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Citrulline as a biomarker of bacteraemia during induction treatment for childhood acute lymphoblastic leukaemia.

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**Abbreviations key**

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<tr>
<th>Abbreviation</th>
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<tr>
<td>ALL</td>
<td>Acute Lymphoblastic Leukemia</td>
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<tr>
<td>NOPHO</td>
<td>Nordic Society Of Paediatric Haematology And Oncology</td>
</tr>
<tr>
<td>ANC</td>
<td>Absolute Neutrophil Count</td>
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<tr>
<td>OUH</td>
<td>Odense University Hospital</td>
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<tr>
<td>RH</td>
<td>Rigshospitalet</td>
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<tr>
<td>CRP</td>
<td>C-Reactive Protein</td>
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<tr>
<td>i.v.</td>
<td>Intravenous</td>
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<td>HR</td>
<td>High Risk</td>
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<td>CVC</td>
<td>Central Venous Catheter</td>
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<td>IQR</td>
<td>Interquartile Range</td>
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<tr>
<td>BSI</td>
<td>Blood Stream Infections</td>
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<tr>
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<td>Hematopoietic Stem Cell Transplantation</td>
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<td>CoNS</td>
<td>Coagulase-Negative Staphylococci</td>
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<td>OM</td>
<td>Oral Mucositis</td>
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<td>IM</td>
<td>Intestinal Mucositis</td>
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<tr>
<td>NCI-CTCAE</td>
<td>National Cancer Institute Common Terminology Criteria for Adverse Events</td>
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Abstract

**Background:** Systemic infections are a major cause of morbidity in children with acute lymphoblastic leukaemia (ALL). However, identification of patients at increased risk is still a challenge. Knowing that both neutropenia and gastrointestinal toxicity are risk factors for bacteraemia, we aimed at comparing neutrophil counts (ANC) and plasma citrulline levels (indicating enterocyte loss) in children with ALL with and without bacteraemia during induction treatment.

**Procedure:** We prospectively included 61 children with ALL treated according to the Nordic Society of Pediatric Hematology and Oncology (NOPHO) ALL2008 protocol. ANC and plasma CRP were measured on treatment days 1, 8, 15, 22 and 29. Plasma citrulline was measured on days 1, 8, 15, and 29. Bacteraemia episodes during induction treatment were recorded retrospectively.

**Results:** 19/61 (31%) patients experienced bacteraemia occurring on median day 13 (range 5–20). Patients with bacteraemia during induction treatment had lower citrulline level on day 15 (P<0.01) compared to patients without bacteraemia, indicating more severe enterocyte loss. Nevertheless, ANC was similar in the two patient groups on days 8 and 15. CRP was negatively correlated with same-day citrulline (P<0.03 for all) and ANC (P<0.04 for all).

**Conclusions:** During chemotherapy-induced neutropenia, plasma citrulline may help identify patients at increased risk of bacteraemia.
Introduction

Infections are a major cause of morbidity and mortality in children with acute lymphoblastic leukaemia (ALL). Although treatment-related mortality is reported as low as 2–4% in current ALL treatment protocols\(^1,2\), most treatment-related deaths are caused by systemic bacterial infections\(^1\). These infections occur mainly during induction treatment\(^3\), when patients experience profound neutropenia from intense chemotherapy and leukemic bone marrow infiltration.

Prompt empirical treatment with intravenous broad-spectrum antibiotics at the occurrence of fever is the standard of care for these patients to prevent rapid progression to septic complications\(^4,5\). Nevertheless, only around 50% of neutropenic fever episodes in children with cancer are reported as microbiologically or clinically documented infections\(^6,7\) and only 10–30% present with bacteraemia\(^7-10\). This implies an excessive use of antibiotics and prolonged hospital admissions in these patients, which favour the emergence of resistant pathogens\(^11\), nosocomial infections\(^12\) and drug-related side effects. Moreover, the extensive use of antibiotics induces imbalances in the gut microbial community, which may hamper intestinal functions and exacerbate the toxic effect of chemotherapy on the gut\(^13\). Therefore, identification of patients at high risk of systemic infections may help to risk-stratify antibiotic use and reduce overtreatment in these patients.

Mucositis of the gastrointestinal tract is a common side effect of chemotherapy that can affect both the oral and intestinal mucosa\(^14\). The severity of oral and/or intestinal mucositis has been positively correlated with the risk of infections in patients receiving chemotherapy\(^15,16\) due to translocation of bacteria across the damaged mucosal barrier\(^17\). In children with ALL, severe oral mucositis assessed by clinical scoring was associated with higher incidence of bacteraemia during induction\(^18\). Nevertheless, the ability of clinical mucositis scoring to objectively and precisely quantify mucosal damage is limited, especially in the case of...
intestinal mucositis. Previous studies suggested that intestinal mucositis severity can be reliably and non-invasively assessed by measuring plasma citrulline level\textsuperscript{19}. Citrulline is an amino acid produced almost exclusively by small-bowel enterocytes, with reduced circulating levels reflecting the loss of functional enterocytes\textsuperscript{20}. Based on the notion that neutropenia and mucositis are both risk factors for infections, we hypothesized that decreased citrulline levels would be associated with bacteraemia and increased inflammation during induction treatment for childhood ALL. Accordingly, our aim was to compare citrulline levels and neutrophil counts between patients with and without bacteraemia.

**Methods**

**Study population**

We included 61 children (1–18 years old) diagnosed with ALL who completed induction treatment according to the Nordic Society of Paediatric Haematology and Oncology (NOPHO) ALL2008 protocol between March 2013 and November 2016 at the H.C. Andersen Children’s Hospital, Odense University Hospital (OUH), Odense, and Rigshospitalet (RH), University Hospital of Copenhagen, Denmark. All patients participated in a randomized, placebo-controlled clinical trial investigating the effect of dietary supplementation with bovine colostrum against gastrointestinal toxicity\textsuperscript{18,21}. In these patients, colostrum was not found to influence citrulline levels or intestinal mucositis scores significantly.

The NOPHO ALL2008 induction treatment has been described in detail\textsuperscript{22}. Briefly, it lasts 29 days and consists of intravenous (i.v.) vincristine (2 mg/m\textsuperscript{2}) on days 1 (diagnosis), 8, 15, 22, and 29, i.v. doxorubicin (40 mg/m\textsuperscript{2}) on days 1 and 22, intrathecal methotrexate on days 1, 8,
15, and 29, and daily oral corticosteroids: prednisolone (60 mg/m²/day, days 1–29) in the non-high risk (non-HR) treatment group and dexamethasone (10 mg/m²/day, days 1–21) in the HR group. All patients received prophylactic trimethoprim-sulfamethoxazole twice weekly during induction treatment.

**Bacteraemia and systemic inflammation**

Blood cultures were routinely collected at the occurrence of fever (a single temperature measurement ≥ 38.5 °C or sustained temperature ≥ 38.0°C for more than one hour) from the central venous catheter (CVC) before initiation of i.v. broad-spectrum antibiotics. Data on positive blood cultures and i.v. antibiotics during induction treatment were retrospectively collected from the patients’ medical records. The occurrence of bacteraemia was defined as the presence of at least one blood culture positive for bacteria or fungi during induction treatment. Plasma C-reactive protein (CRP) was measured as part of the study protocol on treatment days 1, 8, 15, 22, and 29 (+/- 3 days from the scheduled time-point) by automated immuno-turbidimetric assay, with a median of 5 (range2-5) measurements per patient.

**Plasma citrulline, gastro-intestinal mucositis and neutrophil count**

Plasma citrulline level was measured as a marker of enterocyte loss on treatment days 1 (range -2-6), 8 (range 5–11), 15 (range 12–18), and 29 (range 26–33), with a median of 4 (range 1–4) measurements per patient. Plasma was separated from EDTA-anticoagulated blood samples by centrifugation at 1100xg for 10 minutes at +4°C and stored at -80°C within two hours from sample collection. Plasma citrulline analysis was performed using a Waters Acquity™ Ultra-Performance Liquid Chromatography system with an integrated photodiode array detector and the MassTrak™ Amino Acids Solution Kit (Waters Corporation, Milford, MA, USA) according to standard protocols, with the modifications described by Peake *et al*.
Inter-assay coefficient of variation was <10% for tested concentrations (range 4.5–350 µM). Oral mucositis (OM) and intestinal mucositis (IM) were scored by a study nurse on treatment days 1, 8, 15, 22, and 29. OM was scored using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) 4.0 criteria. IM was scored as Grade 0, I or II based on the severity of diarrhoea and abdominal pain according to the NCI-CTCAE 4.0 criteria, as described earlier. Absolute neutrophil count (ANC) was measured on treatment days 1, 8, 15, 22, and 29 (+/- 3 days from the scheduled time-point) by routine methods, with a median of 5 (range 3–5) measurements per patient. ANC values of 0.00×10^9/L were substituted with 0.001×10^9/L to allow for log-transformation. Neutropenia was defined as ANC<0.50×10^9/L.

**Statistical analyses**

Median and interquartile range (IQR) were used for summarizing non-normally distributed continuous variables. Comparisons of categorical variables between independent groups were performed by Chi-square or Fisher’s exact test, while comparisons of continuous variables were performed by Wilcoxon rank sum test. Changes over time in IM severity were investigated by generalized linear mixed model with cumulative log-link for ordinal outcomes with a random intercept by patient. Changes in citrulline levels, CRP levels after diagnosis, and ANC (all log10-transformed due to skewed distributions) over time were investigated by response profile analysis with unstructured variance-covariance matrix. Similarly, differences in these three outcomes between patients with and without bacteraemia during induction treatment were investigated by the same analysis but including the interaction between time and patient group. Differences in the outcomes between baseline and each following time-points and between the two groups at each time-point were evaluated by Wald tests. The proportions of patients with CRP>10 mg/L and ANC...
<0.5×10^9/L in patients with and without bacteraemia during induction treatment were compared by Fisher’s exact test at each time-point. The overall median citrulline level of all measurements taken from day 8 to day 29 was calculated for each patient. Spearman’s rank correlation test was used to investigate the correlation between age and overall median citrulline and between same-day measurements of post-diagnosis citrulline and CRP, and ANC and CRP assuming a monotonic relationship. Logistic regression analysis was used to investigate if an early drop in citrulline level (defined as any decrease in citrulline level from day 1 to day 8) was associated with increased risk of bacteraemia during the rest of induction treatment (first positive blood culture on days 9–29) or during the following week (first positive blood culture on days 9–15). Patients with bacteraemia before day 9 were excluded from this analysis. Two-sided P-values <0.05 were considered statistically significant.

Statistical analyses were performed using the statistical software R version 3.5.1.

**Ethics**

The study was approved by the Regional Scientific Ethics Committee for Southern Denmark (S-20120168) and the Danish Data Protection Agency (2008-58-0035). Written informed consent was collected from parents/legal guardians of all participating patients.

**Results**

We included 61 children newly diagnosed with ALL who completed induction treatment according to the NOPHO ALL2008 protocol. General characteristics of the patients as well as occurrence of OM and IM are presented in Table 1.
Bacteraemia and systemic inflammation

During induction treatment, 19/61 (31%) patients experienced bacteraemia with the first positive blood culture occurring on median day 13 (range 5–20) (Fig. 1). A total of 23 microorganisms were identified: 16 (69.6%) gram-positive bacteria, 6 (26.1%) gram-negative bacteria, and 1 (4.3%) fungus (Table 2). In four patients, two different microorganisms were isolated one, three, nine, and 11 days apart (Table 2). The occurrence of bacteraemia was not associated with age, sex, induction treatment regimen, ALL phenotype, treatment centre, administration of i.v. antibiotics at diagnosis, maximum OM or IM severity during days 8–29.

At diagnosis, 29/61 (47.5%) patients had CRP level above the upper normal limit (10 mg/L). Compared with day 8 (median CRP 1.0 mg/L, IQR 1.0–2.2), CRP levels increased reaching a peak on day 15 (median 7.5 mg/L, IQR 1.0–30.2, P<0.001), remained increased on day 22 (median 2.0 mg/L, IQR 1.0–10.0, P=0.04), and then recovered on day 29 (median 1.0 mg/L, IQR 1.0–1.6, P=0.80). Median CRP levels were higher in patients with bacteraemia during induction treatment compared to patients without bacteraemia during induction treatment; on day 8 (1 mg/L, IQR 1–9.3, vs. 1 mg/L, IQR 1–1.5, P=0.03), day 15 (39 mg/L, IQR 12–89, vs. 2 mg/L, IQR 1–18, P<0.001), and day 22 (11 mg/L, IQR 1.3–55.5, vs. 1.3 mg/L, IQR 1–6, P=0.004). Among patients experiencing bacteraemia, CRP was >10 mg/L in 3/19 (15.8%) on day 8, 15/19 (78.9%) on day 15, and 9/18 (50.0%) on day 22, while among patients not experiencing bacteraemia, CRP was >10 mg/L in 3/39 (7.7%) patients on day 8, 12/41 (29.2%) on day 15 and 4/35 (11.4%) on day 22. The proportions of patients with CRP>10 mg/L in patients with vs. without bacteraemia during induction treatment were compared at each time-point, with significant differences on day 15 (P<0.001) and day 22 (P=0.005).
Intestinal toxicity and neutropenia during induction

Median citrulline level was highest on day 1 (18.4 µM, IQR 15.0–24.3), decreased from day 1 to day 8 (13.6 µM, IQR 10.7–17.8, P<0.001) and day 15 (13.9 µM, IQR 8.6–18.7, P<0.001) indicating maximum intestinal toxicity, and then increased again on day 29 although remaining significantly reduced compared to day 1 (16.0 µM, IQR 12.6–20.5, P=0.01). Compared with day 1, IM severity increased on day 15 (P<0.001) and day 22 (P<0.001) and then decreased on day 29 (P=0.03) (Figure S1). Citrulline level did not differ between patients with IM (grade ≥1) and those without IM on day 8 and 15, while patients with IM had significantly lower citrulline levels compared to patients without IM on day 29 (13.1 µM, IQR 9.3–18.5, vs 17 µM, IQR 14.1–21.1, P=0.03). At diagnosis, median ANC was 0.40×10^9/L (IQR 0.15–0.97) and 35/61 (57%) patients were neutropenic. Compared to diagnosis, ANC decreased on day 8 (median 0.19×10^9/L, IQR 0.05–0.50, P<0.001), reached a nadir on day 15 (0.01×10^9/L, IQR 0.00–0.10, P<0.001), when all patients were neutropenic, then increased on day 22 (0.40×10^9/L, IQR 0.05–1.56, P=0.04) and recovered on day 29 (0.73×10^9/L, IQR 0.18–1.77, P=0.60).

Citrulline and neutropenia in relation to bacteraemia

We observed significantly lower citrulline levels on day 15 for patients with bacteraemia during induction treatment compared to patients without bacteraemia (P<0.01, Fig. 2). From diagnosis to day 15, 38/61 (62.3%) patients had decreasing citrulline levels, 18/61 (29.5%) had constant or rising citrulline levels, and 5/61 (8.2%) had missing citrulline measurements on either day. Of the 38 patients with a drop in citrulline level, 15 (39.5%) experienced bacteraemia at some time during induction treatment, while only 1/18 (5.6%) patient with constant/rising citrulline level experienced bacteraemia (P=0.01) (Table 2). To address whether a drop in citrulline level could predict subsequent infections we excluded the three
patients who had bacteraemia before day 9. Patients with a drop in citrulline level between day 1 and day 8 did not present an increased risk of bacteraemia during the following week (P=0.26) or the rest of induction treatment (P=0.66).

ANC was significantly reduced on day 1 and day 22 in patients with bacteraemia during induction treatment compared to those without (P<0.05, Fig. 3). Hence, during the period when most episodes of bacteraemia were observed (days 8–15) we found no significant difference in ANC between patients with and without bacteraemia during induction treatment. Neutropenia at diagnosis was more frequent in patients with bacteraemia during induction treatment compared to patients without (16/19, 84.2% vs. 19/42, 45.2%, P=0.005), while no difference in occurrence of neutropenia was found at the following time-points.

Reduced citrulline levels were significantly correlated with same-day increased CRP levels indicating an association between the severity of the intestinal damage and the degree of systemic inflammation (P<0.03 for all, Fig. 4). When excluding patients with bacteraemia during induction treatment, the correlation remained significant on day 15 (Spearman’s r=-0.38, P=0.01). Similarly, same-day correlations between ANC and CRP levels showed a significant association between reduced ANC and increased CRP (day 8 r=-0.27, P=0.04; day 15 r= -0.31, P=0.02; day 22 r= -0.63, P<0.001; day 29 r= -0.36, P=0.01). When excluding patients with bacteraemia during induction treatment, the correlation remained significant on day 15 (r=-0.37, P=0.02) and day 29 (r= -0.46, P=0.004). Overall median citrulline during induction treatment was not associated with treatment regimen, sex, age, or ALL phenotype.

**Discussion**

In this study, one third of patients experienced bacteraemia after a median of two weeks from start of chemotherapy, coinciding with the time point of maximum neutropenia and enterocyte loss. Nevertheless, at this time-point, patients experiencing bacteraemia had
significantly lower citrulline levels compared to patients without bacteraemia, despite similar neutropenia severity.

The incidence of bacteraemia around 30% in this study corresponds to the incidence of bloodstream infections (BSI) previously reported in Denmark during NOPHO ALL-2008 or earlier NOPHO induction protocols with similar trimethoprim-sulfamethoxazole prophylaxis. The incidence of bacteraemia or BSI during ALL induction treatment has been reported between 10–45% in other studies from high-income countries. Nevertheless, comparisons are limited by differences in chemotherapy protocols, antimicrobial prophylaxis regimens and definitions of reported outcomes. In our study, bacteraemia occurred after a median of two weeks from start of chemotherapy, as reported by other studies in ALL induction treatment, coinciding with the time-point of maximum enterocyte loss and neutropenia. Nevertheless, plasma citrulline level on day 15 was significantly lower in patients with bacteraemia compared to patients without, while ANC did not differ significantly between the two groups. Moreover, nearly all bacteraemia episodes occurred among patients experiencing a drop in citrulline level during the first two weeks of treatment. These data suggest that, in the context of severe immunosuppression, intestinal barrier injury with bacterial translocation may be a crucial event for the development of BSI. Accordingly, studies in patients undergoing hematopoietic stem cell transplantation (HSCT) have shown a temporal association between bacteraemia and nadir of neutrophil count and citrulline level, with patients developing bacteraemia being characterized by lower citrulline levels rather than more severe or prolonged neutropenia. Outside the haematological setting, patients treated for solid tumours with clinical signs of intestinal mucositis presented increased risk of infections despite similar depth and duration of neutropenia compared to patients without infections. The severity and duration of neutropenia were identified as risk factors for infection in patients with acute leukaemia over...
50 years ago by Bodey et al. Since fever is often the only sign of infection in these patients, empirical treatment with broad-spectrum antibiotics at the occurrence of neutropenic fever has become the standard of care to reduce septic mortality. Nevertheless, only around half of neutropenic fevers in children with cancer can be attributed to an underlying infection. Given the current challenge of increasing antimicrobial resistance and to limit drug related side effects, it is important to better identify patients at increased risk of infections to target antibiotic administration. Our data suggest that citrulline may be a useful marker for this purpose in children with ALL, although a prospective study with sampling at the onset of febrile neutropenia is needed to test this hypothesis. Accordingly, Herbers et al showed that citrulline level measured on the first day of febrile neutropenia was lower in patients with bacteraemia compared to patients without bacteraemia in HSCT receivers. Moreover, a recent study using non-targeted metabolomic analyses has identified citrulline as a marker of bacteraemia or sepsis in haematological patients with febrile neutropenia.

At the timepoint of maximum intestinal toxicity, IM severity was not associated with citrulline level. This may reflect inter-individual variations in IM clinical presentation, as well as limitations of the scoring system. In fact, assessing abdominal pain and diarrhoea in these children may be limited by their ability to communicate and by the large use of laxatives during induction treatment. These limitations underline the need for a biological marker of intestinal toxicity in the paediatric population.

However, we were not able to predict the occurrence of bacteraemia from an early drop in citrulline level between day 1 and day 8. Other innate immunity markers associated with intestinal barrier injury, such as chemokines or the complement cascade, may present earlier changes in their circulating levels and therefore be more useful for early prediction of BSI. The majority of microorganisms isolated in blood cultures were gram-positive bacteria, which is in line with previous studies in children with ALL receiving induction.
treatment and in paediatric oncological patients in general. A third of positive isolates were represented by coagulase-negative staphylococci (CoNS). CoNS bacteraemia is usually assumed to derive from CVC contamination with CoNS colonizing the skin. Nevertheless, there is evidence suggesting that CoNS also reside in the gastrointestinal tract and can translocate into the bloodstream causing bacteraemia. In patients undergoing HSCT, CoNS and oral viridans streptococci bacteraemia have been reported to coincide with increased gut permeability and to occur more frequently following conditioning regimens with high intestinal toxicity. Using molecular relatedness analyses, Costa et al. found that the nasal and alimentary mucosa, rather than the skin at the CVC insertion site, were the most likely sources of CoNS BSI in patients with cancer. Therefore, some of the CoNS bacteraemia in our study may have been caused by translocation of these bacteria through the damaged oral or intestinal mucosa. However, a single positive blood culture was used to define bacteraemia and no peripheral blood cultures were routinely taken. Therefore, we cannot exclude that some of the gram-positive isolates may have derived from blood culture contamination.

As expected, patients with bacteraemia had higher level of systemic inflammation. Nevertheless, a mild plasma CRP elevation during chemotherapy mirroring enterocyte loss and a significant correlation between reduced citrulline and increased CRP levels were present also among patients without bacteraemia. This is in line with the knowledge that systemic inflammation during chemotherapy is not only induced by infection but may also result from gastrointestinal toxicity. In fact, chemotherapy-induced epithelial damage activates a first inflammatory response driven by epithelial cells and tissue monocytes and macrophages. Subsequently, inflammation-induced tissue damage leads to increased permeability and ulceration of the intestinal mucosa, with translocation of bacteria and pathogen-associated molecular patterns, which trigger a second systemic inflammatory
response possibly resulting in fever\(^{43-46}\). To emphasize this close link between mucositis and fever, the term “febrile mucositis” was introduced by Walter, et al\(^{45}\) as a complementary paradigm to febrile neutropenia. Recognizing mucositis as a cause of fever and infections is crucial to direct new preventive strategies.

This study was limited by the sample size, which may have prevented us from identifying a significant difference in neutrophil counts at the time of maximum intestinal toxicity between patients with and without bacteraemia. Moreover, blood cultures were only taken from the CVC, limiting our possibility to discriminate primary BSI from central line-associated BSI. Finally, blood sampling at the onset of febrile neutropenia would have allowed us to investigate if citrulline level could predict systemic infection within each episode.

In conclusion, our data suggest that, in a context of chemotherapy-induced neutropenia, plasma citrulline may constitute an additional marker to identify patients at increased risk of systemic infections and potentially guide antibiotic treatment strategies. Accordingly, future prospective studies in children with ALL should investigate if systemic infections can be predicted by plasma citrulline level, innate immune markers and clinical characteristics at the onset of febrile neutropenia.

**Data availability statement**

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

**Conflicts of interest statement**

The authors declare no conflict of interest.

**Acknowledgments**

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Figure legends:

Figure 1. Timing of first positive blood culture in the 19 patients experiencing bacteraemia during induction treatment. Median day 13 (range 5–20).
Figure 2. Citrulline level in patients with and without bacteraemia during induction treatment. Asterisks above the squared brackets indicate statistically significant differences between the two groups at each time-point. Asterisks above each box indicate statistically significant differences between baseline level and each of the following time-points within each group. **P<0.01, ***P<0.001. Horizontal lines inside the boxes represent the median; the upper and lower limits of the boxes represent the 75th and 25th percentiles, respectively. The Y-axis is not log-transformed for readability.
Figure 3. Absolute neutrophil count (ANC) in patients with and without bacteraemia during induction treatment. Asterisks above the squared brackets indicate statistically significant differences between the two groups at each time-point. Asterisks above each box indicate statistically significant differences between baseline level and each of the following time-points within each group. *P<0.05, **P<0.01, ***P<0.001. The dashed horizontal line represents the ANC value below which neutropenia was defined. Horizontal lines inside the boxes represent the median; the upper and lower limits of the boxes represent the 75th and 25th percentiles, respectively.
Figure 4. Correlations between same-day plasma citrulline and CRP levels. Spearman’s rank correlation coefficients $r$ and $P$-values are showed in the legend.
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<td>Male</td>
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<tr>
<td>Final risk group day 79, N (%)</td>
<td></td>
</tr>
<tr>
<td>SR</td>
<td>20 (32.8)</td>
</tr>
<tr>
<td>IR</td>
<td>32 (52.5)</td>
</tr>
<tr>
<td>Treatment center, N (%)</td>
<td></td>
</tr>
<tr>
<td>Odense</td>
<td>22 (36.1)</td>
</tr>
<tr>
<td>Copenhagen</td>
<td>39 (63.9)</td>
</tr>
<tr>
<td>Maximum OM severity on days 8-29, N (%)&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>25 (42.4)</td>
</tr>
<tr>
<td>Mild</td>
<td>19 (32.2)</td>
</tr>
<tr>
<td>Moderate</td>
<td>7 (11.9)</td>
</tr>
<tr>
<td>Severe</td>
<td>8 (13.6)</td>
</tr>
<tr>
<td>Maximum IM severity on days 8-29, N (%)&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Grade 0</td>
<td>31 (52.5)</td>
</tr>
<tr>
<td>Grade I</td>
<td>21 (35.6)</td>
</tr>
<tr>
<td>Grade II</td>
<td>7 (11.9)</td>
</tr>
</tbody>
</table>

IQR, interquartile range; SR, standard risk; IR, intermediate risk; HR, high risk; OM, oral mucositis; IM, intestinal mucositis

<sup>a</sup>Changed from high risk to non-high risk induction due to t(12;21)

<sup>b</sup>Patients with Ph+ ALL, who received non-HR induction treatment with daily Imatinib from day 15

<sup>c</sup>Died after induction treatment before final risk-group assignment

<sup>d</sup>Out of 59 patients having at least one valid scoring on days 8-29
TABLE 2 Bacterial and fungal agents isolated in patients with bacteremia and drop in citrulline level between day 1 and 15

<table>
<thead>
<tr>
<th>Microorganisms N (%)</th>
<th>Citrulline drop day 1–15 N=18</th>
<th>No citrulline drop day 1–15 N=1</th>
<th>Missing citrulline data N=4</th>
<th>Total N=23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>13 (72)</td>
<td>0 (0)</td>
<td>3 (75)</td>
<td>16 (70)</td>
</tr>
<tr>
<td>Coagulase-negative <em>Staphylococci</em></td>
<td>6 (33)</td>
<td>0 (0)</td>
<td>1 (25)</td>
<td>7 (30)</td>
</tr>
<tr>
<td><em>Staphylococcus warneri</em></td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (4)</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>3 (17)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (13)</td>
</tr>
<tr>
<td>Unclassified coagulase-negative <em>Staphylococci</em></td>
<td>2 (11)</td>
<td>0 (0)</td>
<td>1 (25)</td>
<td>3 (13)</td>
</tr>
<tr>
<td>Viridans group <em>Streptococci</em></td>
<td>3 (17)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (13)</td>
</tr>
<tr>
<td><em>Streptococcus oralis</em></td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (4)</td>
</tr>
<tr>
<td><em>Streptococcus mitis group</em></td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Non-haemolytic <em>Streptococcus salivarius group</em></td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Unclassified non-haemolytic <em>Streptococcus</em></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (25)</td>
<td>1 (4)</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (4)</td>
</tr>
<tr>
<td><em>Propionibacterium acnes</em></td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (4)</td>
</tr>
<tr>
<td><em>Micrococcus luteus</em></td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Gram-negative</td>
<td>4 (22)</td>
<td>1 (100)</td>
<td>1 (25)</td>
<td>6 (26)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>1 (25)</td>
<td>2 (9)</td>
</tr>
<tr>
<td><em>Capnocytophaga sputigena</em></td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (4)</td>
</tr>
<tr>
<td><em>Neisseria lactamica</em></td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (4)</td>
</tr>
<tr>
<td><em>Corynebacterium species</em></td>
<td>0 (0)</td>
<td>1 (100)</td>
<td>0 (0)</td>
<td>1 (4)</td>
</tr>
<tr>
<td><em>Stenotrophomonas maltophilia</em></td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Fungi</td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>

Bold numbers indicate the number of positive isolates (%) consisting of gram-positive bacteria, gram-negative bacteria and fungi.

*Isolated one day apart in the same patient

*Isolated 11 days apart in the same patient

*Isolated three days apart in the same patient

*Isolated nine days apart in the same patient.