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ACUTE LYMPHOBLASTIC LEUKEMIA

Markers of intestinal mucositis to predict blood stream infections at the onset of fever during treatment for childhood acute leukemia

Sarah Weischendorff^{1,2}✉, Mathias Rathe^{3,4}, Malene Johanne Petersen¹, Allan Weimann¹, Christian Enevold², Claus H. Nielsen², Bodil Als-Nielsen¹, Ulrikka Nygaard^{1,5}, Claus Moser^{6,7} and Klaus Müller^{1,2,5}

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Despite chemotherapy-induced intestinal mucositis being a main risk factor for blood stream infections (BSIs), no studies have investigated mucositis severity to predict BSI at fever onset during acute leukemia treatment. This study prospectively evaluated intestinal mucositis severity in 85 children with acute leukemia, representing 242 febrile episodes (122 with concurrent neutropenia) by measuring plasma levels of citrulline (reflecting enterocyte loss), regenerating islet-derived-protein 3α (REG3α, an intestinal antimicrobial peptide) and CCL20 (a mucosal immune regulatory chemokine) along with the general neutrophil chemo-attractants CXCL1 and CXCL8 at fever onset. BSI was documented in 14% of all febrile episodes and in 20% of the neutropenic febrile episodes. In age-, sex-, diagnosis- and neutrophil count-adjusted analyses, decreasing citrulline levels and increasing REG3α and CCL20 levels were independently associated with increased odds of BSI (OR = 1.6, 1.5 and 1.7 per halving/doubling, all $p < 0.05$). Additionally, higher CXCL1 and CXCL8 levels increased the odds of BSI (OR = 1.8 and 1.7 per doubling, all $p < 0.0001$). All three chemokines showed improved diagnostic accuracy compared to C-reactive protein and procalcitonin. These findings underline the importance of disrupted intestinal integrity as a main risk factor for BSI and suggest that objective markers for monitoring mucositis severity may help predicting BSI at fever onset.

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INTRODUCTION

Overall survival of childhood acute leukemia has increased remarkably during the last decades [1–3], but the treatment remains challenged by severe acute toxicities [4]. Chemotherapy-induced neutropenia renders the patients highly susceptible to blood stream infections (BSI) and severe septic progression, accounting for most of the treatment-related mortality [5–7]. Accordingly, broad-spectrum antibiotics are routinely initiated at the onset of fever in patients with neutropenia, even though only 10–30% of these episodes are caused by a verifiable bacterial infection [8–12].

The identification of infections is particularly challenging in patients undergoing high-intensity, myelosuppressive chemotherapy in combination with high-dose glucocorticoids, as this may blur clinical signs of infection. Furthermore, the established biochemical markers of infections, including C-reactive protein (CRP) and procalcitonin (PCT), may also be elevated during non-bacterial infectious toxicities such as drug reactions, inflammatory toxicities, viral infections, and tumor lysis [8–10]. Therefore, more

accurate diagnostic tools and better risk management are warranted to improve early diagnosis and to limit the excessive use of antibiotics and the associated adverse effects including multidrug resistant bacteria, nosocomial infections, prolonged hospitalization and microbial dysbiosis [13, 14].

Gastrointestinal mucositis constitutes a main risk factor for BSI due to translocation of bacteria through the disrupted epithelial barrier as supported by studies in patients undergoing hematopoietic stem cell transplantation [15]. Additionally, studies in children with acute lymphoblastic leukemia (ALL) have revealed a close relation between the severity and timing of intestinal mucositis and the occurrence of BSI [16, 17]. However, studies in this patient population are sparse and to date, no studies have evaluated mucositis severity at the onset of fever.

Monitoring intestinal mucositis is challenging due to the lack of validated and robust objective measures of mucositis severity, but recently new, easily measurable markers have been introduced. These include plasma citrulline, a marker of functional enterocytes [18–20], and chemokine (C-C motif) ligand 20 (CCL20),

¹Department of Pediatrics and Adolescent Medicine, Rigshospitalet, Copenhagen, Denmark. ²Institute for Inflammation Research, Center for Rheumatology and Spine Disease, Rigshospitalet, Copenhagen, Denmark. ³Hans Christian Andersen Children's Hospital, Odense University Hospital, Odense, Denmark. ⁴Department of Clinical Research, University of Southern Denmark, Odense, Denmark. ⁵Institute of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark. ⁶Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark. ⁷Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark. ✉email: sarah.wegener.weischendorff@regionh.dk

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which is a chemokine responsible for mucosal immune regulation, being highly upregulated during mucosal inflammation at various sites to increase the recruitment of lymphocytes [21–23]. Additionally, regenerating islet-derived protein 3 α (REG3 α), an antimicrobial peptide, can be measured at high levels systemically during gastrointestinal toxicity including inflammatory bowel disease and gastrointestinal acute graft-versus-host disease [24–26]. Nevertheless, to date, no studies have evaluated these markers as a diagnostic approach at the onset of fever to predict BSI in patients undergoing chemotherapy.

The aim of this study was to determine intestinal mucositis severity during febrile episodes based on the hypothesis that more severe intestinal mucositis constituted an increased risk of BSI and that objective markers of mucositis could serve as a diagnostic approach, regardless of the presence of neutropenia.

We investigated quantitative markers of mucositis including citrulline, CCL20 and REG3 α in children with acute leukemia at the onset of fever. Additionally, the study examined markers of neutrophil chemotaxis (chemokine (C-X-C motif) ligand 1 and 8; CXCL1 and CXCL8, respectively), which have previously been independently associated with both mucositis and BSI [27–29].

METHODS

Study population

A total of 85 children (1–18 years) representing 242 febrile episodes during treatment for either acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML) at the Department of Pediatrics, Rigshospitalet, Copenhagen, Denmark were enrolled between June 2019 and December 2021. Neutropenia (defined as an absolute neutrophil count (ANC) $<0.5 \times 10^9$ cells/l or ANC $<1.0 \times 10^9$ cells/l with decreasing levels to $<0.5 \times 10^9$ cells/l within 2 days) was observed in 122 of the 242 episodes. Fever was defined as a single temperature measurement $\geq 38.5^\circ\text{C}$ or sustained temperature measurements $\geq 38.0^\circ\text{C}$ for more than 1 h.

Patient characteristics are listed in Table 1. All patients received trimethoprim-sulfamethoxazole twice weekly as prophylaxis for *Pneumocystis jirovecii* infection, while patients with AML or biphenotypical leukemia also typically received voriconazole or equal alternatives as standard antifungal prophylaxis.

Blood stream infections

Blood for cultures were collected during all febrile episodes from the patients' central venous catheter (CVC). For patients with a bodyweight <20 kg, 10 ml of blood for one aerobic tube was drawn, and for patients >20 kg, both an aerobic (drawn first) and an anaerobic tube with 10 ml of blood were collected. Microbial identification was performed at the discretion of the regional diagnostic medical microbiological laboratory, and information regarding BSI was collected from the patients' medical records. The occurrence of BSI was defined as the isolation of one or more bacterial or fungal pathogens from the blood culture. However, two positive blood cultures taken either simultaneously or within 72 h with identical species were required for common skin contaminants including coagulase-negative staphylococci (CoNS), *Cutibacterium acnes* (previously *Propionibacterium acnes*), *Micrococcus* species, and *Corynebacterium* species (except *Corynebacterium jeikeium* and *Corynebacterium diphtheria*). BSI with two or more pathogens isolated in the same blood culture or in different blood cultures taken simultaneously were considered polymicrobial.

Laboratory analyses

EDTA anti-coagulated blood was collected at each episode immediately upon the onset of fever. Plasma was isolated by centrifugation at $2000 \times g$ for 10 min and stored at -80°C within 2 h after sample collection.

Plasma citrulline concentrations were determined by liquid chromatography-tandem mass spectrometry using an ExionLC AD Ultra-High-Performance Liquid Chromatography system coupled to a Sciex QTRAP 6500+ mass spectrometer (AB Sciex, Framingham, MA, USA) with L-citrulline (4,4,5,5-D4) (Cambridge Isotope Laboratories, MA, USA) as the internal standard. Further details can be found in Supplementary Methods.

Plasma levels of CXCL8, CXCL1 and CCL20 were measured using the Bio-Plex Pro Human Chemokine Assay (Bio-Rad, Hercules, CA, USA) with a

Table 1. Patient characteristics.

Characteristic	All patients	Patients with neutropenia
Number of patients, no. (%)	85 (100)	63 (100)
Number of males, no. (%)	54 (64)	39 (62)
Age at diagnosis, years (range)	4.0 (1.0–16.5)	4.2 (1.0–16.5)
Diagnosis, no. (%)		
Pre-B ALL	61 (72)	40 (63)
Ph+ ALL	5 (6)	5 (8)
T-ALL	7 (8)	6 (10)
AML	9 (11)	9 (14)
Biphenotypical acute leukemia	3 (3)	3 (5)
Time from diagnosis/relapse, days (range)	87 (6–870)	78 (6–770)
Treatment protocol, no. (%)		
NOPHO ALL 2008	18 (21)	8 (13)
ALLTogether1	44 (52)	35 (55)
NOPHO-DBH AML 2012	8 (9)	8 (13)
EsPhALL	5 (6)	4 (6)
Other ^a	10 (12)	8 (13)

ALL acute lymphoblastic leukemia, AML acute myeloid leukemia, EsPhALL Philadelphia-chromosome-positive acute lymphoblastic leukemia, NOPHO Nordic Society for Pediatric Hematology and Oncology, DBH Belgian Society of Pediatric Hematology Oncology, The Dutch Childhood Oncology Group, Estonia, and Hong Kong.

^aIncluding relapse protocols and iBFM AMBI2018.

plasma dilution factor of 1:2 on the Luminex platform (Luminex Corporation, Austin, TX, USA) according to the manufacturer's instructions.

Plasma levels of REG3 α (only analyzed in the 122 samples with neutropenia) were measured by enzyme-linked immunosorbent assay (ELISA) using the Ab-Match Assembly Human PAP1 kit with the Ab-Match Universal kit (MBL International, Woburn, MA, USA), according to the manufacturer's protocol with a plasma dilution of 1:10.

Plasma CRP, PCT and ANC were measured as part of the clinical routine for all patients. The reported values correspond to the initial measurement taken upon the recognition of fever. CRP was analyzed by an automated immunoturbidimetric assay using the cobas c 702 module (Roche, Rotkreuz, Switzerland), PCT by an electrochemiluminescence immunoassay using the Elecsys BRAHMS PCT assay (Roche Diagnostics Corporation, IN, USA), and ANC by flow cytometry (Sysmex XN, Norderstedt, Germany).

Statistics

To account for the intraindividual correlation among potentially repeating fever episodes for each patient, a mixed model repeated measures analysis with a compound symmetry covariance structure was used to compare levels of citrulline, CCL20, CXCL1, CXCL8 and REG3 α between groups. All variables were log₂ transformed due to skewed distributions. In addition, a generalized estimating equation with a compound symmetry covariance structure for within-subject dependencies was used for dichotomized outcomes. Fisher's exact probability test was used for comparing dichotomized outcomes between groups.

Determination of the sensitivity and specificity for each marker was derived from the receiver operation characteristic (ROC) curve, ignoring the correlation of repeated measurements. The cut-off value was determined by the minimum distance from the left-upper corner of the unit square (sensitivity and specificity equal to 1.0).

Continuous variables are summarized as median (range) unless otherwise stated, and a two-sided *p* value <0.05 was considered statistically significant. All analyses were performed using the statistical software SAS version 9.4 (SAS Institute, Cary, NC, USA).

RESULTS

Febrile episodes and blood stream infections

The median number of febrile episodes per patient was 2 (1–16), and a total number of 59 patients (69%) had two or more episodes. The number of febrile episodes was not associated with either sex, age, or diagnosis.

In 35 of the 242 febrile episodes (14%), BSI was documented by a positive blood culture. In the subgroup of patients with accompanying neutropenia (122 febrile episodes), a BSI was confirmed in 24 of the episodes (20%). Accordingly, only 11 BSI episodes were confirmed in the 120 non-neutropenic febrile episodes (9%). Six patients were represented by two separate BSI episodes. The presence of neutropenia was associated with increased the risk of BSI (OR = 2.35, 95% CI: 1.14–4.82, $p = 0.020$).

Twenty-two different pathogens accounting for a total of 50 positive isolates were identified (Table 2). Eleven BSI episodes were polymicrobial.

The occurrence of BSI was not associated with sex or age, but patients with AML or biphenotypical acute leukemia tended to have a higher prevalence of BSI as compared to patients with ALL during the study period (6/12 (50%) vs. 17/73 (23%), $p = 0.078$).

Table 2. Bacterial and fungal pathogens isolated in patients with bacteremia.

	All patients	Patients with neutropenia
Positive isolates, no (%)	50 (100)	25 (100)
Gram-positive	18 (34)	9 (36)
<i>Staphylococcus aureus</i>	4 (8)	2 (8)
Coagulase-negative Staphylococci	4 (8)	2 (8)
<i>Staphylococcus epidermidis</i>	4 (8)	2 (8)
Viridans group Streptococci	4 (8)	1 (4)
<i>Streptococcus mitis</i> group	3 (6)	0 (0)
<i>Streptococcus salivarius</i> group	1 (2)	1 (4)
<i>Enterococcus faecalis</i>	1 (2)	0 (0)
<i>Enterococcus faecium</i>	2 (4)	2 (8)
<i>Enterococcus casseliflavus</i>	1 (2)	0 (0)
<i>Bacillus cereus</i>	2 (4)	2 (8)
Gram-negative	32 (60)	16 (64)
Unclassified Gram-negative bacilli	1 (2)	0 (0)
<i>Acinetobacter pittii</i>	1 (2)	0 (0)
<i>Elizabethkingia meningoseptica</i>	1 (2)	0 (0)
<i>Pseudomonas species</i>	2 (4)	0 (0)
<i>Pseudomonas aeruginosa</i>	5 (9)	4 (16)
<i>Pseudomonas putida</i>	1 (2)	0 (0)
<i>Pseudomonas stutzeri</i>	2(4)	1 (4)
<i>Moraxella osloensis</i>	1 (2)	1 (4)
<i>Neisseria subflava</i>	1 (2)	0 (0)
<i>Stenotrophomonas maltophilia</i>	4 (8)	1 (4)
<i>Enterobacter cloacae</i>	2 (4)	1 (4)
<i>Escherichia coli</i>	3 (6)	3 (12)
<i>Citrobacter Braakii</i>	1 (2)	1 (4)
<i>Klebsiella pneumonia</i>	7 (13)	5 (20)

Markers of intestinal mucositis in relation to BSI occurrence

Febrile patients with BSI had significantly lower levels of citrulline (15.7 $\mu\text{mol/l}$ (1.3–30.4) vs. 19.4 $\mu\text{mol/l}$ (1.1–61.0), $p = 0.0094$) and increased levels of CCL20 (70.1 pg/ml (1.3–1797.4) vs. 15.6 pg/ml (1.1–1073.1), $p < 0.0001$) compared to febrile patients without BSI.

When investigating neutropenic febrile episodes alone, these associations were even more pronounced for citrulline and CCL20. In addition, REG3 α was significantly elevated in patients with BSI (Fig. 1).

The risk of BSI increased with decreasing citrulline levels and increasing levels of CCL20 and REG3 α . In multivariate analyses adjusted for sex, age, neutropenia, and diagnosis these associations remained significant (Table 3).

Markers of neutrophil chemotaxis

Both CXCL8 and CXCL1 were significantly increased in patients with BSI compared to those without BSI (87.5 pg/ml (2.1–7640) vs. 18.2 pg/ml (1.5–2117.1), $p < 0.0001$ and 216.2 pg/ml (4.2–12,114.2) vs. 24.2 pg/ml (4.2–1726.3), $p < 0.0001$, respectively). Similarly, elevated CXCL8 and CXCL1 levels were also demonstrated in patients with BSI when only investigating neutropenic febrile episodes as well (Fig. 1).

High levels of CXCL8 and CXCL1 were associated with increased risk of BSI both in univariate and multivariate analyses adjusted for sex, age, neutropenia, and diagnosis (Table 3).

C-reactive protein and procalcitonin

At the onset of fever, CRP levels were slightly increased in BSI episodes compared to non-BSI-related febrile episodes (22.0 mg/l (1.0–295) vs. 14 mg/l (1.0–305), $p = 0.045$), but this was insignificant for the subgroup with concurrent neutropenia (Fig. 1).

PCT was higher in BSI-related febrile episodes than non-BSI febrile episodes (0.50 $\mu\text{g/l}$ (0.02–32.5) vs. 0.29 $\mu\text{g/l}$ (0.06–27.8), $p < 0.0001$), and this also applied when investigating neutropenic febrile episodes alone (Fig. 1).

Elevated levels of both CRP and PCT were associated with an increased risk of BSI in univariate analyses, but only PCT remained significant in multivariate analyses adjusting for sex, age, neutropenia, and diagnosis (Table 3).

Evaluation of the diagnostic accuracy for predicting blood stream infections

ROC curves were established for each univariate model (Fig. 2), estimating the sensitivity and specificity for each parameter at the optimal cut-off value for maximized diagnostic accuracy (Table 4). Furthermore, Supplementary Table 1 details cut-off values for each parameter requiring a reasonable sensitivity of ≥ 0.75 .

When combining the cut-off value for each parameter with the cut-off value for PCT and CRP in the group of patients with febrile neutropenia, a significant improvement in sensitivity was achieved (0.91 for citrulline, CCL20 and CXCL8 and 0.96 for REG3 α and CXCL1 with specificities of 0.27 for citrulline and CXCL8, 0.31 for REG3 α and 0.29 for CCL20 and CXCL1, respectively). Including all patients, the combinations reached a sensitivity of 0.85 for CCL20, CXCL1 and CXCL8 (specificities of 0.38, 0.36 and 0.39, respectively) and 0.88 for citrulline (specificity of 0.26).

DISCUSSION

Improved strategies are needed to promote a more targeted use of antimicrobial therapy without compromising safety in children undergoing chemotherapy. This likely requires a comprehensive, individual approach with effective risk stratification and better diagnostic tools to assist decisions concerning the initiation and duration of antibiotic treatment.

In the present study, we investigated if markers of intestinal mucositis and neutrophil chemotaxis could help differentiate

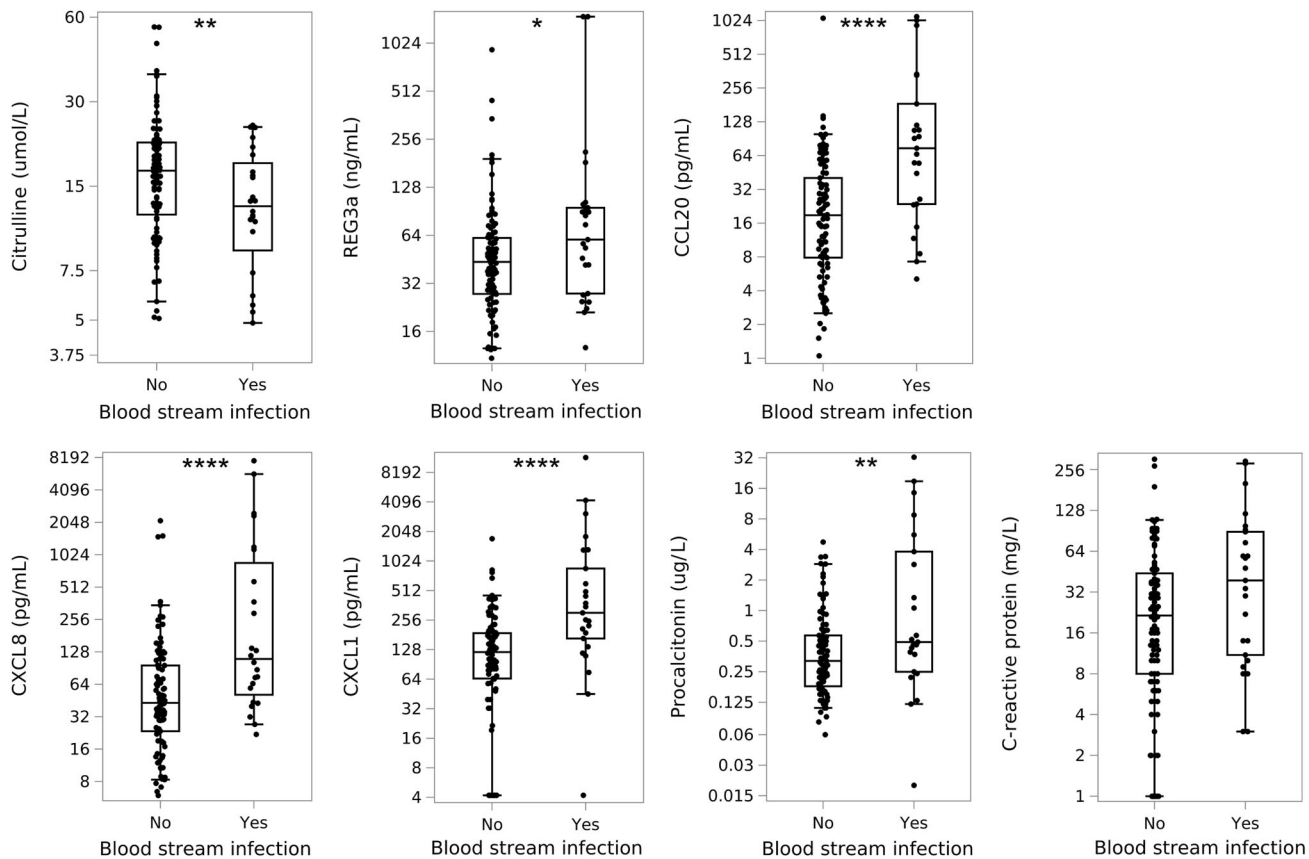


Fig. 1 Plasma levels of citrulline, REG3 α , CCL20, CXCL8, CXCL1, procalcitonin and C-reactive protein during febrile neutropenia in patients with and without blood stream infections. Boxes show the median level with 25th and 75th percentiles; whiskers represent the 5th and 95th percentiles. Asterisks represent comparison between groups. Note: these plots illustrate the raw data, but statistical analyses accounted for repeating fever episodes among patients. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Table 3. Risk of blood stream infection in univariate- and multivariate analyses.

Parameter	Univariate analysis			Multivariate analysis ^a		
	OR	95% CI	<i>p</i> value	OR	95% CI	<i>p</i> value
Citrulline	1.8 ^b	1.2–2.6	0.0037	1.6 ^b	1.1–2.4	0.014
CCL20	1.6	1.3–2.0	<0.0001	1.7	1.3–2.2	<0.0001
REG3 α	1.5	1.1–2.0	0.010	1.5	1.01–2.2	0.045
CXCL8	1.5	1.3–1.8	<0.0001	1.7	1.4–2.0	<0.0001
CXCL1	1.5	1.3–1.9	<0.0001	1.8	1.4–2.2	<0.0001
CRP	1.2	1.02–1.5	0.034	1.2	0.9–1.6	0.19
PCT	1.4	1.2–1.8	0.0009	1.4	1.2–1.8	0.0012

OR odds ratio, CI confidence interval, REG3 α Regenerating Islet-Derived Protein 3 α , IL-8 interleukin 8, CRP C-reactive protein, PCT procalcitonin.

^aAdjusted for sex, age, presence of neutropenia, and diagnosis (acute lymphoblastic leukemia vs. acute myeloid leukemia/biphenotypical leukemia).

^bOR for citrulline is calculated as per halving in contrast the other parameters, where OR is calculated as per doubling.

between BSI and non-BSI-related fever episodes in children undergoing treatment for acute leukemia.

We found significant differences in citrulline, REG3 α , and CCL20 in patients with BSI, indicating more severe intestinal mucositis in BSI-related fever episodes, emphasizing the serious implications of bacterial translocation through an injured intestinal epithelium. Moreover, the data suggest that mucositis and neutropenia are independent risk-factors for BSI. These results not only validate previous findings in both pediatric and adult patients with hematological cancer, suggesting that citrulline and CCL20 could be valuable in identifying individuals at higher risk of developing

BSI during chemotherapy [16, 30–33], but more importantly, they also propose that markers of mucositis can serve as predictive indicators for BSI when patients are admitted with fever.

The significance of a compromised gut barrier integrity as a main site of bacterial translocation was further supported by the fact that many of the BSI episodes were caused by bacteria most likely originating from the oral cavity, the pharynx or the gut, including *Escherichia coli*, *Klebsiella* species, *Enterococcus* species, Viridans group streptococci, *Enterobacter cloacae*, *Citrobacter Braakii*, *Moraxella osloensis* and *Neisseria subflava*. Previous studies found positive cultures with Gram-positive bacteremia (with especially CoNS and

Staphylococcus aureus) relatively more abundant [34–36], but a huge effort has been made in recent years to limit central line associated infections in children with cancer, which could explain this development [37–40]. Furthermore, our strict definition requiring at least two positive cultures for common contaminants (CoNS, microcococcus species etc.) has likely limited false positives.

In addition to citrulline, CCL20 and REG3 α , all of which have been closely related to intestinal damage and inflammation, we also measured the neutrophil chemo-attractants CXCL1 and CXCL8. While CXCL1 has not previously been measured during febrile episodes in children with leukemia, CXCL8 has been extensively studied [27, 41–43]. These chemokines are closely related, originating from similar cellular sources, and being upregulated in response to same immunological stimuli [44]. Both markers were significantly elevated in patients with BSI-related febrile episodes as compared to non-BSI-related febrile

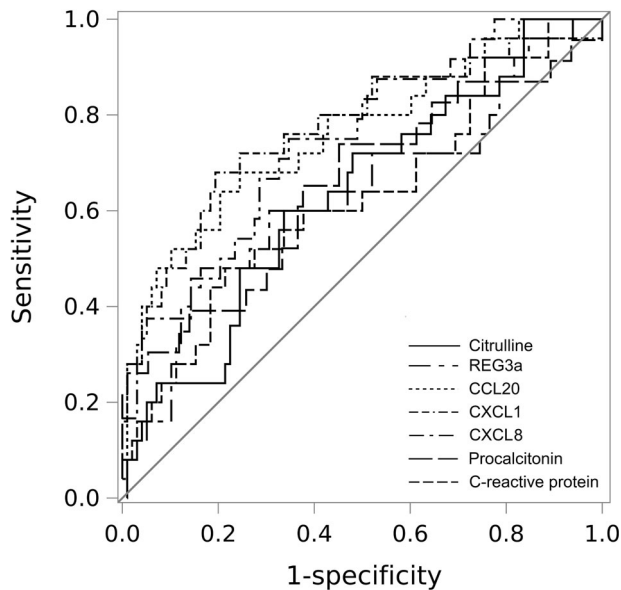


Fig. 2 Receiver operating characteristic (ROC)-curves for citrulline, REG3 α , CCL20, CXCL1, CXCL8, procalcitonin, and C-reactive protein in patients with febrile neutropenia. The reference line (gray) represents random performance.

episodes, confirming previous findings demonstrating increased CXCL1 and CXCL8 levels prior to both clinical symptoms and increasing CRP levels during BSI in children with ALL [29].

For citrulline and REG3 α the diagnostic accuracy was comparable to CRP, while CCL20, CXCL1 and CXCL8 showed better diagnostic accuracy than CRP and PCT. This may likely be related to the fact that febrile mucositis is observed also in the absence of a documented infection [45], and as BSI episodes may also be caused by dissemination of infections unrelated to gut translocation. Accordingly, the superior diagnostic ability of CCL20 in these patients as compared to citrulline and REG3 α could be explained by a release of this chemokine at other mucosal linings including in the respiratory tract.

While both CXCL1 and CXCL8 have been associated with intestinal mucositis, reflecting increased neutrophil recruitment to drive mucosal inflammation [28, 29, 46], both chemokines are more generally upregulated during inflammation with the ability to capture all sources of BSI [44, 47]. This is supported by several studies, suggesting CXCL8 as the most accurate diagnostic marker to differentiate between infectious and non-infectious neutropenic fever episodes during childhood cancer treatment [27, 42]. Nevertheless, defining a reasonable sensitivity level becomes particularly pertinent in the context of highly susceptible patients as in the current setting. Therefore, the cut-off value that maximizes diagnostic accuracy may not align with a clinical operational cut-off value. It is most likely that we will need to combine multiple risk factors to enhance predictive capabilities.

Interestingly, all the studied markers showed comparable ability to detect BSI-related febrile episodes when combined with the currently implemented diagnostic tools (CRP and PCT) with a sensitivity of >0.90 for neutropenic patients. This underlines the potential for risk stratification by biomarkers reflecting the early intestinal injury that initiates and propagates the inflammatory, antimicrobial defense cascade, as previously suggested [16, 29]. The relatively lower specificity observed along this high sensitivity should be considered in the context of current guidelines, which recommend the initiation of empiric intravenous, broad-spectrum antibiotics for all high-risk patients at the onset of neutropenic fever, even though less than half of these patients are ultimately diagnosed with an infection. Even with this limitation, the ability to exclude severe infections in 25% of all febrile neutropenic episodes has the potential to enhance safety when considering early antibiotic cessation. Importantly, these findings are highly exploratory and need to be validated before final conclusions.

Table 4. Diagnostic accuracy for detecting blood stream infections.

Parameter	ROC AUC (95% CI)	Cut-off value	Sensitivity	Specificity
All febrile episodes				
Citrulline	0.60 (0.50–0.70)	16.8 $\mu\text{mol/l}$	0.59	0.58
CCL20	0.73 (0.62–0.84)	40.2 pg/ml	0.65	0.79
CXCL8	0.75 (0.65–0.85)	60.0 pg/ml	0.64	0.80
CXCL1	0.78 (0.68–0.89)	110.6 pg/ml	0.74	0.73
CRP	0.61 (0.50–0.72)	31.0 mg/l	0.45	0.73
PCT	0.66 (0.55–0.78)	0.37 $\mu\text{g/l}$	0.72	0.59
Neutropenic febrile episodes				
Citrulline	0.65 (0.52–0.77)	13.7 $\mu\text{mol/l}$	0.61	0.67
CCL20	0.78 (0.66–0.89)	44.5 pg/ml	0.70	0.76
REG3 α	0.64 (0.51–0.77)	62.0 ng/ml	0.57	0.70
CXCL8	0.75 (0.63–0.86)	59.0 pg/ml	0.77	0.66
CXCL1	0.77 (0.66–0.89)	189.5 pg/ml	0.74	0.76
CRP	0.62 (0.48–0.76)	34.0 mg/l	0.57	0.67
PCT	0.64 (0.50–0.79)	0.37 $\mu\text{g/l}$	0.71	0.54

The main strength of this study is the relatively large, homogenous patient cohort of children with acute leukemia and the inclusion of all febrile episodes as well as the coordinated collection of plasma samples at the first clinical assessment after the onset of symptoms. The strict guidelines requiring blood cultures for all patients presenting with fever makes the outcome of BSI particularly robust, as no missing information is present. However, the large variation in the collection of other microbiological tests and diagnostic imaging, including cultures from alternative foci (urine, tracheal aspirate, stool), and nasopharyngeal swabs for virus detection prevented an appropriate characterization of blood culture negative focal infections due to the large number of untested individuals.

Other important limitations include the observational nature of the study, limiting our ability to infer causal relations and to control for possible interfering parameters such as the use of antibiotics. Moreover, the study would have benefited from repeated measurements of the markers to determine their kinetics and to evaluate the diagnostic accuracy after, e.g., 24–48 h of fever onset. Additionally, having an individual baseline level for citrulline to reflect relative changes during chemotherapy might be of importance in the pediatric setting.

In conclusion, there is growing evidence supporting the present markers as robust, objective and minimally invasive tools to evaluate mucositis severity, which definitely increases feasibility, validity and reproducibility in both research and clinical settings. Our results further indicate that a dysregulated intestinal barrier is a significant risk factor for invasive infections and that monitoring intestinal mucositis by quantitative markers may serve as a potential diagnostic approach to detect BSI-related fever episodes. These findings also emphasize the importance of protecting the gastrointestinal barrier during chemotherapy to reduce infectious morbidity, which merits increased clinical and scientific priority. Controlled, clinical trials are needed to investigate whether these markers can be used clinically to guide antimicrobial therapy.

DATA AVAILABILITY

The data supporting these findings are available from the corresponding author upon reasonable request and after regulatory approval.

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AUTHOR CONTRIBUTIONS

SW contributed to project design, data collection, performed the statistical analyses and interpretation and drafted the manuscript. MR, MJP, AW, CHN, CE, BAN, UN and CM contributed to data collection and data interpretation. KM designed the project, established the collaboration, and contributed to data interpretation. All authors critically revised the manuscript and approved the final version to be published.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICAL APPROVAL

Written and oral informed consent was obtained from the parents/legal guardians before inclusion, and the study was approved by the local scientific ethics committee (H-16016942 and H-2-2010-002) and conducted in accordance with the Declaration of Helsinki.

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Correspondence and requests for materials should be addressed to Sarah Weischendorff.

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