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Evaluation of biomarkers for intestinal damage in pediatric acute lymphoblastic leukemia

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Intestinal damage (ID) leads to bacterial translocation and bloodstream infections—the common cause of non-relapse mortality in childhood acute lymphoblastic leukemia (ALL). This study evaluated ID over ALL induction and the significance of body mass index (BMI) for its development and identified biomarkers reflecting chemotherapy-induced ID. The composite biomarker panel included 37 plasma amino acids, urea, ammonia, fecal calprotectin (fCLP), absolute neutrophil count (ANC), C-reactive protein, and albumin. We prospectively assessed 45 children treated according to the ALLTogether protocol in 2020–2024. Analysis and sample collection were performed on days 1, 8, 15, 22, and 29 of the protocol. The obtained values were compared between the ID and non-ID groups. 40% of patients (18/45) had grade I–III ID which was more pronounced on day 22 of induction when the ANC increased from its lowest point. Age younger than 5.5 years at a diagnosis was a significant prognostic factor for ID. Decreasing BMI and concentrations of citrulline, taurine, cystine, phosphoethanolamine, A-aminobutyric acid, B-alanine, and albumin suggest progressive ID in children treated due to ALL. No difference in ANC and fCLP was found between patients with and without ID, but fCLP levels start to rise simultaneously as the most intense ID is observed. In conclusion, assessing nutritional status and prospective evaluation of biomarkers may provide valuable information on treatment-related ID.

Keywords Intestinal damage, Biomarkers, Pediatric acute lymphoblastic leukemia, Malnutrition

Acute lymphoblastic leukemia (ALL) is one of the most frequent pediatric malignancies, comprising 25% of the total. Although survival rates reach 90%[1], the incidence of treatment-related mortality (TRM) in ALL ranges from 2 to 4%, with 1% occurring during the disease's induction[2–4].

Chemotherapy-provoked neutropenia, intestinal mucosal barrier disruption, microbiome imbalance, and translocation of intestinal Gram-negative bacteria can result in an irreversible systemic inflammatory response, bloodstream infections, multiorgan damage, and fatal outcomes in 2% of children treated for ALL[5,6]. Thus, the early detection of intestinal damage (ID) may be crucial to identify patients who are at the highest risk of complications or need prompt antibiotic administration during chemotherapy.

Patients treated for ALL most often experience gastrointestinal tract disturbances during the induction course. High-dose glucocorticoids alter the mucosal barrier by decreasing microbiota diversity, immune cells, villus height, and IgA production[7–9].

Owing to the activation of proinflammatory cytokines, the catabolic state prevails in the acute phase of a disease leading to the phenomenon of "autocannibalism"[10]. Amino acids (AAs) are precursors and metabolites of proteins; therefore, they emerge as a promising tool enabling the prediction or evaluation of the clinical course of an acute disease. AAs are indispensable for epithelial cells to maintain intestinal mucosa integrity and immune homeostasis, along with the decrease in oxidative stress and proinflammatory cytokine formation by modulating immune and inflammatory responses with the involvement of the latter[11]. As gut injury is associated with infections and increased complication rates, particularly in patients with immunocompromised status, the incipient loss of intestinal cells must be determined.

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Measuring changes in AA concentrations is commonly used in monitoring the course of intestinal diseases, e.g., Crohn's disease, ulcerative colitis, or even gut injury occurring during bacterial infection or sepsis[10–14]. AA concentration changes have been demonstrated in adults with newly diagnosed acute leukemia, who manifest with febrile neutropenia[9], and new insights in pediatric ALL have been shown; however, more data are required to draw more definite conclusions.

We assumed that AA concentration changes could reflect the intensity of the inflammatory response in a post-chemotherapeutic mucosal injury. To determine the potency of post-treatment inflammation, we tracked levels of CRP, as it is a marker of acute inflammation stimulated by the levels of interleukin (IL)-6[15].

Frequent blood sampling during active treatment can cause iatrogenic anemia, particularly in younger children[16]. Thus, finding a noninvasive biomarker, the levels of which can be measured interchangeably with routine blood tests, is necessary. Fecal calprotectin (fCLP) is a sensitive indicator of inflammatory bowel disease. Thus, we have hypothesized that fCLP may be useful in determining post-chemotherapeutic ID and can be used as a noninvasive biomarker. For this purpose, this study evaluated the association between AAs and fCLP as well as with absolute neutrophil count (ANC), albumin, and CRP.

Malnutrition is linked to a higher risk of complications and lower survival rates in children undergoing chemotherapy or allogeneic hematopoietic stem cell transplantation[17,18]. The incidence of malnutrition in pediatric patients with malignancies, outlined by the World Health Organization as either under or overnutrition, has been demonstrated to reach 75%[19]. In 2021, the percentage of malnourished patients aged > 2 years in Lithuania was 36.3%, with 21.7% being overweight or obese and 14.6% being underweight[20].

Weight loss is indicative of a higher rate of febrile neutropenia and increased mortality risk[21]. Obesity is associated with chronic low-grade systemic inflammation, and the primary involvement of adipose tissue leads to changes in the circulating levels of cytokines and acute-phase reactants[22]. Abnormal body mass index (BMI) z-scores are related to fever at the diagnosis of ALL in the group aged 0–5 years[23].

The evaluation of nutritional status on admission may help predict the disease course in patients in critical conditions[24]. Serum biomarkers, a part of routine examination, may reveal useful information about a patient's metabolism. However, some of these biomarkers (usually, proteins) may cause misinterpretations because of changes in their levels in an acute phase of an illness, as it leads to a catabolic state[24].

The most sensitive biomarker for ID that does not depend on the catabolic state is citrulline[25]. However, data regarding other AAs and their association with malnutrition are still lacking.

This study aimed to analyze ID development over ALL induction in children and determine which biomarkers (plasma AA and fCLP) can best monitor chemotherapy-induced ID in children diagnosed with ALL. In addition, we aimed to prove whether malnutrition affects changes in the levels of prognostic biomarkers and whether malnutrition alone may be a significant negative predictive factor for ID progression.

Patients and methods

Study sample and design

In this prospective cohort study, pediatric patients aged < 18 years diagnosed and treated for ALL at Vilnius University Hospital Santaros Klinikos (VUHSK) Center for Pediatric Oncology and Hematology between December 30, 2020, and March 24, 2024, were enrolled. The diagnostics and treatment of pediatric ALL in Lithuania are centralized at this institution; thus, the cohort was population-based. Children enrolled up to April 2022 were treated according to the ALLTogether Pilot protocol. Since April 24, 2022, children received treatment as per the ALLTogether Master protocol (ClinicalTrials.gov Identifier NCT03911128). The induction of both protocols did not differ and is described below in detail. One of the patients was treated according to the Interfant-21 protocol.

All interventions carried out complied with the ethical guidelines of the institutional and national research committee and the 1964 Helsinki Declaration with its later amendments or compatible guidelines. The study was approved by the Vilnius Regional Committee of Biomedical Research (approval no. 158200-18/12-1073-576). All participants (or caregivers according to age) provided informed consent.

The study is registered at www.clinicaltrials.gov (ID 20BMT96, 19/11/2020).

Methods

ID, BMI-based nutritional status, and a biomarker panel that included plasma AA and urea, CRP, fCLP, albumin, and ANC at disease induction were evaluated prospectively. The baseline characteristics of the enrolled patients were extracted from electronic records. ID and BMI assessments and blood and fecal sample collections were performed at five time points during the induction: at diagnosis (before chemotherapy or on day 1) and on days 8, 15, 22, and 29, as per the treatment protocol. ID group was defined as measurements at any time point when ID was documented, non-ID group comprised measurements of those patients who have never experienced ID (as described below). Biomarker values were compared between the ID groups and the normally nourished (BMI z score > − 2 and < 1) and malnourished (BMI z score < − 2 or > 1) groups. Age and BMI at diagnosis were checked as risk factors for ID development during the induction course.

AA and fCLP analysis

Plasma AA concentration was measured by high-performance liquid chromatography at the Center of Medical Genetics of VUHSK (depicted in detail below). Standard collection of blood samples (5 mL) into tubes having lithium heparin was done for the further quantitative AA analysis by the standardized scheme: separation of plasma from blood was performed by centrifugation at 3,000 rpm at 4 °C for 15 min, followed by deproteinization with 5% sulfosalicylic acid and centrifugation at 3,000 rpm at 4 °C for 15 min. The obtained supernatant (300 µl) was subjected to filtration through 0.2-µm pore size cellulose acetate filters and preserved

at − 80 °C for further quantitative AA analysis. The latter was performed with a Biochrom30 + AA analyzer (ion-exchange chromatography, ninhydrin post-column derivatization, and single-point calibration). Norleucine levels were applied for comparison along with the external quality assurance scheme (ERNDIM; https://erndim.org). Concentrations of 37 AAs were determined and assessed: phosphoserine, taurine, phosphoethanolamine, aspartate, threonine, serine, asparagine, glutamate, glutamine, sarcosine, alpha-aminoadipic acid, glycine, alanine, citrulline, alpha-aminobutyric acid, valine, cystine, methionine, cystathione, isoleucine, leucine, tyrosine, beta-alanine, phenylalanine, beta-aminoisobutyric acid, homocysteine, ethanolamine, hydroxylysine, ornithine, lysine, 1-methylhistidine, histidine, tryptophan, 3-methylhistidine, arginine, hydroxyproline, proline and urea and ammonia.

fCLP was measured by EliA Calprotectin 2 fluorescence enzyme immunoassay (Thermo Fisher Scientific) on ImmunoCAP 200. The protein extraction procedure of the samples was performed using the EliA Stool Extraction Kit 2 according to the manufacturer's (Thermo Fisher Scientific) instructions. Extractions were stored at − 20 °C awaiting analysis, which was performed once a week.

Definition of variables

ID diagnostic criteria included the presence of gastrointestinal pain, diarrhea, or enterocolitis according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events v.5 (CTCAE), where the severity was expressed in grades ranging from 0 to V[26] as described in detail in Supplementary Table 1. In case of symptom discrepancy, the degree of ID was defined according to the highest degree. These symptoms were evaluated daily and recorded by a treating physician.

BMI z-scores were determined using WHO Anthro (v3.2.2) and AnthroPlus (v1.0.4). Obesity in children below 5 years of age was defined as a BMI z score > 3 SD, overweight as a BMI z score > 2 SD, and a risk of overweight as a BMI z score > 1 SD; in children older than 5 years obesity was defined as BMI z score > 2 SD and overweight > 1 SD. To be consistent with definitions we defined overweight status as BMI z score > 1 and obese status as BMI z score > 2. Underweight was defined as BMI z score < -2 in all children. The values between above mentioned were classified as normal nutrition. Malnutrition was defined as overweight, obese, or underweight. Baseline nutritional status was defined by BMI z score before starting chemotherapy. For its calculation, morning weight was evaluated.

ALL therapy

All patients underwent therapy as per ALLTogether Pilot or Master chemotherapy protocols, where the induction did not differ. Children were stratified into different risk groups at induction: (A) The NCI standard-risk group (B-ALL, age at diagnosis < 10 years, and leukocyte count $< 50 \times 10^9$/L) was administered dexamethasone (6 mg/m^2 on days 1–28, tapered), vincristine (1.5 mg/m^2, maximum single dose 2 mg on days 1, 8, 15, and 22), PEG-asparaginase (1500 IU/m^2 dose on days 4 and 18), and intrathecal methotrexate according to age. (B) The NCI high-risk group (all T-ALL, B-ALL if ≥ 10 years, and/or leukocyte count $\geq 50 \times 10^9$/L) was also administered daunorubicin (25 mg/m^2 dose on days 1, 8, 15, and 22). Patients with Down syndrome had induction D (instead of dexamethasone, they received prednisolone 40 mg/ m^2 on days 1–28; daunorubicin 45 mg/ m^2 was added on day 16 in the ALLTogether Pilot protocol, and in a case of poor response on day 15).

A 5-month-old infant was subjected to treatment as per the Interfant-21 protocol. The patient was administered 6 mg/m^2 dexamethasone for 28 days daily (and tapered), vincristine weekly, two doses of daunorubicin, cytarabine for 14 days daily, two doses of PEG-asparaginase every 14 days, and intrathecal methotrexate, cytarabine, and methylprednisolone to achieve remission.

Comparative analysis

The mean level of each biomarker was calculated for each patient at every time point. The obtained values were grouped according to whether a study participant manifested with ID at any time point and compared in between. Non-ID values were taken into analysis only from those patients who have never experienced ID. The BMI and BMI z-scores were evaluated at diagnosis to evaluate baseline nutritional state and then at each time point, biomarkers were compared between the malnourished and normally nourished groups, and evaluation of the association of BMI and ID during the induction was performed.

Statistical analysis

Categorical data were presented as frequencies and percentages and analyzed using Pearson's chi-square test to ascertain differences between patients with and without ID. To test for the normality of variables, the Shapiro-Wilk normality test was employed. For non-normally distributed continuous data, medians and interquartile ranges (IQR) were calculated, and the Wilcoxon rank-sum test was used. For normally distributed continuous variables, the mean and standard deviation (SD) were calculated, with Welch's Two-sample t-test used for comparisons. Pearson's product-moment correlation coefficient was used to assess associations between paired biomarker samples. Linear regression and logistic regression were employed to identify significant biomarkers and other relevant variables, depending on the data type of variable under consideration. The generalized estimating equation (GEE) model was utilized to quantify differences between the two patient groups, accounting for the correlation between repeated measurements from the same patients. Receiver operating characteristic (ROC) curves were used to determine optimal threshold values for biomarkers and assess their diagnostic capability about ID. A p-value of < 0.05 was considered statistically significant. Statistical analysis was performed using R software (version 4.2.2, R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics

The sample included 45 consecutive children diagnosed with ALL between December 2020 and March 2024 at our institution. Table 1 summarizes patients' baseline characteristics.

40% of the patients (18 out of 45) had grade I–III ID at least at one-time point—most often, they had grade I ID (11 out of 18, 61.1%). Most ID cases were documented during the fourth week (day 22) of treatment, as

	All patients ($n=45$)	With ID ($n=18$)	Without ID ($n=27$)	p-value
Age, years, median (Q1, Q3)	4.6 (2.1, 8.9)	3.1 (1.8, 4.6)	6.9 (3.9, 10.3)	< 0.001
Sex, n (%)				
Female	21 (46.7)	8 (44.4)	13 (48.1)	0.807
Male	24 (53.3)	10 (55.6)	14 (51.9)	
ALL induction arm*, n (%)				
A	23 (51.1)	14 (77.8)	9 (33.3)	0.029
B	19 (42.2)	4 (22.2)	15 (55.6)	
D	2 (4.4)	0 (0.0)	2 (7.4)	
Interfant-21	1 (2.2)	0 (0.0)	1 (3.7)	
Maximal ID grade, n (%)				
0	27 (60.0)	N.A.	27 (100.0)	N.A.
I	11 (24.4)	11 (61.1)	N.A.	
II	6 (13.3)	6 (33.3)	N.A.	
III	1 (2.22)	1 (5.56)	N.A.	
IV–V	0 (0.0)	0 (0.0)	N.A.	
ALL risk group at the EOI, n (%)				
Standard	6 (13.3)	4 (22.2)	2 (7.4)	0.031
Intermediate-low	19 (42.2)	10 (55.6)	9 (33.3)	
Intermediate-high	14 (31.1)	2 (11.1)	12 (40.4)	
High	3 (6.7)	0 (0.0)	3 (11.1)	
Death during induction, n (%)	3 (6.7)	2 (11.1)	1 (3.7)	
BMI, median (Q1, Q3)	16.00 (15.1, 17.6)	16.5 (15.6, 18.5)	15.9 (15.0, 17.6)	0.211
BMI z-score at diagnosis, median (Q1, Q3)	0.11 (−0.77, 0.90)	0.41 (−0.11, 1.74)	−0.09 (−1.07, 0.80)	0.026
Children < 5 years				
BMI z-score at diagnosis, n (%)				
> 3	1 (4.17)	0 (0.00)	1 (9.09)	
2–3	1 (4.17)	1 (7.69)	0 (0.00)	
> −2 and < 2	22 (91.67)	12 (92.31)	10 (90.91)	
< −2	0 (0.00)	0 (0.00)	0 (0.00)	
BMI z-score at the EOI, n (%)				
> 3	0 (0.00)	0 (0.00)	0 (0.00)	
2–3	0 (0.00)	0 (0.00)	2 (18.18)	
> −2 and < 2	19 (90.48)	10 (83.33)	9 (81.82)	
< −2	2 (9.52)	2 (16.67)	0 (0.00)	
Children > 5 years				
BMI z-score at diagnosis, n (%)				
> 2	0 (0.00)	0 (0.00)	0 (0.00)	
1–2	4 (19.05)	1 (20.00)	3 (18.75)	
> −2 and < 2	16 (76.19)	4 (80.00)	12 (75.00)	
< −2	1 (4.76)	0 (0.00)	1 (6.75)	
BMI z-score at the EOI, n (%)				
> 2	1 (5.00)	1 (20.00)	0 (0.00)	
1–2	2 (10.00)	0 (0.00)	2 (13.33)	
> −2 and < 2	13 (65.00)	4 (80.00)	9 (60.00)	
< −2	4 (20.00)	0 (0.00)	4 (26.67)	

Table 1. Patients' baseline characteristics. ALL, acute lymphoblastic leukemia; BMI, body mass index; EOI, end of induction; ID, intestinal damage; NA, not applicable. *Refer to the Methods section for the difference in the induction. ˣ comparing normal nutrition (BMI z-score < 2 and > − 2) vs. malnutrition (BMI z-score < − 2 and > 2).

depicted in Fig. 1. Boys and girls were equally distributed in both groups. Compared with the children without ID, patients who experienced ID tended to be younger and had a higher BMI z score at diagnosis. Patients with ID were more often treated according to induction A and allocated to the lower-risk group (standard and intermediate-low) at the end of the induction.

BMI Z score changes during the induction course and impact on ID

The assessment of nutritional status, i.e., BMI with changing values over the evaluation period, revealed that more children became malnourished at the end of the induction than at the time of diagnostics. Before the start of treatment, six patients were overweight/obese, and one was underweight, whereas after the induction course, five were overweight/obese and six were underweight (Table 1). The BMI remained higher during the treatment course in patients with ID than without it (Fig. 2). However, during the treatment course according to the GEE method lower BMI was associated with a higher risk of ID (coeff. -0.349, standard error 0.117, p-value 0.003). Focusing on different age groups, in children younger than 5 years of age, the same association was observed (coeff. -0.418, standard error 0.123, p-value 0.001), but in older children, BMI was not related to the risk of ID (coeff. -0.120, standard error 0.284, p-value 0.673).

Differences in plasma AA and other biomarkers between the ID and non-ID groups

Measurement of plasma AA levels revealed that the ID group had a higher median level of hydroxylysine and lower levels of taurine, glycine, citrulline, cystine, arginine, urea, and albumin, although fCLP, CRP, and ANC were not significantly different (Table 2). Neutropenia did not correlate with ID, as its manifestations usually commenced at day 22 when ANC started to rise (Fig. 2).

The density curves (Fig. 3) and the results of GEE analysis (Table 3) were used to select the most important biomarkers for the early diagnosis of ID: citrulline, taurine, albumin, cystine, phosphoethanolamine, aminobutyric acid, and B-alanine. The density graphs illustrate the probability distributions of biomarker levels and age across two patient groups: those with ID and those without. Each curve represents how frequently specific biomarkers or age values occur within the respective groups. The ROC model revealed possible threshold values for age and these biomarkers (Table 3). Citrulline and albumin concentration differed according to the severity of ID as compared to patients without ID: citrulline coefficient in grade I was − 6.979 [-11.966; -1.992], $p = 0.006$, while in grade II—9.996 [-14.468; -5.524], $p = 0.000$; albumin coefficient in grade I was − 3.430 [-6.002; -0.859], $p = 0.009$; while in grade II it was − 4.608 [-8.401; -0.814], $p = 0.017$ in comparison to patients without ID.

The longitudinal dynamics of these biomarkers are shown in Fig. 2. The most prominent decrease in citrulline was on days 22 (8.8 mcmol/l; IQR, 8.6–19.6 with ID vs. 24.25 mcmol/l; IQR, 19.1–29.3 without ID) and 29 (10.1 mcmol/l; IQR, 9.2–13.1 with ID vs. 26.5 mcmol/l; IQR, 18.9–36.1 without ID) (Supplementary Table 2). During the most intense ID in the third-fourth week, the levels of fCLP were higher in children with ID than in those without it (Fig. 4) and the concentration was nearly double, compared with that on day 29 in the non-ID group (Supplementary Table 2).

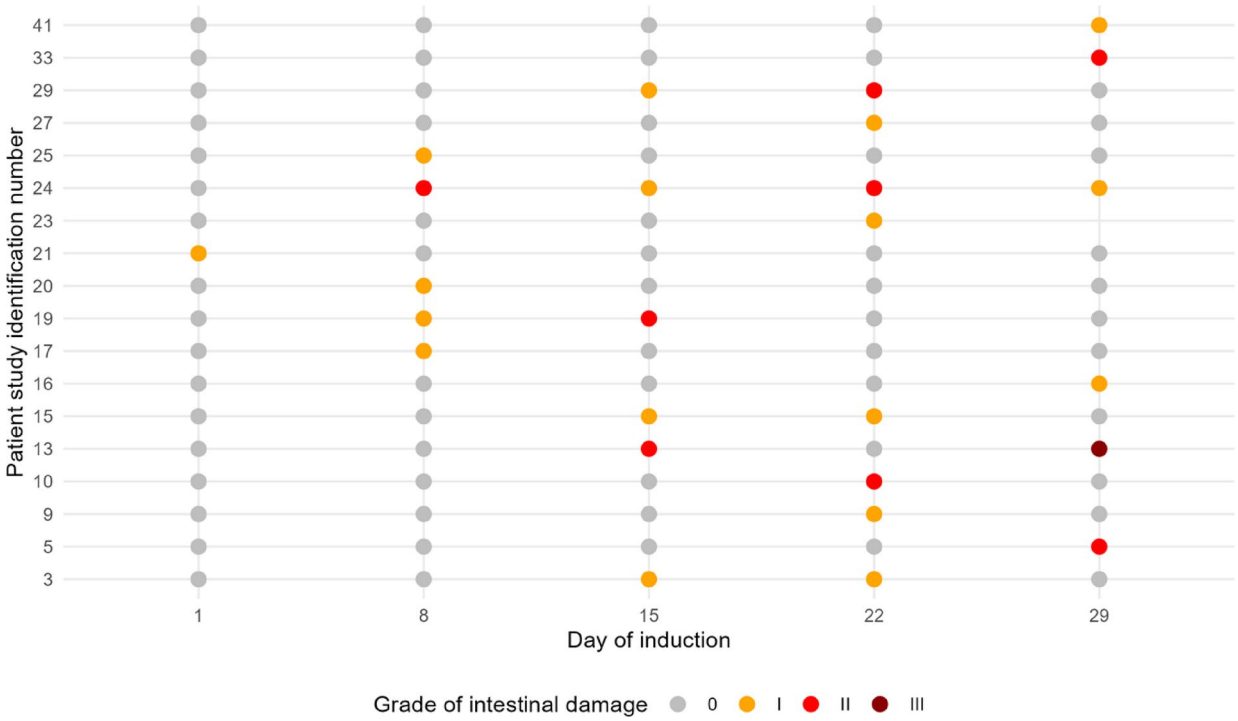

Fig. 1. Onset of intestinal damage during the induction course ($n = 18$).

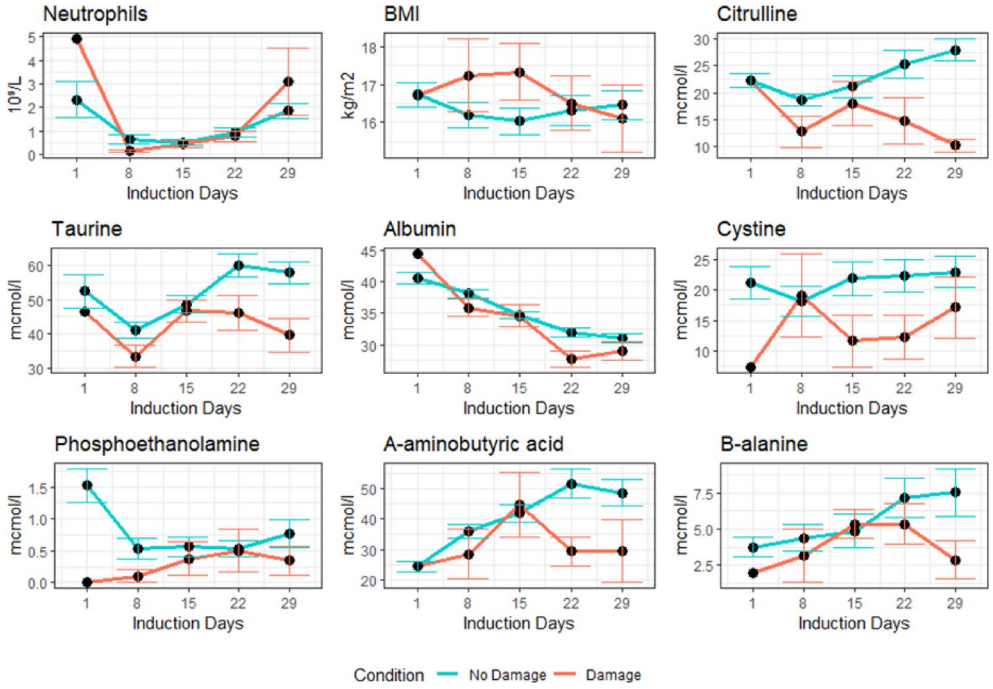

Fig. 2. Changes in mean ANC, BMI, citrulline, taurine, albumin, cystine, phosphoethanolamine, A-aminobutiryc acid, and B-alanine concentrations during the induction course in children with ($n = 18$) and without ($n = 27$) intestinal damage.

The focused analysis of the ID group revealed a negative association between albumin and CRP, taurine and CRP, citrulline and CRP; A-aminobutyric acid and CRP. fCLP did not correlate with ANC (Fig. 5).

Differences in biomarkers between the malnourished and normally nourished groups

According to the GEE model, phosphoethanolamine, glutamine, A-aminoadipic acid, glycine, citrulline, A-aminobutyric acid, methionine, cystathionine, isoleucine, tyrosine, 1-methylhistidine, and proline increased with increasing BMI (Supplementary Table 3).

Age association with ID and biomarkers

According to the density curves (Fig. 3) and the results of the GEE analysis (Table 3), the risk of ID was higher in children younger than 5.5 years.

The concentrations of some biomarkers also varied with age (mostly increasing, except for glutamine): phosphoserine, urea, glutamine, alanine, A-aminobutyric r, valine, methionine, isoleucine, tyrosine, lysine, 1-methyl-histidine, tryptophan, 3-methyl-histidine, hydroxyproline, proline (Supplementary Table 4)[27,28].

Discussion

Myelosuppression and ID during chemotherapy are the main predisposing factors for the development of infectious diseases. Increased intestinal permeability leads to bacterial translocation and bloodstream infection distribution—the most common cause of non-relapse mortality in ALL[29] Even low-grade ID disrupts digestion, absorption, and barrier function[30]. And this is especially important as subclinical ID cannot be foreseen or evaluated clinically, but poses a higher risk for treatment-related complications. Thus, there is a need to find a diagnostic tool that could aid in noticing even a subclinical ID. The induction course of ALL is the most intensive treatment and poses a high risk for ID.

In this study, we were able to identify an ID landscape during the induction in pediatric patients with ALL treated according to the ALLTogether Pilot and Master protocols. ID emerged on the second week of treatment and was mostly expressed on day 22 of induction. This finding has shed new light on the concept of ID as the timing of ID onset has been historically associated with neutropenia, which usually starts within the second week of induction. ID development did not depend on the ANC, although chronic neutropenia was considered the main trigger for ID. As reflected in Fig. 2, the peak of ID coincided with the day when the ANC started to increase. Moreover, no difference in the ANC was found between the ID and non-ID groups. Thus, neutropenia alone is not considered to sufficiently promote intestinal mucosa disruption. The pathophysiology of chemotherapy-associated ID is a complex process involving the damage of intestinal cells and disturbance of the microbiota and AA. The latter interact mutually, ensuring the homeostasis of each other[31]. AAs ensure the synthesis of antibodies and cytokines, stimulating the function of immune cells and keeping the stability of a cell's membrane[32]. They also maintain the integrity of the intestinal barrier and antimicrobial properties[33].

Biomarker, mcmol/L	ID grades I–III, median (IQR) $n = 18$	No ID, median (IQR) $n = 27$	p-value
Phosphoserine	2.26 (1.5–2.9)	2.62 (1.6–4.7)	0.075
Taurine	42.35 (33.3–49.9)	48.60 (38.8–62.6)	0.023
Phosphoethanolamine	0.00 (0.0–0.3)	0.00 (0.0–0.8)	0.209
Aspartate acid	11.73 (0.0–14.3)	7.54 (0.0–12.2)	0.488
Threonine	224.00 (184.8–271.0)	201.00 (144.0–290.0)	0.534
Serine	130.00 (106.8–168.0)	152.00 (124.0–175.0)	0.118
Asparagine	0.00 (0.0–0.0)	0.00 (0.0–3.3)	0.007
Glutamic acid	194.70 (159.1–256.3)	153.70 (95.0–236.6)	0.083
Glutamine	422.50 (301.0–495.5)	470.00 (359.0–579.0)	0.069
Sarcosine	0.29 (0.0–6.8)	1.41 (0.0–5.5)	0.868
A -aminoadipic acid	2.69 (2.3–3.9)	3.00 (1.7–4.2)	0.896
Glycine	236.00 (201.8–287.5)	273.00 (225.0–322.0)	0.045
Alanine	420.50 (333.8–574.5)	452.00 (343.0–610.0)	0.399
Citrulline	13.75 (8.7–20.2)	20.60 (14.9–27.9)	0.001
A-aminobutyric acid	24.00 (18.1–49.3)	38.20 (24.1–46.8)	0.104
Valine	293.00 (241.8–436.5)	347.00 (264.0–437.3)	0.266
Cystine	11.35 (7.4–19.6)	18.30 (6.4–33.1)	0.090
Methionine	27.35 (19.2–39.1)	28.60 (22.8–41.2)	0.366
Cysthathione	1.55 (0.0–2.8)	1.34 (0.0–3.7)	0.587
Isoleucine	99.90 (68.0–141.2)	97.30 (73.9–131.0)	0.843
Leucine	165.00 (131.0–235.2)	183.30 (143.1–247.5)	0.437
Tyrosine	66.00 (50.6–93.9)	76.60 (59.7–101.3)	0.173
B-alanine	4.45 (2.1–5.8)	2.77 (0.0–6.2)	0.137
Phenylalanine	128.55 (99.2–184.9)	115.60 (80.5–177.6)	0.490
B-aminoisobutyric acid	0.00 (0.0–0.3)	0.00 (0.0–0.0)	0.943
Homocysteine	0.00 (0.0–0.0)	0.00 (0.0–0.0)	NA
Ethanolamine	0.00 (0.0–0.0)	0.00 (0.0–0.0)	NA
Ammonia	112.65 (99.7–185.1)	113.00 (79.2–169.9)	0.327
Hydroxylysine	1.50 (0.8–2.3)	0.00 (0.0–1.3)	< 0.001
Ornithine	42.90 (39.0–67.6)	52.00 (42.1–68.2)	0.406
Lysine	167.25 (133.0–209.0)	186.90 (149.1–219.4)	0.344
1-methyl-histidine	7.85 (5.9–10.2)	8.70 (5.0–17.0)	0.401
Histidine	75.40 (61.4–91.6)	71.00 (63.7–83.7)	0.489
Tryptophan	38.80 (31.3–49.5)	45.40 (35.9–55.0)	0.151
3-methyl-histidine	0.00 (0.0–0.0)	0.00 (0.0–0.0)	0.774
Arginine	58.60 (39.4–73.8)	74.40 (54.6–97.5)	0.026
Hydroxyproline	9.75 (5.6–16)	12.10 (8.3–20.6)	0.126
Proline	203.00 (169.0–337.8)	220.00 (159.0–333.0)	0.821
Urea	4947.00 (4178.2–5888.2)	5927.00 (4726.0–7587.0)	0.037
Fecal calprotectin	41.00 (27.2–62.5)	28.00 (12.0–65.0)	0.213
CRP mg/ml	1.69 (0.6–5.4)	0.73 (0.6–4.1)	0.357
ANC $\times 10^9$/L	0.44 (0.1–1.2)	0.43 (0.1–1.1)	0.784
Albumin	31.90 (28.4–35.8)	34.02 (30.8–38.1)	0.024

Table 2. Comparison of plasma AA, fecal CLP, CRP, and ANC levels in patients with and without intestinal damage. *ANC* absolute neutrophil count, *CRP* C-reactive protein, *IQR* interquartile range, *SD* standard deviation.

Age at diagnosis may be an important predictor for ID development—our study revealed that age < 5.5 years was associated with the risk of ID, this might explain the fact, that induction B was related to a lower risk of ID, compared to induction A as younger children without hyperleukocytosis are treated with induction A.

However, during the study, ID manifestations were not consistent—it developed in only 5 out of 18 patients within two consecutive weeks. Our cohort was relatively small, and ID was evaluated only at fixed time points; thus, the ID grades between the time points were not known. Thus, assessing only clinical symptoms made it difficult to predict the course of ID and associated complications, as ID presentation usually coincided with that of systemic inflammation/infection[34]. Although mucositis-related complications most often occur during the first two weeks of treatment[34], there may be late onset. Thus, a biomarker approach may be beneficial in

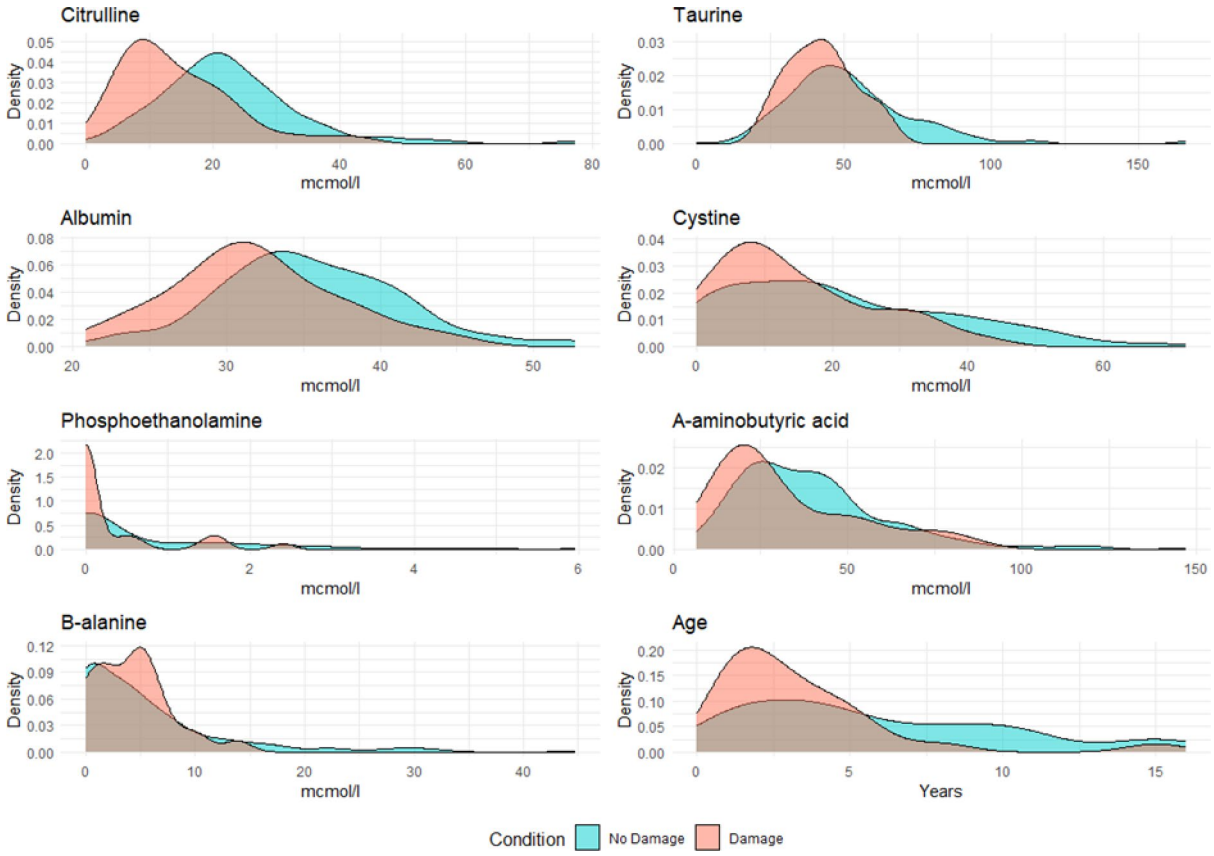

Fig. 3. Density curves for citrulline, taurine, albumin, cystine, phosphoethanolamine, A-aminobutyric acid, B-alanine, and age during ALL induction.

Factor	Coefficient	Threshold	Specificity	Sensitivity
Age, year	-0.013	5.5	0.4685	0.9000
Citrulline, mcmol/l	-9.233	14.65	0.7795	0.6538
Taurine, mcmol/l	-8.975	47.45	0.5385	0.7308
Albumin, g/l	-2.418	32.75	0.6769	0.6538
Cystine, mcmol/l	-7.201	15.35	0.5795	0.6538
A-aminobutyric acid, mcmol/l	-0.395	24.45	0.7385	0.5769
B-alanine, mcmol/l	-10.052	1.18	0.3436	0.8077

Table 3. Threshold values for factors important for the early diagnosis of ID in ALL.

evaluating post-chemotherapy ID in patients with ALL. Defining patients as having low risks of ID and infection may protect them from unnecessary antibiotic therapy and preserve their gut mucosa[35].

AAs are regarded as perfect biomarkers for the prediction or diagnostics of inflammatory diseases; however, the disruption of the microbiota modifies the metabolism of chemotherapeutic drugs and thus may diminish the efficacy of treatment and increase the risk of TRM[36]. Thus, we analyzed the panel of plasma AAs, as they emerge as biomarkers and may be included in a therapeutic scheme as supplements for AA deficiency.

A recent study highlighted the importance of citrulline, an AA produced by intestinal epithelial cells, as an early diagnostic marker for mucositis-associated bloodstream infection during ALL induction in children treated according to the NOPHO 2008 protocol[34]. It has reflected the drop in citrulline concentration in the cases of mucositis[34]. This study also demonstrated that citrulline concentration most prominently declined on days 22 and 29 when clinically symptomatic ID was most frequently observed. In addition, this approach was expanded by covering more AAs in the analyses, concomitantly with CRP, ANC, albumin, and fCLP. In addition, not only citrulline may be effective as an early marker of ID. Differences were also noticed for hydroxylysine, taurine, glycine, citrulline, cystine, arginine, urea, and albumin concentrations. The GEE method revealed that decreasing concentrations of citrulline, taurine, cystine, phosphoethanolamine, A-aminobutyric acid, B-alanine, and albumin are suggestive of progressive ID in children treated due to ALL. Though it is known that treatment with pegylated L-asparaginase by converting asparagine into aspartic acid and ammonia changes the

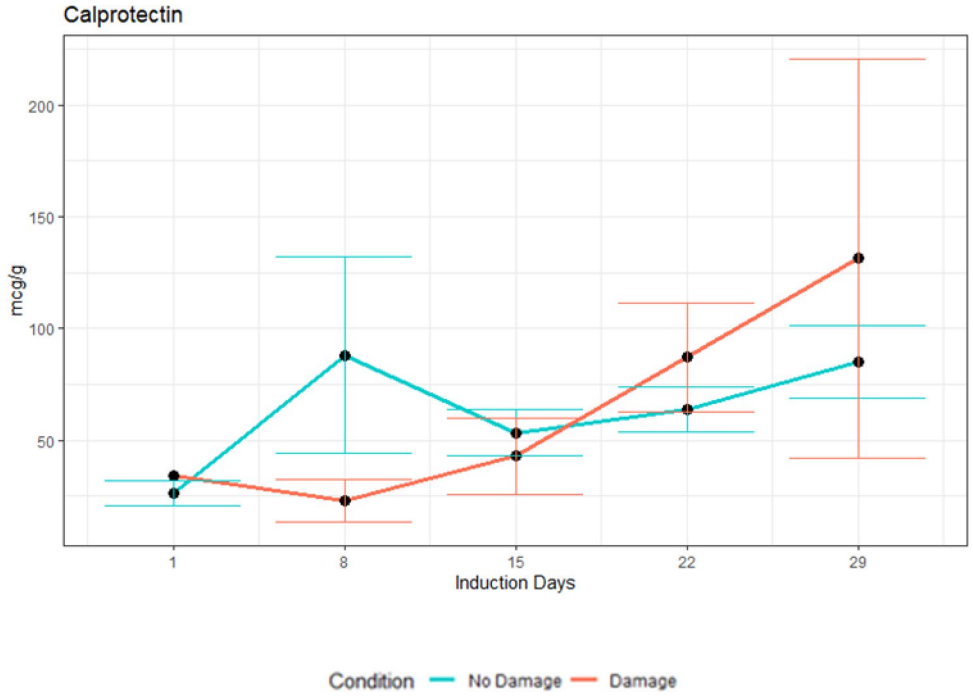

Fig. 4. Dynamics of median fCLP levels in children with and without ID during ALL induction.

concentration of these biomarkers as well as diminishes the concentration of albumin, in our study the treatment was homogenous—all children received pegylated L-asparaginase. Thus, progressive hypoalbuminemia was attributed solely to the ID. Our findings complemented those of the previously mentioned study, as the treatment protocols in Nordic and Baltic countries have been changed from NOPHO 2008 to ALLTogether since 2020.

Citrulline is a non-protein AA, which is generated in the enterocytes of the small bowel[37]. Thus, it is a marker of enterocytes mass and may reflect the severity of inflammatory intestinal diseases. Its concentration tends to decrease without apparent villus and cellular atrophy[25]. This study confirmed the findings concerning decreases in citrulline concentration in ID cases during ALL induction. The most prominent decline in citrulline levels was documented on day 22 of treatment, and it conformed to the time when ID symptoms were the most evident. Importantly, citrulline was not associated with age, though younger age was an important factor for ID.

Nevertheless, the correlation between different biomarkers is also important because they can be used concomitantly and add valuable insights into the clinical situation. CRP and fCLP concentrations did not differ significantly between the ID and non-ID groups. However, CRP levels correlated negatively with those of albumin, assuming that these two parameters may complement each other, as they are routinely used in everyday practice. As pediatric patients often experience iatrogenic anemia, noninvasive markers, such as fCLP, may spare the need for blood samples. With ID development, fCLP levels started to rise, and at the end of induction, the levels were significantly higher than in patients without ID. Thus, it is a promising noninvasive test in the pediatric population. fCLP is secreted by activated macrophages and neutrophils, but this work did not reveal any correlation between fCLP levels and ANC, so this is another important finding supporting fCLP level monitoring even in myelosuppressed patients.

Moreover, the study outcomes emphasize the differences in AA concentrations in malnutrition states. Malnutrition is linked to intestinal inflammation via angiotensin-converting enzyme 2, which regulates intestinal innate immune responses and the microbiota[33]. This study confirmed that malnutrition was associated with higher rates of ID. Thus, when using AAs as diagnostic tools for ID evaluation, it is important to initially evaluate the nutritional state of a pediatric patient. Obesity promotes an inflammatory state, disturbs intestinal metabolomics, and induces oxidative stress[38]. Leptin in children who are obese decreases the number of lymphocytes and phagocyte function[39]. Those with a higher BMI are more likely to be diagnosed with infectious diseases than those with a normal BMI, whereas decreased calorie intake can provoke AA dysregulation because of a catabolic state[39]. Though BMI z score at diagnosis was higher in children who experienced ID, but during the treatment course decreasing BMI was associated with the development of ID.

Overall, malnutrition disrupts micro- and macronutrient balance and alters the immune system and hematopoiesis[32]. In this study we demonstrated, that the concentration of several AA changed with changing BMI, which also affirms the inflammatory effect of nutritional imbalance. Our findings thus emphasize the importance of nutritional status, when interpreting biomarkers.

Poor nutritional status in pediatric patients with malignancies may contribute to a weakened immune function and deteriorated drug metabolism and lead to toxicities and adverse clinical outcomes[40,41]. These nutritional disturbances can be corrected by lifestyle and dietary modifications[42], prompting an idea that the rates of post-chemotherapy complications can be decreased and outcomes improved by modifying nutrition[40,41].

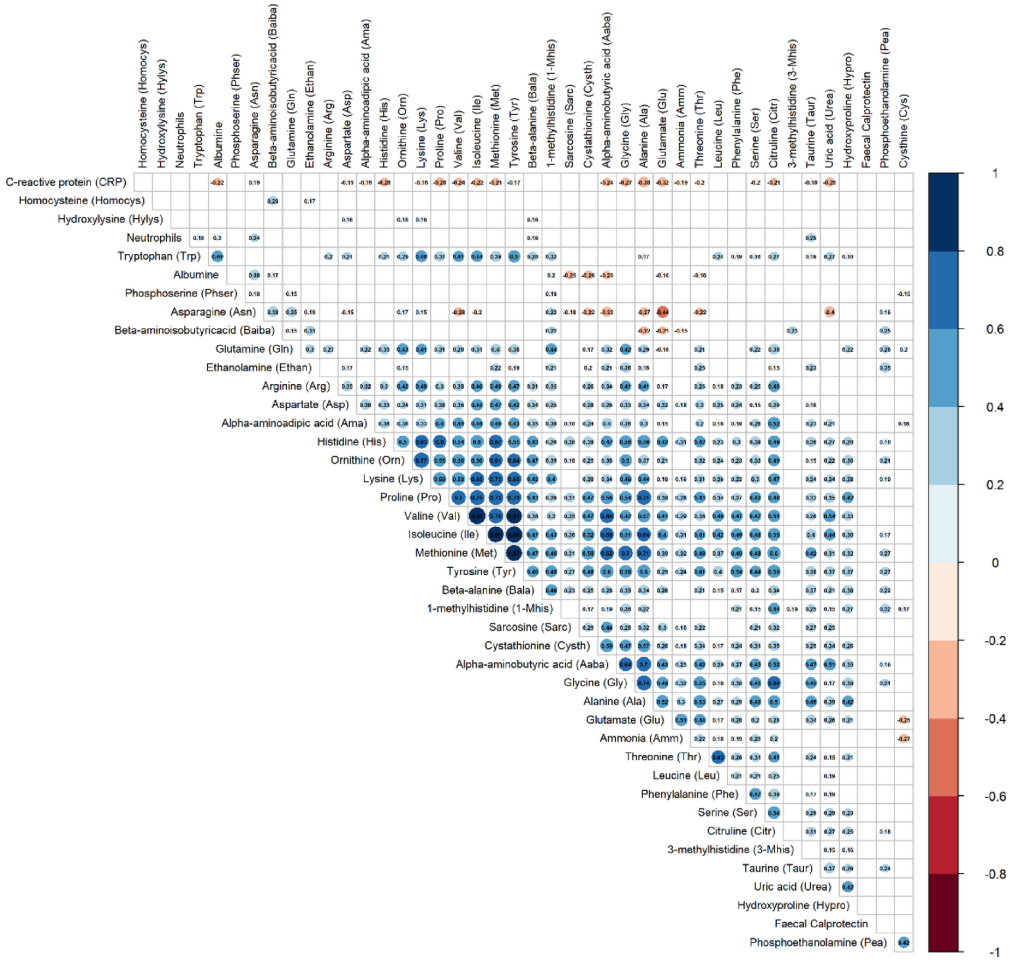

Fig. 5. Biomarker correlation in patients with intestinal damage ($n = 18$).

In summary, this study provides a new insight into the development of ID during induction in children treated for ALL according to the ALLTogether Pilot and Master protocols. A broad panel of biomarkers was monitored in a homogenous cohort using a consistent prospective longitudinal monitoring approach, which is considered a strength of this study. The main limitations are the small number of patients, measurements limited to certain points, ID was defined only clinically using CTCAE. The composition of nutrition and microbiome were not evaluated. Although BMI is the simplest, cheapest, and easiest method of assessing nutritional status in clinical practice, it does not detect changes in body composition. Thus, in case of a significant deviation in the BMI z-score, it is recommended to use other assessment methods (arm circumference, abdominal circumference, skinfold measurement, body composition assessment by various instrumental methods that require the additional skills of medical staff) to assess nutritional status more accurately. Evaluation of BMI z score in children younger than 5 years of age is complicated as new values of overweight and obesity are implemented (BMI z score > 2 and > 3, respectively), thus unification of values in all age groups is needed.

In conclusion, the measurement of citrulline, taurine, albumin, cystine, phosphoethanolamine, A-aminobutyric acid, and B-alanine levels appeared to be a promising tool in monitoring ID in the early phase during ALL induction in pediatric patients, as their concentrations correlated negatively with ID development. fCLP may be a significant noninvasive biomarker that may facilitate the detection of rising concentrations after ID onset. However, neutropenia alone is not associated with ID. Younger age at diagnosis and decreasing BMI during the treatment course are indicative of ID development. Nevertheless, levels of biomarkers should be interpreted with caution when a child is malnourished, as malnutrition may contribute to ID development, where biomarker concentrations are changed. Given the small number of patients analyzed, larger prospective studies are necessary for more definitive conclusions.

Data availability

The date can be accessed upon reasonable request by contacting igne.kairiene@santa.lt.

Received: 22 December 2024; Accepted: 15 April 2025
Published online: 15 May 2025

References

1. Botta, L. et al. Long-term survival and cure fraction estimates for childhood cancer in Europe (EUROCARE-6): results from a population-based study. *Lancet Oncol.* **23**, 1525–1536 (2022).
2. O'Connor, D. et al. Infection-related mortality in children with acute lymphoblastic leukemia: an analysis of infectious deaths on UKALL2003. *Blood* **124**, 1056–1061 (2014).
3. Lund, B. et al. Risk factors for treatment related mortality in childhood acute lymphoblastic leukaemia. *Pediatr. Blood Cancer*. **56**, 551–559 (2011).
4. Toft, N. et al. Results of NOPHO ALL2008 treatment for patients aged 1–45 years with acute lymphoblastic leukemia. *Leukemia* **32**, 606–615 (2018).
5. Brooks, B. R., Jorgenson, J. A., Newhouse, B. J., Shefner, J. M. & Agnese, W. Edaravone in the treatment of amyotrophic lateral sclerosis: efficacy and access to therapy: A roundtable discussion. *Am. J. Manag Care*. **24**, S175–S186 (2018).
6. Cennamo, F. et al. Update on febrile neutropenia in pediatric oncological patients undergoing chemotherapy. *Child. (Basel)*. **8**, 1086 (2021).
7. Tena-Garitaonaindia, M. et al. Modulation of intestinal barrier function by glucocorticoids: lessons from preclinical models. *Pharmacol. Res.* **177**, 106056 (2022).
8. Lima, M. B. D. et al. Gastrointestinal motility, mucosal mast cell, and intestinal histology in rats: Effect of prednisone. *BioMed Res Int* 4637621 (2017).
9. Rashidi, A. et al. Altered microbiota-host metabolic cross talk preceding neutropenic fever in patients with acute leukemia. *Blood Adv.* **5**, 3937–3950 (2021).
10. Su, L. et al. Dynamic changes in amino acid concentration profiles in patients with sepsis. *PLOS ONE*. **10**, e0121933 (2015).
11. Hisamatsu, T. et al. Decreased plasma histidine level predicts risk of relapse in patients with ulcerative colitis in remission. *PLOS ONE*. **10**, e0140716 (2015).
12. Chiba, T., Suzuki, K. & Matsumoto, T. Plasma-free amino acid profiles in Crohn's disease: relationship with the Crohn disease activity index. *Clin. Med. Insights Gastroenterol.* **11**, 1179552218791173 (2018).
13. Ikeda, H. Plasma amino acid levels in individuals with bacterial pneumonia and healthy controls. *Clin. Nutr. ESPEN*. **44**, 204–210 (2021).
14. Kelly, R. S. et al. Integrative omics to detect bacteremia in patients with febrile neutropenia. *PLOS ONE*. **13**, e0197049 (2018).
15. Sproston, N. R. & Ashworth, J. J. Role of C-reactive protein at sites of inflammation and infection. *Front. Immunol.* **9**, 754 (2018).
16. Whitehead, N. S. et al. Interventions to prevent iatrogenic anemia: A laboratory medicine best practices systematic review. *Crit. Care*. **23**, 278 (2019).
17. Zama, D. et al. Enteral nutrition protects children undergoing allogeneic hematopoietic stem cell transplantation from blood stream infections. *Nutr. J.* **19**, 29 (2020).
18. Rogers, P. C. & Barr, R. D. The relevance of nutrition to pediatric oncology: A cancer control perspective. *Pediatr. Blood Cancer*. **67**, e28213 (2020).
19. Joffe, L. & Ladas, E. J. Nutrition during childhood cancer treatment: current Understanding and a path for future research. *Lancet Child. Adolesc. Health*. **4**, 465–475 (2020).
20. Higienos Institutas. *Mokiniu_sveikata_2020.pdf (hi.lt)* (Accessed November, 2023).
21. Diakatou, V. & Vassilakou, T. Nutritional status of pediatric cancer patients at diagnosis and correlations with treatment, clinical outcome and the long-term growth and health of survivors. *Child. (Basel)*. **7**, 218 (2020).
22. Calcaterra, V. et al. Inflammation in obesity-related complications in children: the protective effect of diet and its potential role as a therapeutic agent. *Biomolecules* **10**, 1324 (2020).
23. Gustaitė, S. et al. Changes in nutritional status during induction phase and their association with fever and minimal residual disease in paediatric acute lymphoblastic leukaemia. *Med. (Kaunas)*. **59**, 1008 (2023).
24. Ong, C., Han, W. M., Wong, J. J. M. & Lee, J. H. Nutrition biomarkers and clinical outcomes in critically ill children: A critical appraisal of the literature. *Clin. Nutr.* **33**, 191–197 (2014).
25. Saitoh, W. et al. Plasma citrulline is a sensitive safety biomarker for small intestinal injury in rats. *Toxicol. Lett.* **295**, 416–423 (2018).
26. Common Terminology Criteria for Adverse Events (CTCAE) Version 5. US Department of Health and Human Services, National Institutes of Health, National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE) (cancer.gov) Published November 27, 2017. Accessed November, 2023.
27. James, G., Witten, D., Hastie, T. & Tibshirani, R. *An Introduction To Statistical Learning: with Applications in R* 219 (Springer, 2013).
28. Bruce, P., Bruce, A. & Gedeck, P. *Practical Statistics for Data Scientists: 50 + essential Concepts Using R and Python* (O'Reilly Media, 2017).
29. Song, Y. & Gyarmati, P. Bacterial translocation in acute lymphocytic leukemia. *PLOS ONE*. **14**, e0214526 (2019).
30. Pachauri, K., Vapaatalo, H. & Korpela, R. Even low-grade inflammation impacts on small intestinal function. *World J. Gastroenterol.* **16**(9), 1057–1062. https://doi.org/10.3748/wjg.v16.i9.1057 (2010). PMID: 20205274; PMCID: PMC2835780.
31. Wu, L. et al. Mutual interaction between gut microbiota and protein/amino acid metabolism for host mucosal immunity and health. *Anim. Nutr.* **7**, 11–16 (2021).
32. Munteanu, C. & Schwartz, B. The relationship between nutrition and the immune system. *Front. Nutr.* **9**, 1082500 (2022).
33. He, F. et al. Functions and signaling pathways of amino acids in intestinal inflammation. *BioMed Res Int.* 9171905 (2018).
34. De Pietri, S. et al. Gastrointestinal barrier integrity and mucosal inflammation as risk factors of blood stream infections in children treated for acute lymphoblastic leukaemia. *Int. J. Cancer*. **153**, 1635–1642 (2023).
35. van der Velden, W. J., Herbers, A. H., Netea, M. G. & Blijlevens, N. M. Mucosal barrier injury, fever and infection in neutropenic patients with cancer: introducing the paradigm febrile mucositis. *Br. J. Haematol.* **167**, 441–452 (2014).
36. Zhou, Y., Zhou, C. & Zhang, A. Gut microbiota in acute leukemia: current evidence and future directions. *Front. Microbiol.* **13**, 1045497 (2022).
37. Fragkos, K. C. & Forbes, A. Citrulline as a marker of intestinal function and absorption in clinical settings: A systematic review and meta-analysis. *United Eur. Gastroenterol. J.* **6**, 181–191 (2018).
38. Polidori, N., Grasso, E. A., Chiarelli, F. & Giannini, C. Amino acid-related metabolic signature in obese children and adolescents. *Nutrients* **14**, 1454 (2022).
39. Toaima, N. N., El-Owaidy, R. H., Zaki, D. L. & Eldin, L. B. Infections in children with simple obesity: the relation to phagocytic function and serum leptin. *J. Infect. Public. Health*. **12**, 57–61 (2019).
40. Bauer, J., Jürgens, H. & Frühwald, M. C. Important aspects of nutrition in children with cancer. *Adv. Nutr.* **2**, 67–77 (2011).
41. Dare, J. M., Moppett, J. P., Shield, J. P., Hunt, L. P. & Stevens, M. C. The impact of hyperglycemia on risk of infection and early death during induction therapy for acute lymphoblastic leukemia (ALL). *Pediatr. Blood Cancer*. **60**, E157–E159 (2013).
42. Short, K. R. et al. Effect of obesity and exercise training on plasma amino acids and amino metabolites in American Indian adolescents. *J. Clin. Endocrinol. Metab.* **104**, 3249–3261 (2019).

Acknowledgements

The authors would like to thank Enago (www.enago.com) for the English language review.

Author contributions

I.K. contributed to study design, data collection and interpretation, drafted the manuscript; G.T. and R.V. performed statistical analysis and data interpretation, T.P., A.K. and E.G. participated in data collection and interpretation, A.E. and J.S. contributed to project design and administration, J.R. contributed to study design, data interpretation and supervised the study.

Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

The study was conducted by the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of the Vilnius Regional Committee of Biomedical Research (approval no. 158200-18/12-1073-576). Informed consent was obtained from all subjects involved in the study.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-98947-4.

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