6%)	112 (47.9%)
E3: proximal to the splenic flexure	72 (45.0%)	108 (46.2%)
Weight-adjusted thiopurine dose (mg/kg) [f]	2.07 (1.69 to 2.45)	1.84 (1.48 to 2.19)
Estimate of difference [95% Confidence Interval]	-0.24 [-0.32 to -0.17]	

a values represent: n (%) or median [interquartile range]
b Montreal Classification System from Silverberg MS, Satsangi J, Ahmad T, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Canadian Journal of Gastroenterology 2005;19 Suppl A(5):5A-36A.
c Denominator for affected patients = 229 and for unaffected patients = 300
d Denominator for affected patients = 213 and for unaffected patients = 297
e Denominator for affected patients = 160 and for unaffected patients = 234

f weight adjusted dose represents the maximum azathioprine equivalent dose prior to thiopurine-induced myelosuppression and adjusted for weight (mg/kg): [(mercaptopurine dose (mg) x 2.08) / weight (kg)] or [azathioprine dose (mg) / weight (kg)]

There were no differences in behaviour of IBD when comparing affected and unaffected patients using the Montreal classification of IBD: B1 (non-stricturing and non-penetrating, 57.7% [123/213] vs 58.9% [175/297], respectively), B2 (stricturing, 29.1% [62/213] vs 27.6% [82/297]), and B3 (penetrating, 13.1% [28/213] vs 13.5% [40/297], *P* = .94). There were no differences in the extent of ulcerative colitis and IBD-unclassified when comparing affected and unaffected patients using the Montreal Classification system: E1 (limited to the rectum, 9.4% [15/160] vs 6.0% [14/234], respectively), E2 (distal to the splenic flexure, 45.6% [73/160] vs 47.9% [112/ 234]), and E3 (proximal to the splenic flexure, 45.0% [72/160] vs 46.2% [108/234], *P* = .46). In contrast, affected patients were younger at the time of IBD diagnosis (median, 30.1 years [IQR, 19.3-43.1 years]) com- pared with unaffected patients (median, 31.6 years [IQR, 22.2-44.7 years], P = .02) and received a higher weight- adjusted thiopurine dose (median, 2.07 mg/kg [IQR, 1.69- 2.45 mg/kg] vs 1.84 mg/kg [IQR, 1.48-2.19 mg/kg], respectively, P < .001). In addition, affected patients with Crohn disease were more likely to have colonic or ileo-colonic dis- ease than unaffected patients (L1 [ileal]: 24.9% [57/229] vs 44.0% [132/300], respectively; L2 [colonic]: 32.3% [74/229] vs 26.3% [79/300], and L3 [ileocolonic]: 42.8% [98/229] vs 29.7% [89/300], P < .001).

Among the 398 affected patients, 143 (36%) episodes of TIM occurred within 8 weeks of therapy with the maximum dose of thiopurine (**eTable 4.3-1 in the Supplement**). The median time from commencement of thiopurine to TIM was 28.3 weeks (IQR, 9.0-81.1 weeks) and the median time from maximum dose of thiopurine to TIM was 14.7

weeks (IQR, 5.9-37.9 weeks). Phenotype data for the replication cohort appear in **eTable 4.3-2 in the Supplement**.

4.3.6 Primary Analyses

4.3.6.1 GWAS

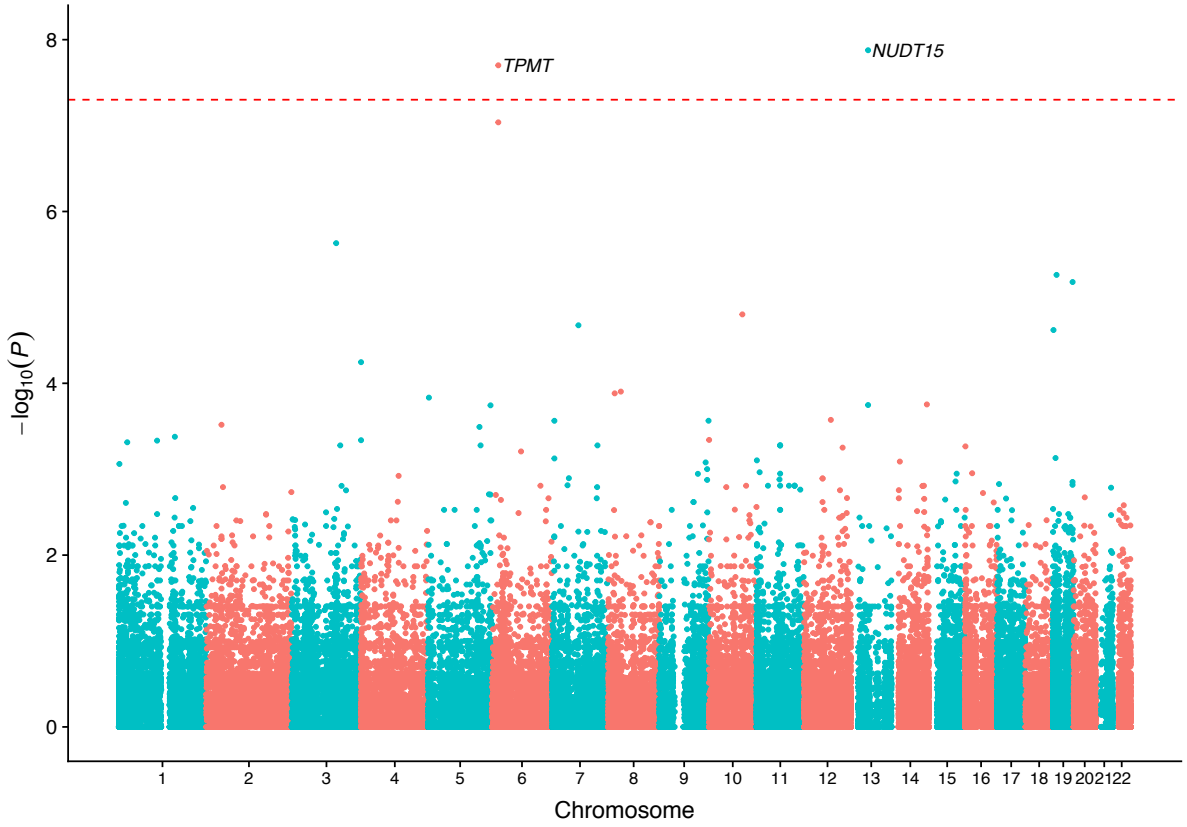
Data from 311 affected and 608 unaffected patients (**eTable 4.3-3 in the Supplement**) were included in the GWAS discovery cohort. The association of TIM with *TPMT* (rs11969064) was confirmed (30.5% [95/311] affected patients versus 16.4% [100/608] unaffected patients; odds ratio [OR] 2.3, 95% CI 1.7 to 3.1; $P = 5.2\times10^{-9}$) (**eFigure 4.3-3 in the Supplement)**. This association was enriched in early (≤ 8 weeks of starting maximum thiopurine dose) affected patients (OR 4.0, 95% CI 2.8 to 5.8; $P = 1.8\times10^{-15}$ in early and OR 1.6, 95% CI 1.1 to 2.2; $P = .01$ in late TIM) (**eFigure 4.3-4 in the Supplement**). No other genetic associations with TIM exceeded the *a priori* threshold for statistical significance.

4.3.6.2 EWAS

Data from 328 affected and 633 unaffected patients were included in the EWAS discovery cohort (**eTable 4.3-4 in the Supplement**). The EWAS, performed to investigate the role of rare coding variants, revealed a TIM association with a 6bp in-frame deletion at position 48611918 of chromosome 13 in exon 1 of *NUDT15* (rs746071566, p.Gly17_Val18del; 5.8% [19/328] of affected patients versus 0.2% [1/633] unaffected patients; OR 38.2, 95% CI 5.1 to 286.1; $P = 1.3\times10^{-8}$) (**Figure 4.3-2**). The odds ratio for affected patients with early-onset TIM versus unaffected patients was 74.2 (95% CI 9.6 to 573.5; $P = 8.2\times10^{-10}$) and late-onset TIM was 20.9 (95% CI 2.6 to 170.1, $P = 4.2\times10^{-4}$); affected patients with early-onset TIM were significantly

enriched for the variant (OR 3.6, 95% CI 1.4 to 9.2, *P* = .005) (**eTable 4.3-5 in the Supplement**).

Figure 4.3-2. Manhattan plot for the discovery exome-wide association study analysis in 328 affected and 633 unaffected patients



Each coloured dot represents a single variant within each respective chromosome. The negative $\log_{10}$ *P* value represents a Fisher's exact analysis between affected and unaffected patients. Red dotted horizontal line indicates genome-wide significance at Fisher's exact *P* = 5.0×10^{-8}. Gene names correspond to the gene in closest proximity to the variant with the lowest *P* value at each locus if within 50kbp.

The association of the p.Gly17_Val18del variant and TIM was confirmed in the replication analysis: 2.7% (2/73) of affected patients versus 0.2% (2/840) thiopurine-exposed unaffected patients with IBD (OR 11.8; 95% CI 1.6 to 85.0; *P* = .03). A duplication at this multi-allelic site within *NUDT15* (rs746071566, p.Gly17_Val18dup – also annotated as p.Val18_Val19insGlyVal) was also noted but did not meet genome wide significance (1.5% [5/328] of affected patients versus 0.3% [2/633] unaffected

patients; OR 5.2, 95% CI 1.0 to 26.6; *P* = .04) (**Table 4.3-2**). The only variant outside of *NUDT15* significantly associated with TIM in the exome sequencing data was rs1800460 in *TPMT* (OR 3.0, 95% CI 2.0 to 4.3; $P = 2.0\times10^{-8}$). Gene burden testing did not identify any novel associations beyond *TPMT* and *NUDT15* (**eTable 4.3-6 in the Supplement**).

Table 4.3-2. Association of genetic variants in NUDT15 with thiopurine-induced myelosuppression in patients with inflammatory bowel disease using data from the Exome Wide Association Study (EWAS)

***NUDT15* Identifiers**					**Thiopurine-Induced Myelosuppression Affected Patients (n = 328)**			**Thiopurine-Tolerant Unaffected Patients (n = 633)**			**Odds Ratio (95% Confidence Interval)**	***P* value [b]**
Position	**rsID**	**Protein Sequence**	**Variant Allele**	**Reference Allele**	**Variant Heterozygote (n)**	**Reference Homozygote (n)**	**Minor Allele Frequency Affected Individuals[a]**	**Variant Heterozygote (n)**	**Reference Homozygote (n)**	**Minor Allele Frequency Unaffected Individuals[a]**		
48611918 [c]	rs746071566	p.Gly17_Val18del	A	AGGAGTC	19	304	0.029	1	630	7.9×10-4	38.2 (5.1 to 286.1)	1.3×10^{-8}
48619855	rs116855232	p.Arg139Cys	T	C	8	320	0.012	0	633	0	NA	1.8×10^{-4}
48611918 [c]	rs746071566	p.Gly17_Val18dup [d]	AGGAGTC GGAGTC	AGGAGTC	5	304	0.008	2	630	0.002	5.2 (1.0 to 26.6)	.04
48611979	rs768057637	p.Lys33Glu	G	A	1	327	0.002	0	633	0	NA	.34
48615121	13:48615121	p.Val75Gly	G	T	1	327	0.002	0	633	0	NA	.34
48611961	rs777311140	p.Cys28GlyfsTer28	CGCGG	C	0	328	0	1	632	7.9×10^{-4}	NA	>.99
48611883	13:48611883	p.Met1?	C	A	0	328	0	1	632	7.9×10^{-4}	NA	>.99

Position, chromosome 13 position; rsID, single nucleotide polymorphism identification number, or chromosome: position if no rsID available; NA, not applicable

[a] Minor Allele Frequency (MAF) = [number of variant alleles in population/(2*number of participants)] noting that no affected patients or unaffected patients were homozygote for *NUDT15* variant alleles

[b] *P* values calculated using Fisher's exact test. A genome-wide significance threshold of $P < 5\times10^{-8}$ was considered significant.

[c] This site is multi-allelic and both of these variants occur at the same chromosome position (48611918): 19 patients were heterozygous for p.Gly17_Val18del, 5 affected patients were heterozygous for p.Gly17_Val18dup and 304 affected patients were homozygous reference = 328 patients in total

[d] previously annotated as p.Val18_Val19insGlyVal

4.3.6.3 Exploratory Analyses

NUDT15 sequence data were next examined for the presence of all coding variants, either previously associated with TIM[362,415,416], or identified in gnomAD[427] and predicted as deleterious in PROVEAN[428] (**Table 4.3-2 and eFigure 4.3-5 in the Supplement**). However, four (p.Lys33Glu, p.Val75Gly, p.Cys28GlyfsTer28 and p.Met1?) of the seven *NUDT15* variants were each only found in a single individual. Therefore, only variants either meeting genome-wide association in this analysis (p.Gly17_Val18del) or previously associated with TIM in other analyses (p.Arg139Cys and p.Gly17_Val18dup) were included for subsequent exploratory analyses.

Overall, 9.5% (31/328) of the non-Finnish European TIM discovery cohort carry any of the three *NUDT15* coding variants, compared with 0.5% (3/633) of unaffected patients (OR 20.9, 95% CI 6.4 to 68.6; $P = 1.5\times10^{-12}$). The association with these *NUDT15* variants was enriched in early versus late TIM affected patients (OR 3.3, 95% CI 1.6 to 6.9, $P < .001$).

75% (717/961) of the study patients had TPMT activity levels available for analysis: all ten patients with ‘absent’, and 73% (80/109) with ‘low’ TPMT activity carried variant *TPMT* haplotypes (**eFigure 4.3-6 and eTables 4.3-7 to 4.3-9 in the Supplement**). Overall, 4.9% (16/328) of affected patients and 0.2% (1/633) of unaffected patients had two TIM-associated *TPMT* variant haplotypes.

4.3.6.4 Genotype-Phenotype Analyses

Among all affected patients in the EWAS analysis, the median time to TIM was 15 weeks (IQR 6 to 41) with 34% (111/328) experiencing early myelosuppression. Of note, 18% (59/328) presented with an opportunistic infection, 23% (77/328) were admitted to hospital with a median length of stay of 6 days (IQR 2 to 9) and 9% (31/328) required granulocyte colony stimulating factor (GCSF) rescue therapy.

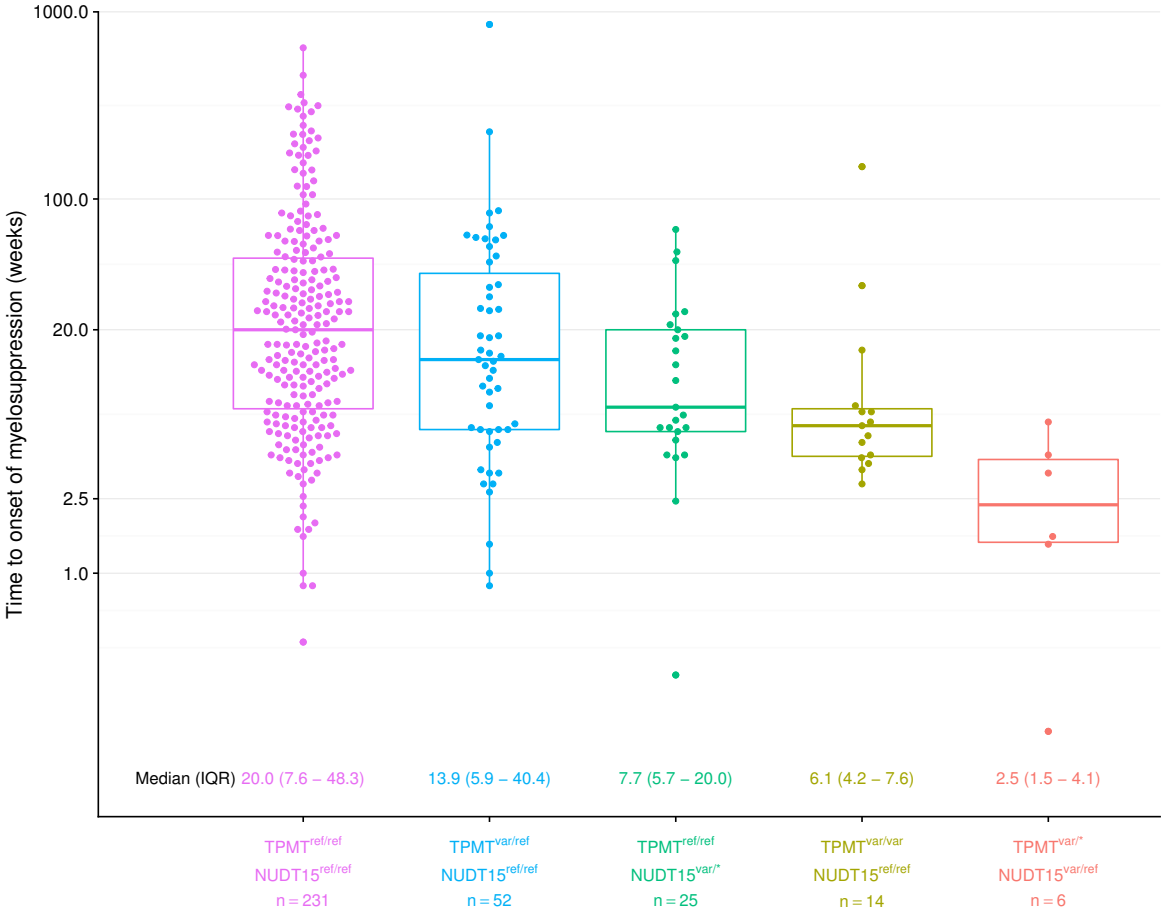
The median time to TIM was shorter in affected patients who carried *NUDT15* variants compared with affected patients without risk variants (7.7 weeks [IQR, 5.7-20.0 weeks] vs 20.0 weeks [IQR, 7.6-48.3 weeks], respectively; $P = .009$) and in those who carried double *TPMT* variants (6.1 weeks [IQR, 4.2-7.6 weeks] vs 20.0 weeks [IQR, 7.6-48.3 weeks], respectively; $P = .002$).

The median time to TIM was shortest in patients with both *TPMT* and *NUDT15* variants compared with affected patients without risk variants (2.5 weeks [IQR, 1.5-4.1 weeks] vs 20.0 weeks [IQR, 7.6-48.3 weeks], respectively, $P < .001$; **Figure 4.3-3 and eFigure 4.3-6 in the Supplement**). No difference in time to TIM was seen in patients carrying one variant *TPMT* haplotype and affected patients without risk variants (13.9 weeks [IQR 5.9 to 40.4] versus 20.0 weeks [IQR 7.6 to 48.3], respectively, $P = .14$).

Patients with *NUDT15* and/or *TPMT* variants developed lower median neutrophil counts than non-variant carrier affected patients (0.8×10^9/L [IQR 0.4 to 1.1] versus 1.0×10^9/L [IQR 0.7 to 1.2], respectively; $P < .001$), were more likely to be admitted to hospital (40% [39/97] versus 17% [38/231], respectively; $P < .001$) and were more

likely to receive GCSF rescue therapy (20% [19/97] versus 5.2% [12/231], respectively; *P* < .001) (**eTables 4.3-10 to 4.3-11 in the Supplement**).

Figure 4.3-3. Boxplot for time to thiopurine-induced myelosuppression (TIM) among affected patients defined by NUDT15 and TPMT genotype



ref, reference genotype/haplotype; var, variant; TIM, thiopurine-induced myelosuppression; IQR, inter-quartile range. Data points are each represented by a dot; the lower and upper boundaries of the box correspond to the first and third quartiles; the line within the box represents the median; the upper whisker extends from the upper boundary of the box to the largest value no further than 1.5 * inter-quartile range (IQR). The lower whisker extends from the lower boundary of the box to the lowest value, at most no further than 1.5 * inter-quartile range (IQR).Time to thiopurine-induced myelosuppression (weeks) calculated using the following formula: [Time to TIM (weeks) = date of meeting entry criteria for TIM - start date of highest dose prior to TIM]. Median and interquartile ranges provided to facilitate interpretation of time to TIM (weeks). One TIM case carried two *NUDT15* variants (rs746071566 [p.Gly17_Val18dup] and rs116855232 [p.Arg139Cys])-it was unknown if this represented a compound heterozygote or a heterozygote (*2 *NUDT15* haplotype). For the purposes of analysis, this patient was grouped with *NUDT15* heterozygotes and annotated as *NUDT15*$^{var/*}$. One TIM case was *TPMT* $^{var/var}$ and *NUDT15* $^{var/ref}$; for the purposes of analysis, this patient was grouped with five others who carried single *NUDT15* and *TPMT* variants (*TPMT* $^{var/ref}$ & *NUDT15* $^{var/ref}$). Compared to leftmost group, *P* values (Mann Whitney-U test) for the time difference were .14, .009, .002 and < .001, respectively.

The success of thiopurine re-challenge according to genotype was then explored: 51% (167/328) of affected patients were re-challenged and 57% (95/167) were able to tolerate a lower dose (median successful rechallenge dose = 1.2 mg/kg [IQR 0.9 to 1.5]). Neither weight-adjusted dose, type of thiopurine drug, patient age, *TPMT* genotype, nor *NUDT15* genotype were associated with subsequent tolerance after re-challenge (**eTable 4.3-12 in the Supplement**).

4.3.6.5 Multivariable Logistic Regression

In a multivariable logistic regression model, the odds of thiopurine-induced myelosuppression among those with variants in *NUDT15* (OR 27.3, 95% CI 9.3 to 116.7; $P = 1.1\times10^{-7}$) and *TPMT* (OR 2.2, 95% CI 1.4 to 3.3; $P = 3.5\times10^{-4}$ in heterozygotes; OR 53.4, 95% CI 10.4 to 980.1; $P = 1.5\times10^{-4}$ in homozygotes) were independent of thiopurine weight adjusted dose (OR 2.2, 95% CI 1.8 to 2.8; $P = 5.3\times10^{-11}$) (**Table 4.3-3**).

Table 4.3-3. Association with TPMT and NUDT15 variants on clinical phenotype: multivariate logistic regression model of genetic and dose related factors associated with thiopurine-induced myelosuppression (n = 919 [a])

Variable	Odds Ratio (95% Confidence Interval)	*P* value [d]
Weight adjusted thiopurine dose [c] For every 1mg/kg increase in azathioprine equivalent dose	2.2 (1.8 to 2.8)	5.3×10^{-11}
***NUDT15* genotype**		
NUDT15 ref/ref	Reference	
NUDT15 var/* [b]	27.3 (9.3 to 116.7)	1.1×10^{-7}
***TPMT* haplotype**		
TPMT ref/ref	Reference	
TPMT ref/var	2.2 (1.4 to 3.3)	3.5×10^{-4}
TPMT var/var	53.4 (10.4 to 980.1)	1.5×10^{-4}

Ref, reference haplotype or genotype; var, variant haplotype or genotype
[a] 42 observations were missing (n = 919)
[b] Carriage of 1 or more of 3 *NUDT15* variants: rs746071566 [p.Gly17_Val18del], rs746071566 [p.Gly17_Val18dup], and rs116855232 [p.Arg139Cys]. One patient with TIM possessed 2 *NUDT15* variants (rs746071566 [p.Gly17_Val18dup] and rs116855232 [p.Arg139Cys]); however, it was not possible to ascertain if this represented a compound heterozygote or 2 variants on the same strand (*2 *NUDT15* haplotype). For the purpose of the analysis, this case was considered as a single *NUDT15* variant carrier (*NUDT15* var/*).
[c] weight-adjusted thiopurine dose represents the maximum azathioprine equivalent dose prior to thiopurine-induced myelosuppression adjusted for weight (mg/kg)
[d] represents *P* value from logistic regression with all three variables included. *P* < .05 deemed clinically significant.

4.3.6.6 Clinical Usefulness

For *NUDT15*, the estimated number of patients needed to genotype to prevent 1 patient from developing TIM was 95 patients (95% CI, 62-143 patients). For every 10000 patients genotyped, 164 would test positive for a *NUDT15* variant, and of these patients, 105 would have developed TIM if they had not received an alternative treatment (positive predictive value, 64% [95% CI, 43%-100%]; **eMethods in Supplement**). Genotyping 10000 patients for *NUDT15* would prevent 105 cases of TIM, which is 95 patients genotyped for every case pre- vented. The number needed

to genotype assumed a cumulative incidence of TIM of 7% (95% CI 6% to 8%) taken from a meta-analysis of 8302 patients[346], drug avoidance strategy in *NUDT15* variant carriers, a population carriage frequency of 1.6% (95% CI 1.5% to 1.8%) and odds ratios derived from bootstrapping our affected and unaffected population (sampling with replacement to estimate the variability of the odds ratio). If a dose reduction strategy were used in *NUDT15* variant carriers instead, thus reducing risk of TIM to that of patients with the reference genotype (absolute risk 6%, 95% CI 5% to 7%), the number needed to genotype was 105 (95% CI 65 to 168).

For *TPMT* the estimated number needed to genotype was 123 (95% CI 75 to 235). For every 10000 patients genotyped, 996 would test positive for a *TPMT* variant and need to receive an alternative therapy to prevent TIM in 81 patients (95% CI, 43-133 patients). Genotyping 10000 patients for *TPMT* would prevent 81 cases of TIM, which is 123 genotyped for every case prevented. This assumed the following for patients carrying two *TPMT* variant haplotypes: drug avoidance, a population carrier frequency of 0.26%[432] (95% CI 0.19% to 0.34%) and an odds ratio of 53.4 (95% CI 10.4 to 980.1); and for patients carrying one *TPMT* haplotype: dose reduction, a population carrier frequency of 9.7% (95% CI 8.4% to 11.0%) and an odds ratio of 2.2 (95% CI 1.4 to 3.3)

In the wider cohort of 398 adjudicated affected patients, including patients of non-European ancestry excluded from GWAS and EWAS analyses, carriage of *NUDT15* variants was more frequent than in patients of non-Finnish European ancestry (South Asian 100% [4/4] versus non-Finnish European 9% [31/328], $P = 1.1\times10^{-4}$; East Asian

56% [23/41] versus non-Finnish European 9% [31/328], $P = 2.0\times10^{-11}$) (**eTable 4.3-13 in the Supplement**).

Estimates of the rate of carrying one or more *NUDT15* risk alleles in general population using gnomAD reference database ranged from 0.7% in people of African ancestry to 29.2% in people of East Asian ancestry (**eTable 4.3-14 in the Supplement**).

4.3.7 Discussion

An association between an *NUDT15* variant (p.Gly17_Val18del) and TIM has been identified and replicated in independent non-Finnish European ancestry cohorts. In total, three *NUDT15* coding variants, including p.Gly17_Val18del, were identified and collectively associated with TIM independent of *TPMT* genotype and thiopurine dose. Patients with *NUDT15* and/or *TPMT* variants had a faster onset, more severe myelosuppression, and had a greater need for GCSF rescue therapy.

This is the first study, to our knowledge, to describe association of an *NUDT15* variant with TIM in patients of European ancestry at genome-wide significance. This extends previous work by Moriyama *et al* who first described this p.Gly17_Val18del variant in two paediatric patients with acute lymphoblastic leukaemia and thiopurine-induced myelosuppression; one of whom was of European, and the second, of African ancestry.[416]

The p.Arg139Cys variant has previously been associated with TIM in an admixed North American IBD cohort study where the minor allele frequency reported was 2.7% in affected and 0.3% in unaffected patients (OR 9.50; $P = 4.6\times10^{-4}$).[362] In contrast,

prior to this study the p.Gly17_Val18dup variant had only been reported in cohorts of East Asian ancestry, to our knowledge.[415]

NUDT15 is hypothesised to hydrolyse nucleoside triphosphate active metabolites (6-thio-dGTP, 6-thio-GTP, and dGTP) thus preventing their incorporation into DNA where they would otherwise lead to futile mismatch repair and apoptosis.[362,415,433] Functional experiments confirm that *NUDT15* variants result in lower enzymatic activity leading to higher levels of thiopurine active metabolites and a greater risk of myelosuppression.[362,415,416,433] The p.Gly17_Val18dup variant reduces *NUDT15* activity to approximately 15% of normal activity whilst p.Gly17_Val18del and p.Arg139Cys are nearly void of enzyme activity, suggesting that patients with these variants may be particularly sensitive to thiopurines.[415,416]

Given the widespread use of the thiopurines, these findings may have ramifications beyond the management of IBD in patients of European ancestry. Indeed, while *NUDT15* variants were first associated with TIM in East Asian patients with IBD[362] this phenomenon has now been demonstrated in oncology and other immune mediated diseases[363,434] as well as other populations.[362,416–420] For population stratification reasons, patients of non-European ancestry were excluded from the genetic analyses of this study. However, it is interesting to note the high frequencies of *NUDT15* variants and absence of *TPMT* variants in these other ethnic groups: in populations of East Asian ancestry the frequency of variant *NUDT15* haplotypes is 29.2% in comparison to Latin American, South Asian and non-Finnish European populations where the frequency of variant carriers is 20.7%, 13.4% and 1.6%, respectively.[427]

As expected, in the wider cohort of adjudicated affected patients, patients of non-European descent demonstrated a higher carriage frequency of *NUDT15* variants and a lower carriage frequency of *TPMT* variants. If replicated in additional studies, these findings suggest that *NUDT15* testing may be considered prior to thiopurine therapy irrespective of the ethnic background of the patient.

The positive predictive value of *NUDT15* genotyping estimated in this study together with the recent development of alternative, but more expensive, therapies, suggests potential clinical utility of pre-treatment testing and drug avoidance in genetically at risk patients. Recommendations regarding pre-treatment *NUDT15* genotyping are under review by the Clinical Pharmacogenetics Implementation Consortium (CPIC) based on data from East Asians.[429] Our data suggest that pre-treatment sequencing of the *NUDT15* gene, including the p.Gly17_Val18del deletion, may also be considered in patients of European ancestry. However, this will not obviate the requirement for regular blood test monitoring for the duration of treatment in patients deemed at low risk of TIM.

The estimated number needed to genotype for *NUDT15* is 95, similar to the number needed to genotype reported here and by others[435] for *TPMT* (123 and 100, respectively). However, further validation studies including a cost effectiveness analysis should be conducted prior to implementation of pre-treatment *NUDT15* genotyping.

4.3.7.1 Limitations

This study has several limitations. First, inclusion was restricted to patients with IBD of non-Finnish European ancestry. Further research is required to evaluate the association of these variants with TIM in other ancestries and disease groups.

Second, the replication cohort was not exclusively recruited from independent sites, as the central site recruited affected and unaffected patients to the discovery cohort and then additional patients to the replication cohorts.

Third, in keeping with all case-control studies, the data are likely to be susceptible to recall bias, with greater recruitment of more severe affected patients. We estimate that our affected patients represent 5% of the total eligible IBD patients with an episode of TIM. This is based on a UK IBD prevalence of 388/100,000[436], a thiopurine exposure rate of 31%[437], and a 7% rate of TIM.[346] This recall bias might explain the IBD phenotype differences observed between cases and controls and over-estimate the risk associated with *NUDT15* variants and TIM.

Fourth, 4.9% (16/328) of affected and 0.2% (1/633) of unaffected patients had two of the known TIM-associated *TPMT* variant haplotypes, despite the recommended practice of pre-treatment measurement of TPMT activity and thiopurine avoidance in TPMT-deficient patients. These patients arguably should not have received treatment with a thiopurine, regardless of the presence of *NUDT15* variants.

Fifth, the proposed mitigation strategy of drug avoidance rather than dose reduction in patients with *NUDT15* coding variants may be over-cautious. Previous studies in

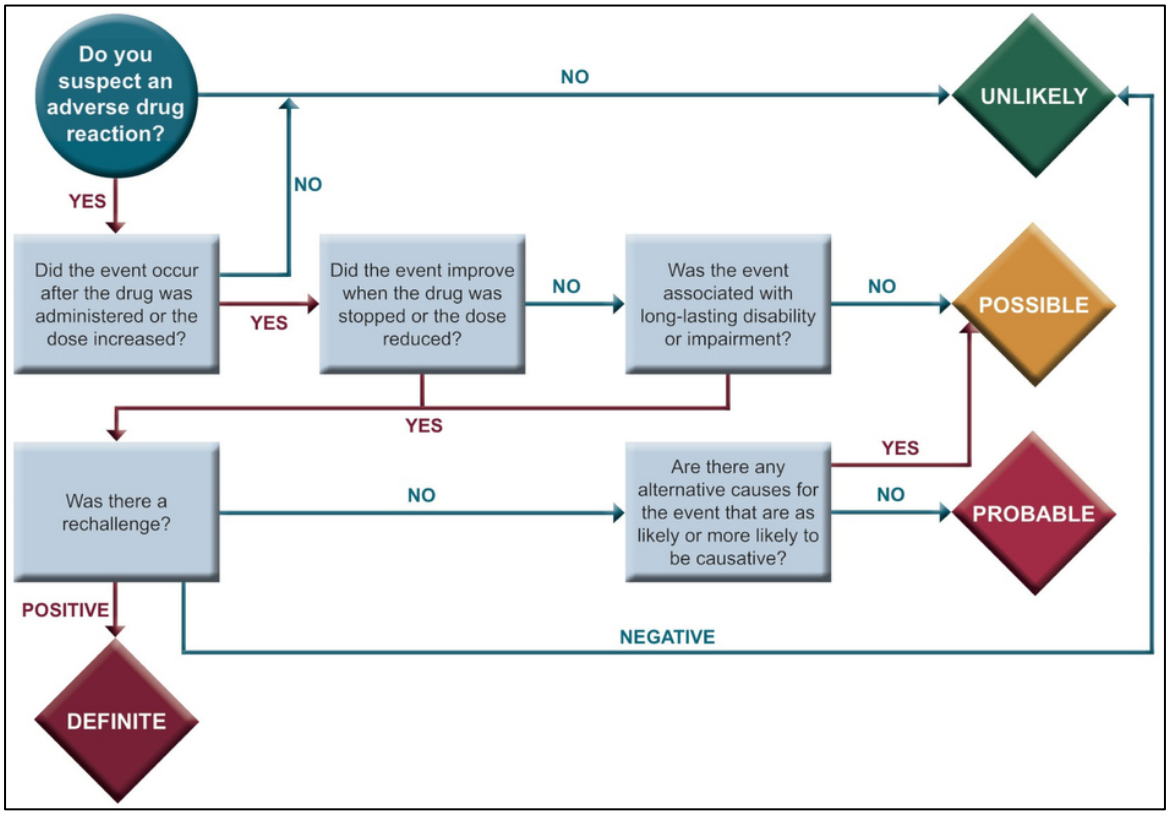
patients of East Asian ancestry have shown that even patients with two low function *NUDT15* alleles may successfully tolerate a 90% thiopurine dose reduction.[415,433,434] Likewise, in *NUDT15* knockout mice models, accumulation of thiopurine metabolites was noted to be in an MP-dose related fashion, suggesting that dose reduction might be an effective strategy.[433] However, as discussed above, not all variants affect *NUDT15* enzymatic function to the same extent and the magnitude of the deleterious effect of individual variants may differ across ethnic groups.[43] Furthermore, it is unknown whether such a marked dose reduction would compromise the therapeutic effect of thiopurines in IBD. In our study of patients of non-Finnish European ancestry, almost 50% of patients with a single variant did not tolerate a thiopurine re-challenge at a lower dose. These arguments may justify the use of alternative, more expensive therapies in this small group of patients at high risk of TIM. However, further data are needed to explore whether dose reduction with enhanced monitoring or drug avoidance is the safer, cheaper and more clinically effective strategy.

4.3.7.2 Conclusions

Among patients of European ancestry with inflammatory bowel disease, variants in *NUDT15* were associated with increased risk of thiopurine-induced myelosuppression. These findings suggest that *NUDT15* genotyping may be considered prior to initiation of thiopurine therapy; however, further study including additional validation in independent cohorts is required.

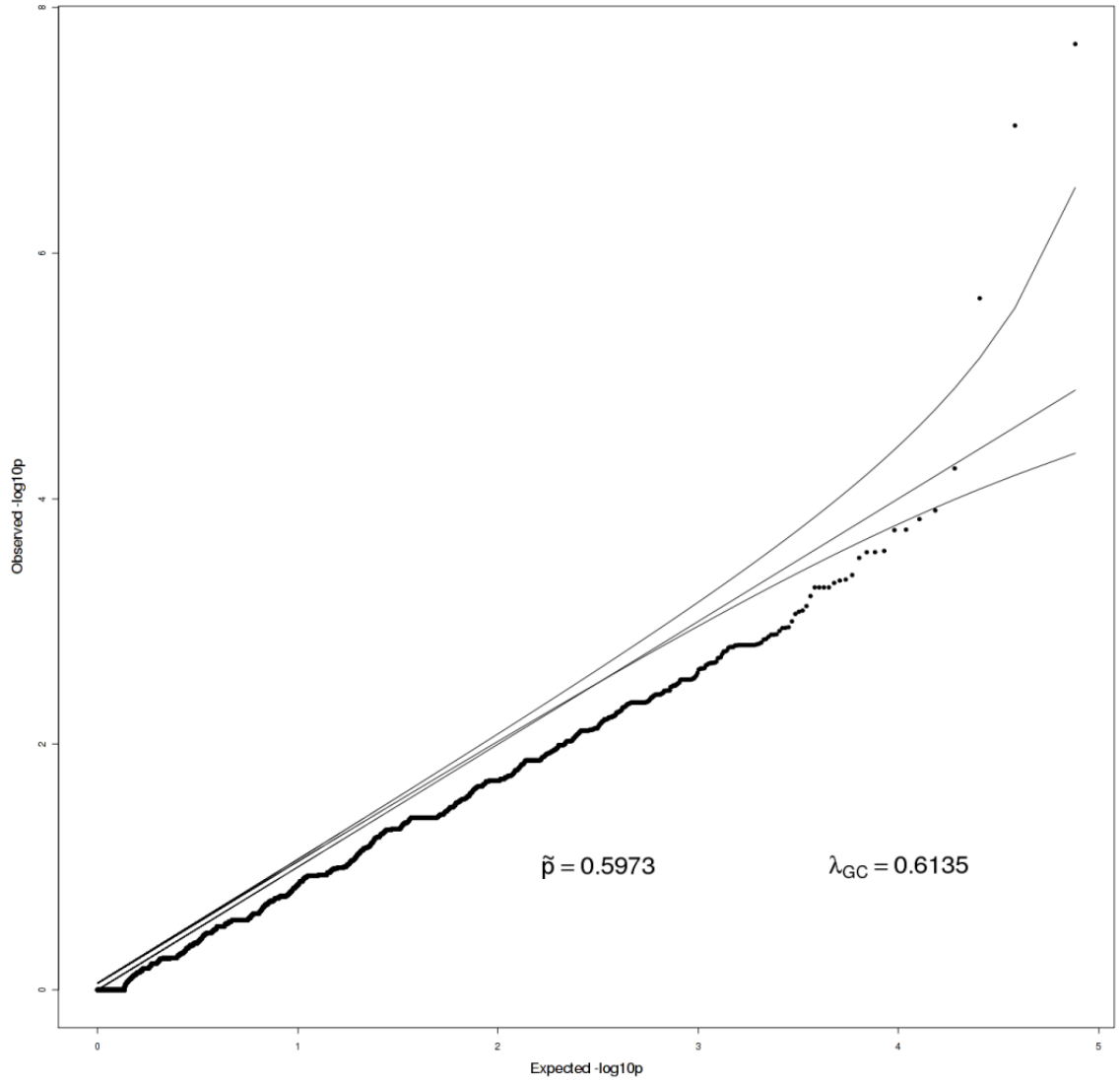
RESEARCH PAPER IV SUPPLEMENT

eFigure 4.3-1. Adjudication assessment tool



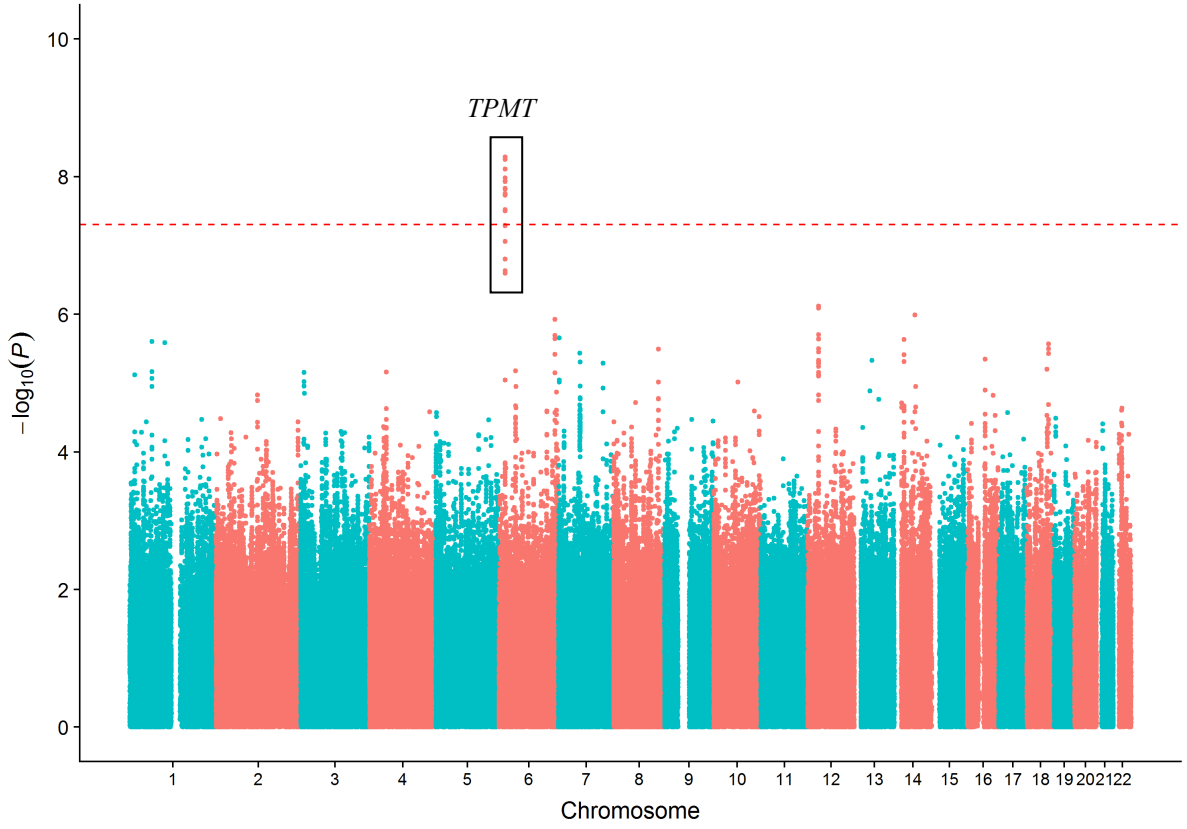
Adapted version of the Liverpool Adverse Drug Reaction Causality Assessment Tool used in the adjudication process. Adapted from Gallagher *et al*. (Gallagher, R.M. *et al*. Development and inter-rater reliability of the Liverpool adverse drug reaction causality assessment tool. *PLoS One*, e28096, 2011).[338]

eFigure 4.3-2. Quantile-Quantile plot demonstrating genomic inflation factor in 328 affected and 633 unaffected individuals used in the primary ExWAS discovery cohort



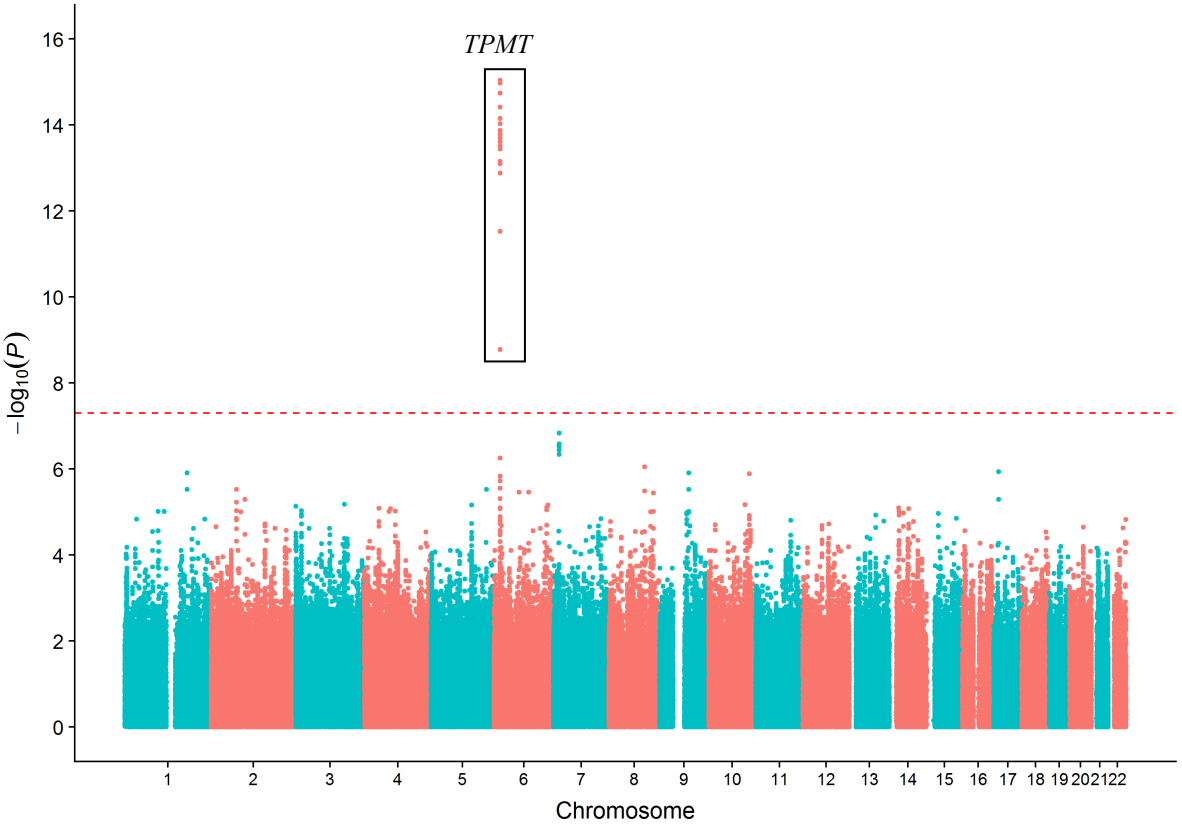
Genomic inflation factor (λ_{GC}) and quantile–quantile (Q–Q) plots are used to compare the genome-wide distribution of the test statistic with the expected null distribution. The Q–Q plot is a useful visual tool to mark deviations of the observed distribution from the expected null distribution. Inflated (λ_{GC} >1.0) values or residual deviations in the Q–Q plot may point to undetected sample duplications, unknown familial relationships, a poorly calibrated test statistic, systematic technical bias or gross population stratification.[438]

eFigure 4.3-3. A Manhattan plot showing genome wide associations (GWA) between single-nucleotide polymorphisms (SNPs) and thiopurine-induced myelosuppression after 1000 Genomes imputation using 311 affected and 608 unaffected individuals



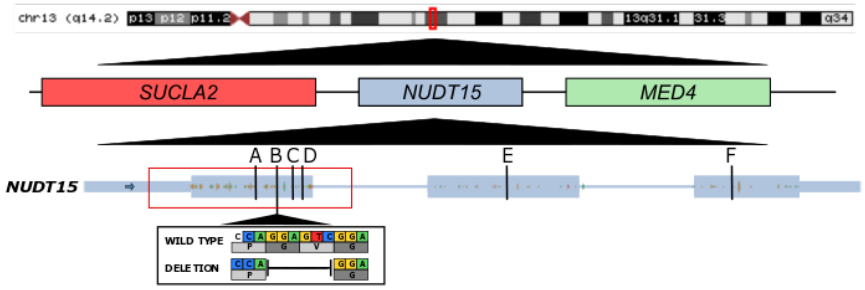
Each dot represents a $-\log_{10} P$ value calculated by Fisher's exact test for the allele frequency in 311 thiopurine-induced myelosuppression affected individuals with inflammatory bowel disease and 608 thiopurine-exposed unaffected individuals with inflammatory bowel disease. The red dotted horizontal line represents at $P = 5.0\times10^{-8}$ represents the genome wide significance level. The gene name corresponds to the gene in closest proximity to the variant with the lowest *P* value at each locus.

eFigure 4.3-4. A Manhattan plot showing genome wide associations (GWA) between single-nucleotide polymorphisms (SNPs) and early (≤ 8 weeks of starting maximum thiopurine dose) thiopurine-induced myelosuppression after 1000 Genomes imputation using 107 affected and 608 unaffected individuals



Each dot represents a $-\log_{10} P$ value calculated by Fisher exact test for the allele frequency in 107 early thiopurine-induced myelosuppression affected individuals with inflammatory bowel disease and 608 thiopurine-exposed unaffected individuals with inflammatory bowel disease. The red dotted horizontal line represents at $P = 5.0\times10^{-8}$ represents the genome wide significance level. Gene name corresponds to the gene in closest proximity to the variant with the lowest *P* value at each locus.

eFigure 4.3-5. Gene map illustrating location (labelled A to F) of 7 deleterious coding NUDT15 variants on chromosome 13 found among 964 exome sequenced thiopurine-exposed patients: 328 affected and 633 unaffected individuals



***NUDT15* identifiers**						***NUDT15* risk allele frequencies reported in this study [b]**		**Estimates of *NUDT15* risk allele frequencies in general population using gnomAD reference database**			
rsID	**Location**	**Reference allele/Variant allele**	**Annotation**	**Position on Gene Map**	**Protein Sequence**	**Risk Allele Frequency Affected Individuals**	**Risk Allele Frequency Unaffected Individuals**	**Carriage Rate of Risk Allele in Non-Finnish Europeans % (95% CI)**	**Risk Allele Count in Non-Finnish Europeans (95% CI)**	**Total Allele Number in Non-Finnish Europeans**	**Homozygote Risk Allele Count in Non-Finnish Europeans**
rs746071566	48611918	AGGAGTC /A	in-frame deletion	B[a]	p.Gly17_Val18 del	0.029	7.9×10^{-4}	0.43 (0.37 - 0.49)	216 (188-246)	101458	0
rs116855232	48619855	C/T	missense	F	p.Arg139Cys	0.012	0	0.71 (0.64 - 0.77)	448 (405-488)	126510	2
rs554405994	48611918	AGGAGTC / AGGAGTC GGAGTC	in-frame insertion	B[a]	p.Gly17_Val18 dup	0.008	0.002	0.52 (0.46 - 0.59)	264 (233-297)	101458	0
rs768057637	48611979	A/G	missense	D	p.Lys33Glu	0.002	0	0.010 (0.002 - 0.020)	5 (1-10)	101230	0
13:48615121	48615121	T/G	missense	E	p.Val75Gly	0.002	0	0.005 (0.000 - 0.012)	3 (0-7)	112164	0
rs777311140	48611961	C/CGCGG	frame-shift	C	p.Cys28GlyfsTer28	0	7.9×10^{-4}	0.041 (0.025 - 0.059)	23 (14-33)	111744	0
13:48611883	48611883	A/C	start lost	A	p.Met1?	0	7.9×10^{-4}	0.003 (0.000 - 0.009)	1 (0-3)	70518	0

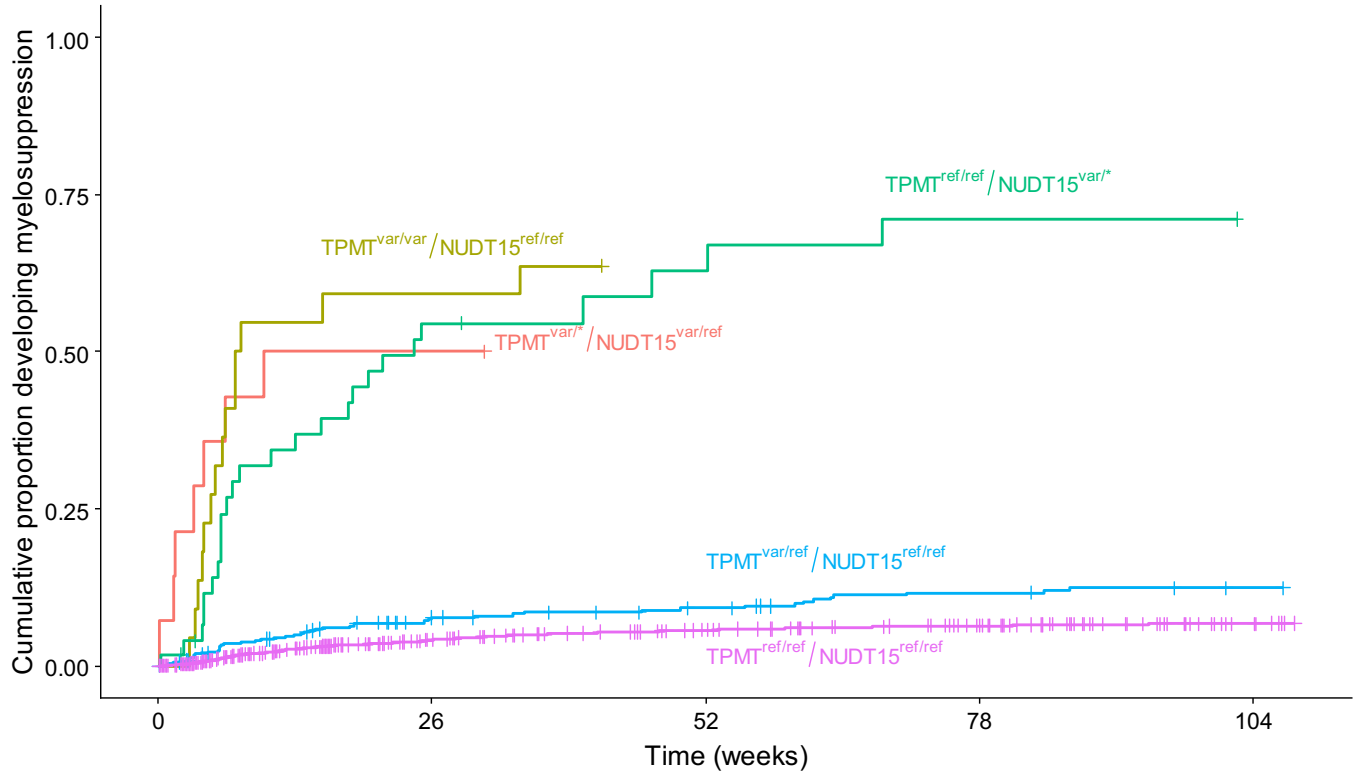
rsID; Single Nucleotide Polymorphism database (dbSNP) ID; Location, chromosome 13 position; gnomAD, Genome Aggregation Database (http://gnomad.broadinstitute.org/); Position, chromosome 13 position; rsID, single nucleotide polymorphism identification number (dbSNP), or chromosome and position if no rsID available

[a] This site is multi-allelic and both of these variants occur at the same chromosome position (48611918): 19 affected individuals were heterozygous for rs746071566, 5 affected individuals were heterozygous for rs554405994 and 304 affected individuals were homozygous reference = 328 individuals in total
[b] Risk Allele Frequency (RAF) = [number of variant alleles in population/(2*number of participants)] noting that no affected or unaffected individuals were homozygote for *NUDT15* variant alleles

eFigure 4.3-6. Relationship between TPMT diplotype and TPMT phenotype-enzyme activity among 328 thiopurine-induced myelosuppression affected individuals and 633 thiopurine-exposed unaffected individuals

Patients with missing TPMT phenotype data excluded. Numbers of patients with respective diplotype reported to the right of bars. Ref, reference genotype; var, variant genotype.

eFigure 4.3-7. Estimated cumulative incidence of thiopurine-induced myelosuppression among patients with NUDT15 and/or TPMT variants



Time = number of weeks after starting maximum dose of thiopurine. Case control exome sequenced dataset of variant frequencies adjusted by duplicating control cohort to match the non-thiopurine-induced myelosuppression frequency (93%) that would be expected in the general inflammatory bowel disease population (7% cumulative incidence of thiopurine-induced myelosuppression reported by Gisbert JP, Gomollón F. Thiopurine-Induced Myelotoxicity in patients with Inflammatory Bowel Disease: A Review. Am J Gastroenterol. 2008;103(7):1783-1800).[346]

eTable 4.3-1. Number of thiopurine-induced myelosuppression affected individuals and thiopurine-exposed unaffected individuals among all patients used in the final analyses subdivided by early (≤ 8 weeks) and late onset myelosuppression

Experiment	Early thiopurine induced myelosuppression affected individuals [a] (≤ 8 weeks) n (%)	Late thiopurine induced myelosuppression affected individuals [b] (> 8 weeks) n (%)	Thiopurine-exposed unaffected individuals N
Adjudicated patients [c]	143 (36%)	255 (65%)	679
Imputed GWAS	107 (34%)	204 (66%)	608
ExWAS	111 (34%)	217 (66%)	633

GWAS, Genome Wide Association Study; ExWAS, Exome Wide Association Study
[a] Early thiopurine induced myelosuppression defined as ≤ 8 weeks from maximum dose of thiopurine to thiopurine-induced myelosuppression
[b] Late thiopurine induced myelosuppression defined as > 8 weeks from maximum dose of thiopurine to thiopurine-induced myelosuppression
[c] Includes patients of all ethnicities prior to selection of Non-Finnish Europeans (NFE) for GWAS and ExWAS analyses

eTable 4.3-2. IBD and drug exposure phenotype in adjudication affected and unaffected individuals in the replication cohort (after quality control)

Characteristic	Thiopurine-induced myelosuppression affected individuals n = 73 [a]	Thiopurine-exposed unaffected individuals n = 840 [a]	*P* value [b]
Centre			
Cedars Sinai	1 (1.4%)	302 (36.0%)	<.001
Additional UK cases	32 (43.8%)	0 (0.0%)	
Massachusetts General Hospital	4 (5.5%)	68 (8.1%)	
Paris	16 (21.9%)	282 (33.6%)	
University Medical Centre Groningen	20 (27.4%)	188 (22.4%)	
Sex			
Female	49 (67.1%)	449 (53.5%)	.03
Male	24 (32.9%)	391 (46.5%)	
Diagnosis			
Crohn's disease	43 (58.9%)	541 (64.4%)	.05
IBD-unclassified (IBD-U)	6 (8.2%)	23 (2.7%)	
ulcerative colitis (UC)	24 (32.9%)	276 (32.9%)	
Age at IBD diagnosis (years)	26.0 [18.9 to 43.0]	23.0 [17.0 to 31.0]	.004
Montreal Crohn's age at diagnosis [c, d]			
A1: <17 yrs	5 (11.4%)	104 (26.1%)	<.001
A2: 17 to 40 yrs	19 (43.2%)	248 (62.2%)	
A3: >40 yrs	20 (45.5%)	47 (11.8%)	
Montreal Crohn's location [c, e]			
L1: ileal	10 (21.7%)	116 (21.6%)	>.99
L2: colonic	11 (23.9%)	126 (23.5%)	
L3: ileocolonic	25 (54.3%)	294 (54.9%)	
Montreal Crohn's behaviour [c, f]			
B1: non-stricturing and non-penetrating	14/44 (31.8%)	186/525 (35.4%)	.49
B2: stricturing	15/44 (34.1%)	134/525 (25.5%)	
B3: penetrating	15/44 (34.1%)	205/525 (39.0%)	
Montreal ulcerative colitis extent [c, g]			
E1: limited to the rectum	1 (3.4%)	12 (4.0%)	.63
E2: distal to the splenic flexure	11 (37.9%)	89 (29.9%)	
E3: proximal to the splenic flexure	17 (58.6%)	197 (66.1%)	

[a] values represent: n (%) or median [interquartile range]
[b] *P* value represents Fisher's exact or Mann-Whitney U test as appropriate with *P* < .05 deemed statistically significant
[c] Montreal Classification System from Silverberg MS, Satsangi J, Ahmad T, *et al*. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Canadian Journal of Gastroenterology 2005;19 Suppl A(5):5A-36A.
[d] Denominator for affected individuals = 44 and for unaffected individuals = 399
[e] Denominator for affected individuals = 46 and for unaffected individuals = 536
[f] Denominator for affected individuals = 44 and for unaffected individuals = 525
[g] Denominator for affected individuals = 29 and for unaffected individuals = 298

eTable 4.3-3. Inflammatory bowel disease and drug exposure phenotype in affected and unaffected individuals used in Genome Wide Association Study (GWAS)

Characteristic	Thiopurine-induced myelosuppression affected individuals n = 311 [a]	Thiopurine-exposed unaffected individuals n = 608 [a]	*P* value [b]
Sex			
Female	174 (55.9%)	296 (48.7%)	.04
Male	137 (44.1%)	312 (51.3%)	
Diagnosis			
Crohn's disease (CD)	182 (58.5%)	333 (54.8%)	.009
IBD-unclassified (IBD-U)	8 (2.6%)	3 (0.5%)	
Ulcerative colitis (UC)	121 (38.9%)	272 (44.7%)	
Age at IBD diagnosis (yrs)	31.3 (21.3 to 46.1)	31.5 (22.2 to 44.7)	.59
Estimate of difference [95% Confidence Interval]	0.6 [-1.5 to 2.7]		
Montreal Crohn's disease classification [c]			
Age at diagnosis [d]			
A1: <17 yrs	36 (19.9%)	17 (6.4%)	<.001
A2: 17-40 yrs	97 (53.6%)	210 (78.7%)	
A3: >40 yrs	48 (26.5%)	40 (15.0%)	
Location [e]			
L1: ileal	44 (24.2%)	116 (43.4%)	<.001
L2: colonic	63 (34.6%)	74 (27.7%)	
L3: ileocolonic	75 (41.2%)	77 (28.8%)	
Behaviour [f]			
B1: non-stricturing and non-penetrating	99 (58.9%)	157 (59.2%)	.69
B2: stricturing	51 (30.4%)	73 (27.5%)	
B3: penetrating	18 (10.7%)	35 (13.2%)	
Montreal ulcerative colitis /inflammatory bowel disease-unclassified extent [c]			
E1: limited to the rectum	13 (10.7%)	14 (6.7%)	.38
E2: distal to the splenic flexure	53 (43.8%)	101 (48.6%)	
E3: proximal to the splenic flexure	55 (45.5%)	93 (44.7%)	
Weight-adjusted thiopurine dose (mg/kg) [h]	2.07 (1.69 to 2.44)	1.84 (1.48 to 2.20)	<.001
Estimate of difference [95% Confidence Interval]	-0.23 (-0.32 to -0.15)		

[a] values represent: n (%) or median [interquartile range]
[b] *P* value represents Fisher's exact or Mann-Whitney U test as appropriate with *P* < .05 deemed statistically significant
[c] Montreal Classification System from Silverberg MS, Satsangi J, Ahmad T, *et al*. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Canadian Journal of Gastroenterology 2005;19 Suppl A(5):5A-36A.
[d] Denominator for affected individuals = 181 and for unaffected individuals = 267
[e] Denominator for affected individuals = 182 and for unaffected individuals = 267
[f] Denominator for affected individuals = 168 and for unaffected individuals = 265
[g] Denominator for affected individuals = 121 and for unaffected individuals = 208

[h] weight adjusted dose represents the maximum azathioprine equivalent dose prior to thiopurine-induced myelosuppression and adjusted for weight (mg/kg): [(mercaptopurine dose (mg) x 2.08) / weight (kg)] or [azathioprine dose (mg) / weight (kg)]

eTable 4.3-4. Inflammatory bowel disease and drug exposure phenotype in affected and unaffected individuals used in Exome Wide Association Study (ExWAS)

Characteristic	Thiopurine-induced myelosuppression affected individuals n = 328 [a]	Thiopurine-exposed unaffected individuals n = 633 [a]	*P* value [b]
Sex			
Female	181 (55.2%)	310 (49.0%)	.08
Male	147 (44.8%)	323 (51.0%)	
Diagnosis			
Crohn's disease (CD)	193 (58.8%)	350 (55.3%)	.01
IBD-unclassified (IBD-U)	8 (2.4%)	3 (0.5%)	
Ulcerative colitis (UC)	127 (38.7%)	280 (44.2%)	
Age at IBD diagnosis (yrs)	31.4 [21.0 to 45.7]	32.0 [22.3 to 45.4]	0.36
Estimate of difference [95% Confidence Interval]	1.0 [-1.1 to 3.1]		
Montreal Crohn's disease classification [c]			
Age at diagnosis [d]			
A1: <17 yrs	40/ (20.8%)	18 (6.4%)	<.001
A2: 17-40 yrs	101 (52.6%)	223 (79.1%)	
A3: >40 yrs	51 (26.6%)	41 (14.5%)	
Location [e]			
L1: ileal	51 (26.4%)	125 (44.3%)	<.001
L2: colonic	64 (33.2%)	76 (27.0%)	
L3: ileocolonic	78 (40.4%)	81 (28.7%)	
Behaviour [f]			
B1: non-stricturing and non-penetrating	104 (58.4%)	164 (58.6%)	.48
B2: stricturing	56 (31.5%)	78 (27.9%)	
B3: penetrating	18 (10.1%)	38 (13.6%)	
Montreal ulcerative colitis /inflammatory bowel disease-unclassified extent [c]			
E1: limited to the rectum	14 (11.0%)	14 (6.5%)	.32
E2: distal to the splenic flexure	57 (44.9%)	105 (49.1%)	
E3: proximal to the splenic flexure	56 (44.1%)	9 (44.4%)	
Weight-adjusted thiopurine dose (mg/kg) [h]	2.07 (1.69 to 2.44)	1.84 (1.47 to 2.20)	<.001
Estimate of difference [95% Confidence Interval]	-0.23 [-0.31 to -0.15]		

[a] values represent: n (%) or median [interquartile range]
[b] *P* value represents Fisher's exact or Mann-Whitney U test as appropriate with *P* < .05 deemed statistically significant
[c] Montreal Classification System from Silverberg MS, Satsangi J, Ahmad T, *et al*. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Canadian Journal of Gastroenterology 2005;19 Suppl A(5):5A-36A.
[d] Denominator for affected individuals = 192 and for unaffected individuals = 282
[e] Denominator for affected individuals = 193 and for unaffected individuals = 282
[f] Denominator for affected individuals = 178 and for unaffected individuals = 280
[g] Denominator for affected individuals = 127 and for unaffected individuals = 214
[h] weight adjusted dose represents the maximum azathioprine equivalent dose prior to TIM and adjusted for weight (mg/kg): [(mercaptopurine dose (mg) x 2.08) / weight (kg)] or [azathioprine dose (mg) / weight (kg)

eTable 4.3-5. Association of genetic variants in NUDT15 with thiopurine-induced myelosuppression in patients with inflammatory bowel disease using data from the Exome Wide Association Study (ExWAS) stratified by time to myelosuppression

***NUDT15* identifiers**				**Early (≤ 8 weeks) thiopurine-induced myelosuppression affected individuals [b] (n = 111)**					**Late (> 8 weeks) thiopurine-induced myelosuppression affected individuals [b] (n = 217)**					**Thiopurine-tolerant unaffected individuals (n = 633)**		
Position	**Reference (Ref) allele**	**Variant (Var) allele**	**Amino acid change**	**Var hom (n)**	**Var het (n)**	**Ref hom (n)**	**Odds Ratio (95% CI)**	***P* value[a]**	**Var hom (n)**	**Var het (n)**	**Ref hom (n)**	**Odds Ratio (95% CI)**	***P* value[a]**	**Var hom (n)**	**Var het (n)**	**Ref hom (n)**
48611918[c]	AGGAGTC	A	p.Gly17_Val18del	0	12	96	74.2 (9.6 to 573.5)	8.2×10^{-10}	0	7	208	20.9 (2.6 to170.1)	4.2×10^{-4}	0	1	630
48619855	C	T	p.Arg139Cys	0	4	107	NA	4.8×10^{-4}	0	4	213	NA	0.004	0	0	633
48611918[c]	AGGAGTC	AGGAGTCGGAGTC	p.Gly17_Val18dup[d]	0	3	96	9.7 (1.6 to 58.5)	0.02	0	2	208	5.1(1.0 to 26.6)	0.26	0	2	630

Position, chromosome position; het, heterozygote; hom, homozygote; var, variant; ref, reference; NA, not applicable; CI, Confidence Interval.
a P values calculated using Fisher's exact test between early (≤ 8 weeks from maximum thiopurine dose to thiopurine-induced myelosuppression) TIM affected vs. thiopurine-exposed unaffected individuals and late (> 8 weeks from maximum dose thiopurine to thiopurine-induced myelosuppression) TIM affected vs. thiopurine-exposed unaffected individuals. P < 5.0×10-8 deemed statistically significant
b Time to myelosuppression (weeks) = [date of maximum thiopurine dose] – [date of meeting inclusion criteria]
c This site is multi-allelic and both of these variants occur at the same chromosome position (48611918): 19 individuals were heterozygous for rs746071566 (p.Gly17_Val18del), 5 TIM affected individuals were heterozygous for rs554405994 (p.Gly17_Val18dup) and 304 TIM affected individuals were homozygous reference (328 individuals in total)
d p.Gly17_Val18dup previously also annotated as p.Val18_Val19insGlyVal

eTable 4.3-6. Genes associated with thiopurine-induced myelosuppression on gene burden testing in discovery and replication cohorts

Gene	NCBI RefSeq identifier	Chromosome number	Number of variants identified	Discovery cohort *P* value [a]	Replication cohort *P* value [a]
TPMT	NM_018283	6	19 variants	2.1×10^{-7}	1.6×10^{-5}
NUDT15	NM_000367	13	64 variants	3.0×10^{-10}	5.1×10^{-5}
CDC37	NM_007065	19	14 variants	5.5×10^{-8}	.51

NCBI, National Center for Biotechnology Information
[a] SKAT *P* value provided with $P < 5.0\times10^{-8}$ deemed statistically significant

eTable 4.3-7. TPMT haplotypes among entire dataset of 328 thiopurine-induced myelosuppression affected individuals and 633 thiopurine-exposed unaffected individuals

Haplotypes	TPMT enzyme activity assigned in this study	TPMT enzyme activity status assigned by CPIC[a] and/or LOVD[b]	dbSNP rsID and chromosome position	Nucleotide change in *TPMT*[c]	Amino acid change
***1**	Normal	Functional/normal activity/wild-type	rs2842934 allele A[d]	Wild type 474T[d]	Not applicable
***2**	Non-functional	CPIC: Non-functional, variant, or mutant/no activity LOVD: Variant affects function	rs1800462	238G>C	Ala80Pro
***3A**	Non-functional	CPIC: Non-functional, variant, or mutant/no activity LOVD: Effect not classified	rs1800460 rs1142345	460G>A 719A>G	Ala154Thr Tyr240Cys
***3C**	Non-functional	CPIC: Non-functional, variant, or mutant/no activity LOVD: Variant affects function	rs1142345	719A>G	Tyr240Cys
***4**	Non-functional	CPIC: Non-functional, variant, or mutant/no activity LOVD: Effect not classified	rs1800584	626-1G>A	Unknown
***8**	Non-functional	CPIC: Probable reduced-function/decreased activity LOVD: Effect unknown	rs56161402	644G>A	Arg215His
***9**	Non-functional	CPIC: Uncertain Function LVID: Effect unknown	rs151149760	356A>C	Lys119Thr
***12**	Non-functional	CPIC: Uncertain Function LOVD: Effect not classified	rs200220210	374C>T	Ser125Leu
***21**	Non-functional	CPIC: Uncertain Function LOVD: Effect not classified	rs200591577	205C>G	Leu69Val
***37**	Non-functional	CPIC: Uncertain Function LOVD: Effect not classified	rs398122996	648T>A	Cys216Ter
***40**	Non-functional	CPIC: Uncertain Function LOVD: Not listed	rs139392616	677G>A	Arg226Gln

a CPIC, Clinical Pharmacogenetics Implementation Consortium (https://cpicpgx.org/). Function listed by curator.
b LOVD, Leiden Open (source) Variation Database (https://databases.lovd.nl/shared/transcripts/TPMT)
c Nucleotide changes in the TPMT gene (given on the negative chromosomal strand, NCBI reference sequence NM_000367.2) are numbered such that the A in the ATG is + 1.
d dbSNP reports G>A at this position: however, the TPMT nomenclature committee has defined wildtype as having allele A at this position (positive chromosomal strand) and the *1S allele as having allele G at this position (positive chromosomal strand)

eTable 4.3-8. TPMT phenotype-enzyme activity among 328 thiopurine-induced myelosuppression affected individuals and 633 thiopurine-exposed unaffected individuals

TPMT phenotype / enzyme activity level [a]	Affected individuals (n)%	Unaffected individuals (n)%	*P* value [b]
absent	10 (3%)	0 (0%)	<.001
low	39 (12%)	70 (11%)	
normal	171 (52%)	427 (68%)	
high	7 (2%)	3 (1%)	
not tested	101 (31%)	133 (21%)	
Grand Total	**328**	**633**	

[a] manufacturer of assay as well as processing laboratory for quantitative TPMT enzyme activity levels differ among participants, therefore, only ordinal data reported.
[b] *P* < .05 deemed statistically significant

eTable 4.3-9. TPMT diplotype and TPMT phenotype-enzyme activity among 328 thiopurine-induced myelosuppression affected individuals and 633 thiopurine-exposed unaffected individuals

TPMT diplotype	Frequency of TPMT phenotype / enzyme activity level [a]					Grand Total	*TPMT* genotype
	'absent'	'low'	'normal'	'high'	test not done		
*2/*3A	2	1	0	0	0	3	Double variant (*TPMT* var/var)
*3A/*21	1	0	0	0	0	1	
*3A/*3A	3	2	0	0	5	10	
*3A/*3C	1	0	0	0	1	2	
*3A/*9	1	0	0	0	0	1	
*1/*12	0	0	1	0	0	1	Single variant (*TPMT* ref/var)
*1/*2	0	5	0	0	6	11	
*1/*21	0	0	1	0	0	1	
*1/*37	0	0	0	0	1	1	
*1/*3A	2	59	6	0	25	92	
*1/*3C	0	12	0	0	3	15	
*1/*4	0	1	0	0	0	1	
*1/*40	0	0	1	0	0	1	
*1/*8	0	0	2	0	0	2	
*1/*1	0	29	587	10	193	819	Reference genotype (*TPMT* ref/ref)
Grand Total	10	109	598	10	234	961	

Ref, reference genotype; var, variant genotype.
a manufacturer of assay as well as processing laboratory for quantitative TPMT enzyme activity levels differ among participants, therefore, only ordinal rather than quantitative data reported.

eTable 4.3-10. Clinical phenotype among 328 thiopurine-induced myelosuppression affected individuals according to NUDT15 and TPMT genotype

Phenotype variable	Thiopurine induced myelosuppression (TIM) affected individuals with reference genotype for both *TPMT* and *NUDT15* (n = 231)	Thiopurine induced myelosuppression affected individuals with *TPMT* and/or *NUDT15* variants (n = 97)	*P* value
Thiopurine / n (%)			.29
Azathioprine (AZA)	157 (68.0%)	72 (74.2%)	
Mercaptopurine (MP)	74 (32.0%)	25 (25.8%)	
Weight adjusted thiopurine dose[a]			
Median [IQR] / (mg/kg)	2.1 [1.7 to 2.5]	1.9 [1.6 to 2.2]	.002
Time to TIM[c]			
Median [IQR] / (weeks)	20.0 [7.6 to 48.3]	7.9 [4.7 to 25.0]	<.001
≤ 8 weeks / n (%)	62 (26.8%)	49 (50.5%)	<.001
Lowest white cell count			
Median [IQR] / ($\times 10^9$/L)	2.2 [1.9 to 2.4]	1.9 [1.3 to 2.3]	<.001
Lowest neutrophil count			
Median [IQR] / ($\times 10^9$/L)	1.0 [0.7 to 1.2]	0.8 [0.4 to 1.1]	<.001
Infective complication of TIM			
Yes / n (%)	38 (16.5%)	21 (21.6%)	.27
Hospital admission required			
Yes / n (%)	38 (16.5%)	39 (40.2%)	<.001
Granulocyte simulating factor (GCSF) required			
Yes / n (%)	12 (5.2%)	19 (19.8%)	<.001
Successfully rechallenged at lower thiopurine dose			
Yes / n (%)	77 (61.1%)	18 (43.9%)	.07
Weight adjusted dose successful rechallenges[a,b]			
Median dose [IQR] / (mg/kg)	1.2 [0.9 to 1.6]	1.2 [0.9 to 1.3]	.43
Weight adjusted dose unsuccessful rechallenges[a,b]			
Median dose [IQR] / (mg/kg)	0.8 [0.6 to 1.5]	1.1 [0.9 to 1.2]	.86

Fisher's exact and Mann-Whitney *U* tests used as appropriate. *P* < .05 deemed statistically significant

[a] Weight adjusted dose represents the maximum azathioprine equivalent dose prior to TIM and adjusted for weight (mg/kg): [(mercaptopurine dose (mg) x 2.08) / weight (kg)] or [azathioprine dose (mg) / weight (kg)]

[b] An unsuccessful drug rechallenge was defined as a second exposure to a thiopurine at the same or lower dose which was then subsequently withdrawn due to any adverse event, or, a second fall in absolute white blood cell count to ≤ 3.0 x10^9/L

[c] Time to TIM (weeks) = [date of meeting entry criteria for TIM - start date of highest dose prior to TIM]

eTable 4.3-11. Clinical phenotype among 328 thiopurine-induced myelosuppression affected individuals according to NUDT15 and TPMT genotype

Phenotype variable	***TPMT*** **ref/ref** ***NUDT15*** **ref/ref** **(n = 231)**	***TPMT*** **var/ref** ***NUDT15*** **ref/ref** **(n = 51)**	***TPMT*** **ref/ref** ***NUDT15*** **var/*** **(n = 25)** [a]	***TPMT*** **var/var** ***NUDT15*** **ref/ref** **(n = 15)**	***TPMT*** **var/*** ***NUDT15*** **var/ref** **(n = 6)** [e]	***P*** **value** [d]
Thiopurine responsible for thiopurine-induced myelosuppression (TIM) / n (%)						
Azathioprine (AZA)	157 (68.0%)	37 (72.5%)	16 (64.0%)	14 (93.3%)	5 (83.3%)	.24
Mercaptopurine (MP)	74 (32.0%)	14 (27.5%)	9 (36.0%)	1 (6.7%)	1 (16.7%)	
Weight adjusted thiopurine dose [b]						
Median [IQR] / (mg/kg)	2.1 [1.7 to 2.5]	2.0 [1.7 to 2.3]	1.9 [1.6 to 2.3]	1.7 [1.4 to 2.0]	2.0 [1.4 to 2.1]	.004
Time to TIM [c]						
Median [IQR] / (weeks)	20.0 [7.6 to 48.3]	13.9 [5.9 to 40.4]	7.7 [5.7 to 20.0]	6.1 [4.2 to 7.6]	2.5 [1.5 to 4.1]	<.001
≤ 8 weeks / n (%)	62 (26.8%)	18 (35.3%)	13 (52.0%)	12 (80.0%)	6 (100.0%)	<.001
Lowest white cell count						
Median [IQR] / ($\times 10^9$/L)	2.2 [1.9 to 2.4]	2.1 [1.5 to 2.3]	2.1 [1.6 to 2.4]	1.1 [0.9 to 1.4]	2.1 [1.8 to 2.2]	<.001
Lowest neutrophil count						
Median [IQR] / ($\times 10^9$/L)	1.0 [0.7 to 1.2]	1.0 [0.6 to 1.2]	0.8 [0.5 to 1.0]	0.2 [0.1 to 0.4]	0.3 [0.2 to 0.8]	<.001

eTable 4.3-11 continued…

…eTable 4.3-11. continued

Phenotype variable	*TPMT* ref/ref *NUDT15* ref/ref (n = 231)	*TPMT* var/ref *NUDT15* ref/ref (n = 51)	*TPMT* ref/ref *NUDT15* var/* (n = 25) [a]	*TPMT* var/var *NUDT15* ref/ref (n = 15)	*TPMT* var/* *NUDT15* var/ref (n = 6) [e]	*P* value [d]
Infective complication of thiopurine induced myelosuppression (TIM)						
Yes / n (%)	38 (16.5%)	9 (17.6%)	5 (20.0%)	5 (33.3%)	2 (33.3%)	.34
Hospital admission required						
Yes / n (%)	38 (16.5%)	18 (35.3%)	6 (24.0%)	13 (86.7%)	2 (33.3%)	<.001
Granulocyte simulating factor (GCSF) required [f]						
Yes / n (%)	12 (5.2%)	7 (14.0%)	4 (16.0%)	7 (46.7%)	1 (16.7%)	<.001
Successfully rechallenged at lower thiopurine dose [g]						
Yes / n (%)	77 (61.1%)	12 (50.0%)	5 (38.5%)	0 (0.0%)	1 (50.0%)	.17
Weight adjusted dose successful rechallenges [b,c]						
Median dose [IQR] / (mg/kg)	1.2 [0.9 to 1.6]	1.0 [0.9 to 1.2]	1.2 [1.0 to 1.3]	NA	1.5 [1.5 to 1.5]	.65
Weight adjusted dose unsuccessful rechallenges [b,c]						
Median dose [IQR] / (mg/kg)	0.8 [0.6 to 1.5]	1.1 [1.1 to 1.2]	0.9 [0.8 to 1.5]	NA	NA	.95

TIM, thiopurine-induced myelosuppression. Values represent: n (%) or median [IQR]. The following were used to define variant subgroups of among TIM affected individuals:
***TPMT* ref/ref *& NUDT15* ref/ref*: TPMT* reference and *NUDT15* reference genotype**
***TPMT* var/ref *& NUDT15* ref/ref*: TPMT* heterozygote variant *and NUDT15* reference genotype**
***TPMT* ref/ref *& NUDT15* var/*/: *TPMT* reference and *NUDT15* heterozygote variant genotype**
***TPMT* var/var *& NUDT15* ref/ref*: TPMT* homozygote variant and *NUDT15* reference genotype**
***TPMT* var/* *& NUDT15* var/ref*: TPMT* heterozygote or homozygote variant and *NUDT15* heterozygote variant genotype**
[a] one TIM case possessed two *NUDT15* variants (rs554405994 [p.Gly17_Val18dup] and rs116855232 [p.Arg139Cys]). It was not possible to ascertain if this represented a compound heterozygote or two variants on the same strand (*2 *NUDT15* haplotype). For the purposes of the analysis, this case was considered as a single *NUDT15* variant carrier (*NUDT15* var/*)
[b] weight adjusted dose represents maximum azathioprine equivalent dose prior to TIM and adjusted for weight (mg/kg): [(mercaptopurine dose (mg) x 2.08) / weight (kg)] or [azathioprine dose (mg) / weight (kg)]

[c] an unsuccessful drug rechallenge was defined as a second exposure to a thiopurine at the same or lower dose which was then subsequently withdrawn due to any adverse event, or, a second fall in absolute white blood cell count to ≤ 3.0 x10^9/L
[d] Fisher's exact and Kruskal-Wallis tests were used for comparison among all groups. *P* values < .05 deemed statistically significant
[e] one TIM case was *TPMT* var/var and *NUDT15* var/ref in order that this patient was neither lost or analyzed alone, they were grouped with five patients who were *TPMT* var/ref and *NUDT15* var/ref
[f] from leftmost group denominators = 230, 50, 25, 16, 6
[g] from leftmost group denominators = 126, 24, 13, 2, 2

eTable 4.3-12. Comparison of phenotype among thiopurine-induced myelosuppression affected individuals with successful and unsuccessful rechallenge with a thiopurine

Variable	Level	Successful thiopurine rechallenge n = 95	Unsuccessful thiopurine rechallenge [c] n = 72	*P* value [b]
Age at inflammatory bowel disease diagnosis	Years	29.8 [18.7 to 43.1]	31.0 [19.0 to 45.3]	.77
Age at time of index myelosuppression	Years	36.8 [24.2 to 52.2]	38.8 [25.4 to 52.0]	.77
Sex	Female	47 (49.5%)	39 (54.2%)	.64
Disease type	Crohn's disease	59 (62.1%)	39 (54.2%)	.53
	IBD-unclassified	2 (2.1%)	2 (2.8%)	
	ulcerative colitis	34 (35.8%)	31 (43.1%)	
TPMT haplotype	*TPMT* ref/ref	82 (86.3%)	57 (79.2%)	.18
	TPMT ref/var	13 (13.7%)	13 (18.1%)	
	TPMT var/var	0 (0.0%)	2 (2.8%)	
NUDT15 haplotype	*NUDT15* ref/var	6 (6.3%)	9 (12.5%)	.18
Thiopurine used in rechallenge	azathioprine	52 (59.8%)	38 (70.4%)	.21
Weight adjusted azathioprine equivalent thiopurine dose of rechallenge [a]	mg/kg	1.2 [0.9 to 1.5]	1.0 [0.6 to 1.5]	.21

values represent: n (%) or median [interquartile range]. ref/ref, reference genotype; ref/var, heterozygote genotype; var/var, homozygote variant genotype

[a] weight adjusted dose represents the maximum azathioprine equivalent dose prior to thiopurine-induced myelosuppression and adjusted for weight (mg/kg): [(mercaptopurine dose (mg) x 2.08) / weight (kg)] or [azathioprine dose (mg) / weight (kg)]

[b] *P* values reflect Fisher's exact or Mann-Whitney U test as appropriate. *P* < .05 deemed statistically significant

[c] an unsuccessful drug rechallenge was defined as a second exposure to a thiopurine at the same or lower dose which was then subsequently withdrawn due to any adverse event, or, a second fall in absolute white blood cell count to ≤ 3.0 x10^9/L

eTable 4.3-13. TPMT and NUDT15 variants in thiopurine-induced myelosuppression affected individuals of non-European and European ancestry (n = 373)

Gene	Variant position/ rsID/ protein sequence	Genotype/ Haplotype	East Asian (n=4)	South Asian (n=41)	Non-Finnish European (n=328)
NUDT15	48611918/ rs746071566 p.Gly17_Val18del	ref/var	0 (0.0%)	0 (0.0%)	19 (5.8%)
	48611918/ rs554405994 p.Gly17_Val18dup	ref/var	1 (25.0%)	2 (4.9%)	5 (1.5%)
	48619855/ rs116855232/ p.Arg139Cys	ref/var	2 (50.0%)	16 (39.0%)	8 (2.4%)
		var/var	2 (50.0%)	6 (14.6%)	0 (0.0%)
	Any of the above *NUDT15* coding variants		4 (100.0%)	23 (56.1%)	31 (10.1%)
TPMT	var [a] /var [a]		0 (0.0%)	0 (0.0%)	16 (4.9%)
	ref [b] /var [a]		1 (25.0%)	1 (2.4%)	56 (17.1%)
	ref [b] /ref [b]		3 (75.0%)	40 (97.6%)	256 (78.0%)

Variant position, chromosome position; rsID, single nucleotide polymorphism identification number; ref, reference haplotype/genotype; var, variant haplotype/genotype
[a] Variant *TPMT* haplotypes include: *2, *3A, *3C, *4, *8, *9, *12, *21, *37, *40
[b] Reference *TPMT* haplotype include: *1

eTable 4.3-14. Genotype frequencies of NUDT15 variants in the gnomADa populations (n = 373)

rsid	genotype	African (n=11164)	Ashkenazi Jewish (n=4548)	East Asian (n=7925)	European (Finnish) (n=10812)	European (Non-Finnish) (n=50729)	Latino (n=14791)	South Asian (n=13166)	Other (n=3179)
48611918/ rs746071566 p.Gly17_Val18del	ref/var	0.0%	0.0%	0.0%	0.1%	0.4%	0.1%	0.1%	0.0%
	var/var	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
48611918/ rs554405994 p.Gly17_Val18dup	ref/var	0.4%	0.1%	11.3%	2.5%	0.5%	9.6%	0.3%	1.9%
	var/var	0.0%	0.0%	0.3%	0.0%	0.0%	0.3%	0.0%	0.0%
48619855/ rs116855232/ p.Arg139Cys	ref/var	0.2%	0.8%	18.6%	4.4%	0.7%	11.5%	12.4%	3.6%
	var/var	0.0%	0.0%	1.2%	0.1%	0.0%	0.4%	0.6%	0.1%
Any of the above *NUDT15* coding variants		0.7%	0.9%	29.2%	6.9%	1.6%	20.7%	13.4%	5.6%

ref, reference haplotype/genotype; var, variant haplotype/genotype
Frequency of carrying one or more variants calculated using the minor allele frequencies for each genomic position and calculating the probability of carrying no minor alleles.
[a] gnomAD, Genome Aggregation Database (http://gnomad.broadinstitute.org/)

eAppendix 4.3-1. Participants of adjudication meetings

Name	Institution
Gareth J Walker	Department of Gastroenterology, Royal Devon and Exeter Hospital NHS Foundation Trust, Exeter, UK
Graham A Heap	Department of Gastroenterology, Royal Devon and Exeter Hospital NHS Foundation Trust, Exeter, UK
Chris Calvert	Department of Gastroenterology, Royal Devon and Exeter Hospital NHS Foundation Trust, Exeter, UK
Andy T Cole	Gastroenterology and Hepatology , Royal Derby Hospital, Derby Teaching Hospitals NHS Foundation Trust, Derby, UK
Tom J Creed	Gastroenterology and Hepatology, University Hospitals Bristol, University Hospitals Bristol NHS Foundation Trust, Bristol, UK
Tawfique K Daneshmend	Department of Gastroenterology, Royal Devon and Exeter Hospital NHS Foundation Trust, Exeter, UK
Anjan Dhar	Department of Gastroenterology, Bishop Auckland General Hospital, County Durham and Darlington NHS Foundation Trust, Darlington, UK
Suranga Dharmasiri	Department of Gastroenterology, Southampton General Hospital, University Hospital Southampton NHS Foundation Trust, Southampton, UK
Daniel R Gaya	Department of Gastroenterology, Glasgow Royal Infirmary, NHS Greater Glasgow and Clyde, Glasgow, UK
John N Gordon	Gastroenterology & Hepatology Services, Royal Hampshire County Hospital, Hampshire Hospitals NHS Foundation Trust, Winchester, UK
Emma Greig	Department of Gastroenterology, Musgrove Park Hospital, Taunton and Somerset NHS Hospitals, Taunton, UK
Ailsa L Hart	Department of Gastroenterology, St Mark's Hospital, London North West Healthcare NHS Trust, Harrow, UK
Neel M Heerasing	Department of Gastroenterology, Royal Devon and Exeter Hospital NHS Foundation Trust, Exeter, UK
Peter Hendy	Department of Gastroenterology, Royal Devon and Exeter Hospital NHS Foundation Trust, Exeter, UK
Peter M Irving	Department of Gastroenterology, Guy's and St Thomas' NHS Foundation Trust, London, UK
Stephen J Lewis	Department of Gastroenterology, Plymouth Hospitals NHS Trust , Plymouth, UK
James Lindsay	Department of Gastroenterology, The Royal London Hospital, Barts Health NHS Trust, London, UK
John C Mansfield	Department of Gastroenterology, Newcastle Upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK
Charles DR Murray	Department of Gastroenterology, Royal Free Hospital, Royal Free London NHS Foundation Trust, London, UK
Timothy R Orchard	Faculty of Medicine, Imperial College Healthcare NHS Trust , London, UK
Richard CG Pollok	Department of Gastroenterology, St George's Healthcare NHS Trust, Tooting, UK

Subramaniam Ramakrishnan	Gastrointestinal and Liver services, Warrington and Halton Hospitals NHS Foundation Trust , Warrington, UK
David S Rampton	Department of Gastroenterology, The Royal London Hospital, Barts Health NHS Trust, London, UK
Richard K Russell	Department of Paediatric Gastroenterology , Royal Hospital for Children, NHS Greater Glasgow and Clyde, Glasgow, UK **CONT….**
Shaji Sebastian	Gastroenterology and Hepatology, Hull and East Yorkshire Hospitals NHS Trust , Hull, UK
Abhey Singh	Department of Gastroenterology, Royal Devon and Exeter Hospital NHS Foundation Trust, Exeter, UK
Anthony Todd	Department of Haematology, Royal Devon and Exeter Hospital NHS Foundation Trust, Exeter, UK
Mark Tremelling	Department of Gastroenterology, Norfolk and Norwich University Hospitals NHS Foundation Trust, Norwich, UK
James R Goodhand	Department of Gastroenterology, Royal Devon and Exeter Hospital NHS Foundation Trust, Exeter, UK
Nicholas A Kennedy	Department of Gastroenterology, Royal Devon and Exeter Hospital NHS Foundation Trust, Exeter, UK
Tariq Ahmad	Department of Gastroenterology, Royal Devon and Exeter Hospital NHS Foundation Trust, Exeter, UK

eAppendix 4.3-2. Rules for adjudication of thiopurine-induced myelosuppression affected individuals

1. A **positive** rechallenge requires a fall in total white cell count or neutrophil count on repeated administration of the same or lower dose. If a rechallenge of a lower dose is tolerated then the patient is classified as a probable, not definite.
2. If a rechallenge is tolerated at the **same or a higher dose** the patient is unlikely irrespective of the time frame of administration.
3. If there have been multiple (greater than 2) rechallenges and some have been tolerated at an **equivalent mg/kg** but others have not, then the patient is classified as unlikely.
4. The role of other leukopenia causing drugs can usually be dismissed in affected individuals which meet the definite criteria for thiopurine induced myelosuppression.
5. Any co-administration of a drug known to cause leukopenia renders the case a possible, irrespective of the temporal relationship unless they continued to take the drug at the same dose.
6. Other confounding leukopenia causing drugs can be excluded as a cause if stopped **more than 3 months before** the episode of leukopenia.
7. Inclusion criteria are absolute (white cell count $\leq 2.5 \times 10^9$/L and/or reduction in neutrophil count to $\leq 1.0 \times 10^9$/L)
8. Infliximab is not a cause of leukopenia and there is insufficient evidence to suggest an interaction with azathioprine. Equally 5ASA drugs have insufficient evidence for an interaction with azathioprine.
9. If we do not have a recorded stop date for thiopurines, we can seek clarification if necessary, but the major criteria require its use within the last 7 days prior to leucopoenia.
10. Concurrent administration of allopurinol categorizes the patient as possible as a result of shunting the metabolic pathway towards 6-TGN and thus increasing the risk of myelosuppression.

eAppendix 4.3-3. Drugs deemed to cause leukopenia for adjudication process

1. Allopurinol
2. Anti-thyroid drugs (thionamides – Methimazole, Carbimazole, Propylthiouracil)
3. Anti-inflammatory drugs (Sulfasalazine, Nonsteroidal anti-inflammatory drugs [NSAIDs], Penicillamine)
4. Psychotropic drugs (Clozapine, Phenothiazines, Tricyclic and tetracyclic antidepressant)
5. Gastrointestinal drugs (Sulfasalazine, Histamine H2- receptor antagonists)
6. Cardiovascular drugs (Antiarrhythmic agents (tocainide, procainamide, flecainide), ACE inhibitors (enalapril, captopril), Propranolol, Dipyridamole, Digoxin)
7. Dermatologic drugs (Dapsone, Isotretinoin)
8. Antibacterial drugs (Macrolides including minocycline, Trimethoprim-sulfamethoxazole, Chloramphenicol, Sulfonamides, Vancomycin, Cephalosporin)
9. Antimalarial drugs
10. Antifungal agents (Amphotericin B, Flucytosine)
11. Anticonvulsants (Carbamazepine, Phenytoin, Ethosuximide, Valproate, lamotrigine)
12. Diuretics (Thiazides, Acetazolamide, Frusemide, Spironolactone)
13. Chlorpropamide
14. Bupropion
15. Immunosuppressive drugs

eAppendix 4.3-4. Case Report Form

International IBD Genetics Consortium

PRED4

Thiopurine Induced Leucopaenia

Case Report Form

Please stick study label here

On completion, please return to:
IBD Pharmacogenetics Research Office
The Research, Innovation, Learning and Development Centre (RILD)
Barrack Road
Exeter
EX2 5DW

Thiopurine Induced Leucopaenia

Section 1 - Inclusion Criteria

Study code ☐

1.1 Major criteria (all must be met)

- ☐ History of inflammatory bowel disease
- ☐ History of thiopurine exposure in the previous 7 days
- ☐ Normal total white cell count and/or neutrophil count at baseline
- ☐ Fall in total white cell count to ≤2.5x10^9/L, or reduction in neutrophil count to ≤1.0x10^9/L
- ☐ Medical opinion implicating thiopurine leads to dose reduction or drug withdrawal (even if temporary)

1.2 Other risk factor(s) or potential causes for leucopaenia (see page 2)*

- ☐ No - Category A
- ☐ Yes - Category B

If yes:

- ☐ Drugs, please specify (* See page 2) and give details in section 7
- ☐ Symptoms suggestive of recent viral infection
- ☐ Myeloproliferative diseases
- ☐ Rheumatoid arthritis, SLE
- ☐ B12 or folate deficiency
- ☐ Hypersplenism
- ☐ Other, please specify ☐

1.3 Minor criteria:

- ☐ Fall in total white cell count or neutrophil count within 12 months of introduction of thiopurines
- ☐ White cell count and neutrophil count returns to normal range after dose reduction or drug withdrawal
- ☐ Recurrence (defined as total white cell count ≤3.5x10^9/L or neutrophil count ≤2.0x10^9/L) on re-challenge with either Azathioprine or Mercaptopurine

1.4 Number of minor criteria ☐

1.5 Participant's eligibility Investigator sign-off

Is the participant eligible to take part in the clinical trial? ☐ Yes ☐ No

If no, please give reason(s) for screen failure:

1. ☐
2. ☐

Investigator's signature ☐ Date dd / mm / yyyy

Investigator's name (print) ☐

Thiopurine Induced Leucopaenia

Section 2 - Patient Details

Study code ☐

2.1 Patient details

Date of Birth dd / mm / yyyy Sex: M ☐ F ☐

Weight at time of leucopaenia (or nearest estimate) ☐ kg

Date of weight dd / mm / yyyy Height ☐ cm

2.2 Ethnicity - *Please tick as appropriate*

White

- ☐ British
- ☐ Irish
- ☐ Any other White background

Black or Black British

- ☐ Caribbean
- ☐ African
- ☐ Any other Black background

Mixed

- ☐ White and Black Caribbean
- ☐ White and Black African
- ☐ White and Asian
- ☐ Any other Mixed background

Chinese or Other Ethnic Group

- ☐ Chinese
- ☐ Any other ethnic group (*please specify*)

  ☐
- ☐ Not stated

Asian or Asian background

- ☐ Indian
- ☐ Pakistani
- ☐ Bangladeshi
- ☐ Any other Asian background

2.3 Participant informed consent

Date participant signed written consent form dd / mm / yyyy

Date of blood sample taken dd / mm / yyyy

Thiopurine Induced Leucopaenia

Section 3 - Medical History

Study code

3.1 Hospital Details

3.1.1 Consultant Gastroenterologist

Hospital

Hospital address

Consultant telephone

Consultant email

3.1.2 Consultant Haematologist

Hospital

Hospital address

Consultant telephone

Consultant email

3.2 Other significant medical history

☐ Yes ☐ No

If yes, please give details here

Thiopurine Induced Leucopaenia

Section 4 - Diagnosis & Classification of IBD Study code

4.1 Diagnosis and classification of IBD

- ☐ Crohn's disease — Date of diagnosis: dd / mm / yyyy
- ☐ Ulcerative Colitis — Date of diagnosis: dd / mm / yyyy
- ☐ IBD unclassified — Date of diagnosis: dd / mm / yyyy

4.2 Smoking history

4.2.1 Start date: dd / mm / yyyy 4.2.2 End date: dd / mm / yyyy

4.2.3 Maximum number of cigarettes per day

4.3 Ulcerative colitis

4.3.1 The extent of ulcerative colitis can be classified as:

- ☐ E1 Ulcerative proctitis - inflammation is limited to the rectum (proximal extent of inflammation is distal to the rectosigmoid junction)
- ☐ E2 Left sided UC (distal UC) - inflammation limited to a proportion of the colorectum up to the splenic flexure
- ☐ E3 Extensive UC (pancolitis) - inflammation extends beyond the splenic flexure
- ☐ Ex Unknown

4.3.2 Disease severity in 2 years prior to development of leucopaenia

- ☐ DS0 Clinical remission. Asymptomatic; no escalation of treatment
- ☐ DS1 Mild relapses – managed with oral or rectal aminosalicylates and/or rectal steroids: **no oral steroids** required
- ☐ DS2 Moderate relapses requiring oral steroids and/or addition of immunomodulator
- ☐ DS3 Severe or refractory disease requiring inpatient admission or colectomy

4.4 Crohn's disease

4.4.1 Location

- ☐ L1 Ileal
- ☐ L2 Colonic
- ☐ L3 Ileocolonic
- ☐ L4 Isolated upper disease

4.4.2 Behaviour - the behaviour can be defined by looking at reports from Barium enema, colonoscopy, MRI, CT

- ☐ B1 Non stricturing, non-penetrating
- ☐ B2 Stricturing
- ☐ B3 Internal penetrating
- ☐ p Perianal disease modifier

Thiopurine Induced Leucopaenia

Section 5 - Leucopaenia History

Study code

5.1 Which thiopurine was suspected of causing leucopaenia?

☐ Azathioprine ☐ Mercaptopurine

5.2 Date thiopurine first commenced dd / mm / yyyy

5.3 Maximum dose of thiopurine in 8 weeks prior to episode of leucopaenia

5.3.1 Date when this maximum dose of thiopurine started dd / mm / yyyy

5.4 Were TGN levels measured within 2 months of detecting the leucopaenia

☐ Yes ☐ No ☐ Unknown

If yes, what was the level (pmol/8 x 10^8 RBC)?

5.5 Presentation

Did the patient present because of:

☐ Routine monitoring ☐ Sepsis

☐ Opportunistic blood test ☐ Other

5.6 Leucopaenia

	Date	Total white cell count	Neutrophil count	Haemoglobin	Platelet count
Normal range for lab	dd/mm/yyyy				
Last blood test prior to commencing thiopurine	dd/mm/yyyy				
First blood test demonstrating leucopaenia (below normal range for your lab)	dd/mm/yyyy				
Blood test demonstrating lowest total white cell count	dd/mm/yyyy				
Blood test demonstrating lowest neutrophil count	dd/mm/yyyy				

Thiopurine Induced Leucopaenia

Section 5 - Leucopaenia History

Study code

5.7 Action taken

☐ Drug withdrawn Date dd / mm / yyyy

☐ Dose decreased Date dd / mm / yyyy

Reduced dose

☐ Tolerated (normal WCC on this dose) ☐ Not tolerated ☐ Not known

5.8 Recovered cell counts

	Date	Cell count
Best recovered total white cell count within 8 weeks of dose reduction/withdrawal	dd / mm / yyyy	
Best recovered neutrophil count within 8 weeks of dose reduction/withdrawal	dd / mm / yyyy	

Time to best recovered total white cell count/neutrophil count (days)

5.9 Did the patient require hospital admission at any stage due to leucopaenia

☐ Yes ☐ No

If yes, date of admission dd / mm / yyyy

Date of discharge dd / mm / yyyy

5.10 Complications

Any infections? ☐ Yes ☐ No

If yes, please give details

5.11 Was the individual ever re-challenged with thiopurine?

☐ Yes ☐ No ☐ Unknown

Outcome:

☐ Tolerated Dose tolerated

☐ Not tolerated Lowest WCC (with date of test)

Date of drug withdrawal dd / mm / yyyy

5.12 Was a bone marrow biopsy done?

☐ Yes ☐ No ☐ Unknown

If yes, please give results

Thiopurine Induced Leucopaenia

Section 5 - Leucopaenia History

Study code

5.13 Was the patient ever treated with G-CSF?

☐ Yes ☐ No ☐ Unknown

If yes, what was the start date? dd / mm / yyyy

What was the end date? dd / mm / yyyy

Did the patient respond?

Did the patient receive any other treatment for leucopaenia?

Section 6 - Supplementary Information

6.1 What is the individual's thiopurine methytransferase (TPMT) genotype/ activity ?

Genotype

Activity: ☐ Absent Level (U/ml)

☐ Low (carrier)

☐ Normal

☐ High

6.2 Has the individual experienced any other adverse effects attributable to azathioprine/mercaptopurine?

☐ Yes ☐ No ☐ Unknown

If yes:

6.2.1 Abnormal LFTs (please give peak ALT/AST and laboratory reference range)

6.2.2 Pancreatitis (please state peak serum amylase/lipase and laboratory reference)

6.2.3 Other (please state):

Thiopurine Induced Leucopaenia

Section 6 - Supplementary Information

Study code

6.3 Family history

Family history of thiopurine induced leucopaenia ☐ Yes ☐ No ☐ Unknown

If yes, give details

Section 7 - Other Drug History

7.1 Did the patient receive steroids in the 3 months prior to recognition of leucopaenia?

☐ Yes ☐ No ☐ Unknown

If yes, type of steroid | Dose

Date commenced dd / mm / yyyy | Date ceased dd / mm / yyyy

7.2 Other drugs in 3 months prior to development of leucopaenia

Drug name	Dose and Route	Start date	Stop date
		dd / mm / yyyy	dd / mm / yyyy
		dd / mm / yyyy	dd / mm / yyyy
		dd / mm / yyyy	dd / mm / yyyy
		dd / mm / yyyy	dd / mm / yyyy
		dd / mm / yyyy	dd / mm / yyyy
		dd / mm / yyyy	dd / mm / yyyy
		dd / mm / yyyy	dd / mm / yyyy
		dd / mm / yyyy	dd / mm / yyyy
		dd / mm / yyyy	dd / mm / yyyy
		dd / mm / yyyy	dd / mm / yyyy
		dd / mm / yyyy	dd / mm / yyyy
		dd / mm / yyyy	dd / mm / yyyy

eAppendix 4.3-5. IBD Pharmacogenetics Study Group Members

Name	Institution	City	Country
Prof Peter Bampton	Flinders Medical Centre, Flinders University of South Australia	Adelaide	Australia
Prof Jane Andrews	Royal Adelaide Hospital	Adelaide	Australia
Assoc Prof Graham Radford-Smith	Royal Brisbane & Women's Hospital	Brisbane	Australia
Dr Kavitha Subramaniam	Canberra Hospital	Canberra	Australia
Dr Susan Connor	Liverpool Hospital, New South Wales	Liverpool	Australia
Prof Timothy H Florin	Mater Research Institute – University of Queensland	South Brisbane	Australia
Dr Marianne Mortimore	Mater Research Institute – University of Queensland	South Brisbane	Australia
Dr Sally Bell	St Vincent's Hospital	Sydney	Australia
Dr Alissa Walsh	St Vincent's Hospital	Sydney	Australia
Prof Ian C Lawrance	Fremantle Hospital, Fremantle	Western Australia	Australia
Dr Richard N Fedorak	University of Alberta	Edmonton	Canada
Dr Richard N Fedorak	University of Alberta	Edmonton	Canada
Dr Mark Silverberg	Mount Sinai Hospital	Toronto	Canada
Prof Vibeke Andersen	Regional Hospital Viborg	Viborg	Denmark
Prof Laurent Beaugerie	Saint-Antoine Hospital and Sorbonne Universite	Paris	France
Dr Philippe Seksik	Saint-Antoine Hospital and Sorbonne Universite	Paris	France
Prof Harry Sokol	Saint-Antoine Hospital and Sorbonne Universite	Paris	France
Dr Andre Franke	Christian-Albrechts-University of Kiel	Kiel	Germany
Dr Stefan Schreiber	Christian-Albrechts-University of Kiel	Kiel	Germany
Dr Sebastian Zeissig	University Medical Center Schleswig-Hostein	Kiel	Germany
Prof Epameinondas Tsianos	University Hospital of Ioannina	Ioannina	Greece
Dr Konstantinos H Katsanos	University of Ioannina	Ioannina	Greece
Dr Amir Karban	Rambam Health Care Campus	Haifa	Israel
Dr Raffi Lev-Tzion	Shaare Zedek Medical Centre	Jerusalem	Israel
Dr Vito Annese	Azienda Ospedaliero Universitaria Careggi	Florence	Italy
Dr Vito Annese	Azienda Ospedaliero Universitaria Careggi	Florence	Italy
Dr Giacomo Sturniolo	Univerita di Padova	Padova	Italy
Dr Renata D'Inca	University Hospital of Padova	Padua	Italy

Dr Pierre Ellul	Mater Dei Hospital	Msida	Malta
Dr Eleonora AM Festen	University Medical Center Groningen	Groningen	Netherlands
Dr Michiel D Voskuil	University Medical Center Groningen	Groningen	Netherlands
Prof Rinse K Weersma	University Medical Center Groningen	Groningen	Netherlands
Assoc Prof Michael Schultz	Dunedin Hospital	Dunedin	New Zealand
Dr Gillian Watermeyer	Groote Schuur Hospital	Cape Town	South Africa
Assoc Prof Jonas Halfvarson	Örebro University	Örebro	Sweden
Dr Mauro D'Amato	Karolinska Institute	Stockholm	Sweden
Dr Malcolm Smith	Aberdeen Royal Infirmary	Aberdeen	UK
Dr Karen Yearsley	Nevill Hall Hospital	Abergavenny	UK
Dr Vinod Patel	Tameside and Glossop Integrated Care NHS Foundation Trust	Ashton-under-Lyne	UK
Dr Dipak Roy	Tameside and Glossop Integrated Care NHS Foundation Trust	Ashton-under-Lyne	UK
Dr Sue Cullen	Stoke Mandeville Hospital	Aylesbury	UK
Dr David Gorard	Stoke Mandeville Hospital	Aylesbury	UK
Dr Elaine Spalding	University Hospital Crosshouse	Ayrshire & Arran	UK
Dr Steve Mann	Barnet & Chase Farm Hospitals, Royal Free London NHS Foundation Trust	Barnet	UK
Dr Alex Moran	Northern Devon Healthcare Trust	Barnstaple	UK
Dr Zia Mazhar	Basildon and Thurrock University Hospitals NHS Foundation Trust	Basildon	UK
Dr Matthew Brown	Basingstoke and North Hampshire Hospital	Basingstoke	UK
Dr Rebecca Saich	Basingstoke and North Hampshire Hospital	Basingstoke	UK
Dr Ben Colleypriest	Royal United Hospitals Bath NHS Foundation Trust	Bath	UK
Dr Jonathan Quinlan	Royal United Hospitals Bath NHS Foundation Trust	Bath	UK
Dr Rafeeq Muhammed	Birmingham Children's Hospital	Birmingham	UK
Dr Naveen Sharma	Birmingham Heartlands Hospital	Birmingham	UK
Dr Mark Thomas	Heart of England NHS Foundation Trust	Birmingham	UK
Dr Tariq Iqbal	Queen Elizabeth Hospital	Birmingham	UK
Dr Rachel Cooney	Sandwell Hospital	Birmingham	UK
Dr David Grimes	Royal Blackburn Hospital	Blackburn	UK
Dr Salil Singh	Bolton NHS Foundation Trust	Bolton	UK

Dr Sean Weaver	Royal Bournemouth General Hospital	Bournemouth	UK
Dr Cathryn Preston	Bradford Royal Infirmary	Bradford	UK
Dr Alan Ireland	Royal Sussex County Hospital	Brighton	UK
Dr Melissa Smith	Royal Sussex County Hospital	Brighton	UK
Dr James Kennedy	Southmead Hospital, North Bristol NHS Trust	Bristol	UK
Dr Melanie Lockett	Southmead Hospital, North Bristol NHS Trust	Bristol	UK
Dr Dharam Basude	University Hospitals Bristol, University Hospitals Bristol NHS Foundation Trust	Bristol	UK
Dr Tom J Creed	University Hospitals Bristol, University Hospitals Bristol NHS Foundation Trust	Bristol	UK
Dr Miles Parkes	Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust	Cambridge	UK
Dr Franco Torrente	Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust	Cambridge	UK
Dr Michael Delaney	Kent & Canterbury Hospital	Canterbury	UK
Dr John Green	University Hospital Llandough	Cardiff	UK
Dr Barney Hawthorne	University Hospital of Wales	Cardiff	UK
Dr Theresa Barnes	Countess of Chester Hospital	Chester	UK
Dr Andy Milestone	Countess of Chester Hospital	Chester	UK
Dr David Elphick	Chesterfield Royal Hospital NHS Foundation Trust	Chesterfield	UK
Dr Acuth Shenoy	Colchester Hospital University NHS Foundation Trust	Colchester	UK
Dr Anjan Dhar	Bishop Auckland General Hospital, County Durham and Darlington NHS Foundation Trust	Darlington	UK
Dr Andy T Cole	Royal Derby Hospital	Derby	UK
Dr Kathleen Holding	Royal Derby Hospital	Derby	UK
Dr Andy T Cole	Royal Derby Hospital, Derby Teaching Hospitals NHS Foundation Trust	Derby	UK
Dr James Shutt	Dorset County Hospital NHS Foundation Trust	Dorchester	UK
Dr Jo Taylor	Dorset County Hospital NHS Foundation Trust	Dorchester	UK
Dr Shanika Silva	Russells Hall Hospital	Dudley	UK
Dr Sheldon Cooper	The Dudley Group NHS Foundation Trust	Dudley	UK
Dr Shanika deSilva	The Dudley Group NHS Foundation Trust	Dudley	UK

Dr Craig Mowat	Ninewells Hospital, NHS Tayside	Dundee	UK
Dr Sam Panthakalam	Eastbourne District General Hospital	Eastbourne	UK
Dr Charlie W Lees	Western General Hospital, NHS Lothian	Edinburgh	UK
Prof Jack Satsangi	Western General Hospital, NHS Lothian	Edinburgh	UK
Dr Pritash Patel	Epsom & St. Helier University Hospital NHS Trust	Epsom	UK
Dr Deb Ghosh	Princess Alexandra Hospital	Essex	UK
Dr Rosemary Philiips	Princess Alexandra Hospital	Essex	UK
Dr Rosemary Philips	Princess Alexandra Hospital	Essex	UK
Dr Tariq Ahmad	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Dr Chris Calvert	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Dr Neil Chanchlani	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Dr Helen Clarke	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Dr Richard D'Souza	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Dr Tawfique K Daneshmend	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Dr Naomi Edney	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Dr James R Goodhand	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Dr James Goodhands	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Dr James Hart	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Dr Graham A Heap	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Dr Neel M Heerasing	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Dr Neel M Heerasing	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK

Dr Peter Hendy	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Dr Nicholas A Kennedy	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Dr Simeng Lee	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Dr Abhey Singh	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Dr Lucy Smyth	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Dr Kenji So	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Dr Amanda Thomas	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Dr Anthony Todd	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Dr Gareth J Walker	Royal Devon and Exeter Hospital NHS Foundation Trust	Exeter	UK
Ms Claire M Bewshea	University of Exeter	Exeter	UK
Mrs Hanlie Olivier	University of Exeter	Exeter	UK
Dr James W Harrison	University of Exeter Medical School	Exeter	UK
Dr Samuel E Jones	University of Exeter Medical School	Exeter	UK
Miss Rebecca Ward	University of Exeter Medical School	Exeter	UK
Assoc Prof Michael N Weedon	University of Exeter Medical School	Exeter	UK
Dr Natalie Direkze	Frimley Park Hospital	Frimley	UK
Dr Sarah Langlands	Frimley Park Hospital	Frimley	UK
Dr Daniel R Gaya	Glasgow Royal Infirmary, NHS Greater Glasgow and Clyde	Glasgow	UK
Prof Richard K Russell	Royal Hospital for Children, NHS Greater Glasgow and Clyde	Glasgow	UK
Dr Richard Russell	Royal Hospital for Children, NHS Greater Glasgow and Clyde	Glasgow	UK
Dr Paul Dunckley	Gloucestershire Royal Hospital, Gloucestershire Hospitals NHS Foundation Trust	Gloucester	UK
Dr Tariq Mahmood	Grantham & District Hospital	Grantham	UK

Dr Paul Banim	James Paget University Hospital	Great Yarmouth	UK
Dr Sunil Sonwalker	Calderdale Royal Hospital	Halifax	UK
Dr Gareth Davies	Harrogate and District NHS Foundation Trust	Harrogate	UK
Prof Ailsa L Hart	St Mark's Hospital, London North West Healthcare NHS Trust	Harrow	UK
Dr Ailsa L Hart	St Mark's Hospital, London North West Healthcare NHS Trust	Harrow	UK
Dr Theresa Barnes	Hereford County Hospital	Hereford	UK
Dr Andy Milestone	Hereford County Hospital	Hereford	UK
Dr Carl A Anderson	Wellcome Trust Sanger Institute	Hinxton	UK
Dr Jeffrey C Barrett	Wellcome Trust Sanger Institute	Hinxton	UK
Dr Shaji Sebastian	Hull and East Yorkshire Hospitals NHS Trust	Hull	UK
Prof Angus Watson	Raigmore Hospital	Inverness	UK
Dr Daniel Gavin	The Ipswich Hospital NHS Trust	Ipswich	UK
Dr John Wyke	The Ipswich Hospital NHS Trust	Ipswich	UK
Dr Richard Shenderay	Airedale NHS Foundation Trust	Keighley	UK
Dr Debasish Das	Kettering General Hospital	Kettering	UK
NA Francesca Bredin	King's Lynn Hospital	King's Lynn	UK
Dr David Watts	Forth Valley Royal Hospital	Larbert	UK
Dr John Puntis	Leeds General Infirmary	Leeds	UK
Dr Mundre Pradeep	Leeds Teaching Hospitals	Leeds	UK
Dr Simon M Everett	St James's University Hospital, Leeds Teaching Hospitals NHS Trust	Leeds	UK
Dr Pradeep Mundre	St James's University Hospital, Leeds Teaching Hospitals NHS Trust	Leeds	UK
Dr Anne Willmott	Leicester Royal Infirmary - Paediatric	Leicester	UK
Dr David Reffitt	University Hospital Lewisham	Lewisham	UK
Dr Sathish Babu	Lincoln County Hospital	Lincoln	UK
Dr Christopher Goldsmith	Aintree University Hospital NHS Foundation Trust	Liverpool	UK
Dr Asheesh Sharma	Aintree University Hospital NHS Foundation Trust	Liverpool	UK
Dr Marcus Auth	Alder Hey Children's Hospital, Alder Hey Children's NHS Foundation Trust	Liverpool	UK
Dr Sree Subramanian	Royal Liverpool & Broadgreen University Hospitals NHS Trust	Liverpool	UK

Prof James O Lindsay	Blizard Institute, Barts and the London School of Medicine, Queen Mary University of London	London	UK
Dr John Fell	Chelsea and Westminster Hospital NHS Foundation Trust	London	UK
Dr Marcus Harbord	Chelsea and Westminster Hospital NHS Foundation Trust	London	UK
Dr Fevronia Kiparissi	Great Ormond Street Hospital for Children NHS Foundation Trust	London	UK
Dr Neil Shah	Great Ormond Street Hospital for Children NHS Foundation Trust	London	UK
Dr Peter M Irving	Guy's and St Thomas' NHS Foundation Trust	London	UK
Dr Timothy R Orchard	Imperial College Healthcare NHS Trust	London	UK
Dr Guy Chung-Faye	King's College Hospital	London	UK
Dr Alistair McNair	Queen Elizabeth Hospital	London	UK
Dr Charles DR Murray	Royal Free Hospital, Royal Free London NHS Foundation Trust	London	UK
Dr Nick Croft	The Royal London Hospital, Barts Health NHS Trust	London	UK
Prof David S Rampton	The Royal London Hospital, Barts Health NHS Trust	London	UK
Dr Stuart Bloom	University College London Hospital	London	UK
Dr Anita Modi	Luton and Dunstable University Hospital	Luton	UK
Dr Simon Campbell	Manchester Royal Infirmary	Manchester	UK
Dr Scott Levison	Manchester Royal Infirmary	Manchester	UK
Dr Jimmy Limdi	Pennine Trust	Manchester	UK
Dr Anthony Akobeng	Royal Manchester Children's Hospital	Manchester	UK
Dr Joel Mawdsley	West Middlesex University Hospital	Middlesex	UK
Dr George MacFaul	Milton Keynes University Hospital NHS Foundation Trust	Milton Keynes	UK
Dr George McFaul	Milton Keynes University Hospital NHS Foundation Trust	Milton Keynes	UK
Dr Suren Kanegasundaram	Freeman Hospital	Newcastle upon Tyne	UK
Dr John C Mansfield	Newcastle Upon Tyne Hospitals NHS Foundation Trust	Newcastle upon Tyne	UK

Dr John C Mansfield	Newcastle Upon Tyne Hospitals NHS Foundation Trust	Newcastle upon Tyne	UK
Dr Mary-Anne Morris	Norfolk and Norwich University Hospitals NHS Foundation Trust	Norwich	UK
Dr Mark Tremelling	Norfolk and Norwich University Hospitals NHS Foundation Trust	Norwich	UK
Dr Charlie Charlton	Nottingham Children's Hospital	Nottingham	UK
Dr Sian Kirkham	Nottingham Children's Hospital	Nottingham	UK
Prof Chris Hawkey	Nottingham University Hospitals	Nottingham	UK
Dr Alison Simmons	Oxford University Hospitals	Oxford	UK
NA Michael Fung	Plymouth Hospitals NHS Trust	Plymouth	UK
Dr Stephen J Lewis	Plymouth Hospitals NHS Trust	Plymouth	UK
Dr Tony Lopez	University Plymouth NHS Hospitals	Plymouth	UK
Dr Jonathon A Snook	Poole Hospital NHS Foundation Trust	Poole	UK
Dr Mark Tighe	Poole Hospital NHS Foundation Trust	Poole	UK
Dr Pradeep Bhandari	Queen Alexandra Hospital	Portsmouth	UK
Dr Jon Simmons	Royal Berkshire Hospital, Royal Berkshire NHS Foundation Trust	Reading	UK
Dr Jonathan Simmons	Royal Berkshire Hospital, Royal Berkshire NHS Foundation Trust	Reading	UK
Dr Simon Lal	Salford Royal NHS Foundation Trust	Salford	UK
Dr Juliette Loehry	Salisbury District Hospital	Salisbury	UK
Dr Ahmed Dawood	Scunthorpe General Hospital	Scunthorpe	UK
Dr Alan Lobo	Royal Hallamshire Hospital	Sheffield	UK
Dr David Campbell	Sheffield Children's Hospital	Sheffield	UK
Dr Mark Smith	Shrewsbury and Telford Hospital NHS Trust	Shrewsbury	UK
Dr Gill Watts	Wythenshawe Hospital	South Manchester	UK
Dr Helen Dalal	James Cook University Hospital	South Tees	UK
Dr Helen Dallal	James Cook University Hospital	South Tees	UK
Dr Simon Panter	South Tyneside District Hospital	South Tyneside	UK
Dr Fraser R Cummings	Southampton General Hospital, University Hospital	Southampton	UK

	Southampton NHS Foundation Trust		
Dr Fraser JRF Cummings	Southampton General Hospital, University Hospital Southampton NHS Foundation Trust	Southampton	UK
Dr Suranga Dharmasiri	Southampton General Hospital, University Hospital Southampton NHS Foundation Trust	Southampton	UK
Dr Richard Felwick	Southampton General Hospital, University Hospital Southampton NHS Foundation Trust	Southampton	UK
Dr Zahid Mahmood	Stepping Hill Hospital	Stockport	UK
Dr Sandip Sen	Royal Stoke University Hospital	Stoke-on-Trent	UK
Dr Sandip Sen	University Hospitals of North Midlands	Stoke-on-Trent	UK
Dr Stephen Foley	King's Mill Hospital	Sutton-in-Ashfield	UK
Dr Emma Greig	Musgrove Park Hospital, Taunton and Somerset NHS Hospitals	Taunton	UK
Dr Emma Wesley	Musgrove Park Hospital, Taunton and Somerset NHS Hospitals	Taunton	UK
Dr Richard CG Pollok	St George's Healthcare NHS Trust	Tooting	UK
Dr Cathryn Edwards	Torbay and South Devon NHS Foundation Trust	Torquay	UK
Dr Mark Feeney	Torbay and South Devon NHS Foundation Trust	Torquay	UK
Dr John Beckly	Royal Cornwall Hospital	Truro	UK
Dr Praveen Rajasekhar	Northumbria NHS Trust	Tyne and Wear	UK
Dr Deven Vani	Mid Yorkshire Hospitals NHS Trust	Wakefield	UK
Dr Subramaniam Ramakrishnan	Warrington and Halton Hospitals NHS Foundation Trust	Warrington	UK
Dr Basant Chaudhary	Watford General Hospital, West Hertfordshire Hospitals NHS Trust	Watford	UK
Dr Rakesh Chaudhary	Watford General Hospital, West Hertfordshire Hospitals NHS Trust	Watford	UK
Dr Andy Bell	Weston General Hospital	Weston-super-Mare	UK
Dr Chris MacDonald	North Cumbria University Hospitals NHS Trust	Whitehaven	UK
Dr John N Gordon	Royal Hampshire County Hospital, Hampshire	Winchester	UK

	Hospitals NHS Foundation Trust		
Dr Matthew J Brookes	New Cross Hospital, The Royal Wolverhampton NHS Trust	Wolverhampton	UK
Dr Ian Gee	Worcestershire Royal Hospital	Worcester	UK
Dr Ashok Rai	Worcestershire Royal Hospital	Worcester	UK
Dr Andy Li	Worthing Hospital	Worthing	UK
Dr Zinu Philipose	Worthing Hospital	Worthing	UK
Dr Neeraj Prasad	Wrightington Hospital	Wrightington	UK
Dr Steve Gore	Yeovil District Hospital	Yeovil	UK
Dr Dave Border	York Teaching Hospital NHS Foundation Trust	York	UK
Dr Colin Jones	York Teaching Hospital NHS Foundation Trust	York	UK
Assistant Prof Ashwin N Ananthakrishnan	Massachusetts General Hospital	Boston	USA
Prof Mark J Daly	Broad Institute	Harvard	USA
Dr Jukka Koskela	Broad Institute	Harvard	USA
Assistant Prof Manuel A Rivas	Broad Institute	Harvard	USA
Dr Christine R Stevens	Broad Institute	Harvard	USA
Dr Ramnik Xavier	Broad Institute	Harvard	USA
Dr Dermot McGovern	Cedars-Sinai Medical Center	Los Angeles	USA
Mrs Jane F McGovern	Cedars-Sinai Medical Center	Los Angeles	USA

eAppendix 4.3-6. The International Serious Adverse Events Scientific Management Committee Members

Matt Nelson, PhD, GSK

Sally John, PhD, Pfizer

Jeffrey F Waring, PhD, Abbott

Scott Patterson, PhD, Amgen

Bryan J. Barratt, PhD, Astra-Zeneca

Joe Walker, PhD, Daiichi Sankyo

Peter Shaw, PhD, Merck

Steve Lewitzky, PhD, Novartis

Michael Dunn, PhD

eMethods in the Supplement

Identification of thiopurine-exposed unaffected individuals

Thiopurine-exposed patients with IBD were identified as unaffected individuals from the Exeter IBDGEN cohort (IBDGEN CLRN 9073). This cohort includes approximately 85% of patients under the care of the IBD team at The Royal Devon & Exeter (RD&E) NHS Trust; a specialist referral centre for IBD in the South West of England. In order to appropriately identify thiopurine-exposed unaffected individuals, the start and stop dates as well as the doses of thiopurines were extracted from paper and electronic medical records. Full blood count results were interrogated to identify the lowest absolute white blood cell count and absolute neutrophil count during the period of thiopurine therapy. Only patients with an absolute white blood cell count ≥ 3.0×10^9/L and absolute neutrophil count ≥ 1.5×10^9/L throughout their time on a thiopurine were included in the final control cohort.

Adjudication of thiopurine-induced myelosuppression affected individuals

Adjudication of all thiopurine-induced myelosuppression (TIM) affected individuals was undertaken using a series of expert panels to rigorously assess cases using a modified version of the validated Liverpool Adverse Drug Reaction Causality Assessment Tool (**eFigure 4.3-1 and eAppendix 4.3-2 in the Supplement**): "probable" TIM affected individuals must have demonstrated a clear temporal relationship with thiopurine and no other identifiable risk factors for TIM, including the concomitant use of other drugs recognised as causing myelosuppression (**eAppendix 4.3-3 in the Supplement**). In addition to these criteria, "definite" TIM affected individuals also developed a second episode of TIM after thiopurine rechallenge. The collective results from each panel member were collated and the panel discussed

discrepant cases before a final adjudication decision was reached. Only “definite” and “probable” TIM affected individuals were included in the final analyses.

Extraction of DNA

Two 6 mL EDTA blood samples (BD Vacutainer, USA) were taken from each participant in the discovery cohort and DNA was extracted using the Qiagen Autopure LS with Puregene chemistry (Qiagen NV, Venlo, Netherlands).

Genetic Data Sources and Measurement

GWAS: 245,185 variants were genotyped using the Illumina Infinium G4L Genome-Wide Association Study (GWAS) array (Illumina, San Diego, CA, USA) genotype calls were made using Birdsuite[439] at the Broad Institute, Boston, USA. We excluded individuals and variants with a call rate < 98% or minor allele frequency (MAF) < 1%. We also excluded individuals where the sex determined from the genetic data disagreed with the phenotype. The genotyping had been performed in two batches. On initial review of principal component analysis, there was a batch effect evident and so we removed any variants with an uncorrected *P* value of < .05 for association with batch on a chi-squared allelic test. Variants with Hardy Weinberg equilibrium (HWE) $P < 1\times10^{-6}$ in the unaffected individuals were excluded from further analysis. The relatedness of all samples was assessed from the GWAS data using KING 1.9[440] and for any pairs of samples whose scores suggested that they were third degree relatives or closer (kinship coefficient > 0.0442) one of the pair was excluded. Principal component analysis was carried out using Genome-wide Complex Trait Analysis (GCTA) v1.24[421] with data from the 1000 Genomes project.[422] Only individuals clustering with the non-Finnish European (NFE) individuals from the 1000 Genomes project were included. An overview of the numbers and reasons for individuals being excluded are displayed in **Figure 4.3-1**. We imputed the GWAS data into the

Haplotype Reference Consortium panel using EAGLE2 and PBWT (Positional Burrows-Wheeler Transform) and the Wellcome Trust Sanger imputation service.[423,424,441] We excluded SNPs with a post-imputation info score of < 0.85 or MAF < 0.01. Post QC, we had 6,272,335 variants.

ExWAS: Whole exome sequencing was performed in two batches. Exonic sequences were enriched using the Illumina Nextera prep kit and hybrid capture (Illumina Rapid Capture Enrichment - 37Mb target) and sequenced using the Illumina HiSeq platform (150bp paired reads). We processed exome sequencing reads in accordance with the Broad Institute best practice guidelines. Reads were mapped to the human genome reference sequence (GRCh37) using BWA-MEM. Each sample was sequenced to an average depth of 34×, with ~99% of the targeted regions covered by ≥ 1×, ~92% covered by ≥ 10× and ~70% covered by ≥ 25×. The Genome Analysis Toolkit v26[442] was used to call alleles at variant sites and the Variant Quality Score Recalibration (VQSR) pipeline was used to assess the quality of variant calls and only those passing quality control were included in the analyses. Variants with a Hardy Weinberg equilibrium (HWE) $P < 1\times10^{-6}$ were excluded as were any variants with a genotyping success rate of < 0.98. Additionally, we excluded any variants with a read depth of < 10x or with a genotype skew $P < 5\times10^{-9}$ (binomial test). To eliminate a possible batch effect, we compared allele frequencies in TIM affected individuals processed in the two batches and excluded variants that demonstrated an association with batch ($P <$ .05). We also checked for an association between the novel variant and the first five principal components to ensure that this was not due to residual population stratification. The post genotype quality control QQ plot is shown in **eFigure 4.3-2 in the Supplement**.

Sanger sequencing

Exon 1 and the intron/exon boundary of *NUDT15* was amplified by PCR using M13-tailed primers (ctctcgctttgatttcggcg and cacctcacagacgaactccc). The resulting amplicon was sequenced on an ABI 3730 Capillary sequencer (Applied Biosystems, CA, USA) using standard methods. Sequencing products were compared to the published sequence (NM_018283.2) using Mutation Surveyor Software v5.0.1 (SoftGenetics, PA, USA).

Gene Burden Test

We performed a collapsed variant association test using PLINK-seq 0.10[443] and Sequence Kernel Association Test (SKAT) as a SNP-set level test to evaluate if an association existed between sets of rare variants among TIM affected individuals and thiopurine-exposed IBD unaffected individuals. SKAT aggregates individual score test statistics of SNPs in a SNP set and efficiently computes SNP-set level *P*-values, e.g. a gene or a region level *P*-value, while adjusting for covariates, such as principal components to account for population stratification.

Genotype-Phenotype Calculations

We calculated the maximum azathioprine equivalent dose prior to TIM and adjusted this for weight according to the following formula:

- Weight adjusted thiopurine dose (mg/kg) = [mercaptopurine dose (mg)·2.08 / weight (kg)] OR [azathioprine dose (mg) / weight (kg)]
- Time to TIM was calculated using the following formula:
  Time TIM (weeks) = [date first met TIM criteria -
  date commenced maximum dose]

Clinical Usefulness Estimates: *NUDT15*

Although we report data from an observational case-control study, estimates for clinical usefulness are made according to adapted methods based on Tonk *et al*[430] and de Graaff *et al.*[431] For these estimates we assumed an overall risk of TIM of 7% (cumulative incidence),[346] avoidance of drug in individuals carrying *NUDT15* variants, overall population *NUDT15* coding variant carrier frequency of 1.6% (95% CI carriage frequency 1.5%-1.8%)[427] and used the unadjusted odds ratio taken from our multivariable logistic regression model for *NUDT15* variant carriers (OR = 27.3). In brief, we formed a 2×2 contingency table (Table A below), and used the following rearrangement of the odds ratio (OR) formula to derive a, b, c and d from p, q: $OR = \frac{\frac{a}{c}}{\frac{b}{d}}$

Noting also that:

$b = p - a$, c $= q - a$, $d = 1 - (p - a) - (q - a) - a$

a

$$= \frac{(p+q)(OR-1) + 1 - \sqrt{p^2(OR-1)^2 + 2p(-q \cdot OR^2 + q + OR - 1) + (q(OR-1)+1)^2}}{2(OR-1)}$$

Table A: 2x2 contingency tables

		Thiopurine-induced myelosuppression (TIM) Adverse Event		**Total**
		Affected	**Unaffected**	
Presence of one or more *NUDT15* variants	Positive (G1)	*a*	*b*	*p*
	Negative (G0)	*c*	*d*	*(1-p)*
	Total	*q*	*(1-q)*	*1*

		Thiopurine-induced myelosuppression (TIM) Adverse Event		**Total**
		Affected	**Unaffected**	
Presence of one or more *NUDT15* variants	Positive (G1)	0.0105	0.0060	0.0164
	Negative (G0)	0.0595	0.9240	0.9836
	Total	0.0700	0.9300	1.0000

Table B: Clinical validity estimates for TIM in patients with one or more of three deleterious coding variants in NUDT15

Term	Definition	Formula	Estimates for patients with one or more *NUDT15* variants
Carriage frequency (CF)	Population carriage frequency of three *NUDT15* variants (p.Gly17_Val18del, p.Arg139Cys, p.Gly17_Val18dup) from gnomAD[427]	p	0.0164
Sensitivity (Sens)	Probability that the genetic variants are present in those with TIM	$\mathrm{a/q}$	0.15
Specificity (Spec)	Probability that the genetic variants are absent in those without TIM	$\frac{\mathrm{d}}{(1-\mathrm{q})}$	0.99
Positive Predictive Value (PPV)	Probability of TIM when the genetic variants are present	$\mathrm{a/p}$	0.64
Negative Predictive Value (NPV)	Probability of no TIM when the genetic variants are absent	$\frac{\mathrm{d}}{(1-\mathrm{p})}$	0.94
Relative Risk (RR)	Ratio of the probability of TIM if variants present, and probability of TIM if variants absent	$\frac{\mathrm{a}/(\mathrm{a}+\mathrm{b})}{\mathrm{c}/(\mathrm{c}+\mathrm{d})}$	10.53
Absolute Risk Difference (ARD) (also known as attributable risk)	Difference in the probability of TIM if variants are present and the probability of TIM if variants are absent	$\frac{\mathrm{a}}{(\mathrm{a+b})} - \frac{\mathrm{c}}{(\mathrm{c+d})}$	0.58
Population attributable fraction (PAF)	Proportion of TIM that would be eliminated from the population if patients with variants were not exposed to thiopurine treatment	$\frac{\mathrm{p(RR-1)}}{(1+\mathrm{p(RR-1)})}$	0.14
Number needed to receive alternative treatment: Drug avoid	Number of patients with variants who need alternative treatment to prevent one patient from having TIM (**drug avoidance strategy**)	$\frac{1}{\mathrm{PPV}}$	1.57
Number needed to receive alternative treatment: Dose reduce	Number of patients with variants who need alternative treatment to prevent one patient from having TIM (**dose reduction strategy**)	$\frac{1}{\mathrm{ARD}}$	1.73

Number needed to harm (NNH)	Number of patients who need receive a thiopurine to see one case of TIM	$1/\mathrm{q}$	14.29
Number needed to genotype (NNG): **Drug avoid**	Number of patients that have to be genotyped to prevent one patient from having TIM: **drug avoidance** strategy in both heterozygotes and homozygotes reduces risk of TIM to zero	$1/\mathrm{a} \equiv \frac{(1/\mathrm{CF})}{\mathrm{Sens}}$	95.4
Number needed to genotype (NNG): **Dose reduce**	Number of patients that have to be genotyped to prevent one patient from having TIM: **dose reduction strategy** reduces risk of TIM in heterozygotes to that of wild-type affected individuals and avoidance of drug in homozygotes	$\frac{1}{(\mathrm{ARD} \cdot \mathrm{p})}$	105.4

Clinical Usefulness Estimations: *TPMT*

To calculate similar clinical usefulness estimates for *TPMT*, we assumed the following: a population prevalence for homozygotes[352] (G2) p = 0.0033 (1/300); a mitigation strategy of drug avoidance in homozygotes and dose reduction in heterozygotes; odds ratios (OR's) informed by our multivariable logistic regression model for *TPMT* variant carriers (OR = 2.2 and OR = 53.4 in *TPMT* heterozygotes and homozygotes, respectively). We used an iterative process to calculate one cell in the 3x2 table below and derived the remaining five cells from this.

Table C: 3x2 contingency tables

		Thiopurine-induced myelosuppression (TIM) Adverse Event		**Total**
		Affected	**Unaffected**	
TPMT variant status	Positive Homozygote (G2)	*a*	*b*	p
	Positive Heterozygote (G1)	*c*	*d*	$2(\sqrt{p})$-2 p
	Negative (G0)	*e*	*f*	$(1-\sqrt{p})^2$
Total		*q*	*1-q*	1

Note that p = population prevalence of $TPMT$ homozygotes = $1/300$

		Thiopurine-induced myelosuppression (TIM) Adverse Event		Total
		Affected	**Unaffected**	
TPMT variant status	**Positive Homozygote (G2)**	0.0020	0.0006	0.0026
	Positive Heterozygote (G1)	0.0121	0.0849	0.0970
	Negative (G0)	0.0558	0.8446	0.9004
Total		0.0700	0.9300	1

Figures given to 4 decimal places. Exact figures used to calculate clinical validity estimates below

Table D: Clinical validity estimates for TIM in patients with variants in TPMT

Term	Definition	Formula	Estimates for patients with one or more *TPMT* variants
Absolute risk in wild-type/ variants absent (AR_{G0})	Absolute risk of TIM in patients with wild-type/ absent variants genotype (AR_{G0})	$\frac{e}{(e+f)}$	0.06
Absolute risk in heterozygotes (AR_{G1})	Absolute risk of TIM in patients with heterozygote genotype (AR_{G1})	$\frac{c}{(c+d)}$	0.12
Absolute risk in homozygotes (AR_{G2})	Absolute risk of TIM in patients with heterozygote genotype (AR_{G2})	$\frac{a}{(a+b)} \equiv \frac{a}{p}$	0.78
Relative Risk in heterozygotes (RR_{G1})	Ratio of the probability of TIM in heterozygotes (AR_{G1}), and probability of TIM in patients with wild-type genotype (AR_{G0})	$\frac{c/(c+d)}{e/(e+f)}$	2.01
Relative Risk in homozygotes (RR_{G2})	Ratio of the probability of TIM in homozygotes (AR_{G2}), and probability of TIM in patients with wild-type genotype (AR_{G0})	$\frac{a/(a+b)}{e/(e+f)}$	12.56
Attributable risk G1 (=absolute risk difference)(ARD_{G1})	Difference in the probability of TIM if heterozygote (AR_{G1}) and probability of TIM in patients with wild-type genotype (AR_{G0})	$\frac{e}{(e+f)} - \frac{c}{(c+d)}$	0.06
Attributable risk G2 (=absolute risk difference)(ARD_{G2})	Difference in the probability of TIM if homozygote (AR_{G2}) and probability of TIM in patients with wild-type genotype (AR_{G0})	$\frac{e}{(e+f)} - \frac{a}{(a+b)}$	0.72
Population attributable fraction (PAF)	Proportion of TIM that would be eliminated from the population if patients with variant were not exposed to thiopurine treatment	$\frac{(q - [\mathrm{ARG0}])}{q}$	0.11
Number needed to genotype: Drug avoidance	Number of patients that have to be genotyped to prevent one patient from having TIM:	$\frac{1}{(a+c)}$	70.7

heterozygotes (and drug avoidance in homozygotes	**drug avoidance strategy** in both heterozygotes (G1) and homozygotes (G2)		
Number needed to genotype: Dose reduction **in heterozygotes (and drug avoidance in homozygotes)**	Number of patients that have to be genotyped to prevent one patient from having TIM: **dose reduction strategy** reduces risk of TIM in heterozygotes to that of wild-type affected individuals (G0) and avoidance of drug in homozygotes (G2)	See formula below for NNG [dose reduction]	123.0

$$\mathrm{NNG[dose\ reduction]} = \frac{1}{(\mathrm{ARG2} \cdot \mathrm{p}) + \left((\mathrm{ARG1} - \mathrm{ARG0}) \cdot 2\left(\sqrt{p} - p\right)\right)}$$

Estimation of confidence intervals around population carrier frequencies and number needed to genotype calculations

To provide a confidence interval for the number needed to genotype point estimate requires three inputs:

1. Odds ratio of thiopurine-induced myelosuppression for those carrying genetic variant versus patients with reference genotype (taken from the multivariable model in the manuscript)

2. Population probability of carrying one or more *NUDT15* variants (a dominant model was used as it is noted that with a low minor allele frequency (MAF), compound heterozygotes and homozygotes are rare, and no estimate for the effect size in those individuals was available)

3. Population probability of thiopurine-induced myelosuppression

To generate confidence intervals for the external estimates for the minor allele frequency or carriage frequency, 10,000 bootstrapped replicates of our own genotype/phenotype data were generated. For each one, the probability of thiopurine-induced myelosuppression was also randomly simulated using a binomial distribution centered on the best published estimate of 0.07 and a sample size of 8302 (from meta-analysis by Gisbert JP and Gomollón F. Thiopurine-induced myelotoxicity in patients with inflammatory bowel disease: A Review. American Journal of Gastroenterology. 2008).[346] For the genetic data, the minor allele frequency was again simulated for each of our three variants using a binomial distribution and the population size of 101458 or 126510 alleles in gnomAD. Overall rate of carrying one or more relevant *NUDT15* variants = $1 - ((1 - MAF_1 – MAF_2)^2 \times (1 - MAF_3)^2)$. For *TPMT,* the minor allele frequency was calculated using data in a large British cohort.[432]

The final estimates are:

Variable	Point estimate	2.5%tile	97.5%tile
Probability of TIM in all thiopurine-exposed IBD patients	0.070	0.064	0.076
Probability of carrying ≥1 TIM-associated *NUDT15* variants in non-Finnish Europeans	0.016	0.015	0.018
Odds ratio for ≥1 TIM-associated *NUDT15* variants of TIM	27.3	10.9	33805857.1
Positive predictive value of ≥1 TIM-associated *NUDT15* variants for TIM	63.7%	42.7%	100.0%
Number needed to genotype for *NUDT15* (avoidance strategy)	95	62	143
Number needed to genotype for *NUDT15* (dose reduction strategy)	105	65	168
Minor allele frequency of combined *TPMT* loss of function haplotypes	0.051	0.044	0.058
Odds ratio for 1 *TPMT* loss of function haplotype for TIM	2.16	1.40	3.29
Odds ratio for 2 *TPMT* loss of function haplotypes for TIM	53.4	14.6	54566558.2
Positive predictive value for 1 *TPMT* loss of function haplotype for TIM	12.5%	8.8%	16.8%
Positive predictive value for 2 *TPMT* loss of function haplotypes for TIM	77.9%	49.5%	100.0%
Number needed to genotype for *TPMT* (avoidance strategy)	71	52	98
Number needed to genotype for *TPMT* (dose reduction strategy for 1 loss of function haplotype, avoidance for 2)	123	75	235

Note that occasionally the bootstrapping resulted in a very large odds ratio as one may end up with combinations that have probabilities of TIM of 1; however, the NNG is robust to this.

4.3.8.1 Article Information

Corresponding Author:

Dr Tariq Ahmad, DPhil, Department of Gastroenterology, Exeter IBD Research Team, Royal Devon and Exeter Hospital (Wonford), Barrack Road, Exeter, United Kingdom, EX2 5DW

Author Contributions:

G.W. and N.K. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: T.A., G.W., G.H., C.B., K.S., A.S.

Project manager: H.O.

Acquisition, analysis, or interpretation of data: G.W., J.H., G.H., M.V., J.K., M.W., N.K.

Acquisition of replication cohort: R.W., H.S., D.McG., L.B., P.S., R.X., E.F., A.A.

Adjudication of affected patients: T.A., G.W., G.H., J.H., N.K., J.G., N.H., P.H., A.S., J.L., M.T., D.G., S.L., P.I., T.C., E.G., A.T., A.C., R.R., A.D., S.S., A.H., J.M., T.O., S.R., S.D., T.D., R.P., J.G., C.M., D.R., C.C.

Genotyping and exome sequencing: M.D.

Sanger sequencing: Re.W.

Drafting of the manuscript: G.W., J.H., G.H., C.B., J.G., N.K., T.A. D.McG

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: G.W., J.H., M.V., J.K., N.K.

Obtained funding: T.A., G.W., C.B., G.H.

Administrative, technical, or material support: All remaining authors contributed by submitting a substantial number of samples in line with ICMJE criteria

4.4 Discussion

4.4.1 How the chapter addresses the aims and objectives of the thesis

Objective 7: *To investigate the association between novel genetic variants and thiopurine-induced myelosuppression in European patients with IBD*

First, in a genome-wide association study, I confirmed the known association with thiopurine methyltransferase (*TPMT*) and thiopurine-induced myelosuppression (TIM) in European IBD patients: a variant *TPMT* single nucleotide polymorphism (rs11969064) was found in 31% (95/311) of affected patients compared with 16% (100/608) of unaffected patients (OR 2.3 [95% CI, 1.7 to 3.1], $P = 5.2 \times 10^{-9}$). No other genetic associations with TIM exceeded the *a priori* threshold for statistical significance.

TPMT activity is inherited as a monogenic, autosomal co-dominant trait, and its association and importance in thiopurine metabolism and toxicity has long been established.[352] TPMT catabolizes mercaptopurine to an inactive methylmercaptopurine base, leaving less parent drug available for eventual anabolism to active thioguanine nucleotides (TGNs).[429,444] The thioguanine nucleotides are responsible for the immunosuppressive action of thiopurines and inhibit *de novo* purine synthesis.[445] Individuals with one loss-of-function allele (heterozygotes) are at slight increased risk, whilst those with two loss-of-function alleles (homozygotes) are at profound increased risk of myelosuppression unless dosing is reduced accordingly. Pre-treatment testing of TPMT is recommended to identify patients at risk of TIM.[446]

Second, using whole-exome sequence data, which facilitates the detection of rare variants, I also identified an association with a 6-base-pair in-frame deletion (p.Gly17_Val18del) in nudix (nucleoside diphosphate linked moiety X)-type motif 15 (*NUDT15*). This association was then replicated in an independent cohort of European IBD patients. In total I found three coding *NUDT15* variants in our data-set (p.Gly17_Val18del, p.Gly17_Val18dup and p.Arg139Cys) that were present in 9.5% of cases and 0.5% of controls (OR 20.9 [95% CI, 6.4 to 68.6], $P = 1.5 \times 10^{-12}$).

The protein encoded by *NUDT15* is thought to hydrolyse and inactivate one of the thioguanine nucleotide (TGN) active metabolites called thioguanine triphosphate, which when incorporated into DNA leads to futile mismatch repair and apoptosis.[447] Therefore, patients with variants that reduce *NUDT15* activity accumulate higher levels of certain active TGNs that are thought to lead to myelosuppression. However, current clinical assays for TGNs do not distinguish tri- from di- from mono-phosphates, and therefore TGN levels cannot be used to identify low NUDT15 activity.[446] Recent evidence suggests that thioguanine incorporated into red blood cell DNA may be an indicator of NUDT15 status in patients receiving thiopurines, but this assay is not widely available and further work is needed prior to clinical use. [415]

The frequency of *TPMT* variants is lower in East Asian as compared with European populations (~3% versus ~10%), however, the incidence of TIM in Asians is considerably higher.[360,362,448,449] Studies in Asian patients have recently identified variants in *NUDT15* as risk factors for TIM.[362,418,450,451] My study is the first to report association between *NUDT15* variants and TIM at the genome-wide significance level

and suggests that these variants are also clinically relevant in Europeans populations.[416]

Objective 8: *If genetic variants are present, to explore if the frequency of these variants were enriched in those patients with early drug reactions (≤ 8 weeks from start of maximum dose)*

As hypothesised, the association between the p.Gly17_Val18del variant and TIM was enriched in cases occurring within 8 weeks of first commencing the maximum dose of thiopurine. In a meta-analysis of over 8000 patients, Gisbert and Gomollón reported that TIM occurred as soon as 12 days but up to 27 years after commencing treatment.[346] In another study by Lewis *et al* looking at the timing of myelosuppression among 1997 new thiopurine users, the authors found that the median time from onset of therapy to first documentation of severe leukopenia was 25 days (range, 15–53 days).[452] This is much faster than in my study where the median time to TIM was 28 weeks (IQR, 9–81weeks) and the median time from maximum dose of thiopurine to TIM was 15 weeks (IQR, 6–38 weeks). Our definitions of myelosuppression were similar and therefore these differences likely reflect the use of pre-treatment TPMT testing, which was excluded in the Lewis *et al* study, but used in two-thirds of the patients recruited to mine. Lewis *et al* also reported that the majority of TIM events occurred within the first 8 weeks of starting treatment: during this period, the incidence of severe leukopenia per 100 person-months was 0.16 in comparison to 0.01 after week 26.[452] The authors suggested that stable mild leucopenia was likely attributable to changes in drug adherence or body weight, whereas rapid onset severe leucopenia was probably a consequence of either drug interaction or infection.[452]

Whilst *TPMT* (Chromosome 6) and *NUDT15* (Chromosome 13) are inherited separately, the likelihood of an individual being an intermediate metabolizer for both genes depends upon the population frequencies of the variant alleles. Hitherto, it was unclear what effect this compound intermediate genotype would have. In my study I found that the median time to TIM was shortest in patients with *both TPMT* and *NUDT15* variants compared with affected patients without risk variants. Whereas, no difference in time to TIM was seen in patients carrying one variant *TPMT* haplotype compared with affected patients without risk variants.

In the multivariable logistic regression, I demonstrated that weight-adjusted dose, and *NUDT15* and *TPMT* genotypes were independently associated with TIM and that there was no significant interaction between *NUDT15* and *TPMT* in the model.

Together these data endorse the current practice of short interval blood monitoring in the early stages of thiopurine treatment when the risk of myelotoxicity is greatest, however, the occurrence of late toxicity, even in patients with genetic variants means that long-term blood monitoring is still required.

Objective 9: *the clinical phenotype and morbidity related to carriage of a TIM associated genetic variant(s)*

In the genotype-phenotype analysis, I found that patients carrying *NUDT15* and/or *TPMT* variants experienced a more profound myelosuppressive episode than affected patients without these variants with lower neutrophil counts, higher rates of hospitalisation and use of rescue granulocyte colony–stimulating factor (GCSF) therapy. However, my study design precluded more definitive conclusions being

drawn on the genotype-phenotype interaction which would require a prospective cohort design.

Importantly for the consideration of future prescribing in European patients, I found that neither weight-adjusted dose, type of thiopurine, patient age, *TPMT* genotype, nor *NUDT15* genotype were associated with subsequent thiopurine tolerance after drug rechallenge.

The UK IBD pharmacogenetics group have deemed that a drug-avoidance strategy is the most appropriate for European patients, whereas the latest 2018 CPIC guidelines, written prior to our publication, recommend a 30–80% mercaptopurine dose reduction in intermediate NUDT15 metabolisers and only drug-avoidance for non-malignant treatment indications in poor NUDT15 metabolisers. [446]

Objective 10: *To ascertain the clinical validity (e.g. sensitivity, specificity, negative and positive predictive values) of genetic testing to identify patients at risk of TIM*

Although I report data from an observational case-control study, which by design have a different proportion of cases than in the true population of interest, one can still make estimates of clinical utility according to adapted methods based on Tonk *et al*[430] and de Graaff *et al*[431]. Tonk *et al* stated that the clinical validity of a pharmacogenetic test is determined not only by the strength of the association between the genetic variant and the adverse event, but also, by the frequencies of the genetic variant and the adverse event; therefore, a strong association is essential but not a sufficient condition to ensure good clinical validity.

The clinical validity then in turn influences the *clinical utility* of the test, which in my study is the ability of *NUDT15* genotyping to prevent TIM through the use of mitigating strategies including dose reduction or drug avoidance. Therefore, an assessment of clinical utility of *NUDT15* testing needs to go beyond the strength of the association alone.

In order to aid translation of pharmacogenetic testing I calculated clinical validity estimates for *NUDT15* in accordance with CPIC guidelines.[395] Accordingly, I used the genetic variant frequency for the total population (p) from the gnomAD reference database[453] (1.6%, 95% CI, 1.5%-1.8%) and the cumulative incidence for TIM (7%, 95% CI, 6%-8%) based on a meta- analysis of 8302 patients by Gisbert *et al*[346] to enable completion of cells a to d in the 2x2 contingency table (see Figures 4-1 and 4-2).

Figure 4.4-1. Blank 2x2 contingency table

		Adverse Event		Total
		Affected	**Unaffected**	
Presence of one or more *NUDT15* variant	**Positive (G1)**	a	b	p
	Negative (G0)	c	d	(1-p)
	Total	q	(1-q)	1

Figure 4.4-2. Completed 2x2 contingency table

		Adverse Event		Total
		Affected	**Unaffected**	
Presence of one or more *NUDT15* variant	**Positive (G1)**	1.05	0.60	1.64
	Negative (G0)	5.95	92.40	98.36
	Total	7.00	93.00	1.00

These tables can be constructed using empirical data or using hypothetical data calculated from summary statistics and association measures, such as odds ratios derived from observational studies with a case–control design in combination with the frequencies of the genetic variant and the adverse event. The following formulae can be used to derive cells a to d in accordance with methods by Tonk *et al* [430]

p = variant frequency (taken from reference database-gnomAD)

q = adverse event frequency (taken from meta-analysis by Gisbert el al)

$$a = \left(\left(\left(p \times OR + (1-p) + q \times (OR-1)\right) - \sqrt{\left(\left(-p \times OR - (1-p) - q \times (OR-1)\right)^{2} - 4 \times (OR-1) \times p \times q \times OR\right)}\right)\right) / \left(2 \times (OR-1)\right)$$

$$b = p - a$$

$$c = q - a$$

$$d = 1 - a - b - c$$

For *NUDT15*, the estimated number of patients needed to genotype (NNG) to prevent one patient from developing TIM was 95 patients (95% CI, 62-143 patients). That is, for every 10,000 patients genotyped, 164 would test positive for a *NUDT15* variant, and of these patients, 105 would have developed TIM if they had not received an alternative treatment (PPV = 64%). Genotyping 10,000 patients for *NUDT15* would therefore prevent 105 cases of TIM, which is 95 patients genotyped for every case prevented. Finally, I estimated that introduction *NUDT15* testing would reduce TIM by 14% (population attributable fraction). Even if one uses a dose-reduction rather than drug-avoidance strategy the NNG only increases marginally to 105.

My clinical validity estimates for *NUDT15* are similar to those previously reported by others for *TPMT*[435] (NNG = 100), and therefore support the extension of *NUDT15* pre-treatment genetic testing to European patients.

4.4.2 The Implications for future practice

Whilst *NUDT15* variants have previously been shown to be important in patients of East Asian[362,418,450], South Asian[451] and South American[419] ancestry who suffer TIM, this is the first time that a genetic association has been described in a large European cohort at the genome-wide significance level. In order to bridge the gap from 'bench-to-bedside' and translate this discovery into clinical practice several factors need to be considered.

Traditionally, a prospective randomised-control trial (RCT) is next required to assess the clinical utility and cost-effectiveness of a drug or biomarker. However, it would be ethically unacceptable to randomize patients with and without *TPMT* and *NUDT15* variants to receive thiopurines given the risks of myelosuppression and potentially fatal consequences.[346] An alternative methodology utilizes a 'historic-cohort study' design, where historical data relating to disease outcome and costs of current best practice are compared with prospective data captured after the introduction of pharmacogenetic testing. This study design focuses recruitment on just one arm of the study, thus maximising the power to detect outcome differences in patients with rare variants. It is worth noting in the case of *NUDT15*, one would need to recruit an estimated 10000 IBD patients to find 164 variant carriers.

Experts have argued that to hold biomarkers to the same level of evidence as required for a new drug unjustifiably slows progress in the field of precision medicine.[408] Advocates of this argument might cite the widespread successful implementation of pre-abacavir treatment HLA-B*5701 testing as an example of a pharmacogenetic test that was adopted without RCT data.[329]

The next decision to be made prior to rolling-out *NUDT15* testing, is whether to recommend a dose-reduction or drug-avoidance strategy. The Clinical Pharmacogenetics Implementation Consortium (CPIC) have recently released their guidance for thiopurine-dosing based on *TPMT* and *NUDT15* genetics in 2018.[446] The publication of these recommendations, predated our own publication and were largely based on data in patients of Asian ancestry with Acute Lymphoblastic Leukaemia. The CPIC authors have recommended a 30-80% mercaptopurine dose-reduction in 'intermediate' heterozygote *NUDT15* metabolizers; whilst *NUDT15* homozygotes with 'poor' functional enzyme activity may tolerate a 10-fold dose reduction.[446] Whether to risk myelosuppression in this latter group is dependent on the indication for thiopurine treatment; a risk justified perhaps in patients with malignant disease. In IBD we propose a drug-avoidance strategy for patients with *NUDT15* variants as two-thirds of European patients carrying one of three *NUDT15* variants developed TIM, and rechallenge with a thiopurine was rarely successful even at a lower dose. The consequences of our drug-avoidance strategy are more profound for the large number of South Asians living in the UK; 14% of whom carry an *NUDT15* variant (see Appendix B). However, in contrast to even a few years ago, there are now a growing number of safe and arguably more effective alternative IBD therapies.[454–456] Furthermore, I feel that it would introduce unnecessary complexity to offer different advice to patients of different ethnicities, not least because self-reported ethnicity is notoriously unreliable.

Following consultation at the Personalised Medicine NHS review board in 2018 no further prospective studies were deemed necessary prior to rolling out *NUDT15* genotyping to the UK and this service will shortly be rolled-out to the NHS, starting in Exeter.

Chapter 5

5 Conclusion

Individual patient variability may explain why despite a growing number of therapeutic options in the treatment of inflammatory bowel disease (IBD) many patients still suffer disabling disease. Precision medicine aims to address this dilemma by improving the timing and delivery of healthcare for each patient by targeting treatment according to the application of biomarkers. I focused on two key components of precision IBD medicine: accelerating the time to diagnosis through biomarker application and the use of pharmacogenetics to facilitate safe drug prescribing.

I found that faecal calprotectin was a clinically useful biomarker that helped General Practitioners (GPs) identify IBD in young adults and children. In adults, raising the cut-off threshold used to distinguish positive from negative calprotectin tests from 50μg/g to 100μg/g doubled the positive predictive value with a negligible loss in negative predictive value. However, there was a 14% false negative rate which requires GPs to safety-net calprotectin negative patients. Among paediatric IBD patients, there were no false negative calprotectin tests and this test was superior to both symptoms and CRP in distinguishing IBD from non-IBD. This makes it an attractive non-invasive alternative method to conventional bloods tests for the targeted assessment of paediatric patients. In adults over the first three years following introduction of the test to primary care, I estimated that calprotectin saved 200 referrals- and £52,000 -per year.

However, further significant savings are achievable if primary and secondary care physicians take greater confidence in this test.

The median time to diagnosis in adults and children was 4 and 2 months, respectively; the greatest component of which in both cohorts was the time it took patients to present to their GP. A positive primary care calprotectin was neither associated with a reduction in the time to GP referral nor the time to secondary care diagnosis. However, uptake in adults and children was low and only used prior to a new IBD diagnosis in approximately one-quarter of patients. In adults, one-fifth of patients were diagnosed with IBD following an emergent presentation, which was associated with a higher inflammatory burden, more severe and extensive disease and a greater need for hospitalisation and biologic treatment in the first year after diagnosis as compared with non-emergent IBD diagnoses. Conversely, a delayed diagnosis was not associated with a complicated disease course.

Using agnostic genome-wide and exome-wide methodologies to explore thiopurine-induced myelosuppression. I discovered a novel association with a variant in *NUDT15* and reported that patients with *TPMT* and/or *NUDT15* variants experience a quicker and more severe myelosuppressive phenotype. Thiopurine dose-reduction was not associated with subsequent rechallenge success and therefore a drug-avoidance rather than dose-reduction strategy in patients carrying *NUDT15* variants should be encouraged. The clinical validity estimate for the number needed to genotype is similar to that of *TPMT*, which is already commonly measured prior to initiating thiopurine treatment and supports extension of pre-treatment testing to *NUDT15*.

The actionable findings reported in this thesis have led to changes in clinical practice locally and nationally and will help deliver precision medicine in the field of IBD. The primary care calprotectin study has demonstrated how a faecal biomarker can help prioritise outpatient referrals and deliver cost-savings, leading to the adoption of our clinical pathway across several UK sites. Our new revised pathway has now been rolled-out locally and uses a single calprotectin cut-off of 100µg/g, an 8-week GP safety-net review of patient symptoms, a direct-to-test option in patients with a calprotectin of ≥ 250µg/g, and a ring-fenced dietician who offers specialist dietetic advice for patients with functional gut disorders. Further work is needed to promote both a greater public awareness of the need for patients to seek medical help with persistent or worrisome new lower GI symptoms and to encourage greater use of calprotectin among primary care physicians. Furthermore, we also need to evaluate whether use of calprotectin actually delivers on our predicted savings with a measurable reduction in endoscopy referrals and increase in diagnostic yield. Locally, faecal immunohistochemical testing (qFIT) has been introduced for patients greater than 50 years old with new lower GI symptoms not meeting two-week wait criteria. We are in the process of working with the qFIT steering group in order to ensure that GPs have a clear coherent strategy for testing and referring patients of all ages.

The identification of *NUDT15* variants as determinants of thiopurine-induced myelosuppression in European individuals has led to the rapid development of an NHS clinical service from the Exeter molecular genetics laboratory and in due course adoption of the test to the National Genomic Test Directory. In time further work is needed to evaluate the impact of this pharmacogenetic test on disease-

related outcomes and treatment costs. Prospective data collection after commencing the *NUDT15* testing service will hopefully enable identification of further *NUDT15* variants

REFERENCES

1. Denson LA, Curran M, McGovern DPB, et al. Challenges in IBD Research: Precision Medicine. *Inflammatory Bowel Diseases*. 2019;25(Supplement_2):S31-S39. doi:10.1093/ibd/izz078

2. Burisch J, Zammit SC, Ellul P, et al. Disease course of inflammatory bowel disease unclassified in a European population-based inception cohort: An Epi-IBD study. *Journal of gastroenterology and hepatology*. December 2018. doi:10.1111/jgh.14563

3. Loftus E V, Sandborn WJ. Epidemiology of inflammatory bowel disease. *Gastroenterology clinics of North America*. 2002;31(1):1-20.

4. Ng SC, Shi HY, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet (London, England)*. 2018;390(10114):2769-2778. doi:10.1016/S0140-6736(17)32448-0

5. Molodecky NA, Soon IS, Rabi DM, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology*. 2012;142(1):46-54.e42; quiz e30. doi:10.1053/j.gastro.2011.10.001

6. Shivananda S, Lennard-Jones J, Logan R, et al. Incidence of inflammatory bowel disease across Europe: is there a difference between north and south? Results of the European Collaborative Study on Inflammatory Bowel Disease (EC-IBD). *Gut*. 1996;39(5):690-697.

7. Hamilton B, Heerasing N, Hendy PJ, et al. PTU-010 Prevalence and phenotype of IBD across primary and secondary care: implications for colorectal cancer surveillance. *Gut*. 2018;67(Suppl 1):A67-A67. doi:10.1136/gutjnl-2018-bsgabstracts.132

8. Jones G-R, Lyons M, Plevris N, et al. IBD prevalence in Lothian, Scotland, derived by capture-recapture methodology. *Gut*. 2019;68(11):1953-1960. doi:10.1136/gutjnl-2019-318936

9. Shah SC, Khalili H, Gower-Rousseau C, et al. Sex-Based Differences in Incidence of Inflammatory Bowel Diseases-Pooled Analysis of Population-Based Studies From Western Countries. *Gastroenterology*. 2018;155(4):1079-1089.e3. doi:10.1053/j.gastro.2018.06.043

10. Ortizo R, Lee SY, Nguyen ET, Jamal MM, Bechtold MM, Nguyen DL. Exposure to oral contraceptives increases the risk for development of inflammatory bowel disease. *European Journal of Gastroenterology & Hepatology*. 2017;29(9):1064-1070. doi:10.1097/MEG.0000000000000915

11. Rolston VS, Boroujerdi L, Long MD, et al. The Influence of Hormonal Fluctuation on Inflammatory Bowel Disease Symptom Severity—A Cross-Sectional Cohort Study.

Inflammatory Bowel Diseases. 2018;24(2):387-393. doi:10.1093/ibd/izx004

12. Torres J, Mehandru S, Colombel J-F, Peyrin-Biroulet L. Crohn's disease. *Lancet (London, England)*. 2017;389(10080):1741-1755. doi:10.1016/S0140-6736(16)31711-1

13. Becker HM, Grigat D, Ghosh S, et al. Living with inflammatory bowel disease: A Crohn's and Colitis Canada survey. *Canadian journal of gastroenterology & hepatology*. 2015;29(2):77-84.

14. van der Valk ME, Mangen M-JJ, Leenders M, et al. Risk factors of work disability in patients with inflammatory bowel disease--a Dutch nationwide web-based survey: work disability in inflammatory bowel disease. *Journal of Crohn's & colitis*. 2014;8(7):590-597. doi:10.1016/j.crohns.2013.11.019

15. Loftus E V, Skup M, Ozbay AB, et al. The impact of moderate-to-severe Crohn's Disease on employees' salary growth. *Inflammatory bowel diseases*. 2014;20(10):1734-1738. doi:10.1097/MIB.0000000000000133

16. Solberg IC, Vatn MH, Høie O, et al. Clinical course in Crohn's disease: results of a Norwegian population-based ten-year follow-up study. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association*. 2007;5(12):1430-1438. doi:10.1016/j.cgh.2007.09.002

17. Burisch J, Kiudelis G, Kupcinskas L, et al. Natural disease course of Crohn's disease during the first 5 years after diagnosis in a European population-based inception cohort: an Epi-IBD study. *Gut*. 2018;68(3):423-433. doi:10.1136/gutjnl-2017-315568

18. Burisch J, Katsanos KH, Christodoulou DK, et al. Natural Disease Course of Ulcerative Colitis during the First Five Years of Follow-up in a European Population-based Inception Cohort - An Epi-IBD Study. *Journal of Crohn's and Colitis*. 2019;13(2):198-208. doi:10.1093/ecco-jcc/jjy154

19. Parragi L, Fournier N, Zeitz J, et al. Colectomy Rates in Ulcerative Colitis are Low and Decreasing: 10-year Follow-up Data From the Swiss IBD Cohort Study. *Journal of Crohn's and Colitis*. 2018;12(7):811-818. doi:10.1093/ecco-jcc/jjy040

20. Eaden JA, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut*. 2001;48(4):526-535. doi:10.1136/GUT.48.4.526

21. Satsangi J, Grootscholten C, Holt H, Jewell DP. Clinical patterns of familial inflammatory bowel disease. *Gut*. 1996;38(5):738-741. doi:10.1136/gut.38.5.738

22. Thompson NP, Driscoll R, Pounder RE, Wakefield AJ. Genetics versus environment in inflammatory bowel disease: results of a British twin study. *BMJ (Clinical research ed)*. 1996;312(7023):95-96.

23. Halfvarson J, Bodin L, Tysk C, Lindberg E, Järnerot G. Inflammatory bowel disease in a Swedish twin cohort: a long-term follow-up of concordance and clinical characteristics. *Gastroenterology*. 2003;124(7):1767-1773.

24. Roth MP, Petersen GM, McElree C, Vadheim CM, Panish JF, Rotter JI. Familial empiric risk estimates of inflammatory bowel disease in Ashkenazi Jews. *Gastroenterology*. 1989;96(4):1016-1020.

25. Halme L, Paavola-Sakki P, Turunen U, Lappalainen M, Farkkila M, Kontula K. Family and twin studies in inflammatory bowel disease. *World journal of gastroenterology*. 2006;12(23):3668-3672. doi:10.3748/WJG.V12.I23.3668

26. Uhlig HH, Schwerd T. From Genes to Mechanisms: The Expanding Spectrum of Monogenic Disorders Associated with Inflammatory Bowel Disease. *Inflammatory Bowel Diseases*. 2015;22(1):202-212. doi:10.1097/mib.0000000000000614

27. Cleynen I, Boucher G, Jostins L, et al. Inherited determinants of Crohn's disease and ulcerative colitis phenotypes: a genetic association study. *Lancet (London, England)*. 2016;387(10014):156-167. doi:10.1016/S0140-6736(15)00465-1

28. de Lange KM, Moutsianas L, Lee JC, et al. Genome-wide association study implicates immune activation of multiple integrin genes in inflammatory bowel disease. *Nature Genetics*. 2017;49(2):256-261. doi:10.1038/ng.3760

29. Hugot J-P, Laurent-Puig P, Gower-Rousseau C, et al. Mapping of a susceptibility locus for Crohn's disease on chromosome 16. *Nature*. 1996;379(6568):821-823. doi:10.1038/379821a0

30. Mirza MM, Lee J, Teare D, et al. Evidence of linkage of the inflammatory bowel disease susceptibility locus on chromosome 16 (IBD1) to ulcerative colitis. *Journal of Medical Genetics*. 1998;35(3):218-221. doi:10.1136/jmg.35.3.218

31. Cooney R, Baker J, Brain O, et al. NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. *Nature Medicine*. 2010;16(1):90-97. doi:10.1038/nm.2069

32. Satsangi J, Parkes M, Louis E, et al. Two stage genome–wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. *Nature Genetics*. 1996;14(2):199-202. doi:10.1038/ng1096-199

33. Verstockt B, Smith KG, Lee JC. Genome-wide association studies in Crohn's disease: Past, present and future. *Clinical & Translational Immunology*. 2018;7(1):e1001. doi:10.1002/cti2.1001

34. Hampe J, Franke A, Rosenstiel P, et al. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. *Nature Genetics*. 2007;39(2):207-211. doi:10.1038/ng1954

35. Parkes M, Barrett JC, Prescott NJ, et al. Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. *Nature Genetics*. 2007;39(7):830-832. doi:10.1038/ng2061

36. McGuckin MA, Eri R, Simms LA, Florin THJ, Radford-Smith G. Intestinal barrier dysfunction in inflammatory bowel diseases. *Inflammatory Bowel Diseases*.

2009;15(1):100-113. doi:10.1002/ibd.20539

37. Ellinghaus D, Jostins L, Spain SL, et al. Analysis of five chronic inflammatory diseases identifies 27 new associations and highlights disease-specific patterns at shared loci. *Nature genetics*. 2016;48(5):510-518. doi:10.1038/ng.3528

38. Rennick D, Davidson N, Berg D. Interleukin-10 gene knock-out mice: a model of chronic inflammation. *Clinical immunology and immunopathology*. 1995;76(3 Pt 2):S174-8.

39. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. I NTERLEUKIN -10 AND THE I NTERLEUKIN -10 R ECEPTOR. *Annual Review of Immunology*. 2001;19(1):683-765. doi:10.1146/annurev.immunol.19.1.683

40. Jostins L, Ripke S, Weersma RK, et al. Host–microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature*. 2012;491(7422):119-124. doi:10.1038/nature11582

41. Franke A, McGovern DPB, Barrett JC, et al. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nature Genetics*. 2010;42(12):1118-1125. doi:10.1038/ng.717

42. Anderson CA, Boucher G, Lees CW, et al. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nature genetics*. 2011;43(3):246-252. doi:10.1038/ng.764

43. Ye BD, McGovern DPB. Genetic variation in IBD: progress, clues to pathogenesis and possible clinical utility. *Expert review of clinical immunology*. 2016;12(10):1091-1107. doi:10.1080/1744666X.2016.1184972

44. WGS vs. WES - AllSeq. https://allseq.com/kb/wgsvswes/. Accessed June 9, 2019.

45. Luo Y, de Lange KM, Jostins L, et al. Exploring the genetic architecture of inflammatory bowel disease by whole-genome sequencing identifies association at ADCY7. *Nature genetics*. 2017;49(2):186-192. doi:10.1038/ng.3761

46. Hunt KA, Mistry V, Bockett NA, et al. Negligible impact of rare autoimmune-locus coding-region variants on missing heritability. *Nature*. 2013;498(7453):232-235. doi:10.1038/nature12170

47. Misra R, Faiz O, Munkholm P, Burisch J, Arebi N. Epidemiology of inflammatory bowel disease in racial and ethnic migrant groups. *World journal of gastroenterology*. 2018;24(3):424-437. doi:10.3748/wjg.v24.i3.424

48. Jayanthi V, Probert CS, Pinder D, Wicks AC, Mayberry JF. Epidemiology of Crohn's disease in Indian migrants and the indigenous population in Leicestershire. *The Quarterly journal of medicine*. 1992;82(298):125-138.

49. Fellows IW, Freeman JG, Holmes GK. Crohn's disease in the city of Derby, 1951-85. *Gut*. 1990;31(11):1262-1265.

50. Probert CS, Jayanthi V, Hughes AO, Thompson JR, Wicks AC, Mayberry JF. Prevalence and family risk of ulcerative colitis and Crohn's disease: an epidemiological study among Europeans and south Asians in Leicestershire. *Gut*. 1993;34(11):1547.

51. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*. 2014;505(7484):559. doi:10.1038/NATURE12820

52. Parada Venegas D, De la Fuente MK, Landskron G, et al. Short Chain Fatty Acids (SCFAs)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases. *Frontiers in Immunology*. 2019;10:277. doi:10.3389/fimmu.2019.00277

53. Berni Canani R, Di Costanzo M, Leone L. The epigenetic effects of butyrate: Potential therapeutic implications for clinical practice. *Clinical Epigenetics*. 2012;4(1):4. doi:10.1186/1868-7083-4-4

54. Flanagan PK, Campbell BJ, Rhodes JM. Lessons from Diversion Studies and Antibacterial Interventions. *Digestive Diseases*. 2012;30(4):347-350. doi:10.1159/000338122

55. Ashton JJ, Gavin J, Beattie RM. Exclusive enteral nutrition in Crohn's disease: Evidence and practicalities. *Clinical nutrition (Edinburgh, Scotland)*. 2019;38(1):80-89. doi:10.1016/j.clnu.2018.01.020

56. Sigall Boneh R, Sarbagili Shabat C, Yanai H, et al. Dietary Therapy With the Crohn's Disease Exclusion Diet is a Successful Strategy for Induction of Remission in Children and Adults Failing Biological Therapy. *Journal of Crohn's and Colitis*. 2017;11(10):1205-1212. doi:10.1093/ecco-jcc/jjx071

57. Bernstein CN. Antibiotics, Probiotics and Prebiotics in IBD. In: *Nestle Nutrition Institute Workshop Series*. Vol 79. ; 2014:83-100. doi:10.1159/000360713

58. Paramsothy S, Paramsothy R, Rubin DT, et al. Faecal Microbiota Transplantation for Inflammatory Bowel Disease: A Systematic Review and Meta-analysis. *Journal of Crohn's and Colitis*. 2017;11(10):1180-1199. doi:10.1093/ecco-jcc/jjx063

59. Kugathasan S, Denson LA, Walters TD, et al. Prediction of complicated disease course for children newly diagnosed with Crohn's disease: a multicentre inception cohort study. *The Lancet*. 2017;389(10080):1710-1718. doi:10.1016/S0140-6736(17)30317-3

60. GEM Project: A Multidisciplinary Human Study on the Genetic, Environmental, and Microbial Interactions that Cause Inflammatory Bowel Disease | Crohn's & Colitis Foundation. https://www.crohnscolitisfoundation.org/research/clinical-trials-community/featured-research/clinical-trials/gem-project-a.html. Accessed April 25, 2019.

61. Waddington CH. The epigenotype. 1942. *International journal of epidemiology*. 2012;41(1):10-13. doi:10.1093/ije/dyr184

62. Jenke AC, Zilbauer M. Epigenetics in inflammatory bowel disease. *Current Opinion in Gastroenterology*. 2012;28(6):577-584. doi:10.1097/MOG.0b013e328357336b

63. Youngson NA, Whitelaw E. Transgenerational Epigenetic Effects. *Annual Review of Genomics and Human Genetics*. 2008;9(1):233-257. doi:10.1146/annurev.genom.9.081307.164445

64. Portela A, Esteller M. Epigenetic modifications and human disease. *Nature Biotechnology*. 2010;28(10):1057-1068. doi:10.1038/nbt.1685

65. Lim U, Song M-A. Dietary and Lifestyle Factors of DNA Methylation. In: *Methods in Molecular Biology (Clifton, N.J.)*. Vol 863. ; 2012:359-376. doi:10.1007/978-1-61779-612-8_23

66. Tsaprouni LG, Yang T-P, Bell J, et al. Cigarette smoking reduces DNA methylation levels at multiple genomic loci but the effect is partially reversible upon cessation. *Epigenetics*. 2014;9(10):1382-1396. doi:10.4161/15592294.2014.969637

67. Nimmo ER, Prendergast JG, Aldhous MC, et al. Genome-wide methylation profiling in Crohn's disease identifies altered epigenetic regulation of key host defense mechanisms including the Th17 pathway. *Inflammatory Bowel Diseases*. 2012;18(5):889-899. doi:10.1002/ibd.21912

68. Marcin W, Michael S. Genetics and epigenetics of inflammatory bowel disease. *Swiss Medical Weekly*. 2018;148(37-38). doi:10.4414/smw.2018.14671

69. Maaser C, Sturm A, Vavricka SR, et al. ECCO-ESGAR Guideline for Diagnostic Assessment in IBD Part 1: Initial diagnosis, monitoring of known IBD, detection of complications. *Journal of Crohn's and Colitis*. 2018;13(2):144-164K. doi:10.1093/ecco-jcc/jjy113

70. Vermeire S, Van Assche G, Rutgeerts P. Laboratory markers in IBD: useful, magic, or unnecessary toys? *Gut*. 2006;55(3):426-431. doi:10.1136/gut.2005.069476

71. Silverberg MS, Satsangi J, Ahmad T, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Canadian Journal of Gastroenterology*. 2005;19 Suppl A(5):5A-36A. doi:10.1155/2005/269076

72. Wright R, Truelove SR. Serial rectal biopsy in ulcerative colitis during the course of a controlled therapeutic trial of various diets. *The American journal of digestive diseases*. 1966;11(11):847-857.

73. Riley SA, Mani V, Goodman MJ, Dutt S, Herd ME. Microscopic activity in ulcerative colitis: what does it mean? *Gut*. 1991;32(2):174-178.

74. Bryant R V, Burger DC, Delo J, et al. Beyond endoscopic mucosal healing in UC: histological remission better predicts corticosteroid use and hospitalisation over 6 years of follow-up. *Gut*. 2016;65(3):408-414. doi:10.1136/gutjnl-2015-309598

75. Kopylov U, Yung DE, Engel T, et al. Diagnostic yield of capsule endoscopy versus magnetic resonance enterography and small bowel contrast ultrasound in the evaluation of small bowel Crohn's disease: Systematic review and meta-analysis. *Digestive and Liver Disease*. 2017;49(8):854-863. doi:10.1016/j.dld.2017.04.013

76. Langner C, Magro F, Driessen A, et al. The histopathological approach to inflammatory bowel disease: a practice guide. *Virchows Archiv*. 2014;464(5):511-527. doi:10.1007/s00428-014-1543-4

77. Prenzel F, Uhlig HH. Frequency of indeterminate colitis in children and adults with IBD — a metaanalysis. *Journal of Crohn's and Colitis*. 2009;3(4):277-281. doi:10.1016/j.crohns.2009.07.001

78. Washington K, Greenson JK, Montgomery E, et al. Histopathology of ulcerative colitis in initial rectal biopsy in children. *The American journal of surgical pathology*. 2002;26(11):1441-1449.

79. Panés J, López-Sanromán A, Bermejo F, et al. Early azathioprine therapy is no more effective than placebo for newly diagnosed Crohn's disease. *Gastroenterology*. 2013;145(4):766-74.e1. doi:10.1053/j.gastro.2013.06.009

80. Cosnes J, Bourrier A, Laharie D, et al. Early administration of azathioprine vs conventional management of Crohn's Disease: a randomized controlled trial. *Gastroenterology*. 2013;145(4):758-65.e2; quiz e14-5. doi:10.1053/j.gastro.2013.04.048

81. D'Haens G, Baert F, van Assche G, et al. Early combined immunosuppression or conventional management in patients with newly diagnosed Crohn's disease: an open randomised trial. *Lancet*. 2008;371(9613):660-667. doi:10.1016/S0140-6736(08)60304-9

82. Baert F, Moortgat L, Van Assche G, et al. Mucosal healing predicts sustained clinical remission in patients with early-stage Crohn's disease. *Gastroenterology*. 2010;138(2):463-468; quiz e10-1. doi:10.1053/j.gastro.2009.09.056

83. Colombel JF, Sandborn WJ, Reinisch W, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. *The New England journal of medicine*. 2010;362(15):1383-1395. doi:10.1056/NEJMoa0904492

84. Kennedy NA, Heap GA, Green HD, et al. Predictors of anti-TNF treatment failure in anti-TNF-naive patients with active luminal Crohn's disease: a prospective, multicentre, cohort study. *The Lancet Gastroenterology & Hepatology*. 2019;4(5):341-353. doi:10.1016/S2468-1253(19)30012-3

85. Beaugerie L, Brousse N, Bouvier AM, et al. Lymphoproliferative disorders in patients receiving thiopurines for inflammatory bowel disease: a prospective observational cohort study. *Lancet*. 2009;374(9701):1617-1625. doi:10.1016/S0140-6736(09)61302-7

86. Peyrin-Biroulet L, Khosrotehrani K, Carrat F, et al. Increased risk for nonmelanoma skin cancers in patients who receive thiopurines for inflammatory bowel disease. *Gastroenterology*. 2011;141(5):1621-28.e1-5. doi:10.1053/j.gastro.2011.06.050

87. Lichtenstein GR, Feagan BG, Cohen RD, et al. Serious infections and mortality in association with therapies for Crohn's disease: TREAT registry. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association*. 2006;4(5):621-630. doi:10.1016/j.cgh.2006.03.002

88. Long MD, Martin CF, Pipkin CA, Herfarth HH, Sandler RS, Kappelman MD. Risk of Melanoma and Nonmelanoma Skin Cancer Among Patients With Inflammatory Bowel Disease. *Gastroenterology*. 2012;143(2):390-399.e1. doi:10.1053/j.gastro.2012.05.004

89. Siegel CA, Marden SM, Persing SM, Larson RJ, Sands BE. Risk of Lymphoma Associated With Combination Anti–Tumor Necrosis Factor and Immunomodulator Therapy for the Treatment of Crohn's Disease: A Meta-Analysis. *Clinical Gastroenterology and Hepatology*. 2009;7(8):874-881. doi:10.1016/j.cgh.2009.01.004

90. Khanna R, Bressler B, Levesque BG, et al. Early combined immunosuppression for the management of Crohn's disease (REACT): a cluster randomised controlled trial. *Lancet (London, England)*. 2015;386(10006). doi:10.1016/S0140-6736(15)00068-9

91. Heerasing N, Thompson B, Hendy P, et al. Exclusive enteral nutrition provides an effective bridge to safer interval elective surgery for adults with Crohn's disease. *Alimentary Pharmacology & Therapeutics*. 2017;45(5):660-669. doi:10.1111/apt.13934

92. Cullen G, O'toole A, Keegan D, Sheahan K, Hyland JM, O'donoghue DP. Long-term clinical results of ileocecal resection for Crohn's disease. *Inflammatory bowel diseases*. 2007;13(11):1369-1373. doi:10.1002/ibd.20220

93. Aratari A, Papi C, Leandro G, Viscido A, Capurso L, Caprilli R. Early versus late surgery for ileo-caecal Crohn's disease. *Alimentary pharmacology & therapeutics*. 2007;26(10):1303-1312. doi:10.1111/j.1365-2036.2007.03515.x

94. Wynn TA, Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nature Medicine*. 2012;18(7):1028-1040. doi:10.1038/nm.2807

95. Chen W, Lu C, Hirota C, Iacucci M, Ghosh S, Gui X. Smooth Muscle Hyperplasia/Hypertrophy is the Most Prominent Histological Change in Crohn's Fibrostenosing Bowel Strictures: A Semiquantitative Analysis by Using a Novel Histological Grading Scheme. *Journal of Crohn's and Colitis*. 2017;11(1):92-104. doi:10.1093/ecco-jcc/jjw126

96. Rönnblom A, Holmström T, Karlbom U, Tanghöj H, Thörn M, Sjöberg D. Clinical course of Crohn's disease during the first 5 years. Results from a population-based cohort in Sweden (ICURE) diagnosed 2005–2009. *Scandinavian Journal of Gastroenterology*. 2017;52(1):81-86. doi:10.1080/00365521.2016.1230777

97. Fiorino G, Morin M, Bonovas S, et al. Prevalence of Bowel Damage Assessed by Cross-Sectional Imaging in Early Crohn's Disease and its Impact on Disease Outcome. *Journal of Crohn's & colitis*. 2017;11(3):274-280. doi:10.1093/ecco-jcc/jjw185

98. EDWARDS FC, TRUELOVE SC. THE COURSE AND PROGNOSIS OF ULCERATIVE COLITIS. III. COMPLICATIONS. *Gut*. 1964;5:1-22.

99. Gumaste V, Sachar DB, Greenstein AJ. Benign and malignant colorectal strictures in ulcerative colitis. *Gut*. 1992;33(7):938-941.

100. Pariente B, Cosnes J, Danese S, et al. Development of the Crohn's disease digestive

damage score, the Lémann score. *Inflammatory Bowel Diseases*. 2011;17(6):1415-1422. doi:10.1002/ibd.21506

101. Scheibe K, Kersten C, Schmied A, et al. Inhibiting Interleukin 36 Receptor Signaling Reduces Fibrosis in Mice With Chronic Intestinal Inflammation. *Gastroenterology*. 2019;156(4):1082-1097.e11. doi:10.1053/j.gastro.2018.11.029

102. Holvoet T, Devriese S, Castermans K, et al. Treatment of Intestinal Fibrosis in Experimental Inflammatory Bowel Disease by the Pleiotropic Actions of a Local Rho Kinase Inhibitor. *Gastroenterology*. 2017;153(4):1054-1067. doi:10.1053/j.gastro.2017.06.013

103. Van Assche G, Thienpont C, D'Hoore A, et al. Long-term outcome of endoscopic dilatation in patients with Crohn's disease is not affected by disease activity or medical therapy. *Gut*. 2010;59(3):320-324. doi:10.1136/gut.2009.180182

104. Ding NS, Yip WM, Choi CH, et al. Endoscopic Dilatation of Crohn's Anastomotic Strictures is Effective in the Long Term, and Escalation of Medical Therapy Improves Outcomes in the Biologic Era. *Journal of Crohn's & colitis*. 2016;10(10):1172-1178. doi:10.1093/ecco-jcc/jjw072

105. Fumery M, Pineton de Chambrun G, Stefanescu C, et al. Detection of Dysplasia or Cancer in 3.5% of Patients With Inflammatory Bowel Disease and Colonic Strictures. *Clinical Gastroenterology and Hepatology*. 2015;13(10):1770-1775. doi:10.1016/j.cgh.2015.04.185

106. Hellers G, Bergstrand O, Ewerth S, Holmstrom B. Occurrence and outcome after primary treatment of anal fistulae in Crohn's disease. *Gut*. 1980;21(6):525-527. doi:10.1136/gut.21.6.525

107. Coulie B, Camilleri M, Bharucha AE, Sandborn WJ, Burton D. Colonic motility in chronic ulcerative proctosigmoiditis and the effects of nicotine on colonic motility in patients and healthy subjects. *Alimentary pharmacology & therapeutics*. 2001;15(5):653-663.

108. Al-Shboul OA. The importance of interstitial cells of cajal in the gastrointestinal tract. *Saudi journal of gastroenterology : official journal of the Saudi Gastroenterology Association*. 2013;19(1):3-15. doi:10.4103/1319-3767.105909

109. Geboes K, Collins S. Structural abnormalities of the nervous system in Crohn's disease and ulcerative colitis. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society*. 1998;10(3):189-202.

110. Torres J, Billioud V, Sachar DB, Peyrin-Biroulet L, Colombel J-F. Ulcerative Colitis as A Progressive Disease: The Forgotten Evidence. *Inflammatory Bowel Diseases*. 2012;18(7):1356-1363. doi:10.1002/ibd.22839

111. Mowat C, Cole A, Windsor A, et al. Guidelines for the management of inflammatory bowel disease in adults. *Gut*. 2011;60(5):571-607. doi:doi:10.1136/gut.2010.224154

112. Dignass A1, Van Assche G, Lindsay JO, Lémann M, Söderholm J, Colombel JF, Danese S,

D'Hoore A, Gassull M, Gomollón F, Hommes DW, Michetti P, O'Morain C, Oresland T, Windsor A, Stange EF TSEC and CO (ECCO)., Dignass A, Van Assche G, et al. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Current management. *Journal of Crohn's & colitis*. 2010;4(1):28-62. doi:10.1016/j.crohns.2009.12.002

113. Xie J, Itzkowitz SH. Cancer in inflammatory bowel disease. *World Journal of Gastroenterology : WJG*. 2008;14(3):378. doi:10.3748/WJG.14.378

114. Itzkowitz SH, Harpaz N. Diagnosis and management of dysplasia in patients with inflammatory bowel diseases. *Gastroenterology*. 2004;126(6):1634-1648.

115. Laine L, Kaltenbach T, Barkun A, et al. SCENIC International Consensus Statement on Surveillance and Management of Dysplasia in Inflammatory Bowel Disease. *Gastroenterology*. 2015;148(3):639-651.e28. doi:10.1053/j.gastro.2015.01.031

116. Overview | Colorectal cancer prevention: colonoscopic surveillance in adults with ulcerative colitis, Crohn's disease or adenomas | Guidance | NICE.

117. Ibraheim H, Dhillon AS, Koumoutsos I, Gulati S, Hayee B. Curriculum review: colorectal cancer surveillance and management of dysplasia in IBD. *Frontline Gastroenterology*. 2018;9(4):271-277. doi:10.1136/flgastro-2017-100919

118. Sykiotis GP, Kalliolias GD, Papavassiliou AG. Pharmacogenetic Principles in the Hippocratic Writings. *The Journal of Clinical Pharmacology*. 2005;45(11):1218-1220. doi:10.1177/0091270005281091

119. Roses AD. Pharmacogenetics and the practice of medicine. *Nature*. 2000;405(6788):857-865. doi:10.1038/35015728

120. Vester-Andersen MK, Prosberg M V, Jess T, et al. Disease course and surgery rates in inflammatory bowel disease: a population-based, 7-year follow-up study in the era of immunomodulating therapy. *The American journal of gastroenterology*. 2014;109(5):705-714. doi:10.1038/ajg.2014.45

121. Cosnes J, Gower-Rousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. *Gastroenterology*. 2011;140(6):1785-1794. doi:10.1053/j.gastro.2011.01.055

122. Schleidgen S, Klingler C, Bertram T, Rogowski WH, Marckmann G. What is personalized medicine: sharpening a vague term based on a systematic literature review. *BMC medical ethics*. 2013;14(1):55. doi:10.1186/1472-6939-14-55

123. Hingorani AD, Windt DA van der, Riley RD, et al. Prognosis research strategy (PROGRESS) 4: stratified medicine research. *BMJ (Clinical research ed)*. 2013;346(feb05_1):e5793. doi:10.1136/bmj.e5793

124. Mosli MH, Sandborn WJ, Kim RB, Khanna R, Al-Judaibi B, Feagan BG. Toward a personalized medicine approach to the management of inflammatory bowel disease. *The American journal of gastroenterology*. 2014;109(7):994-1004. doi:10.1038/ajg.2014.110

125. Schreiber S, Reinisch W, Colombel JF, et al. Subgroup analysis of the placebo-controlled CHARM trial: Increased remission rates through 3years for adalimumab-treated patients with early Crohn's disease. *Journal of Crohn's and Colitis*. 2013;7(3):213-221. doi:10.1016/j.crohns.2012.05.015

126. Schreiber S, Reinisch W, Colombel JF et al. Early Crohn's disease shows high levels of remission to therapy with adalimumab: Sub-analysis of CHARM. *Gastroenterology*. 2007;132(4):Suppl 2; A985. doi:10.1016/S0016-5085(07)60009-2

127. Schreiber S, Colombel J-F, Bloomfield R, et al. Increased Response and Remission Rates in Short-Duration Crohn's Disease With Subcutaneous Certolizumab Pegol: An Analysis of PRECiSE 2 Randomized Maintenance Trial Data. *The American Journal of Gastroenterology*. 2010;105(7):1574-1582. doi:10.1038/ajg.2010.78

128. Colombel J-F, Reinisch W, Mantzaris GJ, et al. Randomised clinical trial: deep remission in biologic and immunomodulator naïve patients with Crohn's disease - a SONIC *post hoc* analysis. *Alimentary Pharmacology & Therapeutics*. 2015;41(8):734-746. doi:10.1111/apt.13139

129. Panaccione R, Löfberg R, Rutgeerts P, et al. Efficacy and Safety of Adalimumab by Disease Duration: Analysis of Pooled Data From Crohn's Disease Studies. *Journal of Crohn's & colitis*. February 2019. doi:10.1093/ecco-jcc/jjy223

130. VENY M, Esteller M, RICART E, Piqué JM, PANÉS J, Salas A. Late Crohn's disease patients present an increase in peripheral Th17 cells and cytokine production compared with early patients. *Alimentary pharmacology & therapeutics*. 2010;31(5):561-572. doi:10.1111/j.1365-2036.2009.04209.x

131. Kugathasan S, Saubermann LJ, Smith L, et al. Mucosal T-cell immunoregulation varies in early and late inflammatory bowel disease. *Gut*. 2007;56(12):1696-1705. doi:10.1136/gut.2006.116467

132. Rivera–Nieves J, Ho J, Bamias G, et al. Antibody Blockade of CCL25/CCR9 Ameliorates Early but not Late Chronic Murine Ileitis. *Gastroenterology*. 2006;131(5):1518-1529. doi:10.1053/j.gastro.2006.08.031

133. Ordás I, Feagan BG, Sandborn WJ. Early use of immunosuppressives or TNF antagonists for the treatment of Crohn's disease: time for a change. *Gut*. 2011;60(12):1754-1763. doi:10.1136/gutjnl-2011-300934

134. Sandborn WJ, Hanauer S, Van Assche G, et al. Treating beyond symptoms with a view to improving patient outcomes in inflammatory bowel diseases. *Journal of Crohn's & colitis*. 2014;8(9):927-935. doi:10.1016/j.crohns.2014.02.021

135. Lakatos PL, Czegledi Z, Szamosi T, et al. Perianal disease, small bowel disease, smoking, prior steroid or early azathioprine/biological therapy are predictors of disease behavior change in patients with Crohn's disease. *World journal of gastroenterology*. 2009;15(28):3504-3510.

136. Beaugerie L, Seksik P, Nion-Larmurier I, et al. Predictors of Crohn's disease.

Gastroenterology. 2006;130(3):650-656. doi:10.1053/j.gastro.2005.12.019

137. Zallot C, Peyrin-Biroulet L. Clinical risk factors for complicated disease: how reliable are they? *Digestive diseases (Basel, Switzerland)*. 2012;30 Suppl 3:67-72. doi:10.1159/000342608

138. Pigneur B, Seksik P, Viola S, et al. Natural history of Crohn's disease: comparison between childhood- and adult-onset disease. *Inflammatory bowel diseases*. 2010;16(6):953-961. doi:10.1002/ibd.21152

139. Henriksen M, Jahnsen J, Lygren I, Vatn MH, Moum B. Are there any differences in phenotype or disease course between familial and sporadic cases of inflammatory bowel disease? Results of a population-based follow-up study. *The American journal of gastroenterology*. 2007;102(9):1955-1963. doi:10.1111/j.1572-0241.2007.01368.x

140. Yamamoto T, Keighley MR. Smoking and disease recurrence after operation for Crohn's disease. *The British journal of surgery*. 2000;87(4):398-404. doi:10.1046/j.1365-2168.2000.01443.x

141. Solberg IC, Lygren I, Jahnsen J, et al. Clinical course during the first 10 years of ulcerative colitis: results from a population-based inception cohort (IBSEN Study). *Scandinavian journal of gastroenterology*. 2009;44(4):431-440. doi:10.1080/00365520802600961

142. Munkholm P, Langholz E, Davidsen M, Binder V. Disease activity courses in a regional cohort of Crohn's disease patients. *Scandinavian journal of gastroenterology*. 1995;30(7):699-706.

143. Langholz E, Munkholm P, Davidsen M, Binder V. Course of ulcerative colitis: analysis of changes in disease activity over years. *Gastroenterology*. 1994;107(1):3-11.

144. Hoie O, Wolters FL, Riis L, et al. Low colectomy rates in ulcerative colitis in an unselected European cohort followed for 10 years. *Gastroenterology*. 2007;132(2):507-515. doi:10.1053/j.gastro.2006.11.015

145. Romberg-Camps MJL, Dagnelie PC, Kester ADM, et al. Influence of phenotype at diagnosis and of other potential prognostic factors on the course of inflammatory bowel disease. *The American journal of gastroenterology*. 2009;104(2):371-383. doi:10.1038/ajg.2008.38

146. Beaugerie L, Massot N, Carbonnel F, Cattan S, Gendre JP, Cosnes J. Impact of cessation of smoking on the course of ulcerative colitis. *The American journal of gastroenterology*. 2001;96(7):2113-2116. doi:10.1111/j.1572-0241.2001.03944.x

147. Ayres RC, Gillen CD, Walmsley RS, Allan RN. Progression of ulcerative proctosigmoiditis: incidence and factors influencing progression. *European journal of gastroenterology & hepatology*. 1996;8(6):555-558.

148. Farmer RG, Easley KA, Rankin GB. Clinical patterns, natural history, and progression of ulcerative colitis. A long-term follow-up of 1116 patients. *Digestive diseases and sciences*. 1993;38(6):1137-1146.

149. Tyler AD, Milgrom R, Stempak JM, et al. The NOD2insC polymorphism is associated with worse outcome following ileal pouch-anal anastomosis for ulcerative colitis. *Gut*. 2013;62(10):1433-1439. doi:10.1136/gutjnl-2011-301957

150. Louis E, Mary J-Y, Vernier-Massouille G, et al. Maintenance of remission among patients with Crohn's disease on antimetabolite therapy after infliximab therapy is stopped. *Gastroenterology*. 2012;142(1):63-70.e5; quiz e31. doi:10.1053/j.gastro.2011.09.034

151. Kennedy NA, Warner B, Johnston EL, et al. Relapse after withdrawal from anti-TNF therapy for inflammatory bowel disease: an observational study, plus systematic review and meta-analysis. *Alimentary Pharmacology & Therapeutics*. 2016;43(8):910-923. doi:10.1111/apt.13547

152. SPARE | The University of Edinburgh. https://www.ed.ac.uk/usher/edinburgh-clinical-trials/our-studies/ukcrc-studies/spare. Accessed May 10, 2019.

153. Costa F, Mumolo MG, Ceccarelli L, et al. Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. *Gut*. 2005;54(3):364-368. doi:10.1136/gut.2004.043406

154. Vos M De, Louis EJ, Jahnsen J, et al. Consecutive Fecal Calprotectin Measurements to Predict Relapse in Patients with Ulcerative Colitis Receiving Infliximab Maintenance Therapy. *Inflammatory Bowel Diseases*. 2013;19(10):2111-2117. doi:10.1097/MIB.0b013e31829b2a37

155. Rutgeerts P, Geboes K, Vantrappen G, Beyls J, Kerremans R, Hiele M. Predictability of the postoperative course of Crohn's disease. *Gastroenterology*. 1990;99(4):956-963.

156. Wright EK, Kamm MA, De Cruz P, et al. Measurement of Fecal Calprotectin Improves Monitoring and Detection of Recurrence of Crohn's Disease After Surgery. *Gastroenterology*. 2015;148(5):938-947.e1. doi:10.1053/j.gastro.2015.01.026

157. D'Haens G, Ferrante M, Vermeire S, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. *Inflammatory Bowel Diseases*. 2012;18(12):2218-2224. doi:10.1002/ibd.22917

158. Tham YS, Yung DE, Fay S, et al. Fecal calprotectin for detection of postoperative endoscopicrecurrence in Crohn's disease: systematic review andmeta-analysis. *Therapeutic Advances in Gastroenterology*. 2018;11:1756284818785571. doi:10.1177/1756284818785571

159. Abreu MT. Serologies in Crohn's disease: can we change the gray zone to black and white? *Gastroenterology*. 2006;131(2):664-667. doi:10.1053/j.gastro.2006.06.040

160. Saxon A, Shanahan F, Landers C, Ganz T, Targan S. A distinct subset of antineutrophil cytoplasmic antibodies is associated with inflammatory bowel disease. *The Journal of allergy and clinical immunology*. 1990;86(2):202-210.

161. Main J, McKenzie H, Yeaman GR, et al. Antibody to Saccharomyces cerevisiae (bakers' yeast) in Crohn's disease. *BMJ (Clinical research ed)*. 1988;297(6656):1105-1106.

162. Li X, Conklin L, Alex P. New serological biomarkers of inflammatory bowel disease. *World journal of gastroenterology : WJG*. 2008;14(33):5115-5124.

163. Zholudev A, Zurakowski D, Young W, Leichtner A, Bousvaros A. Serologic testing with ANCA, ASCA, and anti-OmpC in children and young adults with Crohn's disease and ulcerative colitis: diagnostic value and correlation with disease phenotype. *The American journal of gastroenterology*. 2004;99(11):2235-2241. doi:10.1111/j.1572-0241.2004.40369.x

164. Dubinsky MC, Lin Y-C, Dutridge D, et al. Serum immune responses predict rapid disease progression among children with Crohn's disease: immune responses predict disease progression. *The American journal of gastroenterology*. 2006;101(2):360-367. doi:10.1111/j.1572-0241.2006.00456.x

165. Targan SR, Landers CJ, Yang H, et al. Antibodies to CBir1 flagellin define a unique response that is associated independently with complicated Crohn's disease. *Gastroenterology*. 2005;128(7):2020-2028.

166. Mow WS, Vasiliauskas EA, Lin Y-C, et al. Association of antibody responses to microbial antigens and complications of small bowel Crohn's disease. *Gastroenterology*. 2004;126(2):414-424.

167. Solberg IC, Cvancarova M, Vatn MH, Moum B, IBSEN Study Group. Risk matrix for prediction of advanced disease in a population-based study of patients with Crohn's Disease (the IBSEN Study). *Inflammatory bowel diseases*. 2014;20(1):60-68. doi:10.1097/01.MIB.0000436956.78220.67

168. Vermeire S, Van Assche G, Rutgeerts P. Role of genetics in prediction of disease course and response to therapy. *World journal of gastroenterology : WJG*. 2010;16(21):2609-2615.

169. Alonso A, Domènech E, Julià A, et al. Identification of risk loci for Crohn's disease phenotypes using a genome-wide association study. *Gastroenterology*. 2015;148(4):794-805. doi:10.1053/j.gastro.2014.12.030

170. Jostins L, Ripke S, Weersma RK, et al. 1. Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature. 2012;491(7422):119–24. doi:10.1038/nature11582.Host-microbe interactions have shaped the genetic architecture o. *Nature*. 2012;491(7422):119-124. doi:10.1038/nature11582

171. Annese V, Lombardi G, Perri F, et al. Variants of CARD15 are associated with an aggressive clinical course of Crohn's disease--an IG-IBD study. *The American journal of gastroenterology*. 2005;100(1):84-92. doi:10.1111/j.1572-0241.2005.40705.x

172. Alvarez-Lobos M, Arostegui JI, Sans M, et al. Crohn's disease patients carrying Nod2/CARD15 gene variants have an increased and early need for first surgery due to stricturing disease and higher rate of surgical recurrence. *Annals of surgery*. 2005;242(5):693-700.

173. Seiderer J, Brand S, Herrmann KA, et al. Predictive value of the CARD15 variant 1007fs for the diagnosis of intestinal stenoses and the need for surgery in Crohn's disease in clinical practice: results of a prospective study. *Inflammatory bowel diseases*. 2006;12(12):1114-1121. doi:10.1097/01.mib.0000235836.32176.5e

174. Cleynen I, González JR, Figueroa C, et al. Genetic factors conferring an increased susceptibility to develop Crohn's disease also influence disease phenotype: results from the IBDchip European Project. *Gut*. 2013;62(11):1556-1565. doi:10.1136/gutjnl-2011-300777

175. Ahmad T, Armuzzi A, Bunce M, et al. The molecular classification of the clinical manifestations of Crohn's disease. *Gastroenterology*. 2002;122(4):854-866.

176. Lee JC, Espéli M, Anderson CA, et al. Human SNP links differential outcomes in inflammatory and infectious disease to a FOXO3-regulated pathway. *Cell*. 2013;155(1):57-69. doi:10.1016/j.cell.2013.08.034

177. Lee JC. Predicting the course of IBD: light at the end of the tunnel? *Digestive diseases (Basel, Switzerland)*. 2012;30 Suppl 1(s1):95-99. doi:10.1159/000341132

178. Xie J, Itzkowitz SH. Cancer in inflammatory bowel disease. *World Journal of Gastroenterology*. 2008;14(3):378. doi:10.3748/wjg.14.378

179. Watanabe T, Kobunai T, Toda E, et al. Gene expression signature and the prediction of ulcerative colitis-associated colorectal cancer by DNA microarray. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2007;13(2 Pt 1):415-420. doi:10.1158/1078-0432.CCR-06-0753

180. Wheeler JM, Kim HC, Efstathiou JA, Ilyas M, Mortensen NJ, Bodmer WF. Hypermethylation of the promoter region of the E-cadherin gene (CDH1) in sporadic and ulcerative colitis associated colorectal cancer. *Gut*. 2001;48(3):367-371.

181. Sato F, Shibata D, Harpaz N, et al. Aberrant methylation of the HPP1 gene in ulcerative colitis-associated colorectal carcinoma. *Cancer research*. 2002;62(23):6820-6822.

182. Pekow J, Dougherty U, Huang Y, et al. Gene Signature Distinguishes Patients with Chronic Ulcerative Colitis Harboring Remote Neoplastic Lesions. *Inflammatory Bowel Diseases*. 2013;19(3):461-470. doi:10.1097/MIB.0b013e3182802bac

183. Rubin CE, Haggitt RC, Burmer GC, et al. DNA aneuploidy in colonic biopsies predicts future development of dysplasia in ulcerative colitis. *Gastroenterology*. 1992;103(5):1611-1620.

184. Brentnall TA, Crispin DA, Rabinovitch PS, et al. Mutations in the p53 gene: an early marker of neoplastic progression in ulcerative colitis. *Gastroenterology*. 1994;107(2):369-378.

185. Pediatric RISK Stratification Study | Crohn's & Colitis Foundation. https://www.crohnscolitisfoundation.org/science-and-professionals/research/current-research-studies/pediatric-risk-successes.html. Accessed May 31, 2019.

186. Orlando A, Colombo E, Kohn A, et al. Infliximab in the treatment of Crohn's disease: Predictors of response in an Italian multicentric open study. *Digestive and Liver Disease*. 2005;37(8):577-583. doi:10.1016/j.dld.2005.01.019

187. Arnott IDR, McNeill G, Satsangi J. An analysis of factors influencing short-term and sustained response to infliximab treatment for Crohn's disease. *Alimentary pharmacology & therapeutics*. 2003;17(12):1451-1457.

188. Vermeire S, Louis E, Carbonez A, et al. Demographic and clinical parameters influencing the short-term outcome of anti-tumor necrosis factor (infliximab) treatment in Crohn's disease. *The American Journal of Gastroenterology*. 2002;97(9):2357-2363. doi:10.1111/j.1572-0241.2002.05991.x

189. Parsi MA, Achkar J-P, Richardson S, et al. Predictors of response to infliximab in patients with Crohn's disease. *Gastroenterology*. 2002;123(3):707-713.

190. Siegel CA, Melmed GY. Predicting Response to Anti-TNF Agents for the Treatment of Crohn's Disease. *Therapeutic advances in gastroenterology*. 2009;2(4):245-251. doi:10.1177/1756283X09336364

191. Cucchiara S, Latiano A, Palmieri O, et al. Polymorphisms of tumor necrosis factor-alpha but not MDR1 influence response to medical therapy in pediatric-onset inflammatory bowel disease. *Journal of pediatric gastroenterology and nutrition*. 2007;44(2):171-179. doi:10.1097/MPG.0b013e31802c41f3

192. Gerich ME, McGovern DPB. Towards personalized care in IBD. *Nature reviews Gastroenterology & hepatology*. 2014;11(5):287-299. doi:10.1038/nrgastro.2013.242

193. Wang M-H, Friton JJ, Raffals LE, et al. Novel Genetic Risk Variants Can Predict Anti-TNF Agent Response in Patients With Inflammatory Bowel Disease. *Journal of Crohn's and Colitis*. January 2019. doi:10.1093/ecco-jcc/jjz017

194. Arijs I, Li K, Toedter G, et al. Mucosal gene signatures to predict response to infliximab in patients with ulcerative colitis. *Gut*. 2009;58(12):1612-1619. doi:10.1136/gut.2009.178665

195. Atreya R, Neumann H, Neufert C, et al. In vivo imaging using fluorescent antibodies to tumor necrosis factor predicts therapeutic response in Crohn's disease. *Nature Medicine*. 2014;20(3):313-318. doi:10.1038/nm.3462

196. Naisbitt DJ, Nattrass RG, Ogese MO. In vitro diagnosis of delayed-type drug hypersensitivity: mechanistic aspects and unmet needs. *Immunology and allergy clinics of North America*. 2014;34(3):691-705, x. doi:10.1016/j.iac.2014.04.009

197. Steenholdt C, Palarasah Y, Bendtzen K, et al. Pre-existing IgG antibodies cross-reacting with the Fab region of infliximab predict efficacy and safety of infliximab therapy in inflammatory bowel disease. *Alimentary Pharmacology & Therapeutics*. 2013;37(12):1172-1183. doi:10.1111/apt.12330

198. Kennedy NA, Heap GA, Green HD, et al. Predictors of anti-TNF treatment failure in anti-

TNF-naive patients with active luminal Crohn's disease: a prospective, multicentre, cohort study. *The lancet Gastroenterology & hepatology*. 2019;4(5):341-353. doi:10.1016/S2468-1253(19)30012-3

199. Vermeire S, O'Byrne S, Keir M, et al. Etrolizumab as induction therapy for ulcerative colitis: a randomised, controlled, phase 2 trial. *Lancet*. 2014;384(9940):309-318. doi:10.1016/S0140-6736(14)60661-9

200. Bouguen G, Levesque BG, Feagan BG, et al. Treat to Target: A Proposed New Paradigm for the Management of Crohn's Disease. *Clinical Gastroenterology and Hepatology*. 2015;13(6):1042-1050.e2. doi:10.1016/j.cgh.2013.09.006

201. Peyrin-Biroulet L, Sandborn W, Sands BE, et al. Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE): Determining Therapeutic Goals for Treat-to-Target. *The American Journal of Gastroenterology*. 2015;110(9):1324-1338. doi:10.1038/ajg.2015.233

202. Bryant R V, Winer S, Travis SPL, Riddell RH. Systematic review: histological remission in inflammatory bowel disease. Is "complete" remission the new treatment paradigm? An IOIBD initiative. *Journal of Crohn's & colitis*. 2014;8(12):1582-1597. doi:10.1016/j.crohns.2014.08.011

203. Colombel J-F, Panaccione R, Bossuyt P, et al. Effect of tight control management on Crohn's disease (CALM): a multicentre, randomised, controlled phase 3 trial. *Lancet (London, England)*. 2018;390(10114):2779-2789. doi:10.1016/S0140-6736(17)32641-7

204. Yzet C, Ungaro R, Bossuyt P, et al. OP35 Endoscopic and deep remission at 1 year prevents disease progression in early Crohn's disease: long-term data from CALM. *Journal of Crohn's and Colitis*. 2019;13(Supplement_1):S024-S025. doi:10.1093/ecco-jcc/jjy222.032

205. Røseth AG, Fagerhol MK, Aadland E, Schjønsby H. Assessment of the neutrophil dominating protein calprotectin in feces. A methodologic study. *Scandinavian journal of gastroenterology*. 1992;27(9):793-798.

206. Kalla R, Kennedy NA, Ventham NT, et al. Serum Calprotectin: A Novel Diagnostic and Prognostic Marker in Inflammatory Bowel Diseases. *The American Journal of Gastroenterology*. September 2016. doi:10.1038/ajg.2016.342

207. Kopylov U, Rosenfeld G, Bressler B, Seidman E. Clinical Utility of Fecal Biomarkers for the Diagnosis and Management of Inflammatory Bowel Disease. *Inflammatory Bowel Diseases*. 2014;20(4):742-756. doi:10.1097/01.MIB.0000442681.85545.31

208. Lamb CA, Mohiuddin MK, Gicquel J, et al. Faecal calprotectin or lactoferrin can identify postoperative recurrence in Crohn's disease. *British Journal of Surgery*. 2009;96(6):663-674. doi:10.1002/bjs.6593

209. Schoepfer AM, Beglinger C, Straumann A, et al. Fecal calprotectin correlates more closely with the Simple Endoscopic Score for Crohn's disease (SES-CD) than CRP, blood leukocytes, and the CDAI. *The American journal of gastroenterology*. 2010;105(1):162-

169. doi:10.1038/ajg.2009.545

210. Tibble J, Teahon K, Thjodleifsson B, et al. A simple method for assessing intestinal inflammation in Crohn's disease. *Gut*. 2000;47(4):506-513.

211. Lasson A, Stotzer P-O, Öhman L, Isaksson S, Sapnara M, Strid H. The intra-individual variability of faecal calprotectin: A prospective study in patients with active ulcerative colitis. *Journal of Crohn's and Colitis*. July 2014. doi:10.1016/j.crohns.2014.06.002

212. Whitehead SJ, French J, Brookes MJ, Ford C, Gama R. Between-assay variability of faecal calprotectin enzyme-linked immunosorbent assay kits. *Annals of Clinical Biochemistry*. 2013;50(1):53-61. doi:10.1258/acb.2012.011272

213. Dhaliwal A, Zeino Z, Tomkins C, et al. Utility of faecal calprotectin in inflammatory bowel disease (IBD): what cut-offs should we apply? 2015;6(1):14-19. doi:10.1136/flgastro-2013-100420

214. NICE DG11. Faecal calprotectin diagnostic tests for inflammatory diseases of the bowel. ISBN: 97831347313032639. https://www.nice.org.uk/guidance/dg11. Published 2013. Accessed September 9, 2015.

215. van der Sluijs Veer G, van den Hoven B, G.V.M. Russel M, A.J.T.M. van den Bergh F. Time-resolved fluorimetric immunoassay of calprotectin: technical and clinical aspects in diagnosis of inflammatory bowel diseases. *Clinical Chemistry and Laboratory Medicine (CCLM)*. 2006;44(3):292-298. doi:10.1515/CCLM.2006.051

216. Time Resolved Fluorescence – Expedeon. https://www.expedeon.com/resources/applications/time-resolved-fluorescence/. Accessed May 10, 2019.

217. Point-of-care and home faecal calprotectin tests for monitoring treatment response in inflammatory bowel disease | Guidance and guidelines | NICE.

218. Common Formats Of Lateral Flow Tests - Creative Diagnostics. https://www.cd-diatest.com/common-formats-of-lateral-flow-tests_d27. Accessed May 10, 2019.

219. van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. *BMJ*. 2010;341(jul15 1):c3369-c3369. doi:10.1136/bmj.c3369

220. Holtedahl K, Vedsted P, Borgquist L, et al. Abdominal symptoms in general practice: Frequency, cancer suspicions raised, and actions taken by GPs in six European countries. Cohort study with prospective registration of cancer. *Heliyon*. 2017;3(6):e00328. doi:10.1016/j.heliyon.2017.e00328

221. Thompson WG, Heaton KW, Smyth GT, Smyth C. Irritable bowel syndrome in general practice: prevalence, characteristics, and referral. *Gut*. 2000;46(1):78-82. doi:10.1136/gut.46.1.78

222. Gavin DR, Valori RM, Anderson JT, Donnelly MT, Williams JG, Swarbrick ET. The national colonoscopy audit: a nationwide assessment of the quality and safety of colonoscopy in the UK. *Gut*. 2013;62(2):242-249. doi:10.1136/gutjnl-2011-301848

223. Stapley SA, Rubin GP, Alsina D, Shephard EA, Rutter MD, Hamilton WT. Clinical features of bowel disease in patients aged <50 years in primary care: A large case-control study. *British Journal of General Practice*. 2017;67(658):e336-e344. doi:10.3399/bjgp17X690425

224. Yang D-H, Yang S-K, Park SH, et al. Usefulness of C-Reactive Protein as a Disease Activity Marker in Crohn's Disease according to the Location of Disease. *Gut and Liver*. 2015;9(1):80. doi:10.5009/GNL13424

225. Holtman GA, Lisman-van Leeuwen Y, Reitsma JB, Berger MY. Noninvasive Tests for Inflammatory Bowel Disease: A Meta-analysis. *Pediatrics*. 2015;137(1):e20152126. doi:10.1542/peds.2015-2126

226. Tibble JA, Sigthorsson G, Bridger S, Fagerhol MK, Bjarnason I. Surrogate markers of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease. *Gastroenterology*. 2000;119(1):15-22.

227. Holtman GA, Lisman-van Leeuwen Y, Kollen BJ, et al. Diagnostic test strategies in children at increased risk of inflammatory bowel disease in primary care. Ro S, ed. *PloS one*. 2017;12(12):e0189111. doi:10.1371/journal.pone.0189111

228. Poullis A, Foster R, Northfield TC, Mendall MA. Review article: faecal markers in the assessment of activity in inflammatory bowel disease. *Alimentary pharmacology & therapeutics*. 2002;16(4):675-681.

229. https://www.nhs.uk/conditions/bowel-cancer-screening/.../Having-a-colonoscopy.pdf. https://www.nhs.uk/conditions/bowel-cancer-screening/.../Having-a-colonoscopy.pdf.

230. Vavricka SR, Spigaglia SM, Rogler G, et al. Systematic evaluation of risk factors for diagnostic delay in inflammatory bowel disease. *Inflammatory bowel diseases*. 2012;18(3):496-505. doi:10.1002/ibd.21719

231. Schoepfer AM, Dehlavi M-A, Fournier N, et al. Diagnostic delay in Crohn's disease is associated with a complicated disease course and increased operation rate. *The American journal of gastroenterology*. 2013;108(11):1744-1753; quiz 1754. doi:10.1038/ajg.2013.248

232. Timmer A, Behrens R, Buderus S, et al. Childhood onset inflammatory bowel disease: predictors of delayed diagnosis from the CEDATA German-language pediatric inflammatory bowel disease registry. *The Journal of pediatrics*. 2011;158(3):467-473.e2. doi:10.1016/j.jpeds.2010.09.014

233. Frøslie KF, Jahnsen J, Moum BA, Vatn MH, IBSEN Group. Mucosal healing in inflammatory bowel disease: results from a Norwegian population-based cohort. *Gastroenterology*. 2007;133(2):412-422. doi:10.1053/j.gastro.2007.05.051

234. Rutter M, Saunders B, Wilkinson K, et al. Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. *Gastroenterology*. 2004;126(2):451-459.

235. Ghosh S, Mitchell R. Impact of inflammatory bowel disease on quality of life: Results of the European Federation of Crohn's and Ulcerative Colitis Associations (EFCCA) patient survey. *Journal of Crohn's and Colitis*. 2007;1(1):10-20. doi:10.1016/j.crohns.2007.06.005

236. Irvine EJ. Quality of life of patients with ulcerative colitis: past, present, and future. *Inflammatory bowel diseases*. 2008;14(4):554-565. doi:10.1002/ibd.20301

237. BÜHLMANN fCAL® ELISA - Calprotectin. https://www.calprotectin.co.uk/calprotectin-products/calprotectin-for-laboratories/fcal-elisa/. Accessed May 11, 2019.

238. Faecal Calprotectin: Immundiagnostik AG. http://www.immundiagnostik.com/en/home/products/kits-assays/oncology.html?tx_mokom01immunprodukte_pi1%5Ban%5D=K6927&tx_mokom01immunprodukte_pi1%5Bag%5D=406&cHash=740f70a652. Accessed May 11, 2019.

239. What is the faecal calprotectin test? *Drug and therapeutics bulletin*. 2014;52(9):102-104. doi:10.1136/dtb.2014.9.0276

240. Pavlidis P, Chedgy FJ, Tibble JA. Diagnostic accuracy and clinical application of faecal calprotectin in adult patients presenting with gastrointestinal symptoms in primary care. *Scandinavian journal of gastroenterology*. 2013;48(9):1048-1054. doi:10.3109/00365521.2013.816771

241. Turvill J, O'Connell S, Brooks A, et al. Evaluation of a faecal calprotectin care pathway for use in primary care. *Primary Health Care Research & Development*. 2016;17(05):428-436. doi:10.1017/S1463423616000049

242. Faecal Calprotectin testing in Primary Care - Calprotectin. https://www.calprotectin.co.uk/portfolio/faecal-calprotectin-testing-in-primary-care/. Accessed May 12, 2019.

243. Waugh N, Cummins E, Royle P, et al. Faecal calprotectin testing for differentiating amongst inflammatory and non-inflammatory bowel diseases: systematic review and economic evaluation. *Health technology assessment (Winchester, England)*. 2013;17(55):xv-xix, 1-211. doi:10.3310/hta17550

244. Sawczenko A, Sandhu BK. Presenting features of inflammatory bowel disease in Great Britain and Ireland. *Arch Dis Child*. 2003;88:995-1000. doi:10.1136/ADC.88.11.995

245. Davidson F, Lock RJ. Paediatric reference ranges for faecal calprotectin: a UK study. *Annals of Clinical Biochemistry*. 2017;54(2):214-218. doi:10.1177/0004563216639335

246. Rugtveit J, Fagerhol MK. Age-dependent variations in fecal calprotectin concentrations in children. *Journal of pediatric gastroenterology and nutrition*. 2002;34(3):323-324; author reply 324-5.

247. Mowat C, Digby J, Strachan JA, et al. Faecal haemoglobin and faecal calprotectin as indicators of bowel disease in patients presenting to primary care with bowel symptoms. *Gut*. 2016;65(9):1463-1469. doi:10.1136/gutjnl-2015-309579

248. Turvill J, Aghahoseini A, Sivarajasingham N, et al. Faecal calprotectin in patients with suspected colorectal cancer: a diagnostic accuracy study. *British Journal of General Practice*. 2016;66(648):e499-e506. doi:10.3399/bjgp16X685645

249. Endorsed resource – The use of faecal calprotectin in primary care as a decision diagnostic for inflammatory bowel disease and irritable bowel syndrome | Faecal calprotectin diagnostic tests for inflammatory diseases of the bowel | Guidance | NICE.

250. Crosland A, Jones R. Rectal bleeding: prevalence and consultation behaviour. *BMJ*. 1995;311(7003):486-488. doi:10.1136/bmj.311.7003.486

251. Black TP, Manolakis CS, Di Palma JA. “Red flag” evaluation yield in irritable bowel syndrome. *Journal of Gastrointestinal and Liver Diseases*. 2012;21(2):153-156.

252. Cancer Research UK, Bowel cancer incidence statistics 2012-2014. http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/bowel-cancer/incidence#heading-One. Accessed July 24, 2017.

253. NICE NG12. Suspected cancer | recognition and referral. https://www.nice.org.uk/guidance/ng12. Published 2015. Accessed July 25, 2017.

254. Eastern Devon – Northern, Eastern and Western Devon Clinical Commissioning Group. https://www.newdevonccg.nhs.uk/your-ccg/eastern-devon-100113. Accessed October 13, 2017.

255. Shinkins B, Thompson M. Diagnostic accuracy studies: how to report and analyse inconclusive test results. *BMJ: British Medical …*. 2013;2778(May):1-11. doi:10.1136/bmj.f2778

256. Lennard-Jones JE. Classification of inflammatory bowel disease. *Scandinavian journal of gastroenterology Supplement*. 1989;170:2-6; discussion 16-9. doi:10.3109/00365528909091339

257. National tariff payment system 2017/18 and 2018/19 | NHS Improvement. https://improvement.nhs.uk/resources/national-tariff-1719/. Accessed August 5, 2017.

258. Youden WJ. Index for rating diagnostic tests. *Cancer*. 1950;3(1):32-35. doi:10.1002/1097-0142(1950)3:1<32::AID-CNCR2820030106>3.0.CO;2-3

259. Health Research Authority - Protecting and promoting the interests of patients and the public in health research. http://www.hra.nhs.uk/. Accessed August 13, 2017.

260. SIPPONEN T, KÄRKKÄINEN P, SAVILAHTI E, et al. Correlation of faecal calprotectin and lactoferrin with an endoscopic score for Crohn’s disease and histological findings. *Alimentary Pharmacology & Therapeutics*. 2008;28(10):1221-1229. doi:10.1111/j.1365-

2036.2008.03835.x

261. Dolwani S, Metzner M, Wassell JJ, Yong A, Hawthorne AB. Diagnostic accuracy of faecal calprotectin estimation in prediction of abnormal small bowel radiology. *Alimentary Pharmacology and Therapeutics*. 2004;20(6):615-621. doi:10.1111/j.1365-2036.2004.02128.x

262. Conroy S, Hale MF, Cross SS, et al. Unrestricted faecal calprotectin testing performs poorly in the diagnosis of inflammatory bowel disease in patients in primary care. *Journal of Clinical Pathology*. August 2017:jclinpath-2017-204506. doi:10.1136/jclinpath-2017-204506

263. NICE CG61. Irritable bowel syndrome in adults: diagnosis and management. https://www.nice.org.uk/guidance/cg61. Published 2008. Accessed February 15, 2017.

264. Widlak MM, Thomas CL, Thomas MG, et al. Diagnostic accuracy of faecal biomarkers in detecting colorectal cancer and adenoma in symptomatic patients. *Alimentary Pharmacology & Therapeutics*. 2017;45(2):354-363. doi:10.1111/apt.13865

265. Kok L, Elias SG, Witteman BJM, et al. Diagnostic accuracy of point-of-care fecal calprotectin and immunochemical occult blood tests for diagnosis of organic bowel disease in primary care: The cost-effectiveness of a decision rule for abdominal complaints in primary care (CEDAR) study. *Clinical Chemistry*. 2012;58(6):989-998. doi:10.1373/clinchem.2011.177980

266. Spee LAA, Lisman-Van Leeuwen Y, Benninga MA, Bierma-Zeinstra SMA, Berger MY. Prevalence, characteristics, and management of childhood functional abdominal pain in general practice. *Scandinavian journal of primary health care*. 2013;31(4):197-202. doi:10.3109/02813432.2013.844405

267. Holtman GA, Lisman-van Leeuwen Y, Kollen BJ, et al. Challenges in diagnostic accuracy studies in primary care: the fecal calprotectin example. *BMC family practice*. 2013;14(1):179. doi:10.1186/1471-2296-14-179

268. Gieteling MJ, Lisman-van Leeuwen Y, van der Wouden JC, Schellevis FG, Berger MY. Childhood nonspecific abdominal pain in family practice: incidence, associated factors, and management. *Annals of family medicine*. 2011;9(4):337-343. doi:10.1370/afm.1268

269. Van de Vijver E, Schreuder AB, Cnossen WR, Muller Kobold AC, van Rheenen PF, North Netherlands Pediatric IBD Consortium. Safely ruling out inflammatory bowel disease in children and teenagers without referral for endoscopy. *Archives of disease in childhood*. 2012;97(12):1014-1018. doi:10.1136/archdischild-2011-301206

270. Holtman GA, Lisman-van Leeuwen Y, van Rheenen PF, et al. Evaluation of point-of-care test calprotectin and lactoferrin for inflammatory bowel disease among children with chronic gastrointestinal symptoms. *Family Practice*. 2016;34(4):cmw079. doi:10.1093/fampra/cmw079

271. Henderson P, Anderson NH, Wilson DC. The Diagnostic Accuracy of Fecal Calprotectin During the Investigation of Suspected Pediatric Inflammatory Bowel Disease: A

Systematic Review and Meta-Analysis. *The American Journal of Gastroenterology*. 2014;109(5):637-645. doi:10.1038/ajg.2013.131

272. Walker GJ, Moore L, Heerasing N, et al. Faecal calprotectin effectively excludes inflammatory bowel disease in 789 symptomatic young adults with/without alarm symptoms: a prospective UK primary care cohort study. *Alimentary Pharmacology & Therapeutics*. 2018;47(8):1103-1116. doi:10.1111/apt.14563

273. Turvill J. High negative predictive value of a normal faecal calprotectin in patients with symptomatic intestinal disease. *Frontline Gastroenterology*. 2012;3(1):21-28. doi:10.1136/flgastro-2011-100011

274. WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System.

275. Levine A, Griffiths A, Markowitz J, et al. Pediatric modification of the Montreal classification for inflammatory bowel disease. *Inflammatory Bowel Diseases*. 2011;17(6):1314-1321. doi:10.1002/ibd.21493

276. Levine A, Koletzko S, Turner D, et al. ESPGHAN revised porto criteria for the diagnosis of inflammatory bowel disease in children and adolescents. *Journal of Pediatric Gastroenterology and Nutrition*. 2014;58(6):795-806. doi:10.1097/MPG.0000000000000239

277. Ramraj R, Garcia A, Mosen D, Waiwaiole L, Smith N. Utility of Fecal Calprotectin in Evaluation of Chronic Gastrointestinal Symptoms in Primary Care. *Clinical Pediatrics*. 2018;57(9):1058-1063. doi:10.1177/0009922817744607

278. Carroccio A, Iacono G, Cottone M, et al. Diagnostic accuracy of fecal calprotectin assay in distinguishing organic causes of chronic diarrhea from irritable bowel syndrome: a prospective study in adults and children. *Clinical chemistry*. 2003;49(6 Pt 1):861-867.

279. Yang Z, Clark N, Park KT. Effectiveness and Cost-effectiveness of Measuring Fecal Calprotectin in Diagnosis of Inflammatory Bowel Disease in Adults and Children. *Clinical Gastroenterology and Hepatology*. 2014;12(2):253-262.e2. doi:10.1016/j.cgh.2013.06.028

280. Kawada PS, O'Loughlin E V, Stormon MO, Dutt S, Lee CH, Gaskin KJ. Are We Overdoing Pediatric Lower Gastrointestinal Endoscopy? *Journal of pediatric gastroenterology and nutrition*. 2017;64(6):898-902. doi:10.1097/MPG.0000000000001192

281. Hradsky O, Ohem J, Mitrova K, et al. Fecal calprotectin levels in children is more tightly associated with histological than with macroscopic endoscopy findings. *Clinical laboratory*. 2014;60(12):1993-2000.

282. Roca M, Rodriguez Varela A, Donat E, et al. Fecal Calprotectin and Eosinophil-derived Neurotoxin in Healthy Children Between 0 and 12 Years. *Journal of Pediatric Gastroenterology and Nutrition*. 2017;65(4):394-398. doi:10.1097/MPG.0000000000001542

283. Zhu Q, Li F, Wang J, Shen L, Sheng X. Fecal Calprotectin in Healthy Children Aged 1-4 Years. Ginsberg SD, ed. *PLOS ONE*. 2016;11(3):e0150725. doi:10.1371/journal.pone.0150725

284. Oord T, Hornung N. Fecal calprotectin in healthy children. *Scandinavian Journal of Clinical and Laboratory Investigation*. 2014;74(3):254-258. doi:10.3109/00365513.2013.879732

285. Oliveira SB, Monteiro IM. Diagnosis and management of inflammatory bowel disease in children. *BMJ*. May 2017:j2083. doi:10.1136/bmj.j2083

286. Brown JSS, Humphreys WGG, Parks TGG. Some clinical aspects of Crohn's disease in Northern Ireland: an aid to earlier diagnosis? 1988;38(317):549-551.

287. Nahon S, Lahmek P, Lesgourgues B, et al. Diagnostic delay in a French cohort of Crohn's disease patients. *Journal of Crohn's and Colitis*. 2014;8(9):964-969. doi:10.1016/j.crohns.2014.01.023

288. Burisch J, Pedersen N, Čuković-Čavka S, et al. East–West gradient in the incidence of inflammatory bowel disease in Europe: the ECCO-EpiCom inception cohort. *Gut*. 2014;63(4):588-597. doi:10.1136/gutjnl-2013-304636

289. Safroneeva E, Vavricka SR, Fournier N, et al. Impact of the early use of immunomodulators or TNF antagonists on bowel damage and surgery in Crohn's disease. *Alimentary Pharmacology & Therapeutics*. 2015;42(8):977-989. doi:10.1111/apt.13363

290. Lee D-W, Koo JS, Choe JW, et al. Diagnostic delay in inflammatory bowel disease increases the risk of intestinal surgery. *World journal of gastroenterology*. 2017;23(35):6474-6481. doi:10.3748/wjg.v23.i35.6474

291. Jellema P, van der Windt DAWM, Schellevis FG, van der Horst HE. Systematic review: accuracy of symptom-based criteria for diagnosis of irritable bowel syndrome in primary care. *Alimentary pharmacology & therapeutics*. 2009;30(7):695-706. doi:10.1111/j.1365-2036.2009.04087.x

292. Referral Support in Devon – Northern, Eastern and Western Devon Clinical Commissioning Group. https://www.newdevonccg.nhs.uk/information-for-patients/devon-referral-support-services-your-right-to-choose-100178. Accessed June 15, 2019.

293. MHCLG Open Data. http://opendatacommunities.org/home. Accessed June 15, 2019.

294. NHS Health Research Authority. Is my study research? http://www.hra-decisiontools.org.uk/research/index.html. Accessed December 22, 2017.

295. IBD organisational audit - Adult report - round four 2014 | RCP London. https://www.rcplondon.ac.uk/projects/outputs/ibd-organisational-audit-adult-report-round-four-2014. Published 2014. Accessed June 16, 2019.

296. Quarterly performance of the NHS provider sector: quarter 3 2018/19 | NHS

Improvement. https://improvement.nhs.uk/resources/quarterly-performance-nhs-provider-sector-quarter-3-201819/. Accessed June 16, 2019.

297. Seneviratne A. Diagnostic safety-netting. *British Journal of General Practice*. 2010;60(573):293.1-293. doi:10.3399/bjgp10x483986

298. Florkowski CM. Sensitivity, specificity, receiver-operating characteristic (ROC) curves and likelihood ratios: communicating the performance of diagnostic tests. *The Clinical biochemist Reviews*. 2008;29 Suppl 1(Suppl 1):S83-7.

299. Statistics » Monthly Diagnostics Data 2019-20. https://www.england.nhs.uk/statistics/statistical-work-areas/diagnostics-waiting-times-and-activity/monthly-diagnostics-waiting-times-and-activity/monthly-diagnostics-data-2019-20/. Accessed June 17, 2019.

300. Bekkink OM, McCowan C, Falk GA, Teljeur C, Van De Laar FA, Fahey T. Diagnostic accuracy systematic review of rectal bleeding in combination with other symptoms, signs and tests in relation to colorectal cancer. *British Journal of Cancer*. 2010;102(1):48-58. doi:10.1038/sj.bjc.6605426

301. Williams M, Barclay Y, Benneyworth R, et al. Using best practice to create a pathway to improve management of irritable bowel syndrome: aiming for timely diagnosis, effective treatment and equitable care. *Frontline gastroenterology*. 2016;7(4):323-330. doi:10.1136/flgastro-2016-100727

302. Watson J, Salisbury C, Banks J, Whiting P, Hamilton W. Predictive value of inflammatory markers for cancer diagnosis in primary care: a prospective cohort study using electronic health records. *British Journal of Cancer*. 2019;120(11):1045-1051. doi:10.1038/s41416-019-0458-x

303. Vuik FE, Nieuwenburg SA, Bardou M, et al. Increasing incidence of colorectal cancer in young adults in Europe over the last 25 years. *Gut*. May 2019:gutjnl-2018-317592. doi:10.1136/gutjnl-2018-317592

304. Sewell JL, Velayos FS. Systematic Review. *Inflammatory Bowel Diseases*. 2013;19(3):627-643. doi:10.1002/ibd.22986

305. Zaharie R, Tantau A, Zaharie F, et al. Diagnostic Delay in Romanian Patients with Inflammatory Bowel Disease: Risk Factors and Impact on the Disease Course and Need for Surgery. *Journal of Crohn's & colitis*. 2016;10(3):306-314. doi:10.1093/ecco-jcc/jjv215

306. Cantoro L, Di Sabatino A, Papi C, et al. The Time Course of Diagnostic Delay in Inflammatory Bowel Disease Over the Last Sixty Years: An Italian Multicentre Study. *Journal of Crohn's and Colitis*. 2017;11(8):975-980. doi:10.1093/ecco-jcc/jjx041

307. Van den Houte K, Carbone F, Pannemans J, et al. Prevalence and impact of self-reported irritable bowel symptoms in the general population. *United European Gastroenterology Journal*. 2019;7(2):307-315. doi:10.1177/2050640618821804

308. NHS England » Achieving World-Class Cancer Outcomes: progress report 2016-2020.

https://www.england.nhs.uk/cancer/strategy/achieving-world-class-cancer-outcomes-progress-report-2016-2020/. Accessed June 18, 2019.

309. Banerjea A, Voll J, Chowdhury A, et al. Straight-to-test colonoscopy for 2-week-wait referrals improves time to diagnosis of colorectal cancer and is feasible in a high-volume unit. *Colorectal Disease*. 2017;19(9):819-826. doi:10.1111/codi.13667

310. Christopher J, Flint T, Ahmed H, et al. Straight-to-test for the two-week-wait colorectal cancer pathway under the updated NICE guidelines reduces time to cancer diagnosis and treatment. *The Annals of The Royal College of Surgeons of England*. 2019;101(5):333-339. doi:10.1308/rcsann.2019.0022

311. Zhang W, Wong CH, Chavannes M, Mohammadi T, Rosenfeld G. Cost-effectiveness of faecal calprotectin used in primary care in the diagnosis of inflammatory bowel disease. *BMJ open*. 2019;9(4):e027043. doi:10.1136/bmjopen-2018-027043

312. Henriksen M, Jahnsen J, Lygren I, et al. Clinical course in Crohn's disease: results of a five-year population-based follow-up study (the IBSEN study). *Scandinavian journal of gastroenterology*. 2007;42(5):602-610. doi:10.1080/00365520601076124

313. TRUELOVE SC, WITTS LJ. Cortisone in ulcerative colitis; final report on a therapeutic trial. *British medical journal*. 1955;2(4947):1041-1048.

314. NHS Businesss Service Authority Pacific Team: calprotectin. https://www.nhsbsa.nhs.uk/sites/default/files/2018-06/CCG Faecal Calprotectin case study %28V2%29 4 pager - 06.2018.pdf.

315. Turvill J, Turnock D, Holmes H, et al. Evaluation of the clinical and cost-effectiveness of the York Faecal Calprotectin Care Pathway. *Frontline Gastroenterology*. 2018;9(4):285-294. doi:10.1136/flgastro-2018-100962

316. Quantitative faecal immunochemical tests to guide referral for colorectal cancer in primary care | Guidance and guidelines | NICE.

317. NHS England and NHS Improvement South West » Projects. https://www.england.nhs.uk/south/south-west-clinical-network/our-networks/cancer-network/projects/. Accessed May 12, 2019.

318. Elias SG, Kok L, de Wit NJ, et al. Is there an added value of faecal calprotectin and haemoglobin in the diagnostic work-up for primary care patients suspected of significant colorectal disease? A cross-sectional diagnostic study. *BMC medicine*. 2016;14(1):141. doi:10.1186/s12916-016-0684-5

319. Aronson JK, Ferner RE. Clarification of terminology in drug safety. *Drug safety*. 2005;28(10):851-870.

320. Bouvy JC, De Bruin ML, Koopmanschap MA. Epidemiology of adverse drug reactions in Europe: a review of recent observational studies. *Drug safety*. 2015;38(5):437-453. doi:10.1007/s40264-015-0281-0

321. Pirmohamed M, James S, Meakin S, et al. Adverse drug reactions as cause of admission to hospital: prospective analysis of 18 820 patients. *BMJ (Clinical research ed)*. 2004;329(7456):15-19. doi:10.1136/bmj.329.7456.15

322. Bruce C. Carleton, PharmD, M. Anne Smith Ms. Drug safety: Side effects and mistakes or adverse reactions and deadly errors? *BC MEDICAL JOURNAL*. 2006;48(329).

323. Lazarou J, Pomeranz BH, Corey PN, et al. Incidence of Adverse Drug Reactions in Hospitalized Patients. *JAMA*. 1998;279(15):1200. doi:10.1001/jama.279.15.1200

324. Uetrecht J, Naisbitt DJ. Idiosyncratic adverse drug reactions: current concepts. *Pharmacological reviews*. 2013;65(2):779-808. doi:10.1124/pr.113.007450

325. Heap GA, Weedon MN, Bewshea CM, et al. HLA-DQA1-HLA-DRB1 variants confer susceptibility to pancreatitis induced by thiopurine immunosuppressants. *Nature genetics*. 2014;46(10):1131-1134. doi:10.1038/ng.3093

326. Anderson JA, Adkinson NF. Allergic reactions to drugs and biologic agents. *JAMA*. 1987;258(20):2891-2899.

327. deShazo RD, Kemp SF. Allergic Reactions to Drugs and Biologic Agents. *JAMA: The Journal of the American Medical Association*. 1997;278(22):1895. doi:10.1001/jama.1997.03550220101014

328. Demoly P, Bousquet J. *Current Opinion in Allergy and Clinical Immunology.* Lippincott Williams & Wilkins; 2001.

329. Mallal S, Nolan D, Witt C, et al. Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *Lancet*. 2002;359(9308):727-732.

330. McCormack M, Alfirevic A, Bourgeois S, et al. HLA-A*3101 and carbamazepine-induced hypersensitivity reactions in Europeans. *The New England journal of medicine*. 2011;364(12):1134-1143. doi:10.1056/NEJMoa1013297

331. Ozeki T, Mushiroda T, Yowang A, et al. Genome-wide association study identifies HLA-A*3101 allele as a genetic risk factor for carbamazepine-induced cutaneous adverse drug reactions in Japanese population. *Human molecular genetics*. 2011;20(5):1034-1041. doi:10.1093/hmg/ddq537

332. Chung W-H, Hung S-I, Hong H-S, et al. Medical genetics: a marker for Stevens-Johnson syndrome. *Nature*. 2004;428(6982):486. doi:10.1038/428486a

333. Pirmohamed M, Naisbitt DJ, Gordon F, Park BK. The danger hypothesis--potential role in idiosyncratic drug reactions. *Toxicology*. 2002;181-182:55-63.

334. UK PGx Network. http://www.uk-pgx-stratmed.co.uk/. Accessed March 1, 2018.

335. IBDGenetics - Home page. https://www.ibdgenetics.org/. Accessed February 4, 2018.

336. iSAEC | International SAE Consortium. https://www.saeconsortium.org/. Accessed February 4, 2018.

337. Pirmohamed M, Aithal GP, Behr E, Daly A, Roden D. The phenotype standardization project: improving pharmacogenetic studies of serious adverse drug reactions. *Clinical pharmacology and therapeutics*. 2011;89(6):784-785. doi:10.1038/clpt.2011.30

338. Gallagher RM, Kirkham JJ, Mason JR, et al. Development and inter-rater reliability of the Liverpool adverse drug reaction causality assessment tool. *PloS one*. 2011;6(12):e28096. doi:10.1371/journal.pone.0028096

339. Chhaya V, Saxena S, Cecil E, et al. Steroid dependency and trends in prescribing for inflammatory bowel disease - a 20-year national population-based study. *Alimentary Pharmacology & Therapeutics*. 2016;44(5):482-494. doi:10.1111/apt.13700

340. Gisbert JP, Gomollon F. Thiopurine-Induced Myelotoxicity in Patients With Inflammatory Bowel Disease: A Review. *The American Journal of Gastroenterology*. 2008;103(7):1783-1800. doi:10.1111/j.1572-0241.2008.01848.x

341. Chaparro M, Ordás I, Cabré E, et al. Safety of thiopurine therapy in inflammatory bowel disease: long-term follow-up study of 3931 patients. *Inflammatory bowel diseases*. 2013;19(7):1404-1410. doi:10.1097/MIB.0b013e318281f28f

342. Chaparro M, Ordás I, Cabré E, et al. Safety of thiopurine therapy in inflammatory bowel disease: long-term follow-up study of 3931 patients. *Inflammatory bowel diseases*. 2013;19(7):1404-1410. doi:10.1097/MIB.0b013e318281f28f

343. Gearry RB, Barclay ML, Burt MJ, Collett JA, Chapman BA. Thiopurine drug adverse effects in a population of New Zealand patients with inflammatory bowel disease. *Pharmacoepidemiology and Drug Safety*. 2004;13(8):563-567. doi:10.1002/pds.926

344. Bastida G, Nos P, Aguas M, et al. Incidence, risk factors and clinical course of thiopurine-induced liver injury in patients with inflammatory bowel disease. *Alimentary pharmacology & therapeutics*. 2005;22(9):775-782. doi:10.1111/j.1365-2036.2005.02636.x

345. Gisbert JP, González-Lama Y, Maté J. Thiopurine-Induced Liver Injury in Patients With Inflammatory Bowel Disease: A Systematic Review. *The American Journal of Gastroenterology*. 2007;102(7):1518-1527. doi:10.1111/j.1572-0241.2007.01187.x

346. Gisbert JP, Gomollón F. Thiopurine-induced myelotoxicity in patients with inflammatory bowel disease: A Review. *American Journal of Gastroenterology*. 2008;103(7):1783-1800. doi:10.1111/j.1572-0241.2008.01848.x

347. SIEGEL CA, Sands BE. Review article: practical management of inflammatory bowel disease patients taking immunomodulators. *Alimentary Pharmacology and Therapeutics*. 2005;22(1):1-16. doi:10.1111/j.1365-2036.2005.02520.x

348. Schwab M, Schäffeler E, Marx C, et al. Azathioprine therapy and adverse drug reactions in patients with inflammatory bowel disease: impact of thiopurine S-methyltransferase

polymorphism. *Pharmacogenetics*. 2002;12(6):429-436.

349. Goldberg R, Irving PM. Toxicity and response to thiopurines in patients with inflammatory bowel disease. *Expert Review of Gastroenterology & Hepatology*. 2015;9(7):891-900. doi:10.1586/17474124.2015.1039987

350. Elkjaer M, Shuhaibar M, Burisch J, et al. E-health empowers patients with ulcerative colitis: a randomised controlled trial of the web-guided "Constant-care" approach. *Gut*. 2010;59(12):1652-1661. doi:10.1136/gut.2010.220160

351. González-Lama Y, Gisbert JP. Monitoring thiopurine metabolites in inflammatory bowel disease. *Frontline Gastroenterology*. 2016;7(4):301-307. doi:10.1136/flgastro-2015-100681

352. Weinshilboum RM, Sladek SL. Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. *American journal of human genetics*. 1980;32(5):651-662.

353. Lennard L, Van Loon JA, Lilleyman JS, Weinshilboum RM. Thiopurine pharmacogenetics in leukemia: correlation of erythrocyte thiopurine methyltransferase activity and 6-thioguanine nucleotide concentrations. *Clinical pharmacology and therapeutics*. 1987;41(1):18-25.

354. Lennard L. TPMT in the treatment of Crohn's disease with azathioprine. *Gut*. 2002;51(2):143-146. doi:10.1136/gut.51.2.143

355. European Medicines Agency - Find medicine - Xaluprine. http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/002022/human_med_001490.jsp&mid=WC0b01ac058001d124. Accessed March 1, 2018.

356. FDA approved drug products. www.fda.gov.cder/orange/default.htm. Published 2008. Accessed March 1, 2018.

357. Colombel JF, Ferrari N, Debuysere H, et al. Genotypic analysis of thiopurine S-methyltransferase in patients with Crohn's disease and severe myelosuppression during azathioprine therapy. *Gastroenterology*. 2000;118(6):1025-1030.

358. Teml A, Schaeffeler E, Herrlinger KR, Klotz U, Schwab M. Thiopurine Treatment in Inflammatory Bowel Disease. *Clinical Pharmacokinetics*. 2007;46(3):187-208. doi:10.2165/00003088-200746030-00001

359. Lee HJ, M.D.*, Yang S-K, et al. The Safety and Efficacy of Azathioprine and 6-Mercaptopurine in the Treatment of Korean Patients with Crohn's Disease. *Intestinal Research*. 2009;7(1):22-31.

360. Takatsu N, Matsui T, Murakami Y, et al. Adverse reactions to azathioprine cannot be predicted by thiopurine S-methyltransferase genotype in Japanese patients with inflammatory bowel disease. *Journal of Gastroenterology and Hepatology*. 2009;24(7):1258-1264. doi:10.1111/j.1440-1746.2009.05917.x

361. Jung YS, Cheon JH, Park JJ, et al. Correlation of genotypes for thiopurine methyltransferase and inosine triphosphate pyrophosphatase with long-term clinical outcomes in Korean patients with inflammatory bowel diseases during treatment with thiopurine drugs. *Journal of Human Genetics*. 2010;55(2):121-123. doi:10.1038/jhg.2009.125

362. Yang S-K, Hong M, Baek J, et al. A common missense variant in NUDT15 confers susceptibility to thiopurine-induced leukopenia. *Nature Genetics*. 2014;advance on(9):1017-1020. doi:10.1038/ng.3060

363. Moriyama T, Nishii R, Lin T-N, et al. The effects of inherited NUDT15 polymorphisms on thiopurine active metabolites in Japanese children with acute lymphoblastic leukemia. *Pharmacogenetics and Genomics*. 2017;27(6):236-239. doi:10.1097/FPC.0000000000000282

364. Bollen TL, van Santvoort HC, Besselink MG, et al. The Atlanta Classification of acute pancreatitis revisited. *The British journal of surgery*. 2008;95:6-21. doi:10.1097/00006676-200611000-00047

365. Wilson A, Jansen LE, Rose R V., et al. HLA-DQA1-HLA-DRB1 polymorphism is a major predictor of azathioprine-induced pancreatitis in patients with inflammatory bowel disease. *Alimentary Pharmacology & Therapeutics*. 2018;47(5):615-620. doi:10.1111/apt.14483

366. Björnsson ES, Bergmann OM, Björnsson HK, Kvaran RB, Olafsson S. Incidence, Presentation, and Outcomes in Patients With Drug-Induced Liver Injury in the General Population of Iceland. *Gastroenterology*. 2013;144(7):1419-1425.e3. doi:10.1053/j.gastro.2013.02.006

367. Search Livertox Database. LIVERTOX®. https://livertox.nih.gov/index.html. Published 2016. Accessed January 16, 2017.

368. Marubbio AT, Danielson B. Hepatic veno-occlusive disease in a renal transplant patient receiving azathioprine. *Gastroenterology*. 1975;69(3):739-743.

369. Dubinsky MC, Vasiliauskas EA, Singh H, et al. 6-thioguanine can cause serious liver injury in inflammatory bowel disease patients. *Gastroenterology*. 2003;125(2):298-303.

370. Gearry RB, Barclay ML. Azathioprine and 6-mercaptopurine pharmacogenetics and metabolite monitoring in inflammatory bowel disease. *Journal of Gastroenterology and Hepatology (Australia)*. 2005;20(8):1149-1157. doi:10.1111/j.1440-1746.2005.03832.x

371. Dubinsky MC, Yang H, Hassard P V, et al. 6-MP metabolite profiles provide a biochemical explanation for 6-MP resistance in patients with inflammatory bowel disease. *Gastroenterology*. 2002;122(4):904-915.

372. Warner B, Johnston E, Arenas-Hernandez M, Marinaki A, Irving P, Sanderson J. A practical guide to thiopurine prescribing and monitoring in IBD. *Frontline Gastroenterology*. 2018;9(1):10-15. doi:10.1136/flgastro-2016-100738

373. Meijer B, Wilhelm AJAJ, Mulder CJJCJJ, Bouma G, van Bodegraven AAAA, De Boer NKHNKH. Pharmacology of Thiopurine Therapy in Inflammatory Bowel Disease and Complete Blood Cell Count Outcomes: A 5-Year Database Study. *Therapeutic Drug Monitoring*. 2017;39(4):399-405. doi:10.1097/FTD.0000000000000414

374. McGovern DPBPB, Travis SPLPL, Duley J, Shobowale-Bakre EM, Dalton HRR. Azathioprine intolerance in patients with IBD may be imidazole-related and is independent of TPMT activity. *Gastroenterology*. 2002;122(3):838-839. doi:10.1053/gast.2002.32124

375. Fangbin Z, Xiang G, Liang D, et al. Prospective Evaluation of Pharmacogenomics and Metabolite Measurements upon Azathioprine Therapy in Inflammatory Bowel Disease: An Observational Study. *Medicine*. 2016;95(15):e3326. doi:10.1097/MD.0000000000003326

376. Marinaki AM, Ansari A, Duley JA, et al. Adverse drug reactions to azathioprine therapy are associated with polymorphism in the gene encoding inosine triphosphate pyrophosphatase (itpase). *Pharmacogenetics*. 2004;14(3):181-187.

377. Van Staa TP, Travis S, Leufkens HGM, Logan RF. 5-aminosalicylic acids and the risk of renal disease: a large British epidemiologic study. *Gastroenterology*. 2004;126(7):1733-1739.

378. Kornbluth A, Sachar DB, Practice Parameters Committee of the American College of Gastroenterology. Ulcerative Colitis Practice Guidelines in Adults: American College of Gastroenterology, Practice Parameters Committee. *The American Journal of Gastroenterology*. 2010;105(3):501-523. doi:10.1038/ajg.2009.727

379. Heap GA, So K, Weedon M, et al. Clinical features and HLA association of 5-Aminosalicylate (5-ASA) induced nephrotoxicity in inflammatory bowel disease. *Journal of Crohn's & colitis*. November 2015. doi:10.1093/ecco-jcc/jjv219

380. Das KM, Dubin R. Clinical pharmacokinetics of sulphasalazine. *Clinical pharmacokinetics*. 1(6):406-425.

381. Wiese MD, Alotaibi N, O'Doherty C, et al. Pharmacogenomics of NAT2 and ABCG2 influence the toxicity and efficacy of sulphasalazine containing DMARD regimens in early rheumatoid arthritis. *Pharmacogenomics Journal*. 2014;14(4):350-355. doi:10.1038/tpj.2013.45

382. Okkels H, Sigsgaard T, Wolf H, et al. Arylamine N-acetyltransferase 1 (NAT1) and 2 (NAT2) polymorphisms in susceptibility to bladder cancer: the influence of smoking. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 1997;6(4):225-231.

383. Yamasaki Y, Ieiri I, Kusuhara H, et al. Pharmacogenetic Characterization of Sulfasalazine Disposition Based on NAT2 and ABCG2 (BCRP) Gene Polymorphisms in Humans. *Clinical Pharmacology & Therapeutics*. 2008;84(1):95-103. doi:10.1038/sj.clpt.6100459

384. Knight RA, Selin MJ HH et al. Genetic factors influencing isoniazid blood levels in humans. *Trans Conf Chemother Tuberc*. 1959;8:52-6.

385. Evans DA. Survey of the human acetylator polymorphism in spontaneous disorders. *Journal of medical genetics*. 1984;21(4):243-253.

386. Genetic Basis of Drug Metabolism. https://www.medscape.com/viewarticle/444804_4. Accessed January 14, 2018.

387. Wadelius M, Stjernberg E, Wiholm BE, Rane A. Polymorphisms of NAT2 in relation to sulphasalazine-induced agranulocytosis. *Pharmacogenetics*. 2000;10(1):35-41.

388. SPARROW MP, HANDE SA, FRIEDMAN S, et al. Allopurinol safely and effectively optimizes tioguanine metabolites in inflammatory bowel disease patients not responding to azathioprine and mercaptopurine. *Alimentary Pharmacology and Therapeutics*. 2005;22(5):441-446. doi:10.1111/j.1365-2036.2005.02583.x

389. Sparrow MP. Use of allopurinol to optimize thiopurine immunomodulator efficacy in inflammatory bowel disease. *Gastroenterology & hepatology*. 2008;4(7):505-511.

390. Arellano F, Sacristán JA. Allopurinol Hypersensitivity Syndrome: A Review. *Annals of Pharmacotherapy*. 1993;27(3):337-343. doi:10.1177/106002809302700317

391. Zineh I, Mummaneni P, Lyndly J, et al. Allopurinol pharmacogenetics: assessment of potential clinical usefulness. *Pharmacogenomics*. 2011;12(12):1741-1749. doi:10.2217/pgs.11.131

392. Tassaneeyakul WW, Jantararoungtong T, Chen P, et al. Strong association between HLA-B*5801 and allopurinol-induced Stevens–Johnson syndrome and toxic epidermal necrolysis in a Thai population. *Pharmacogenetics and Genomics*. 2009;19(9):704-709. doi:10.1097/FPC.0b013e328330a3b8

393. HLA-B*5801 Screening to Prevent Allopurinol-induced Severe Cutaneous Adverse Reaction - Full Text View - ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT03046914. Accessed March 1, 2018.

394. Hershfield MS, Callaghan JT, Tassaneeyakul W, et al. Clinical Pharmacogenetics Implementation Consortium Guidelines for Human Leukocyte Antigen-B Genotype and Allopurinol Dosing. *Clinical Pharmacology & Therapeutics*. 2013;93(2):153-158. doi:10.1038/clpt.2012.209

395. CPIC. https://cpicpgx.org/. Accessed August 31, 2017.

396. Naganuma M, Fujii T, Watanabe M. The use of traditional and newer calcineurin inhibitors in inflammatory bowel disease. *Journal of Gastroenterology*. 2011;46(2):129-137. doi:10.1007/s00535-010-0352-z

397. Moes AD, Hesselink DA, Zietse R, van Schaik RH, van Gelder T, Hoorn EJ. Calcineurin inhibitors and hypertension: a role for pharmacogenetics? *Pharmacogenomics*. 2014;15(9):1243-1251. doi:10.2217/pgs.14.87

398. Ferraresso M, Turolo S, Ghio L, et al. Association Between CYP3A5 Polymorphisms and

Blood Pressure in Kidney Transplant Recipients Receiving Calcineurin Inhibitors. *Clinical and Experimental Hypertension*. 2011;33(6):359-365. doi:10.3109/10641963.2011.561896

399. Birdwell KA, Decker B, Barbarino JM, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for CYP3A5 Genotype and Tacrolimus Dosing. *Clinical pharmacology and therapeutics*. 2015;98(1):19-24. doi:10.1002/cpt.113

400. Cleynen I, Van Moerkercke W, Billiet T, et al. Characteristics of Skin Lesions Associated With Anti–Tumor Necrosis Factor Therapy in Patients With Inflammatory Bowel Disease. *Annals of Internal Medicine*. 2016;164(1):10. doi:10.7326/M15-0729

401. Toussirot É, Aubin F. Paradoxical reactions under TNF-α blocking agents and other biological agents given for chronic immune-mediated diseases: an analytical and comprehensive overview. *RMD open*. 2016;2(2):e000239. doi:10.1136/rmdopen-2015-000239

402. Tillack C, Ehmann LM, Friedrich M, et al. Anti-TNF antibody-induced psoriasiform skin lesions in patients with inflammatory bowel disease are characterised by interferon-γ-expressing Th1 cells and IL-17A/IL-22-expressing Th17 cells and respond to anti-IL-12/IL-23 antibody treatment. *Gut*. 2014;63(4):567-577. doi:10.1136/gutjnl-2012-302853

403. Guerra I, Pérez-Jeldres T, Iborra M, et al. Incidence, Clinical Characteristics, and Management of Psoriasis Induced by Anti-TNF Therapy in Patients with Inflammatory Bowel Disease. *Inflammatory Bowel Diseases*. 2016;22(4):894-901. doi:10.1097/MIB.0000000000000757

404. Pugliese D, Guidi L, Ferraro PM, et al. Paradoxical psoriasis in a large cohort of patients with inflammatory bowel disease receiving treatment with anti-TNF alpha: 5-year follow-up study. *Alimentary Pharmacology & Therapeutics*. 2015;42(7):880-888. doi:10.1111/apt.13352

405. Cullen G, Kroshinsky D, Cheifetz AS, Korzenik JR. Psoriasis associated with anti-tumour necrosis factor therapy in inflammatory bowel disease: a new series and a review of 120 cases from the literature. *Alimentary Pharmacology & Therapeutics*. 2011;34(11-12):1318-1327. doi:10.1111/j.1365-2036.2011.04866.x

406. P588 Ustekinumab for the treatment of Crohn's disease patients with TNF antagonist induced psoriasis. Real life experience from a tertiary referral center through week 54. https://www.ecco-ibd.eu/publications/congress-abstract-s/abstracts-2017/item/p588-ustekinumab-for-the-treatment-of-crohn-s-disease-patients-with-tnf-antagonist-induced-psoriasis-real-life-experience-from-a-tertiary-referral-center-through-week-54-2.html. Accessed March 1, 2018.

407. Münger C JP and NJ. Paradoxical Inflammation in IBD Patients Treated with Anti-Tumor Necrosis Factor Alpha Therapy. *Austin Journal Gastroenterology*. 2017;4(2): 1081.

408. Pirmohamed M. Acceptance of biomarker-based tests for application in clinical practice: criteria and obstacles. *Clinical pharmacology and therapeutics*. 2010;88(6):862-866. doi:10.1038/clpt.2010.245

409. The Cost of Sequencing a Human Genome - National Human Genome Research Institute (NHGRI). https://www.genome.gov/sequencingcosts/. Accessed February 7, 2018.

410. Schwarze K, Buchanan J, Taylor JC, Wordsworth S. Are whole-exome and whole-genome sequencing approaches cost-effective? A systematic review of the literature. *GENETICS in MEDICINE*. February 2018. doi:10.1038/gim.2017.247

411. Whirl-Carrillo M, McDonagh EM, Hebert JM, et al. Pharmacogenomics knowledge for personalized medicine. *Clinical pharmacology and therapeutics*. 2012;92(4):414-417. doi:10.1038/clpt.2012.96

412. Lakatos PL, Kiss LS. Current status of thiopurine analogues in the treatment in Crohn's disease. *World Journal of Gastroenterology*. 2011;17(39):4372. doi:10.3748/wjg.v17.i39.4372

413. Moran GW, Dubeau M-F, Kaplan GG, et al. Clinical predictors of thiopurine-related adverse events in Crohn's disease. *World journal of gastroenterology*. 2015;21(25):7795-7804. doi:10.3748/wjg.v21.i25.7795

414. Dewit O, Moreels T, Baert F, et al. Limitations of extensive TPMT genotyping in the management of azathioprine-induced myelosuppression in IBD patients. *Clinical biochemistry*. 2011;44(13):1062-1066. doi:10.1016/j.clinbiochem.2011.06.079

415. Moriyama T, Nishii R, Perez-Andreu V, et al. NUDT15 polymorphisms alter thiopurine metabolism and hematopoietic toxicity. *Nature genetics*. 2016;48(4):367-373. doi:10.1038/ng.3508

416. Moriyama T, Yang YL, Nishii R, et al. Novel variants in NUDT15 and thiopurine intolerance in children with acute lymphoblastic leukemia from diverse ancestry. *Blood*. 2017;130(10):1209-1212. doi:10.1182/blood-2017-05-782383

417. Kakuta Y, Naito T, Onodera M, et al. NUDT15 R139C causes thiopurine-induced early severe hair loss and leukopenia in Japanese patients with IBD. *The Pharmacogenomics Journal*. 2016;16(3):280-285. doi:10.1038/tpj.2015.43

418. Zhu X, Wang X-D, Chao K, et al. NUDT15 polymorphisms are better than thiopurine S-methyltransferase as predictor of risk for thiopurine-induced leukopenia in Chinese patients with Crohn's disease. *Alimentary pharmacology & therapeutics*. 2016;44(9):967-975. doi:10.1111/apt.13796

419. Soler AM, Olano N, Méndez Y, et al. *TPMT* and *NUDT15* genes are both related to mercaptopurine intolerance in acute lymphoblastic leukaemia patients from Uruguay. *British Journal of Haematology*. 2018;181(2):252-255. doi:10.1111/bjh.14532

420. Shah SA V, Paradkar M, Desai D, Ashavaid TF. Nucleoside diphosphate-linked moiety X-type motif 15 C415T variant as a predictor for thiopurine-induced toxicity in Indian patients. *Journal of Gastroenterology and Hepatology*. 2017;32(3):620-624. doi:10.1111/jgh.13494

421. Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: A Tool for Genome-wide Complex Trait

Analysis. *The American Journal of Human Genetics*. 2011;88(1):76-82. doi:10.1016/j.ajhg.2010.11.011

422. 1000 Genomes Project Consortium A, Auton A, Brooks LD, et al. A global reference for human genetic variation. *Nature*. 2015;526(7571):68-74. doi:10.1038/nature15393

423. Loh P-R, Danecek P, Palamara PF, et al. Reference-based phasing using the Haplotype Reference Consortium panel. *Nature genetics*. 2016;48(11):1443-1448. doi:10.1038/ng.3679

424. Durbin R. Efficient haplotype matching and storage using the positional Burrows-Wheeler transform (PBWT). *Bioinformatics*. 2014;30(9):1266-1272. doi:10.1093/bioinformatics/btu014

425. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics (Oxford, England)*. 2009;25(14):1754-1760. doi:10.1093/bioinformatics/btp324

426. Ionita-Laza I, Lee S, Makarov V, Buxbaum JD, Lin X. Sequence Kernel Association Tests for the Combined Effect of Rare and Common Variants. *The American Journal of Human Genetics*. 2013;92(6):841-853. doi:10.1016/j.ajhg.2013.04.015

427. Lek M, Karczewski KJ, Minikel E V., et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016;536(7616):285-291. doi:10.1038/nature19057

428. Choi Y, Sims GE, Murphy S, Miller JR, Chan AP. Predicting the functional effect of amino acid substitutions and indels. de Brevern AG, ed. *PloS one*. 2012;7(10):e46688. doi:10.1371/journal.pone.0046688

429. CPIC Clinical Pharmacogenetics Implementation Consortium.

430. Tonk ECM, Gurwitz D, Maitland-van der Zee A-H, Janssens ACJW. Assessment of pharmacogenetic tests: presenting measures of clinical validity and potential population impact in association studies. *The Pharmacogenomics Journal*. May 2016. doi:10.1038/tpj.2016.34

431. de Graaff L, van Schaik R, van Gelder T. A clinical approach to pharmacogenetics. - PubMed - NCBI. *Neth J Med*. 2013;71(3):145-152.

432. Lennard L, Cartwright CS, Wade R, Vora A. Thiopurine methyltransferase and treatment outcome in the UK acute lymphoblastic leukaemia trial ALL2003. *British Journal of Haematology*. 2015;170(4):550-558. doi:10.1111/bjh.13469

433. Nishii R, Moriyama T, Janke LJ, et al. Preclinical evaluation of *NUDT15* -guided thiopurine therapy and its effects on toxicity and antileukemic efficacy. *Blood*. 2018;131(22):2466-2474. doi:10.1182/blood-2017-11-815506

434. Yang JJ, Landier W, Yang W, et al. Inherited NUDT15 variant is a genetic determinant of mercaptopurine intolerance in children with acute lymphoblastic leukemia. *Journal of*

Clinical Oncology. 2015;33(11):1235-1242. doi:10.1200/JCO.2014.59.4671

435. Winter J, Walker A, Shapiro D, Gaffney D, Spooner RJ, Mills PR. Cost-effectiveness of thiopurine methyltransferase genotype screening in patients about to commence azathioprine therapy for treatment of inflammatory bowel disease. *Alimentary Pharmacology and Therapeutics*. 2004;20(6):593-599. doi:10.1111/j.1365-2036.2004.02124.x

436. Rubin GP, Hungin AP, Kelly PJ, Ling J. Inflammatory bowel disease: epidemiology and management in an English general practice population. *Alimentary pharmacology & therapeutics*. 2000;14(12):1553-1559.

437. Kirchgesner J, Lemaitre M, Carrat F, Zureik M, Carbonnel F, Dray-Spira R. Risk of Serious and Opportunistic Infections Associated With Treatment of Inflammatory Bowel Diseases. *Gastroenterology*. 2018;155(2):337-346.e10. doi:10.1053/j.gastro.2018.04.012

438. Genomic inflation factor and population structure. http://rstudio-pubs-static.s3.amazonaws.com/9743_8a5f7ba3aa724d4b8270c621fdf6d06e.html. Accessed January 12, 2018.

439. Korn JM, Kuruvilla FG, McCarroll SA, et al. Integrated genotype calling and association analysis of SNPs, common copy number polymorphisms and rare CNVs. *Nature genetics*. 2008;40(10):1253-1260. doi:10.1038/ng.237

440. KING: Relationship Inference Software. http://people.virginia.edu/~wc9c/KING/. Accessed October 14, 2018.

441. Consortium the HR, McCarthy S, Das S, et al. A reference panel of 64,976 haplotypes for genotype imputation. *Nature Genetics*. 2016;48(10):1279-1283. doi:10.1038/ng.3643

442. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome research*. 2010;20(9):1297-1303. doi:10.1101/gr.107524.110

443. PLINK/SEQ genetics library. https://atgu.mgh.harvard.edu/plinkseq/index.shtml. Accessed June 9, 2018.

444. Dubinsky MC, Lamothe S, Yang HY, et al. Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease. *Gastroenterology*. 2000;118(4):705-713.

445. Maybaum J, Mandel HG, Krynetski EY, Vanin EF, Roussel MF, Relling M V. Unilateral chromatid damage: a new basis for 6-thioguanine cytotoxicity. *Cancer research*. 1983;43(8):3852-3856.

446. Relling M V., Schwab M, Whirl-Carrillo M, et al. Clinical Pharmacogenetics Implementation Consortium Guideline for Thiopurine Dosing Based on TPMT and NUDT15 Genotypes: 2018 Update. *Clinical Pharmacology and Therapeutics*. 2019;105(5):1095-1105. doi:10.1002/cpt.1304

447. Valerie NCK, Hagenkort A, Page BDG, et al. NUDT15 Hydrolyzes 6-Thio-DeoxyGTP to Mediate the Anticancer Efficacy of 6-Thioguanine. *Cancer Research*. 2016;76(18):5501-5511. doi:10.1158/0008-5472.CAN-16-0584

448. Collie-Duguid ES, Pritchard SC, Powrie RH, et al. The frequency and distribution of thiopurine methyltransferase alleles in Caucasian and Asian populations. *Pharmacogenetics*. 1999;9(1):37-42.

449. Kim JH, Cheon JH, Hong SS, et al. Influences of thiopurine methyltransferase genotype and activity on thiopurine-induced leukopenia in Korean patients with inflammatory bowel disease: a retrospective cohort study. *Journal of clinical gastroenterology*. 2010;44(10):e242-8. doi:10.1097/MCG.0b013e3181d6baf5

450. Sato T, Takagawa T, Kakuta Y, et al. NUDT15, FTO, and RUNX1 genetic variants and thiopurine intolerance among Japanese patients with inflammatory bowel diseases. *Intestinal research*. 2017;15(3):328-337. doi:10.5217/ir.2017.15.3.328

451. Kakuta Y, Kinouchi Y, Shimosegawa T. Pharmacogenetics of thiopurines for inflammatory bowel disease in East Asia: prospects for clinical application of NUDT15 genotyping. *Journal of Gastroenterology*. 2018;53(2):172-180. doi:10.1007/s00535-017-1416-0

452. Lewis JD, Abramson O, Pascua M, et al. Timing of myelosuppression during thiopurine therapy for inflammatory bowel disease: implications for monitoring recommendations. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association*. 2009;7(11):1195-1201; quiz 1141-1142. doi:10.1016/j.cgh.2009.07.019

453. gnomAD browser. http://gnomad.broadinstitute.org/. Accessed June 25, 2018.

454. Sands BE, Peyrin-Biroulet L, Loftus E V., et al. 416a – Vedolizumab Shows Superior Efficacy Versus Adalimumab: Results of Varsity—The First Head-To-Head Study of Biologic Therapy for Moderate-To-Severe Ulcerative Colitis. *Gastroenterology*. 2019;156(6):S-81. doi:10.1016/S0016-5085(19)36989-6

455. Hazlewood GS, Rezaie A, Borman M, et al. Comparative Effectiveness of Immunosuppressants and Biologics for Inducing and Maintaining Remission in Crohn's Disease: A Network Meta-analysis. *Gastroenterology*. 2015;148(2):344-354.e5. doi:10.1053/j.gastro.2014.10.011

456. Vermeire S, Loftus E V., Colombel J-F, et al. Long-term Efficacy of Vedolizumab for Crohn's Disease. *Journal of Crohn's and Colitis*. 2016;11(4):jjw176. doi:10.1093/ecco-jcc/jjw176

Appendices

Appendix A: Faecal calprotectin pathway in adults

Irritable Bowel Syndrome (IBS) Diagnosis and Management for adults under the age of 50

Irritable bowel syndrome can be difficult to diagnose, and it is important to reach the correct diagnosis while striking the right balance between too few and too many investigations.

This IBS pathway aims to provide a patient focused and cost effective diagnostic and management pathway for people with irritable bowel syndrome.

The objectives are:

- Support healthcare professionals to make a positive diagnosis of IBS and to manage patients in primary care, if no red flag indicators are present and investigations are normal. A positive diagnosis will help to reduce unnecessary anxiety in people with symptoms of irritable bowel syndrome and to start effective treatment.
- Where there is diagnostic uncertainty in diagnosing IBS versus inflammatory bowel disease (IBD), to support healthcare professionals in diagnosis with use of faecal calprotectin, reducing avoidable invasive endoscopic procedures
- Provide earlier access to specialist dietetic support and improve patient experience

Key Messages

Scope

This guidance refers to:

- Patients aged 18-49 years who present with lower gastrointestinal symptoms in whom you suspect IBS or IBD

Out of scope

This guidance does not cover:

- Patients under the age of 18 or over the age of 50 (for patients ≥ 50 years old please see local FIT testing guidelines)
- Patients where colorectal cancer is suspected (see red flags)
- Patients in whom there is diagnostic certainty of an IBS diagnosis

Assessment

Signs and Symptoms

Consider IBS when the patient presents with:

- Abdominal pain
- Bloating

Version 14.3 Locality: Eastern
Date last edited: April 2019 Amendments by: Joe Mays/Chris Calvert

- Change of bowel habit

This is usually accompanied by at least two of the following:

- Related to defecation
- Associated with a change in bowel habit
- Associated with a change in stool form (appearance)

Common additional symptoms include abdominal bloating and distension.

Other features such as lethargy, nausea, depression/anxiety, fibromyalgia, backache & bladder symptoms are common in people with IBS, and may be used to support the diagnosis.

History and Examination

Based on the history, IBS can be divided into:

- IBS with diarrhoea (IBS-D) = loose (mushy) or watery stools for ≥25% of bowel movements and hard or lumpy stool for ≤25% of bowel movements.
- IBS with constipation (IBS-C) = hard or lumpy stools for ≥25% of bowel movements and loose (mushy) or watery stools for ≤25% of bowel movements.
- Mixed IBS (IBS-M) = hard or lumpy stools for ≤25% of bowel movements and loose (mushy) or watery stools for ≤25% of bowel movements.
- Unspecified IBS: insufficient abnormality of stool consistency to meet criteria for IBS-C, IBS-D, or IBS-M

The classification of IBS patients into sub-groups is useful for clinical practice, but it is common for IBS patients to switch from one subtype to another over time. More than 75% of IBS patients change to either of the other 2 subtypes at least once over a 1-year period.

Differential Diagnoses

Differential diagnoses may include:

- Inflammatory Bowel Disease (IBD)
- Coeliac disease
- Chronic pancreatitis or pancreatic insufficiency (perform faecal elastase)
- Bile acid malabsorption (common following cholecystectomy)
- Malignancy
- Infection

Red Flags

Please see the suspected cancer NICE guidelines NG12 and the latest local DG30 guidelines for faecal immunochemical testing (FIT)in patients ≥50 years. Patients meeting these criteria should be referred via the lower GI 2ww pathway:

Referral

IBS is a condition to be primarily managed in the community. In patients with symptoms of IBS and that have not responded to simple lifestyle, dietary and pharmacological therapy as recommended by NICE consider referral to the Specialist IBS Dietetic services.

See:

- IBS Investigation flow chart ***LINK***
- IBS Management ***LINK TO MANAGEMENT PAGE ON FORMULARY***

Referrals should only go on to secondary care gastroenterology with a negative faecal calprotectin (<100 µg/g) if there remains a significant doubt of the diagnosis of IBS and in severe refractory cases that have not responded to specialist IBS dietary changes and first- and second-line medical treatment. Note referrals to Gastroenterology that have not been managed as per this guideline will be rejected.

Referral Instructions

Pathway 1: Specialist Dietician pathway

e-Referral Service Selection
Specialty: Dietetics
Clinic Type: Gastroenterology
Service: DRSS-Eastern-Dietetic-Devon CCG -15N

Pathway 2: Suspected IBD for luminal Gastroenterology (to be seen within 2 weeks)

In an unwell patient with acute abdominal pain or significant bloody diarrhoea and possible IBD, due not let primary care investigations delay appropriate urgent assessment, please contact the on-call Consultant Gastroenterologist or use the electronic advice and guidance service.

Pathway 3: Gastroenterology refractory IBS pathway

e-Referral Service Selection
Specialty: GI & Liver
Clinic Type: Lower GI (medical) excl IBD
Service: DRSS-Eastern-GI & Liver (Medicine & Surgery)-Devon CCG -15N

*****Please highlight on the referral form that the referral is in relation to refractory IBS*****

Investigations

LINK TO FLOWCHART

In adults 18-49 years old, with symptoms suggestive of IBS please organise:

- Full Blood Count (FBC)
- Coeliac serology - **please do not repeat if previous performed in last 3 years**
- C-reactive Protein (CRP)
- **If diarrhoea**, Stool for culture and sensitivity

A positive diagnosis of IBS always helps management: patients without 'red flags' and with normal tests should be managed in primary care. Please see IBS management flowchart.

Diagnostic uncertainty between IBS and IBD

If the above bloods tests are normal but you still suspect IBD please organise:

- Stool faecal calprotectin – sampling from the first bowel movement of the day when the patient is most symptomatic is recommended. This may increase the diagnostic yield.
- Please ensure off NSAID and PPIs for 2 weeks prior to testing

IBS symptoms and signs versus IBD

IBS Symptoms	IBD- Ulcerative colitis	IBD- Crohns disease
Abdominal Pain	Blood mixed in stool	Abdominal Pain
Bloating	Diarrhoea including nocturnal	Weight loss
Change in bowel habit - Typically alternating	Urgency/incontinence	Diarrhoea
Other features: mood, backache, bladder symptoms Fibromyalgia, headaches	Family history IBD	Family history of IBD
	Erythema nodosum, uveitis	Erythema nodosum, uveitis

About the Calprotectin stool test

- Calprotectin is a protein released into the gastrointestinal tract when it is inflamed, such as in inflammatory bowel disease (IBD; Crohn's disease and ulcerative colitis) it is stable protein, so can be detected in the stool by laboratory assay.
- Elevated levels of faecal calprotectin are found in IBD.
- By contrast, in functional disorders of the gastrointestinal tract, such as the irritable bowel syndrome (IBS) faecal calprotectin levels are normal.
- Clinically, it can be difficult to be able to distinguish IBS from IBD based on symptoms, signs and blood tests. Here, faecal calprotectin can be used as a biomarker to support your assessment.

- No biomarker test is 100% accurate but this IBS care pathway has been shown to be effective and safe in supporting your clinical decision making. (J. Turvill *et al*, Frontline Gastroenterology, 2017)

What do the results mean?

- **Faecal calprotectin <100 µg/g - IBS is 98% likely**

If all blood tests and faecal calprotectin are less than 100 µg/g – reassure and manage as IBS – unless there remains a significant clinical doubt as to the diagnosis.

- **Faecal calprotectin 100-250 µg/g -IBD is 12% likely**

This result is equivocal and the Calprotectin should be repeated in 2 weeks. If the repeat result remains ≥100 µg/g, then refer urgently to gastroenterology highlighting suspected IBD.

In an unwell patient with acute abdominal pain or significant bloody diarrhoea and possible IBD, due not let primary care investigations delay appropriate urgent assessment, please contact the on call Consultant Gastroenterologist or use the electronic advice and guidance service, otherwise:

Before repeating please exclude:

  - Non-steroidal anti-inflammatory drugs (NSAID) ingestion within the last 2 weeks
  - GI Infection (repeat stool culture)

- **Faecal calprotectin >250 µg/g IBD is 46% likely**

In an unwell patient with acute abdominal pain or significant bloody diarrhoea and possible IBD, due not let primary care investigations delay appropriate urgent assessment, please contact the on-call Consultant Gastroenterologist or use the electronic advice and guidance service, otherwise:

More than 250 µg/g – refer **urgently** to gastroenterology highlighting **suspected IBD**. Please ensure a stool sample is sent to rule out infection as a possible cause of the symptoms.

Management

For management guidance please see the 'Symptom management' guidance under the Irritable Bowel Syndrome information in the Formulary section of the Formulary & Referral website here.

PLEASE NOTE: The recommendations in the management guidance are currently under review, with possible changes to come

Appendix B: Frequencies[a] of NUDT15 alleles in major race/ethnic groups[b,f]

NUDT15 Allele[c]	Effect on protein (NP_060753.1)	CPIC Assigned Allele Functional Status	African Allele Frequency	Caucasian (European + North American) Allele Frequency	East Asian Allele Frequency	South/ Central Asian Allele Frequency	Americas Allele Frequency
***1[d]**	NA	Normal	0.997	0.993	0.879	0.930	0.936
***2**	p.Gly17_Val18dup[e] and R139C	Normal function	0.000	0.000	0.035	0.000	0.037
***3**	R139C	No function	0.001	0.002	0.061	0.067	0.008
***4**	R139H	No function	0.000	0.000	0.001	0.000	0.018
***5**	V18I	Uncertain function	0.000	0.000	0.011	0.000	0.000
***6**	p.Gly17_Val18dup[e]	Uncertain Function	0.002	0.003	0.013	0.002	0.002
***7**	R34T	Uncertain Function	0.000	0.000	0.001	0.000	0.000
***8**	K35E	Uncertain Function	n/a	n/a	n/a	n/a	n/a
***9**	G17_V18del	Uncertain Function	0.000	0.002	0.000	0.000	0.000
Total variant carrier allele frequency (i.e. *2 to*9)			**0.003**	**0.007**	**0.121**	**0.070**	**0.064**
Percentage of population that are either heterozygote or homozygote for alleles *2 to*9			**0.6%**	**1.4%**	**22.7%**	**13.5%**	**12.3%**

Table adapted from CPIC guidelines. The allele frequency table was made by searching the PubMed® database (no start date to 1/2018). Allele frequencies reported in the gnomAD browser (http://gnomad.broadinstitute.org/ - exomes and genomes) and ensembl (grch37.ensembl.org - exomes or genomes) were also included.

[a] Average frequencies based on the reported frequencies in one or multiple studies.

[b] Worldwide race/ethnic designations are based on the Human Genome Diversity Project- CEPH).

[c] See allele definition table (https://www.pharmgkb.org/page/nudt15RefMaterials) for allele definitions

[d] Because NUDT15*1 is not genotyped directly, all alleles that are negative for a sequence variation are defaulted to a NUDT15*1 assignment. The inferred frequency for NUDT15*1 is calculated as: 1 - (sum of averaged variant allele frequencies).

[e] p.Gly17_Val18dup is synonymous with V18_V19insGV

[f] insufficient data in Middle eastern, Occeanian and American African American to estimate allele frequencies in these ethnicities.

[g] carriage frequencies were estimated using the equation describing Hardy Weinberg equilibrium (genotype frequency = $p^2 + 2pq + q^2$) based on reported allele frequencies. i.e. percentage of population that are either heterozygote or homozygote for variant = $[1-p^2]*100$ = [1-(*1 allele frequency^2)]*100

THE END