Micronutrient status influences clinical outcomes of paediatric cancer patients during treatment: a prospective cohort study

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Abstract

Background: Research reporting plasma micronutrient status and its impact on clinical outcomes in paediatric cancer is scarce. Therefore, we investigated the prevalence of plasma micronutrient abnormalities and their impact on clinical outcomes and treatment complications.

Methods: A multicentre prospective-cohort study of children aged <18 years diagnosed with cancer was performed between Aug 2010-Jan 2014. Clinical and nutritional data were collected at diagnosis, 3, 6, 9, 12 and 18 months. Micronutrient status was established using in-house laboratory references (vitamin B12, vitamin A and Vitamin E/Ch) and aged adjusted *Z*-scores (Mg, Se, Zn and Cu) generated from a cohort of healthy Scottish children. Clinical outcomes were classified as "event free survival (EFS)" or "event" (relapse, death, new metastasis or becoming palliative) and treatment complications. Descriptive statistics, logistic regression multilevel analysis were performed.

Results: Eighty-two patients [median (IQR) 3.9 (1.9-8.8) years, 56% males] were recruited. Of these, 72 (88%) samples were available, 74% (53/72) patients had micronutrient abnormalities at baseline; deficiencies (25%, 18/72), excesses (19%, 14/72) and a combination of both (29%, 21/72), which continued for 18 months. Vitamin A deficiency (15%, 3/20) and excess (50%, 10/20) were most prevalent at 18 months, whilst vitamin E/Cholesterol and vitamin B12 were mostly within the normal range. Prevalence of Zn deficiency at diagnosis was 36% (16/44 adjusted for CRP), which remained at these levels throughout the study. Reduction in each selenium concentration unit increased the odds of an event by 2% (OR 0.02) and lower Se predicted higher complications at diagnosis [β (-1.2); t (-2.1); 95% CI (-2.9 – (-0.04)); $p = 0.04$], 3 months [β (-3.9); t (-4.2); 95% CI (-5.57 – (-2.02)); $p < 0.001$] and 12 months [β (-2.3); t (-2.4); 95% CI (-4.10 – (-0.34)); $p = 0.02$]

Conclusions: Given the prevalence of micronutrient abnormalities and the negative impact of low selenium on clinical outcome, micronutrient status should be assessed and monitored in paediatric cancer patients. Larger multicentre population based studies and clinical trials are now warranted.

Keywords: childhood cancer, paediatrics, micronutrient, vitamins, minerals

Introduction

Childhood cancer remains the most common disease related cause of death in industrialised societies[1]. Nonetheless, 5-year survival rates have doubled to 82% in some type of cancers in the last 40 years[1]. This improvement is due to more advanced, targeted and intensive therapies, more sophisticated technology and the success of medical clinical trials in combination with a more holistic approach to patient care[2, 3]. Consequently, attention is focused on reduction of treatment related sequelae during and after therapy[4-6]. Childhood cancer associated malnutrition and its impact on clinical outcomes, including, morbidity[7], mortality[8-10], early relapse[11] and the number of complications during treatment[4, 12], has long been recognised in the Western World[4, 5, 8, 13] and in low- and middle-income countries[6]. However, most research has focused on anthropometry, linear growth and body composition to assess nutritional status, whilst vitamin and trace element (VTE) status and its potential association with clinical outcomes have received little attention[14-19].

Malnutrition, defined as a "state of nutrition in which a deficiency, excess, or imbalance of energy, protein, and other nutrients causing measurable adverse effects on tissue, body size, composition and function, and clinical outcome"[20], is multifactorial and at present, there is not a single "gold standard" method that best assesses it in ill children[4]. Micronutrient abnormalities have been reported in up to 96% of paediatric cancer patients receiving treatment and neutropenic diets[21], despite patients meeting the recommended daily intakes[21]. However, evidence is scarce and limited by their small sample sizes. Furthermore, most studies have focused on vitamin D[22, 23], whilst other VTE, including vitamin A[14-17, 21, 24], vitamin E[14-17, 21], selenium[14, 15, 25], zinc[14, 15, 19], vitamin B12[18, 24], copper[15, 19] and iron/ferritin[26], have seldom been investigated.

Micronutrients encompasses vitamins, minerals and trace elements. Vitamins are organic molecules, which play a key role in indispensable body functions. This include, but not limited to, energy metabolism, antioxidant, gene regulation, cellular differentiation and organ function[27]. Likewise, minerals and trace elements (inorganic molecules) are involved in antioxidant and organ function, as well as enzymatic systems[27]. Clinical signs of micronutrient deficiencies can often be masked by treatment induced side-effects[21], and at the same time exacerbate toxicity[15, 17]. Furthermore,

deficiencies can develop from xerostomia, mucositis, nausea and vomiting or hepatotoxicity/ nephrotoxicity caused by chemotherapy and radiotherapy complications. Therefore, micronutrient abnormalities can be masked by a patient's phenotypic nutritional status, placing both normally-nourished and overnourished patients at risk of micronutrient malnutrition (deficiency or excess). This is particularly important since data from healthy obese children and adolescents consistently report micronutrient deficiencies, which have been attributed to a combination of high intake of energy dense foods low in vitamins and minerals, inadequate intake relative to overall body mass and alterations in micronutrient metabolism[28]. Since micronutrient concentration are rarely assessed within paediatric oncology research, the prevalence of micronutrient deficiency or excess at diagnosis and throughout treatment are relatively unknown[24].

Despite the importance of micronutrient status to health, there is a paucity of evidence reporting micronutrient status in paediatric cancer patients during treatment, only two, of small sample size, have investigated their associations with treatment complications and no study has looked at associations with event or event free survival (EFS). To address this clinical question, we aimed to investigate the *(i)* prevalence of micronutrient abnormalities and intakes (vitamin A, E, B12, copper, magnesium, selenium and zinc) in newly diagnosed paediatric cancer patients and during treatment at defined time points for 18 months; *(ii)* to identify changes in micronutrient status during this period; *(iii)* the relationship between micronutrient status and dietary intakes; *(iv)* the impact of micronutrient status on clinical outcome (event v EFS) and *(v)* treatment complications.

Methods

Study design, population and time-line

A prospective cohort study was performed. Eligibility criteria included: children aged <18 years diagnosed with cancer (ICCC-3)[29] or Langerhans Cell Histiocytosis between Aug-2010 and Feb-2014; attending the South East Scotland regional centre for Haematology and Oncology at the Royal Hospital for Sick Children (RHSC), Edinburgh or Ninewells Hospital, Dundee and patients were recruited consecutively. We excluded children who were treated with palliative intent. Children were

recruited continuously during the study period and were monitored for a maximum period of 18 months and a minimum of 3 months. Measurements were obtained at baseline, 3, 6, 9, 12 and 18 months by two trained researchers in clinic or on the ward. Anonymised data were obtained from medical records of patients who met the eligibility criteria but did not consent to the study. This was done to establish whether the cohort was representative of the SE Scottish paediatric oncology population. Aged adjusted *Z*-scores (Mg, Se, Zn and Cu) were generated from a cohort of healthy Scottish children.

Demographics and clinical parameters

Clinical data (diagnosis, treatment protocol and length of treatment) and demographic data (age, gender, ethnicity and socio-economic deprivation) were collected from medical notes. Clinical outcomes were classified as "event free survival (EFS)" or "event" (relapse, death, new metastasis or becoming palliative during the study period) and treatment complications as the total number of complications at each time point. Treatment intensity was classified as low, medium and high according to Kazak et al[30] and the Standard Index of Multiple Deprivation (SIMD) was used as a proxy marker for socio-economic deprivation of individuals[31]. The paediatric cancer cohort was grouped according to the wider definition of solid tumours, haematological cancers, brain tumours and other associated diagnosis.

Ethical approval was granted from NHS Scotland (NHS REC 06-51104-52).

Measurements of nutritional status and reference values

Measurements of weight and height (or length for infants <2 years) at the time of diagnosis were obtained from clinical notes. Following recruitment, all measurements were taken at each follow up. Measurements of growth and body composition were repeated three times. Weight and height or length were obtained following standard procedures[32]. Body mass index (BMI) was calculated and *Z*-scores were obtained from LMS Growth programme[33]. Nutritional status was classified as underweight (-2.0 SD or ≤ 2.3rd centile), normal weight (> -2.0 to ≤1.05 SD or > 2.0nd to < 85th centile), overweight (> 1.05 to ≤1.63 SD or >85th to ≤95th centile) and obese (1.63 SD or >95th centile)[33, 34].

The percentage of fat mass (FM) and fat free mass (FFM) was measured using a calibrated SF-BIA Quantum II RJL System (frequency 50 kHz) following manufacturer's instructions. The estimation of FM and FFM was calculated using Schaefer et al.[35, 36] total body water equation and the reference values used were Fomon et al.[37] for children <10 years old and Wells et al.[38] for children aged 10 – 18.

Blood collection, procedures and reference values

Blood samples were collected by nursing staff and analysed using standard operating procedures by the hospital's own accredited NHS laboratory. Standard sensitivity C-reactive protein (ss-CRP) was analysed using Abbott Architect C8000 Analyser by a Turbidimetric method at the Royal Infirmary of Edinburgh[39]. The intermediate precision expressed as coefficient of variation (CV%) was ≤ 6. Albumin was also analysed using Abbott Architect analyser by Bromcresol Purple method (CV% ≤ 3.5)[39]. All nutritional blood samples, but Magnesium, were analysed in the Royal Infirmary of Glasgow laboratory. Plasma vitamin A (CV% ≤4) and E/Cholesterol (CV% ≤5) were measured by High Performance Liquid Chromatography (HPLC) with UV detection and vitamin B12 (CV% ≤11) by Chemiluminescent immunoassays performed using the Abbott Architect i2000 Analyser[39]. Zinc (CV% ≤ 3), Copper (CV% ≤ 3) and Selenium (CV% ≤ 3) were analysed using Inductively Coupled Plasma Spectrometry (inductively coupled plasma mass spectrometry in helium collision cell mode; Agilent 7900)[40] and plasma Magnesium (CV% ≤ 3) using Abbott Architect c8000 at the RHSC laboratory[39]. Micronutrient status was established using in-house laboratory references (vitamin B12, vitamin A and Vitamin E/Ch)[40] and aged adjusted *Z*-scores (Mg, Se, Zn and Cu) generated from a cohort of healthy Scottish children.

Dietary intake and nutritional treatment

Total energy (TEI) and micronutrient (vitamin B12, vitamin A, vitamin E/Cholesterol (vitamin E/Ch), Mg, Zn, Se and Cu) intakes were assessed using a 24 h multiple-pass recall method[41] and analysed in WinDiets® (Univation Ltd 2018)[42]. Any nutritional treatment and micronutrient supplementation was recorded. Nutritional treatment was prescribed according to Subjective Global Assessment by the

multidisciplinary team and stratified into three groups as previously described[23]; (i) no nutrition treatment; (ii) macronutrient treatment, which consisted of oral nutrition support, enteral and/or parenteral nutrition and (iii) micronutrient supplementation, which consisted of multivitamins and minerals only or a combination of macronutrient and micronutrient supplementation (micronutrients±macronutrients). Estimated total energy requirements (TER) were calculated using Henry's equation[43] and a low physical activity level (PAL) of the 10th centile[13, 44]. Appropriate micronutrient intakes were assessed against UK Reference Nutrient Intake (RNI) and the percentage of RNI was calculated[45].

Statistical analyses

The Statistical Package for Social Science (IBM-SPSS for Windows Statistics, version 21) was used to analyse all data. The *Z*-scores for Mg, Se, Zn and Cu were generated from a contemporary cohort of healthy Scottish children using the GAMLSS package in R®. Normally distributed data are expressed as a mean ± SD and non-normally distributed data as median and interquartile range (IQR). Descriptive statistics were used to evaluate micronutrient intakes presented as the percentage of the RNI, prevalence of micronutrient deficiencies (vitamin B12, vitamin A, vitamin E/Ch, Mg, Se, Zn and Cu) and to present deficits or excesses of micronutrient intakes. To correct for inflammatory response, we removed the micronutrient concentration values that were associated to high CRP levels (>10 mg/L for Cu and Se and >20 mg/L for Zn and vitamin A)[46]. Correlations between each micronutrient concentration and each micronutrient intake, BMI centile and body composition (FFM% and FM%) were performed using Spearman's correlation (non-normally distributed data). Associations between micronutrient status and categorical variables (BMI status, diagnostic criteria, nutritional support and treatment risk) were established by χ^2 test. Kruskal Wallis-test and One-way ANOVA were applied to test for differences in micronutrient intake and plasma micronutrient concentration between groups. We used binary logistic regression analysis to test weather micronutrient concentration was a predictor of clinical outcome (event v EFS). Predictors that were found to be related to the outcome variable ($p < 0.1$) using univariate analysis were included in the multivariate logistic regression model[47]. Multilevel model analysis with

183 micronutrient concentration as predictors and total number of treatment complications as outcome was
184 performed at each time point. $P < 0.05$ was considered statistically significant.

185 We followed the STROBE checklist for the presentation of our data[48].

186 **Results**

187 ***Demographic and Clinical Characteristics***

188 179 patients were diagnosed with paediatric cancer between Aug 2010 and Feb 2014. Of these, 78
189 (43%) were excluded (Figure 1) and 101 were considered eligible. Eighty-two (81%) were recruited,
190 whilst 19 (19%) refused to participate mainly due to parental stress at cancer diagnosis. Demographic
191 and clinical characteristics of the population are presented in Table 1, patient's accrual in Figure 1 and
192 follow up in Figure 2. There were no statistically significant differences between the paediatric cancer
193 cohort and the paediatric cancer controls (refusals). Twenty-four treatment protocols were used to treat
194 the paediatric cancer cohort, the median follow up was 312 (interquartile ranges (IQR) 123.5 – 653.2)
195 days and the time between diagnosis and baseline measurements was 9.5 (IQR 6.0 – 19.5) days. All
196 patients were receiving cancer treatment when the measurements and plasma micronutrients samples
197 were taken at baseline (diagnosis).

198 At the end of the study (May 2014), the survival rate was 90% (74/82), the death rate was 10% (8/82)
199 and the EFS rate was 85% (70/82). Thus, 15% (12/82) of patients had "events" (relapse, cancer
200 metastasis or did not respond to treatment). Of these, 67% (8/12) died, 17% (2/12) continued treatment
201 with palliative intent, 17% (2/12) were receiving second line treatment by the end of the study, of whom
202 8% (1/12) survived. The median (IQR) for treatment complications ranged between 3 (1 – 4) at
203 diagnosis and 0 (0 – 1) at 18 months. Hepatotoxicity was the most common treatment complication
204 [ranged between 36% (9/25) at 12 months to 45% (19/42) at 6 months], followed by nephrotoxicity
205 [ranged between 21% (16/77) at diagnosis to 36% (9/25) at 12 months], other [ranged between 11%
206 (7/62) at 6 months to 37% (30/82) at diagnosis], infections [ranged between 6% (3/49) at 12 months to
207 24% (20/82) at diagnosis], diarrhoea [ranged between 10% (6/58) at 9 months to 23% (19/82) at

diagnosis], mucositis [ranged between 5% (2/41) at 18 months to 12/82 (15%) at diagnosis] and constipation [ranged between 5% (1/41) at 18 months to 16% (13/82)] and other complications.

Fifty-five patients were referred to the Dietitian for nutritional assessment during the study. The reasons for referral were: undernutrition/weight loss (16/55; 25%), reduced oral intake (10/55; 18%), temporary gut failure (10/55; 25%), to prevent weight loss (7/55; 13%), dysphagia (4/55; 7%), steroid induced diabetes (2/55; 4%), mucositis (1/55; 2%) and following parent's request (1/55; 2%). Of these, 50 (61%) were prescribed some form of nutritional treatment and 5 (6%) had general dietary advice. In total, 14/50 (28%) patients received oral nutrition support (ONS), 17/50 (34%), nasogastric tube-feeding (NG), 4/50 (8%) percutaneous endoscopic gastrostomy feeding (PEG), 1/50 (2%) total parenteral nutrition (TPN) and 15/50 (30%) advanced nutritional treatment (NG/PEG and TPN). In all, 19% (16/82) of cancer patients received multivitamin supplements at some point during the study period, which contained the RNI for vitamin A, vitamin E and vitamin B12. Only 2% (1/82) received a mineral supplementation (magnesium).

Total energy intake, micronutrient intakes and plasma micronutrient concentration of the paediatric cancer cohort

Mean percentage TEI of individual TER (TEI% of TER) was 161% (±42%) throughout the study and this was consistently higher than TER at all stages apart from the 3 months follow up, which was lower [82% (±51%)]. TEI% of TER ranged between 155% (±78%) at diagnosis to 182% (±84%) at 9 months and patients on nutritional treatment 152% (±14%) had similar TEI% of TER than patients who were not 157% (±17%). The mean TEI% of TER with data stratified by BMI category was; undernourished children 103 (±47), healthy weight 136 (±57) and overweight and obese 126 (±61) Kcal/day. TEI% of TER in undernourished children ranged between 87 (±36) at 3 months to 108 (±72) at 6 months. No child was classified as undernourished after the 6 months follow up. TEI% of TER in healthy weight and overweight and obese children ranged between 123 (±54) at 3 months to 152 (±41) at 18 months and 127 (±57) at diagnosis to 140 (±63) at 18 months respectively.

Micronutrient intakes and concentration did not statistically differ between any of the time points when the data was analysed all together or stratified by diagnostic criteria. Furthermore, micronutrient intakes did not statistically differ between BMI or treatment intensity categories at any time point. Table 2 shows micronutrient intakes presented as a percentage of the RNI and the prevalence of paediatric cancer patients having intakes below the RNI. The micronutrient's percentage of the RNI (apart from Vitamin E/Cholesterol) with data stratified by nutritional treatment are presented in Figure 3. There was statistically significant differences in the percentages of both vitamin A and vitamin B12 RNI at 6 months ($F(3.5)$; $p=0.03$), 9 months ($F(3.3)$; $p=0.04$) and 18 months ($F(3.1)$; $p=0.05$) and 6 months ($F(3.0)$; $p=0.05$) and 18 months ($F(3.3)$; $p=0.05$) respectively. Following post-hoc analysis, the micronutrient intakes from the macronutrient+micronutrient group was statistically significantly higher than the macronutrient group at all time points. There was no statistically significant differences between the nutritional treatment groups in any of the mineral intakes at any time point.

Plasma micronutrient status and concentration for the entire cohort during the study period are presented in Table 3. Overall, 74% (53/72) patients had micronutrient abnormalities at baseline; deficiencies (25%, 18/72), excesses (19%, 14/72) and a combination of both (29%, 21/72), which continued for 18 months. There was no significant differences between BMI categories at any stage of the study period in the following micronutrient plasma concentration: Vitamin A, Mg and Se. However, there was a trend towards higher vitamin A concentration in overweight and obese children, whilst Mg concentration was lowest in this group at all times but diagnosis.

Plasma vitamin B12 differed between the BMI categories at 3 months ($F(4.9)$; $p=0.01$); whereby overweight and obese ($F(4.9)$; $p=0.01$) children had significantly lower vitamin B12 concentration than healthy ($p= 0.01$; 95% CI (65.5 – 506.6) and underweight ($p=0.01$, 95% CI 115.5 – 986.3) children. Likewise, plasma vitamin E/Ch from overweight and obese children (5.1±0.7) was significantly lower than underweight (6.5±0.9) and healthy (6.4±1.5) children at 3 months ($F(5.7)$, $p=0.006$; 95% CI 0.51 – 2.13) and plasma copper was also significantly lower in overweight and obese children (13.3±7.3) than those who were underweight (17.3±6.2) or healthy weight (20.6±6.3). Figure 4 shows plasma micronutrient concentration with data stratified by nutritional treatment.

Correlation and associations

None of the plasma micronutrients correlated with dietary intakes, BMI z-scores or FFM% and FM% at any time-point. Correlation analysis was then performed after adjusting for elevated CRP and low albumin concentration. Selenium concentration strongly correlated with selenium intake at 6 months (r= 0.8; p<0.001) and 18 months (r=0.6; p=0.01) and moderately correlated at 12 months (r=0.3; p=0.2). Vitamin B12 moderately correlated with FFM% at diagnosis (r=0.5; p=0.002), 3 months (r=0.3, p=0.02) and 12 months (r=0.5, p=0.04).

Overweight paediatric cancer patients were more likely to have the following micronutrient deficiencies; Magnesium at 3 months [χ^2(8.6); p=0.01], 6 months [χ^2 (6.0); p=0.04] and at 9 months [χ^2(6.8); p=0.01]; vitamin A at 12 months [Fisher Exact test (6); p<0.001]; vitamin B12 at diagnosis [χ^2 (154) p<0.001], 6 months [Fisher exact test (6); p<0.04] and 18 months [χ^2 (15) p<0.001]; Zn at diagnosis [χ^2 (52); p<0.001], 3 months [χ^2 (25); p<0.001] and 12 months [χ^2 (4.1); P=0.05]; Se at diagnosis [χ^2 (19.3) p<0.001] and 3 months [χ^2 (44) p<0.001] and finally Cu at diagnosis [χ^2 (61); p<0.001], 3 months [χ^2 (9); p = 0.01], 12 months [χ^2 (8); p=0.005] and 18 months [χ^2 (15); p<0.001]. Whereas, undernourished patients were more likely to have the following micronutrient deficiencies; vitamin A at diagnosis [χ^2 (25); p<0.001] and 3 months [χ^2 (49); p <0.001]; vitamin B12 at 3 months [χ^2 (63); p<0.001] and selenium at 3 months [χ^2(44) p<0.001].

Impact of plasma micronutrient concentration on clinical outcomes

The impact of plasma micronutrient concentration at the time of diagnosis on clinical outcome (event v EFS) and complications during treatment at diagnosis, 3 and 12 months are presented in Tables 4 and 5 respectively. Univariate analysis indicated that an increase in copper concentration increases the odds of an event by 14% [OR 1.14; 95% CI (1.028 - 1.270). In contrast a reduction in selenium concentration increases the odds of an event by 1.6% [OR 0.016; 95% CI (0.001 - 0.380)]. Logistic regression analysis with BMI Z-score and decimal age as predictors (p < 0.1) indicated that a reduction in each selenium concentration unit increases the odds of an event by 2% [OR 0.02; 95% CI (0.0004 - 0.881)]; however, copper did not significantly affect the likelihood of clinical outcome.

Discussion

To our knowledge, this is the first prospective cohort study investigating the impact of micronutrient status at diagnosis on event and EFS and the first in the UK to investigate the impact of micronutrient concentration on the number of treatment complications in paediatric cancer patients following 18 months of treatment. Importantly, our results show that a reduction in plasma Se units increased the odds of an event (relapse, becoming palliative or death) by 2%. Furthermore, lower Se and Mg concentration significantly predicted number of complications at diagnosis, 3 months and 12 months and at diagnosis and 12 months respectively. In contrast, higher vitamin E/Ch and vitamin A significantly predicted complications at diagnosis and 3 months respectively. Overall, most patients had plasma micronutrient abnormalities, deficiencies and excesses. Vitamin A abnormalities and Zn and Cu deficiency were most prevalent; whilst vitamin E/Ch and vitamin B12 were mostly within the normal range. Most plasma micronutrients did not correlated with dietary intakes, BMI *Z*-scores, FFM% or FM%; however, children receiving a combination of macro- and micronutrient nutritional treatment tended to have higher micronutrient intakes, particularly vitamins A and B12, than those receiving either macronutrient alone or no nutritional treatment. Interestingly, overweight and obese patients were more likely to exhibit vitamin B12, Mg, Se, Zn and Cu deficiencies, despite higher energy intakes.

The impact of micronutrient concentration on clinical outcomes in paediatric cancer patients

Se was the most important predictor of clinical outcomes. Lower Se concentration at diagnosis increased the likelihood of an event and the number of treatment complications experienced by our cohort of paediatric cancer at diagnosis and during treatment (3 months and 12 months). Furthermore, lower Mg also predicted an increased number of complications at diagnosis and 12 months. These findings are supported by a Cochrane review[49], in which better Se status in adults diagnosed with cancer was associated with a reduced risk of mortality (OR 0.55; 95% CI 0.36 – 0.83). Similarly, an earlier study[15] reported that Se significantly contributed to higher total antioxidant status and capacity, measured by ORAC assay, and lower lipid peroxidation, measured by TBARS, also in a paediatric cancer cohort. Biological mechanisms by which Se exerts these effects are still unclear. Nonetheless, experimental studies attribute it to the regulation of both oxidative stress and immune

system and to its counteractive effect on cancer cell growth[49]. For instance, *in vitro* studies demonstrate that Se affects DNA stability, cell proliferation, necrotic and apoptotic cell death of both malignant and healthy cells due to selenoproteins' antioxidant action[49] and suggest that Se maybe used for cancer therapy alongside cancer treatment. However, safe doses should be first established. Our results should be interpreted with caution due to the heterogeneity of our paediatric cohort and the sample size, particularly at later stages. Nevertheless, it is plausible to assume that lower plasma Se increased oxidative stress and inflammatory responses in our cohort and therefore led to poorer outcomes[50].

Interestingly, higher plasma concentration of other micronutrients predicted worse clinical outcomes. Higher Cu increased the likelihood of an event when analysed in isolation. To date, only another study performed in children diagnosed with ALL (n=23)[19], and in adults diagnosed with stage III non-small-cell lung cancer have suggested the use of Cu and Cu/Zn ratio as a prognostic factor of clinical outcome[51]. Like Kennedy et al.[16], we found that higher vitamin E/Ch and vitamin A concentration predicted higher number of complications at diagnosis and 3 months respectively. Many clinical signs of hypervitaminosis A and E mimic those of cancer treatment associated complications, which makes it difficult for clinicians to recognise. For instance, bone pain, anorexia, nausea, vomiting and hepatotoxicity[50] are all clinical signs of both vitamin A toxicity and treatment side-effects[16, 50]. A biological explanation for the increase in treatment complications, particularly hepatotoxicity, seen with higher plasma vitamin A might be related to excess of vitamin A storage in the liver' stellate cells potentially leading to their activation and hypertrophy, excess collagen production and subsequent acute liver injury[52]. Although vitamin E has very low toxicity, a plausible explanation for these findings can be attributed to its pro-oxidant action. Lipoproteins treated *in vitro* suggest that excess of vitamin E may exert a radical chain reaction deeper in lipoproteins and membranes, which may cause damage in the absence of co-oxidants, such as ascorbate and ubiquinone[53].

Our findings have several clinical implications. Firstly, we highly recommend the assessment and monitoring of plasma micronutrient status in all paediatric cancer patients, including overweight and obese. By adopting these measures, complications caused by micronutrient deficiencies or toxicity

should be minimised with appropriate micronutrient supplementation. Secondly, like Mg, clinicians should also consider supplementation of Se and Zn as part of routine practice. We are unable to recommend dosages other than the RNI due to the paucity of clinical trials performed. Finally, markers of inflammation (CRP) and serum albumin should be measured alongside plasma micronutrients to avoid misclassification of status.

Micronutrient intakes and status of paediatric cancer patients

In agreement with two recent small studies from the USA[21] and Brazil[19], our results show a high prevalence of vitamin A (range 9 – 15%), Se (4.5 – 17%), Mg (7 – 15%), Cu (3 – 27%), and Zn (23 – 46%) deficiencies at diagnosis and during treatment even after adjusting for low albumin and elevated CRP. Furthermore, our cohort were not meeting the RNI for these micronutrients, despite TEI being above TER at all-time points, apart from the 3 months follow up. These findings are also in line with two studies in which antioxidant vitamin intakes (vitamin A, vitamin E)[17], Cu and Zn[19] of children diagnosed with ALL were reported at diagnosis[17] and during treatment[17, 19]. Contrary to Morrell et al.[21], Se intakes in our cohort were below the RNI. Of note, only another study has investigated Se changes in a paediatric cancer cohort during the first 6 months of treatment; however, prevalence of deficiencies were not reported[15].

Stratification of the data by nutritional status category and nutritional treatment highlighted that overweight and obese patients have lower vitamin E/Ch, vitamin B12, Mg and Cu concentration than their healthy- and under-weight counterparts 3 months into treatment. Epidemiological data from healthy obese children and adolescents support these findings[54-57]. Aside from lower vitamin and mineral intakes and treatment induced complications, it is plausible that more vitamin E is stored in adipocytes due to an increase in storage capacity; therefore making it less readily available to plasma[55]. Lower vitamin B12 levels may be attributed to a combination of higher intake of carbohydrates and fats and reduced protein from meat sources, which has been reported in healthy obese children and adolescents[56]. Higher intakes of fats, calcium and carbonated drinks can interfere with the absorption of Mg potentially leading to lower plasma Mg concentration[57]. The lower plasma Cu found here contrast with most studies performed in healthy obese children and adults [58] and as we

showed no difference in Cu intakes between the BMI categories, the reasons for these findings are unclear and should be investigated further. Moreover, vitamin intakes and concentration of children receiving a combination of macro- and micronutrients as a form of nutritional treatment are higher than macronutrient alone. This suggests that macronutrient treatment alone (or no form of nutritional support) neither supports micronutrient intake requirements nor appropriate micronutrient status and that overweight and obese patients should also be supplemented and monitored. Furthermore, all supplemented patients, but one, were prescribed vitamin supplementation alone, aka no mineral supplementation, which may explain the similarities in mineral intakes and concentration seen in the three nutritional treatment groups.

None of the micronutrient concentration (vitamin E/Ch, vitamin B12, Cu, Se, Zn Mg), apart from vitamin A, significantly changed over 18 months. Although, no other study has investigated micronutrient status of paediatric cancer patients over this period, our findings echoed the results of two studies performed in a similar population[15] and in children diagnosed with ALL[16]. Like Kennedy et al.[16], but in contrast to a similar cohort from Edinburgh[15], our study showed that vitamin A concentration, alongside higher prevalence of vitamin A excesses, increased at 9, 12 and 18 months, possibly due to the short length of follow up of the latter study (6 month). The increase in vitamin A concentration and higher prevalence of excesses seen in our study may be due to a combination of macronutrient treatment (high energy food sources), vitamin A supplementation and prednisone treatment. There is evidence that the latter increase retinol binding protein (RBP), which in turn would lead to an associated increase in vitamin A concentration[16]. This may also explain the higher plasma vitamin A concentration seen in our overweight and obese cohort compared to healthy or underweight. In contrast, the initial lower levels of vitamin A have been attributed to higher serum-CRP levels and the inevitable acute phase response[59]. It is well established that the synthesis of RBP decreases under systemic inflammatory response, which in turn may lead to false deficiencies. Even after adjusting for serum CRP, our study shows vitamin A deficiencies.

Children treated with chemotherapy, radiotherapy, surgery or treatment combinations experience many complications that affect intake, absorption, metabolism, transport and excretion of nutrients[50]. The

most common complications exhibited in our cohort were diarrhoea, mucositis and vomiting, which all reduce absorption[50]; anorexia, which affects intake, and systemic inflammatory response, hepatotoxicity and nephrotoxicity, which impair transport, metabolism and excretion of nutrients[50]. It is therefore not surprising that most micronutrient intakes did not correlate with micronutrient concentration and that this finding mirrors that of a recent small study (n=23), in which none of the micronutrients measured (vitamin A, C, D, E, β-carotene, Se and Zn) correlated with intakes in a paediatric cancer cohort receiving a neutropenic diet[21].

Limitations of the study and future directions

The reduced sample size at a later stages of the study led to more inaccurate multilevel models and therefore results obtained from 12 months should be interpreted with caution. We originally planned to use a 4 day diet diary[60]; however, this proved to be unfeasible early in the study due to patient's treatment burden. Therefore, we switched to a 24 h multi-pass recall method, which tends to underestimate micronutrient intake[41]; nonetheless, data on micronutrients obtained from nutritional treatment and supplementation were also accounted for. In the future a 24 h multi-pass recall alongside a food frequency questionnaire should be used to improve the estimation of micronutrient intakes assessment. The assessment of Se status under systemic inflammation should be measured (when possible) using red blood cell selenium or whole blood glutathione as biomarkers[27]. Instead, plasma Se was the available assay; however, we adjusted for systemic inflammatory response (high CRP and low albumin) and presented both values. The CRP concentration causing clinically significant effects are >20 mg/L for vitamin A and Zn (underestimation) and >10 mg/L for Cu (overestimate) and Se (underestimation)[46]. Therefore, these should be measured when CRP concentration are below these values. Future research should include large multicentre epidemiological studies to confirm our findings. Moreover, mechanistic and randomised controlled trials investigating the effects of micronutrient supplementation on clinical outcomes and tolerance of cancer treatment are now warranted.

Conclusion

In conclusion, our results highlight that children diagnosed and treated for cancer exhibit high prevalence of micronutrient abnormalities and that overall micronutrient intakes do not appear to meet requirements for most micronutrients, especially in overweight and obese patients. The most important predictor of undesirable clinical outcomes was lower plasma Se, whilst higher concentration of Cu predicted poorer clinical outcomes. Vitamin A and vitamin E/Ch were both associated with more complications during treatment. Importantly, we recommend the assessment and monitoring of micronutrient status during treatment for all paediatric cancer patients at a minimum of 3 months intervals initially and every 6 months thereafter to prevent both deficiencies and toxicity.

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Statement of Authorship

Conceptualization (RRI, IP, DW, MB, JMcK, KG), methodology (RRI, IP, DW, JMcK) Software (NA), validation (DW), formal analysis (RRI, KG), investigation (RRI and IP), resources (MB, DW, JMcK), data curation (RRI, IP) writing-original draft preparation (RRI); writing-review & editing (all authors), visualization (IP, RRI and MB), supervision (DW, MB and JMcK), project administration (DW, JMcK), funding acquisition (DW, MB, JMcK).

Conflict of interest Statement and Funding

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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Table Legends

Table 1. Characteristics of the n=82 Paediatric Oncology cohort and n=22* non-participants)

*N=22: 19 (refused to participate) + 3 (met criteria but were not approached as advised by consultants); **Socio-economic status (SES) I-V where I denotes the most deprived and V the economically most advantageous families; LCH: Langerham's Cell Histiocytosis; [1]Mann-Whitney; [2]Chi square test; [3]Fisher's Exact Test

Table 2. Prevalence of micronutrient intake below the RNI and micronutrient's intake of paediatric cancer patients during the study period presented as a percentage of the RNI

RNI: Reference Nutrient Intake

Table 3. Plasma micronutrient, CRP and albumin concentration and status of paediatric cancer patients during the study period

*One-way ANOVA; F (2.5); *p=0.03;* [1]*p=0.007, 95% CI (-0.7 to -0.12);* [2]*p=0.03*, 95% CI (-0.68 to -0.04)*;* [3]*p=0.01*, 95% CI (-0.79 to -0.1); [1]Values adjusted for Albumin and CRP different from those presented in the table: Vitamin A at diagnosis; deficiency 2%, excess 14%; Cu at diagnosis; deficiency 17%; Zn at diagnosis 36%.

Table 4 (top) Logistic regression analysis to establish the likelihood that each micronutrient concentration (at diagnosis) has on clinical outcome (EFS v Event). (Bottom) Logistic regression analysis to establish the likelihood that Selenium and Copper concentration (at diagnosis) has on clinical outcome (EFS v Event) taking into consideration predictors from univariate analysis ($p<0.01$)

*R value Nagelkerkel.

Table 5. Multilevel analysis showing the impact of micronutrient concentration on the number of complications in paediatric cancer patients at different time points

Only statistically significantly time points from the multilevel model have been presented here.

Figure Legends

Figure 1. Flow diagram showing patient's accrual and sample availability

Figure 2. Patient's follow up at each time point and number of patients having had each type of measurement.

*Drop outs due to: deceased patients, palliative treatment, treatment given in centres other than RHSC, Edinburgh and Ninewells Hospital, Dundee and patients who missed appointments.

Figure 3. Micronutrient intakes of paediatric cancer patients with data stratified by type of nutrition treatment.

Figure 3a. Vitamin A and vitamin B12 intakes; Figure 3b. Zinc and Selenium intakes; Figure 3c. Copper and Magnesium intake.

Figure 4. Mineral concentration of the paediatric cancer cohort against healthy controls.

Figure 4a. Mean vitamin A (µmol/L) and vitamin B12 concentration (ng/L); Figure 4b. Mean Zinc (µmol/L) and Copper concentration (µmol/L); Figure 4c. Mean Selenium (µmol/L) and Magnesium concentration (mmol/L); Figure 4d. Mean vitamin E/Cholesterol concentration (µmol/L).

Figure 5. Micronutrient concentration of paediatric cancer patients with data stratified by type of nutrition treatment

One way ANOVA test; p=0.04, 95% CI (-1.3 to -0.14); p=0.05, 95% CI (-1.7 to -0.15); p <0.01, 95% CI (169 – 743); p=0.01, 95% CI (-7.84 to -0.67)

References

1. UK, C.R., *Childhood Cancer Survival Statistics* 2015.
2. Wallace, W.H., L. Thompson, and R.A. Anderson, Long term follow-up of survivors of childhood cancer: summary of updated SIGN guidance. *BMJ*, 2013. **346**: p. f1190. doi: 10.1136/bmj.f1190.
3. Devidas, M. and J.R. Anderson, Considerations in the design of clinical trials for pediatric acute lymphoblastic leukemia. *Clin investig*, 2013. **3**(9): p. 10/10.4155/cli.13.71. doi: 10.4155/cli.13.71
4. Sala, A., P. Pencharz, and R.D. Barr, *Children, cancer, and nutrition--A dynamic triangle in review.* Cancer, 2004. **100**(4): p. 677-87. doi: 10.1002/cncr.11833.
5. Iniesta, R.R., et al., Effects of pediatric cancer and its treatment on nutritional status: a systematic review. *Nutr Rev*, 2015. **73**(5): p. 276-95. doi: 10.1093/nutrit/nuu062
6. Barr, R.D. and M.C.G. Stevens, The influence of nutrition on clinical outcomes in children with cancer. *Pediatr Blood Cancer*, 2020: p. e28117. doi.org/10.1002/pbc.28117.
7. van Eys, J., et al., A clinical trial of hyperalimentation in children with metastatic malignancies. *Med Pediatr Oncol*, 1980. **8**(1): p. 63-73. doi: 10.1002/mpo.2950080110.
8. Revuelta Iniesta, R., et al., Nutritional status of children and adolescents with cancer in Scotland: A prospective cohort study. *Clin Nutr ESPEN*, 2019. **32**: p. 96-106. doi: 10.1016/j.clnesp.2019.04.006
9. Lange, B.J., et al., Mortality in overweight and underweight children with acute myeloid leukemia. *JAMA*, 2005. **293**(2): p. 203-11. doi: 10.1001/jama.293.2.203.
10. Joffe, L., et al., Nutritional status and clinical outcomes in pediatric patients with solid tumors : A systematic review of the literature. *Seminars in Oncology*, 2019. **46**(1): p. 48-56. doi: 10.1053/j.seminoncol.2018.11.005
11. Viana, M.B., et al., Malnutrition as a prognostic factor in lymphoblastic leukaemia: a multivariate analysis. *Arch Dis Child*, 1994. **71**(4): p. 304-10. doi: 10.1136/adc.71.4.304.
12. Co-Reyes, E., et al., Malnutrition and obesity in pediatric oncology patients: causes, consequences, and interventions. *Pediat Blood Cancer*, 2012. **59**(7): p. 1160-1167. doi: 10.1002/pbc.24272
13. Brinksma, A., et al., Changes in nutritional status in childhood cancer patients: a prospective cohort study. *Clin Nutr*, 2015. **34**(1): p. 66-73. doi: 10.1016/j.clnu.2014.01.013.
14. Malvy, D.J., et al., Serum beta-carotene and antioxidant micronutrients in children with cancer. The 'Cancer in Children and Antioxidant Micronutrients' French Study Group. *Int J Epidemiol*, 1993. **22**(5): p. 761-71. doi: 10.1093/ije/22.5.761.
15. Revuelta Iniesta, R., et al., Assessment of Plasma Antioxidants, Oxidative Stress and Polyunsaturated Fatty Acids in Paediatric Cancer Patients: A Propsective Cohort Pilot Study. *EC Nutrition*, 2015. **2.4**: p. 13. Open access.
16. Kennedy, D.D., et al., Antioxidant status decreases in children with acute lymphoblastic leukemia during the first six months of chemotherapy treatment. *Pediatr Blood Cancer*, 2005. **44**(4): p. 378-385. doi.org/10.1002/pbc.20307.
17. Kennedy, D.D., et al., Low antioxidant vitamin intakes are associated with increases in adverse effects of chemotherapy in children with acute lymphoblastic leukemia. *AJCN*, 2004. **79**(6): p. 1029-1036. doi.org/10.1093/ajcn/79.6.1029
18. Aleksic, D., et al., The importance of the blood levels of homocysteine, folic acid and vitamin B12 in children with malignant diseases. *J BUON*, 2013. **18**(4): p. 1019-25.
19. Sgarbieri, U.R., M. Fisberg, and L.G. Tone, Nutritional assessment and serum zinc and copper concentration in leukemic children. *Sao Paulo Med J*, 1999. **117**: p. 13-18. doi: 10.1590/s1516-31801999000100003.
20. Borum, P.R., Disease-Related Malnutrition: An Evidence-Based Approach To Treatment: edited by Rebecca J Stratton, Ceri J Green, and Marinos Elia, 2003, 824 pages, hardcover, $175. CABI Publishing, Wallingford, United Kingdom. *AJCN*, 2004. **79**(6): p. 1128-1129. doi: doi.org/10.1093/ajcn/79.6.1128

21. Morrell, M.B.G., et al., Dietary intake and micronutrient deficiency in children with cancer. *Pediatr Blood Cancer*, 2019. **66**(10): p. e27895. doi: 10.1002/pbc.27895
22. Revuelta Iniesta, R., et al., Systematic review and meta-analysis: Prevalence and possible causes of vitamin D deficiency and insufficiency in pediatric cancer patients. *Clin Nutr*, 2016. **35**(1): p. 95-108. doi: 10.1016/j.clnu.2014.12.023
23. Iniesta, R.R., et al., 5-Hydroxyvitamin D concentration in paediatric cancer patients from Scotland: a prospective cohort study. *Br J Nutr*, 2016. **116**(11): p. 1926-1934. doi: 10.1017/S0007114516004074
24. Glatt, D., et al., Nutritional screening and assessment of paediatric cancer patients: A quality improvement project (baseline results). *Clin Nutr ESPEN*, 2020. doi: 10.1016/j.clnesp.2020.04.003
25. Mousazadeh, M.H., Level of Selenium of Children with Acute Lymphoblastic Leukemia. *Jacobs journal of cancer science and research*, 2019. Open access, p. 10.
26. Brierley, C.K., et al., Hyperferritinemia in Pediatric Acute Lymphoblastic Leukemia: What Does it Mean? *J Pediatr Hematol Oncol*, 2017. **39**(3): p. 238. doi: 10.1097/MPH.0000000000000712
27. Gerasimidis, K., et al., Assessment and Interpretation of Vitamin and Trace Element Status in Sick Children: A Position Paper From the European Society for Paediatric Gastroenterology Hepatology, and Nutrition Committee on Nutrition. *J Pediatr Gastroenterol Nutr*, 2020. **70**(6): p. 873-881. doi: 10.1097/MPG.0000000000002688.
28. García, O.P., K.Z. Long, and J.L. Rosado, Impact of micronutrient deficiencies on obesity. *Nut Rev*, 2009. **67**(10): p. 559-572. doi: 10.1111/j.1753-4887.2009.00228
29. Steliarova-Foucher, E., et al., International Classification of Childhood Cancer, third edition. *Cancer*, 2005. **103**(7): p. 1457-67. doi: 10.1002/cncr.20910.
30. Kazak, A.E., et al., A revision of the intensity of treatment rating scale: classifying the intensity of pediatric cancer treatment. P*ediatr Blood Cancer*, 2012. **59**(1): p. 96-9. doi: 10.1002/pbc.23320
31. Government, T.S., *Scottish Index of Multiple Deprivation.* 2012.
32. Tanner, J.M., Principles of growth standards. *Acta Paediatr Scand*, 1990. **79**(10): p. 963-7.
33. Cole, T.J., *T*he LMS method for constructing normalized growth standards. *Eur J Clin Nutr*, 1990. **44**(1): p. 45-60.
34. Cole, T.J., et al., Body mass index cut offs to define thinness in children and adolescents: international survey. *BMJ*, 2007. **335**(7612): p. 194. doi: https://doi.org/10.1136/bmj.39238.399444.55
35. Schaefer, F., et al., Usefulness of bioelectric impedance and skinfold measurements in predicting fat-free mass derived from total body potassium in children. *Pediatr Res*, 1994. **35**(5): p. 617-24.
36. Schaefer, F., et al., Assessment of body composition in children with chronic renal failure. *Pediatr Nephrol*, 2000. **14**(7): p. 673-8. doi: 10.1007/s004670000353
37. Fomon, S.J., et al., Body composition of reference children from birth to age 10 years. *Am J Clin Nutr*, 1982. **35**(5 Suppl): p. 1169-75. doi: 10.1093/ajcn/35.5.1169.
38. Wells, J.C., et al., Body-composition reference data for simple and reference techniques and a 4-component model: a new UK reference child. *Am J Clin Nutr*, 2012. **96**(6): p. 1316-26. doi: 10.3945/ajcn.112.036970.
39. Lothian, N. *Edinburgh and Lothians Laboratory Medicine*. 2014 [cited 2021 1/02]; Available from: https://www.edinburghlabmed.co.uk/TestDirectory/Pages/Site.aspx?sID=5&dID=28.
40. Scotland, N.N.S. *Scottish Trace Element and Micronutrient Diagnostic and Research Laboratory. Scotland's specialised laboratory for trace elements and vitamins in health and disease*. 2004 - 2020; Available from: https://www.trace-elements.co.uk/quality.asp.
41. Reilly, J.J., et al., Energy intake by multiple pass 24 h recall and total energy expenditure: a comparison in a representative sample of 3-4-year-olds. *Br J Nutr*, 2001. **86**(5): p. 601-5. doi: 10.1079/bjn2001449.
42. Wise, A., *WinDiets.* Robert Gordon University, 2018.
43. Henry, C.J.K., Basal metabolic rate studies in humans: measurement and development of new equations. *Public Health Nutr*, 2005. **8**(7a): p. 1133-1152. doi: 10.1079/phn2005801.

44. Reilly, J.J., Obesity during and after Treatment for Childhood Cancer. *Endocr Dev*, 2009. **15**: p. 40-58.
45. Health, D.o., *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy*. 1991, Great Britain: TSO.
46. Duncan, A., et al., Quantitative data on the magnitude of the systemic inflammatory response and its effect on micronutrient status based on plasma measurements. *Am J Clin Nutr*, 2012. **95**(1): p. 64-71. doi: 10.3945/ajcn.111.023812.
47. Ranganathan, P., C.S. Pramesh, and R. Aggarwal, Common pitfalls in statistical analysis: Logistic regression. *Perspectives in clinical research*, 2017. **8**(3): p. 148-151. doi: 10.4103/picr.PICR_87_17.
48. Vandenbroucke, J.P., et al., Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. *Int J Surg*, 2014. **12**(12): p. 1500-24. doi.org/10.1371/journal.pmed.0040297
49. Vinceti, M., et al., Selenium for preventing cancer. *Cochrane Database Syst Rev*, 2018. **1**(1): p. CD005195-CD005195. doi: 10.1002/14651858.
50. Gröber, U., et al., Micronutrients in Oncological Intervention. *Nutrients*, 2016. **8**(3): p. 163-163. doi: 10.3390/nu8030163.
51. Chen, H.H.W., et al., Predictive and prognostic value of human copper transporter 1 (hCtr1) in patients with stage III non-small-cell lung cancer receiving first-line platinum-based doublet chemotherapy. *Lung cancer* (Amsterdam, Netherlands), 2012. **75**(2): p. 228-234. doi: 10.1016/j.lungcan.2011.06.011.
52. Cheruvattath, R., et al., Vitamin A toxicity: when one a day doesn't keep the doctor away. *Liver Transpl*, 2006. **12**(12): p. 1888-91. doi: 10.1002/lt.21007.
53. Pearson, P., et al., *The Pro-Oxidant Activity of High-Dose Vitamin E Supplements in Vivo*. BioDrugs, 2006. **20**(5): p. 271-273. doi: 10.2165/00063030-200620050
54. Strauss, R.S., Comparison of serum concentrations of alpha-tocopherol and beta-carotene in a cross-sectional sample of obese and nonobese children (NHANES III). National Health and Nutrition Examination Survey. *J Pediatr*, 1999. **134**(2): p. 160-5. doi: 10.1016/s0022-3476(99)70409-9.
55. Gunanti, I.R., et al., Low Serum Concentrations of Carotenoids and Vitamin E Are Associated with High Adiposity in Mexican-American Children. *J Nutr*, 2014. **144**(4): p. 489-495. doi: 10.3945/jn.113.183137.
56. Pinhas-Hamiel, O., et al., Obese Children and Adolescents: A Risk Group for Low Vitamin B12 Concentration. *Arch Pediatr Adolesc Med*, 2006. **160**(9): p. 933-936. doi:10.1001/archpedi.160.9.933
57. Hassan, S.A.U., et al., Comparison of Serum Magnesium Levels in Overweight and Obese Children and Normal Weight Children. *Cureus*, 2017. **9**(8): p. e1607-e1607.
58. Fan, Y., C. Zhang, and J. Bu, Relationship between Selected Serum Metallic Elements and Obesity in Children and Adolescent in the U.S. *Nutrients*, 2017. **9**(2): p. 104. doi: 10.3390/nu9020104
59. Stephensen, C.B. and G. Gildengorin, Serum retinol, the acute phase response, and the apparent misclassification of vitamin A status in the third National Health and Nutrition Examination Survey. *Am J Clin Nutr*, 2000. **72**(5): p. 1170-1178. doi: 10.1093/ajcn/72.5.1170
60. Caireen Roberts, T.S., Natalie Maplethorpe, Lorna Cox, Sarah Meadows, Sonja Nicholson, Polly Page, and Gillian Swan. National Diet and Nutrition Survey Results from Years 7 and 8 (combined) of the Rolling Programme (2014/2015 to 2015/2016). 2018 [cited 2020 20/06/2020]; 29]. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/699241/NDNS_results_years_7_and_8.pdf.

690 **Table 1.** Characteristics of the n=82 Paediatric Oncology cohort and n=22* non-participants

Patients' characteristics	Cohort			Non-participants			*P*
	Median	**IQR**	**95% CI**	**Median**	**IQR**	**95% CI**	
Age at diagnosis (years)	3.88	1.96-8.83	4.69-6.88	6.52	3.91-10.65	5.37-9.26	0.06[1]
BMI centile							
	N		**%**	**N**		**%**	
Gender							0.5[2]
Male	46		56	10		45.5	
Female	36		44	12		54.5	
Diagnostic criteria							0.9[3]
Solid tumours	39		47	10		45.5	
Haematological Malignancies	36		43	11		50	
Brain tumours	7		8.5	1		4.5	
Other Associated Diagnosis	4		5	0		5	
Diagnosis ICCC-3							
I-Leukaemias	35		43	11		50	
ALL	29		35	11		50	
AML	3		4	0		0	
CML	2		2	0		0	
HLH	1		1	0		0	
II- Lymphoma	10		12	3		14	
III-CNS tumour	5		6	2		9	
IV-Neuroblastoma	6		7	2		9	
V-Retinoblastoma	2		2	0		0	
VI-Renal tumour	6		7	0		0	
VII-Hepatic tumours	1		1	0		0	
VIII-Malignant bone tumours	4		5	3		14	
IX-Soft tissue sarcoma	5		6	1		4	
X-Germ cell tumours	1		1	0		0	
XI-Malignant epithelial neoplasm	4		5	0		0	
XII-Others and unspecified malignant neoplasms	0		0	0		0	
Other associated diagnosis	3		4	0		0	
LCH	3		4	0		0	
Intensity of treatment							0.9[2]
Low	18		22	4		18	
Medium	30		37	8		36	
High	34		41	10		46	
Socioeconomic status**							0.6[2]
I	15		18	7		32	
II	12		15	2		9	
III	15		18	2		9	
IV	24		29	8		36	
V	15		18	3		14	
Ethnicity							0.5[3]
White	80		98	21		95.5	
Non-white	2		2.4	1		4.5	

691 *N=22: 19 (refused to participate) + 3 (met criteria but were not approached as advised by consultants); **Socio-economic status
692 (SES) I-V where I denotes the most deprived and V the economically most advantageous families; LCH: Langerham's Cell
693 Histiocytosis; [1]Mann-Whitney; [2]Chi square test; [3]Fisher's Exact Test

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Table 2. Prevalence of micronutrient intake below the RNI and micronutrient's intake of paediatric cancer patients during the study period presented as a percentage of the RNI

Micronutrient	Diagnosis		3 months		6 months		9 months		12 months		18 months	
	N (%)	Median (IQR)	N (%)	Median (IQR)	N (%)	Median (IQR)	N (%)	Median (IQR)	N (%)	Median (IQR)	N (%)	Median (IQR)
Vitamin A µg/day	38 (49%)	97.3 (48.7 – 140.6)	35 (47%)	102.5 (56.7 – 163.9)	28 (55%)	91.0 (57.8 – 189.2)	25 (51%)	93.0 (69.2 – 164.4)	21 (52.5%)	95.5 (61.1 – 154.9)	12 (35%)	114.6 (85.7 – 155.0)
Vitamin B12 µg/day	12 (15%)	302.9 (137.7 – 830.9)	6 (8%)	357.5 (173.3 – 583.2)	7 (13.5%)	450.5 (160 – 661.4)	3 (6%)	440.0 (205.4 – 660.0)	4 (10%)	335.9 (239.7 – 658.7)	3 (9%)	355.6 (211.6 – 536.2)
Copper mg/day	38 (46%)	110.0 (61.5 – 199.6)	23 (36%)	127.5 (79.9 – 190.0)	22 (42%)	136.2 (75.8 – 194.9)	16 (33%)	142.5 (89.6 – 217.9)	13 (32.5%)	120.0 (77.7 – 141.1)	11 (32%)	128.7 (81.9 – 277.5)
Selenium mg/day	40 (52%)	100.0 (45.8 – 209.2)	39 (53%)	96.7 (62.4 – 170.0)	27 (52%)	106.7 (60.0 – 160.0)	28 (57%)	106.7 (66.7 – 168.6)	16 (40%)	121.7 (48.7 – 158.3)	15 (44%)	110.1 (77.1 – 221.0)
Zinc mg/day	56 (73%)	86.0 (57.8 – 132.0)	48 (66%)	98.0 (60.1 – 144.6)	28 (54%)	94.2 (66.5 – 133.3)	20 (41%)	97.1 (69.6 – 130.4)	18 (45%)	106.1 (66.2 – 146.0)	18 (53%)	95.0 (73.3 – 144.5)
Magnesium mg/day	42 (53%)	96.5 (53.6 – 162.5)	35 (48%)	101.4 (63.7 – 173.7)	20 (38%)	136.7 (79.6 – 194.1)	22 (45%)	118.8 (72.6 – 203.1)	14 (35%)	128.6 (86.7 – 209.1)	14 (41%)	125.0 (78.2 – 219.3)

RNI: Reference Nutrient Intake

Table 3. Plasma micronutrient, CRP and albumin concentration and status of paediatric cancer patients during the study period

Micronutrient	Status	Diagnosis		3 months		6 months		9 months		12 months		18 months	
		N (%)	Mean(±SD)	N (%)	Mean(±SD)	N (%)	Mean(±SD)	N (%)	Mean(±SD)	N (%)	Mean(±SD)	N (%)	Mean(±SD)
Vitamin A* µmol/L	Deficiency	6 (9%)[1]	1.21 ± 0.60	4 (8%)	1.44 ± 0.71	4 (10%)	1.39 ± 0.66	1 (3%)	1.62 ± 0.69[1]	1 (4%)	1.58 ± 0.78[2]	3 (15%)	1.65 ± 0.76[3]
	Excess	8 (12.5%)		14 (23%)		9 (23%)		11 (37%)		7 (29%)		10 (50%)	
Vitamin E/Cholesterol µmol/L	Deficiency	1 (2%)	6.12 ± 1.70	-	6.08 ± 1.45	-	5.85 ± 1.13	-	7.55 ± 7.97	1 (4%)	5.57 ± 1.27	1 (5%)	5.38 ± 1.39
	Excess	3 (5%)		1 (2%)		-		1 (3%)		-		-	
Vitamin B12 ng/L	Deficiency	4 (6%)	574 ± 424	4 (6%)	677 ± 419	2 (5%)	633 ± 436	1 (3%)	640 ± 361	1 (4.5%)	638 ± 312	1 (5%)	611 ± 323
	Excess	5 (8%)		6 (9.5%)		4 (10%)		1 (3%)		1 (4.5%)		1 (5%)	
Copper µmol/L	Deficiency	18 (27%)[1]	16.93 ± 6.80	7 (12%)	17.54 ± 3.98	6 (15%)	18.07 ± 5.58	1 (3%)	17.75 ± 3.76	3 (12.5%)	17.54 ± 4.15	5 (26%)	14.58 ± 3.09
	Excess	8 (12%)		4 (7%)		7 (18%)		3 (10%)		1 (4%)		-	
Selenium µmol/L	Deficiency	3 (4.5%)	0.99 ± 0.40	6 (10%)	0.86 ± 0.20	2 (5%)	0.93 ± 0.23	3 (10%)	0.92 ± 0.30	4 (17%)	0.90 ± 0.25	2 (10.5%)	0.95 ± 0.89
	Excess	7 (11%)		2 (3%)		1 (2.5%)		4 (13%)		-		-	
Zinc µmol/L	Deficiency	29 (46%)[1]	12.16 ± 6.20	13 (23%)	11.02 ± 2.28	13 (36%)	12.64 ± 10.93	7 (25%)	12.00 ± 4.28	9 (39%)	11.74 ± 6.39	5 (26%)	10.66 ± 2.08
	Excess	5 (8%)		-		1 (3%)		2 (7%)		1 (4%)		-	
Magnesium mmol/L	Deficiency	7 (9%)	0.84 ± 0.10	5 (7%)	0.82 ± 0.08	3 (7.5%)	0.82 ± 0.09	3 (10%)	0.84 ± 0.09	3 (12.5%)	0.84 ± 0.10	3 (15%)	0.80 ± 0.09
	Excess	1 (1%)		1 (1%)		-		-		1 (4%)		-	
Albumin (28 – 45) g/L	< 28 g/L	21 (28%)	30.9 ± 6.0	2 (3%)	35.5 ± 5.1	2 (5%)	36.0 ± 5.5	2 (6%)	36.8 ± 4.5	1 (4%)	36.6 ± 5.2	1 (5%)	36.2 ± 4.7
CRP (<10) mg/L	>10mg/L	18 (22%)	18.1 ± 39.6	16 (25%)	11.4 ± 23.9	7 (18%)	13.8 ± 37.7	4 (13%)	4.3 ± 6.9	3 (14%)	12.0 ± 37.9	1 (5%)	2.8 ± 4.1

*One-way ANOVA; F (2.5); *p=0.03;* [1]*p=0.007, 95% CI (-0.7 to -0.12);* [2]*p=0.03*, 95% CI (-0.68 to -0.04)*;* [3]*p=0.01*, 95% CI (-0.79 to -0.1); [1]Values adjusted for albumin and CRP different from those presented in the table: Vitamin A at diagnosis; deficiency 2%, excess 14%; Cu at diagnosis; deficiency 17%; Zn at diagnosis 36%.

Table 4 Logistic regression analysis to establish the likelihood that each micronutrient concentration (at diagnosis) has on clinical outcome (EFS v Event) (top)

Micronutrient concentration	β	SE	*p*	Odds-Ratio	95% CI for Odd-Ratios		R*
					Lower	*Upper*	
Vitamin A* µmol/L	0.256	0.549	0.641	1.292	0.440	3.791	0.06
Vitamin E/Cholesterol µmol/L	0.109	0.189	0.565	1.115	0.770	1.614	0.009
Vitamin B12 ng/L	-0.001	0.001	0.523	0.999	0.997	1.001	0.01
Copper µmol/L	**0.133**	**0.054**	**0.01**	**1.143**	**1.028**	**1.270**	**0.17**
Selenium µmol/L	**-4.153**	**1.625**	**0.01**	**0.016**	**0.001**	**0.380**	**0.24**
Zinc µmol/L	-0.104	0.107	0.334	0.902	0.731	1.112	0.04
Magnesium mmol/L	-3.343	3.083	0.278	0.035	0.000	14.884	0.02

*R value Nagelkerkel.

Table 4. Logistic regression analysis to establish the likelihood that Selenium and Copper concentration (at diagnosis) has on clinical outcome (EFS v Event) taking into consideration predictors from univariate analysis (p<0.01) (bottom)

Micronutrient concentration	β	SE	*p*	Odds-Ratio	95% CI for Odd-Ratios		R*
					Lower	*Upper*	
Selenium µmol/L	**-3.89**	**1.9**	**0.04**	**0.02**	**0.0004**	**0.8810**	**0.33**
BMI *Z*-score	-0.38	0.26	0.1	0.69	0.415	1.136	
Decimal age	-0.07	0.09	0.4	0.92	0.771	1.114	
Copper µmol/L	0.11	0.06	0.06	1.11	0.993	1.244	0.27
BMI *Z*-score	-0.31	0.27	0.2	0.73	0.432	1.233	
Decimal age	-0.14	0.10	0.1	0.87	0.709	1.058	

*R value Nagelkerkel.

Table 5. Multilevel analysis showing the impact of micronutrient concentration on the number of complications in paediatric cancer patients at different time points

Complications during treatment				
Variables	**β**	***t***	**95% CI**	***P***
At diagnosis				
Intercept	6.4	3.5	2.7 – 9.9	0.001
Magnesium	-5.1	-2.9	-8.7 – (-1.6)	0.006
Zinc	0.07	1.9	-0.002 – (0.15)	0.06
Copper	-0.06	-2.0	-0.13 – 0.0002	0.05
Selenium	-1.2	-2.1	-2.9 – (-0.04)	0.04
Vitamin E/Ch	0.3	2.2	0.03 – 0.51	0.03
Vitamin A	0.4	1.1	-0.30 – 1.03	0.3
Vitamin B12	< -0.01	-1.2	-0.001 – 0.0003	0.2
3 months				
Intercept	4.9	2.7	0.57 – 10.44	0.05
Magnesium	-3.2	-1.4	-7.76 – 1.33	0.1
Zinc	0.07	0.9	-0.09 – 0.25	0.4
Copper	0.001	0.02	-0.10 – 0.11	0.9
Selenium	-3.9	-4.2	-5.57 – (-2.02)	<0.001
Vitamin E/Ch	0.05	0.4	-0.21 – 0.32	0.7
Vitamin A	0.9	3.2	0.34 – 1.54	0.003
Vitamin B12	<0.01	1.5	-0.0002 – 0.001	0.1
12 months				
Intercept	4.7	2.8	1.24 – 8.11	0.01
Magnesium	-8.1	-4.6	-11.66 – (-4.46)	<0.001
Zinc	0.01	-0.6	-0.06 – 0.04	0.6
Copper	-0.02	-0.5	-0.10 – 0.06	0.6
Selenium	-2.3	-2.4	-4.10 – (- 0.34)	0.02
Vitamin E/Ch	0.3	1.6	-0.08 – 0.64	0.1
Vitamin A	0.6	2.1	-0.007 – 1.11	0.05
Vitamin B12	<0.001	0.1	-0.002 – 0.0002	0.09

Only statistically significantly time points from the multilevel model have been presented here.

760 **Figure 1.** Flow diagram showing patient's accrual and sample availability

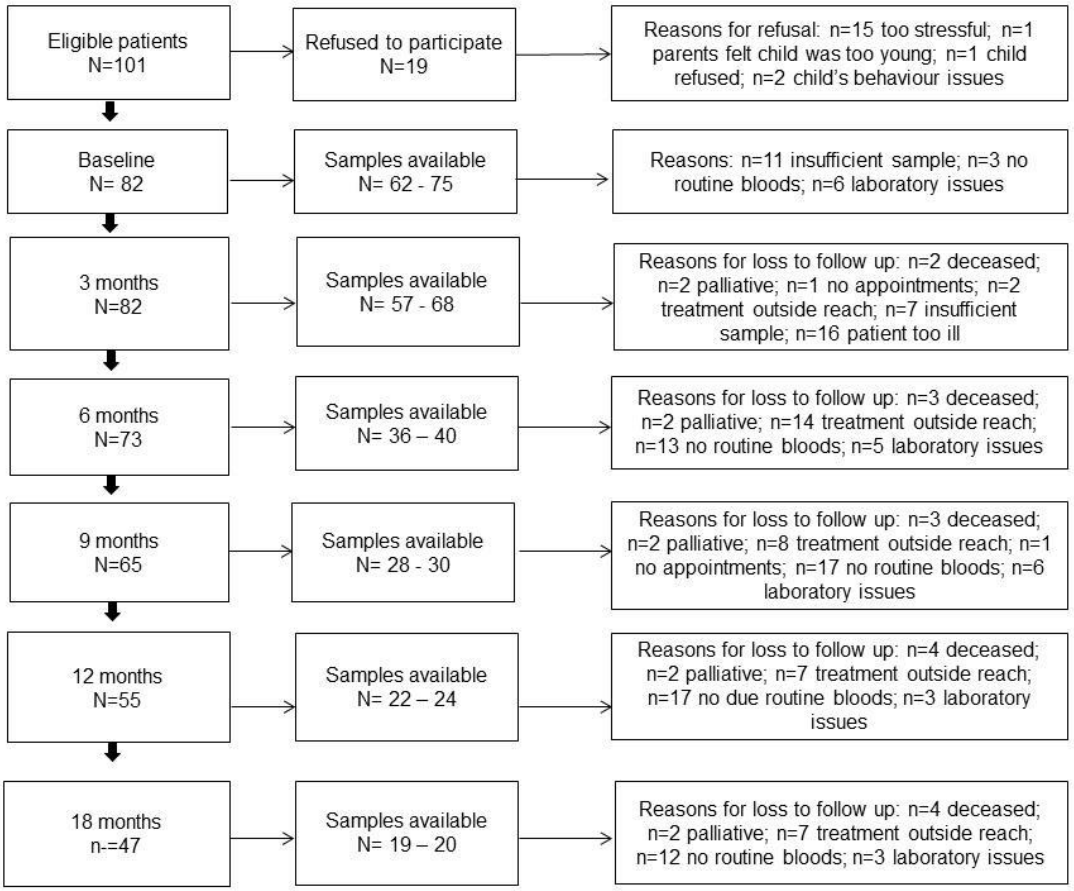

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Figure 2. Patient's follow up at each time point and number of patients having had each type of measurement.

Time point	Patients availability	Drop Outs*	BMI	BIA	Plasma vitamin A	Plasma vitamin E/Ch	Plasma vitamin B 12	Plasma Copper	Plasma Magnesium	Plasma Selenium	Plasma Zinc	Dietary intake
Diagnosis (baseline)	82	0	81	60	64	63	62	67	75	66	63	77
3 months	82	6	75	56	61	57	63	60	68	60	56	75
6 months	73	19	54	38	39	39	39	39	40	39	36	54
9 months	65	14	51	37	30	30	30	31	29	31	28	51
12 months	55	14	42	30	24	24	22	24	24	24	23	42
18 months	47	13	34	28	20	20	19	19	20	19	19	34

*Drop outs due to: deceased patients, palliative treatment, treatment given in centres other than RHSC, Edinburgh and Ninewells Hospital, Dundee and patients who missed appointments.

Figure 3. Micronutrient intakes of paediatric cancer patients with data stratified by type of nutrition treatment.

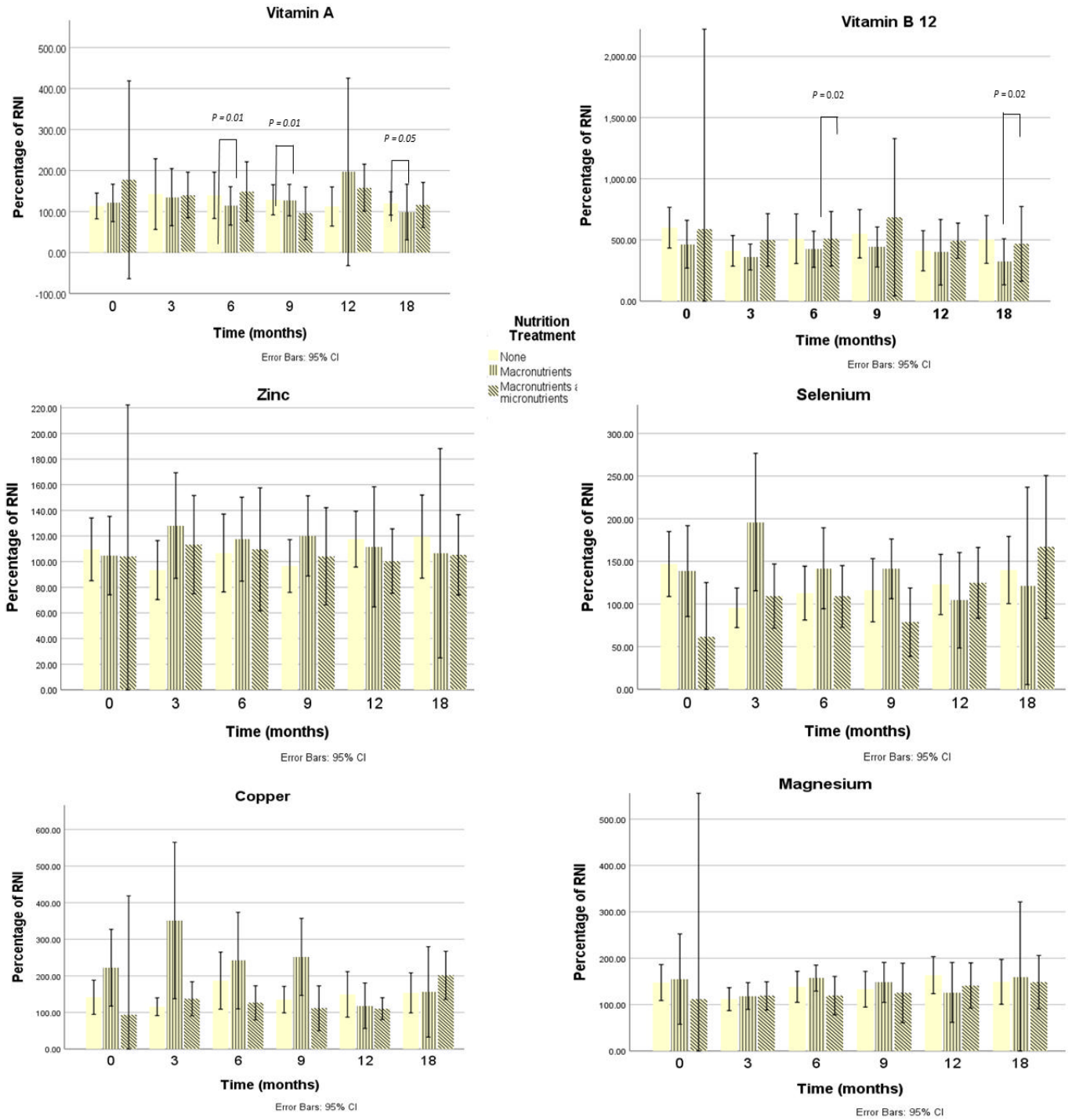
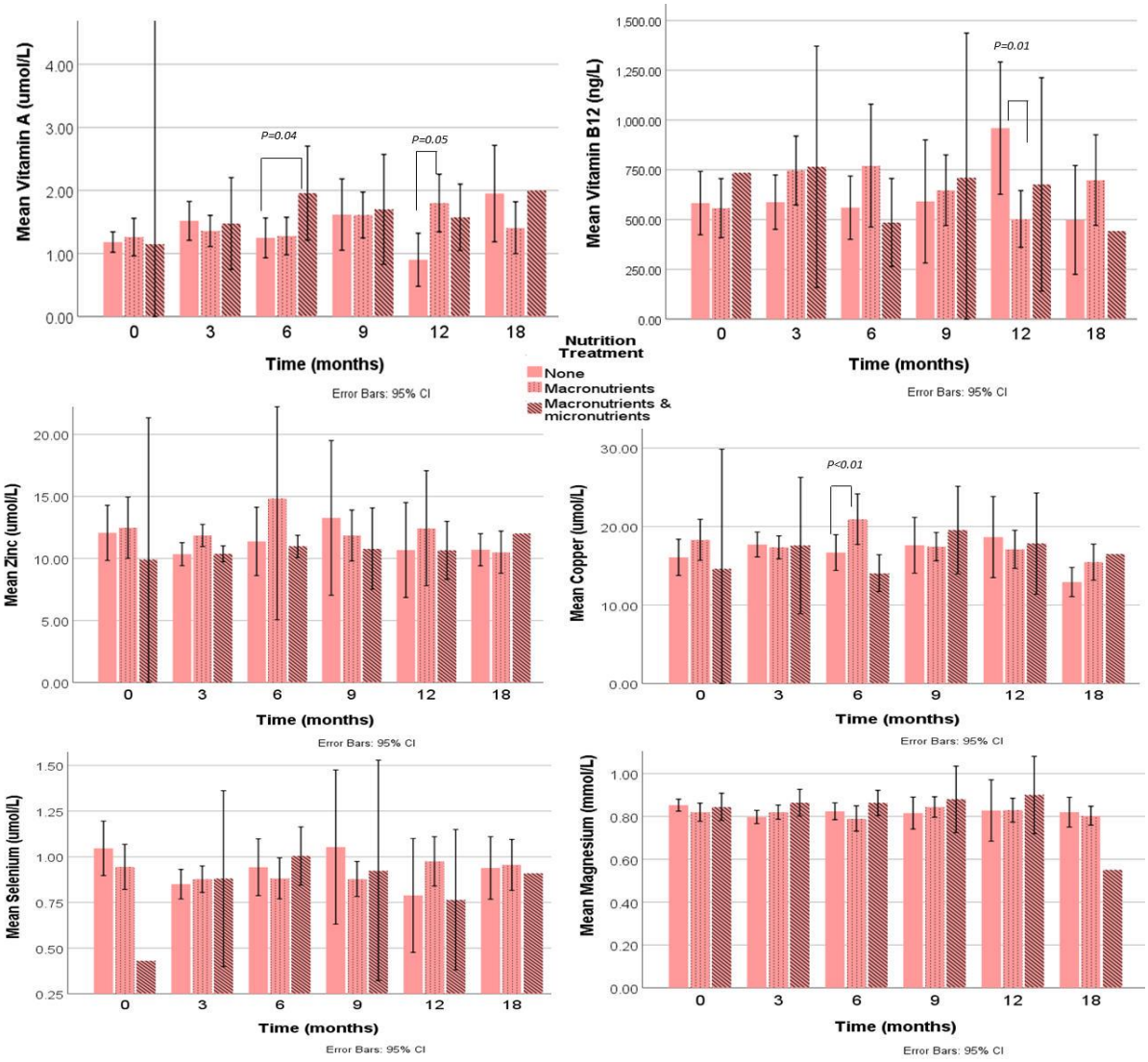
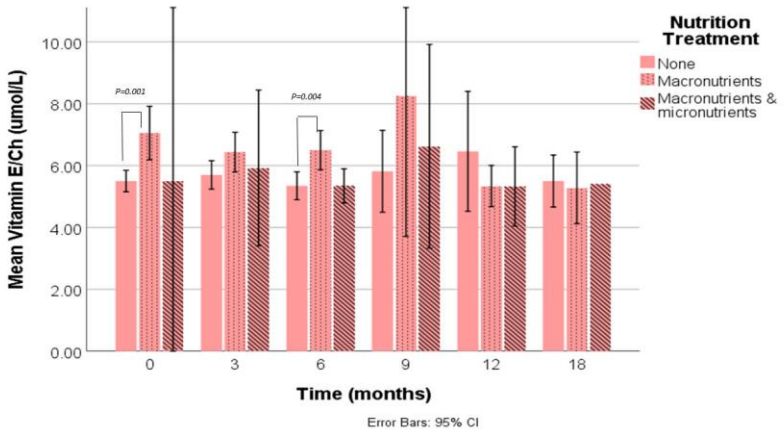


Figure 3a. Vitamin A and vitamin B12 intakes; Figure 3b. Zinc and Selenium intakes; Figure 3c. Copper and Magnesium intake.

Figure 4. Micronutrient concentration of paediatric cancer patients with data stratified by type of nutrition treatment





One way ANOVA test; p=0.04, 95% CI (-1.3 to -0.14); p=0.05, 95% CI (-1.7 to -0.15); p <0.01, 95% CI (169 – 743); p=0.01, 95% CI (-7.84 to -0.67)