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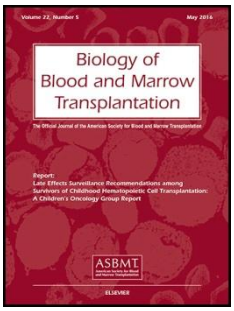

Title: Early Blood Stream Infection after BMT is Associated with Cytokine Dysregulation and Poor Overall Survival

Author: Elizabeth M. Catchpoole, Caitlin E. Thirunavukarasu, Antiopi Varelias, Sanmarié Schlebusch, Stuart Olver, Nienke Zomerdijk, Emma Osland, Glen A. Kennedy, Siok-keen Tey, Geoffrey R. Hill, Kate A. Markey

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Early blood stream infection after BMT is associated with cytokine dysregulation and poor overall survival

Elizabeth M Catchpoole MBBS,[1] Caitlin E Thirunavukarasu MBBS,[2] Antiopi Varelias PhD,[3,5] Sanmarié Schlebusch MBBS,[4,5] Stuart Olver BSc,[3] Nienke Zomerdijk BBus PGDipPsych,[3] Emma Osland AdvAPD,[2] Glen A Kennedy MBBS,[2] Siok-keen Tey MBBS PhD,[2,3,5] Geoffrey R Hill MBBS MD,[2,3,5] Kate A Markey MBBS PhD[2,3,5,6*]

[1] Pathology Queensland, Herston, Brisbane, Australia
[2] Royal Brisbane and Women's Hospital, Herston, Brisbane, Australia
[3] QIMR Berghofer Medical Research Institute, Herston, Brisbane, Australia
[4] Mater Pathology, South Brisbane, Brisbane, Australia
[5] Faculty of Medicine, The University of Queensland, Brisbane, Australia
[6] The Australian Infectious Disease Research Centre, The University of Queensland, Australia

Corresponding author: Dr Kate Markey
QIMR Berghofer Medical Research Institute
300 Herston Road
Herston, Brisbane, Australia, 4006
Ph: +6173362 0290
E: kate.markey@qimr.edu.au

Short title: BSI in BMT patients: risk factors and consequences

Keywords: bone marrow transplantation, blood stream infection, antibiotics, cytokine dysregulation

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Highlights:

- Blood stream infection (BSI) remains an important complication of BMT
- Freedom from BSI was associated with superior overall survival at 2 years
- This effect was most pronounced in older patients receiving RIC allografts
- Pre-engraftment BSI was associated with dysregulation of IL-6 and IL-8

Abstract

The key complications of allogeneic bone marrow transplant (BMT) remain graft-versus-host disease (GVHD) and opportunistic infection. We have analyzed the blood stream infections (BSI) occurring between day -7 and day 100 in a cohort of 184 adult patients undergoing allogeneic BMT in our center. 167 of the 184 patients (91%) had blood cultures collected, and 69 (38%) patients had a confirmed BSI. *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Enterococcus* spp. and viridans *Streptococcus* spp. were the most commonly isolated organisms. Gender, conditioning (myeloablative vs. reduced intensity) and donor type (sibling vs. unrelated) did not differ significantly between those with and without confirmed BSI. Elevated temperature (>38°C) at the time of culture collection was associated with an almost 2-fold increased likelihood of returning a positive blood culture. The absence of a BSI was associated with a significant improvement in overall survival at 2 years, due to a significant reduction in non-relapse mortality predominantly unrelated to the primary BSI. The presence of a BSI prior to engraftment was associated with the dysregulation of IL-6 and IL-8. Our findings suggest that BSI early after BMT defines a group of high-risk patients with enhanced cytokine dysregulation and poor transplant outcome.

Introduction

Allogeneic bone marrow transplantation (BMT) is predominantly performed as curative therapy for high risk hematological malignancies. Graft-versus-host disease (GVHD) and opportunistic infection remain major limitations to successful outcomes. Fevers in conjunction with blood stream infections (BSI) are common in the early period after BMT[1-3] and blood cultures (BC) followed by immediate broad-spectrum empiric antimicrobial therapy remains the standard of care. The reported incidence of BSI following BMT is 12 – 53%,[4, 5] with the majority of infections occurring within the first 100 days.[6, 7]

The epidemiology and resistance patterns of BSI vary significantly between centers. Gram-positive bacteremias are predominantly caused by either coagulase-negative staphylococci (CoNS) or *Enterococcus* spp.[4-6, 8, 9] Gram-negative organisms are isolated in 21 – 50% of BSI in the BMT setting.[4-6, 8, 9] The most common gram-negative organisms are *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*[4, 6-12] Fluoroquinolone resistant gram-negative bacteria are of highest prevalence in centers with high rates of anti-bacterial prophylaxis with these agents (50 – 74%).[9, 12] Fungemia is reported in 0.8 – 10% of BSI, and less than 5% of BSI in cohorts where systemic anti-fungal prophylaxis is used.[4, 6, 8, 9, 12] Established risk factors for BSI after BMT include older age,[10] use of unrelated donors,[13] donor-recipient HLA mismatches, myeloablative conditioning,[13, 14] longer duration of neutropenia,[5, 10] and severe acute GVHD.[11] BSI has been associated with decreased survival in the first 6 months after BMT, particularly when multi-resistant organisms are isolated.[5, 10, 12-15]

Acute GVHD is characterized by an early cytokine storm dominated by IL-6, TNF and IFNγ.[16-18] These cytokines are produced by both donor and recipient cells, and therapies that

inhibit cytokine generation from effector donor T cells are a key component of GVHD prophylaxis and treatment.[19] It is clear that damage and pathogen-associated molecular patterns (DAMP/PAMPs) originating from the microbiome and migrating across a damaged gastrointestinal tract (GI tract) play an important role in promoting these cytokine responses and acute GVHD.[20,21] To date however, this has not been attributed to intact bacteria (i.e. BSI) within the systemic circulation. Here we report the epidemiology of BSI in this patient group in our center and examine the consequences of BSI with regard to cytokine dysregulation and transplant outcome.

Materials and Methods

Patients

Patients underwent BMT at the Royal Brisbane and Women's Hospital (RBWH) between 2009 and 2015, with the majority of patients recruited between 2012 and 2013. Inclusion criteria for the cohort were: age 18 – 75 years, able to provide informed consent, negative for hepatitis B and C, and HIV on routine BMT workup, and not enrolled in another study. Patients underwent T-replete, peripheral blood stem cell (PBSC) allogeneic SCT and from either volunteer unrelated donors (VUD) or matched sibling donors. For unrelated donors, high-resolution sequence-based typing was performed (HLA-A, B, C, DRB1 and DQ). Conditioning was either cyclosphosphamide with total body irradiation (60 mg/kg per day for days –5 and –4, plus 12 Gy TBI over days -3 to -1; Cy TBI, myeloablative conditioning [MAC] protocol) or fludarabine and melphalan (25 mg/m^2 fludarabine from day -7 to -3 and melphalan 120mg/m^2 on d-2; Flu Mel, reduced intensity conditioning [RIC] protocol). Standard GVHD prophylaxis was cyclosporin (5 mg/kg per day on days –1 to +1, then 3 mg/kg per day to maintain therapeutic levels with trough levels of 140–300 ng/mL) for 100

days (with weaning thereafter at clinician discretion) plus methotrexate (15 mg/m^2 on day 1, then 10 mg/m^2 on days 3, 6, and 11). Routine antimicrobial prophylaxis consisted of norfloxacillin (from commencement of conditioning to either neutrophil engraftment or escalation to intravenous antibiotics), trimethoprim/sulfamethoxazole, valaciclovir or acyclovir, and fluconazole. The first-line empiric antimicrobial was piperacillin-tazobactam, with the addition of vancomycin if central venous line associated sepsis was suspected. Adjustment to first-line antibiotics were made in the setting of drug allergy, colonization with resistant organisms, or known previous infection with organisms resistant to piperacillin-tazobactam.

Blood cultures

Blood cultures were collected from each lumen of central lines, and peripherally where possible, at temperatures ≥ 38°C as per unit protocol, and on physician request. Blood cultures were collected using standard clinical procedures, incubated and monitored for positivity using the BacT/Alert 3D system (bioMérieux, Marcy l'Etoile, France). Bacterial susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) standards,[22] transitioning to European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards in 2012.[23] CLSI clinical breakpoints, where available, were used for fungal susceptibility testing. Where patients had multiple consecutive episodes of BC where the same organism was isolated, the organism was included only once in the frequency and susceptibility analysis. Organisms which are common skin contaminants, such as coagulase-negative staphylococcus (CoNS), *Bacillus* spp. and *Cutibacterium (Propionibacterium) acnes*, were only considered significant if they were isolated from all line and peripheral blood cultures collected in a single episode.[24]

Data collection and definitions

Clinical data were collected from transplant protocols and retrospective review of the medical records. Microbiological data were collected from our institutional pathology database. Age, gender, BMT indication, donor type, induction protocol, day of engraftment (defined as 2 consecutive days with ANC >0.5 x 10^9/L), acute GVHD (grading by Seattle criteria[25]), date of GVHD diagnosis, and CMV reactivation (2 consecutive qPCR results of >600 copies/mL) were recorded.

Time and date stamps were used to define separate episodes of BC collection and where BC were collected within 2 hours of index we considered them part of a single clinical episode. Features at the time of the episode were collected from retrospective review, including patient observations, antimicrobial therapy, total parenteral nutrition (TPN) at time of BC collection and ANC. Fever was defined as peripheral body temperature ≥38°C and hypotension defined as systolic blood pressure <90mm Hg.[26]

Cytokine analysis

Cytokines measured were: IL-1, IL-6, IL-8, TNF and IFNγ. Blood samples were collected at day -7, 0, +3, +7, +14, +21, +30, +60, +90 and +120. Serum was prepared and stored at -80°C until time of analysis. Serum cytokines were analyzed using cytokine bead array (CBA; BD Biosciences, Sparks, USA), with data acquired using a BD LSR Fortessa high-throughput system (BD Biosciences, Sparks, USA). Quantification controls for each cytokine were performed for each 96-well sample plate. Some of the cytokine data for these patients has been reported previously, independently of this analysis of BSI.[27]

Statistical analysis

Data were collated using Microsoft Excel. GraphPad Prism (Version 6.00, GraphPad Software, USA) was used for the generation of graphs and for statistical analysis. Contingency analyses (Fisher's exact test) were used to measure associations. Kaplan-Meier time to event analyses were performed (Log-Rank test). Significant differences in cytokine levels in patients with and without BSI were assessed using Mann-Whitney non-parametric analyses at each time point.

Ethics

The observational study protocol was approved by both the QIMR Berghofer Medical Research Institute and RBWH Human Research Ethics Committees with written informed consent obtained from all patients

Results

Patient characteristics and BSI frequency

184 patients were enrolled in our cohort (Table 1), with the majority undergoing transplant for myelodysplastic syndrome (MDS) or acute myeloid leukemia (52%). Between day -7 and day 100, 167 patients (91%) had a clinically indicated BC collected, on 777 separate occasions. On a per-episode basis, 217 (28%) of these yielded positive results, 111 (14%) of which were for clinically significant organisms. On a per-patient basis, 69 patients in our cohort experienced a BSI. Thirty-five patients (51% of the positives) confirmed a BSI prior to neutrophil engraftment, 25 (36%) after engraftment, and 9 patients (13%) had separate BSIs proven both prior to and after engraftment.

From 111 positive blood culture episodes, 93 unique organisms were associated with a BSI. Gram-negative organisms were isolated in 54 cases (58% of total). The most commonly isolated gram-negative bacteria were *Pseudomonas aeruginosa* (12, 22%), *Klebsiella pneumoniae* (9, 17%) and *Escherichia coli* (7, 13%). Among all gram-negative isolates recovered, 21 (38.9%) were resistant to piperacillin-tazobactam and 6 (11.1%) were resistant to meropenem. The presence of extended spectrum beta-lactamase (ESBL) was identified as the mechanism of antimicrobial resistance in 4 (7%) gram-negative isolates. Gram-positive organisms were isolated in 35 (38% of total) cases. The most common gram-positive bacteria identified were *Enterococcus* spp. (12 isolates, 34%) and viridans *Streptococcus* spp. (12 isolates, 34%). Piperacillin-tazobactam resistance was observed in 13 (37%) of the gram-positive isolates, associated with *Enterococcus faecium* (7 episodes), methicillin resistant *Staphylococcus epidermidis* (3 episodes), MRSA (1 episode), and viridans streptococci (2 episodes). Four *vanB* phenotype *E. faecium* were detected. No *vanA* VRE was detected, and

no daptomycin or linezolid resistance was observed. Two fungal BSI occurred, one each with *Lomentospora prolificans* and *Candida glabrata*. Organisms isolated and their resistance patterns are described in Table 2.

Characteristics of BC episodes confirming BSI

Donor source (related vs. unrelated), conditioning regimen (MAC vs RIC) and recipient age had no relationship with BSI incidence (Table 3). The majority of BC were collected in febrile patients (62% of episodes; T≥38˚C) and these yielded significant organisms more frequently than afebrile episodes (17% vs 9%; OR 1.9, 95% CI 1.21 – 3.23; p = 0.0058; Table 3). The organisms isolated from febrile patients were not, however, different to those isolated from afebrile patients. Of note, in this series, neutropenia, hypotension, timing of episode (relative to engraftment) and TPN were not associated with proven BSI. Average time to engraftment was 16.5 ± 4.6 days and was not different between patients with and without BSI (p = 0.65). On a per-patient basis, CMV reactivation at any time before day 100 was not associated with BSI (OR 1.38, 95% CI 0.71 – 2.65; p = 0.39). The most commonly used empiric gram-negative targeted antimicrobials were piperacillin-tazobactam (45%) and meropenem (32%). Empiric gram-positive targeting agents were used frequently, vancomycin in 50% of episodes and teicoplanin in 10 % of episodes.

Patient outcomes associated with BSI

We compared 2-year overall survival (OS) in patients who never experienced a BSI compared with those who did (Fig 1A). BSI-free status was associated with a significant improvement in 2 year overall survival (HR 0.59, 95% CI 0.37-0.95; p = 0.01). Ten patients died within 30 days of BSI. When these patients were excluded from the analysis, BSI-free status still conferred a significant survival benefit (HR 0.50, 95% CI: 0.28 – 0.94; p = 0.023).

This was not due to higher rates of relapse mortality in this group, but rather, attributable entirely to non-relapse mortality (NRM; Fig 1B-C). In patients with a BSI prior to their diagnosis of GVHD, no significant difference in the cumulative incidence of severe aGVHD was seen (Fig 1D), however there was significantly higher mortality attributed to aGVHD (11.6% *vs* 0.9%, $p = 0.0019$) in the patients who experienced a BSI. For patients with BSI any time before day 100, there was a trend towards a higher incidence of extensive chronic GVHD relative to those without confirmed BSI (34.7% in the BSI group *vs* 21.7% in the BSI-free group; $p = 0.06$). There was no significant difference in the relapse rate at 2 years between patients with BSI and those without ($p = 0.47$).

When stratified by conditioning, differences in OS between BSI and BSI-free patients were maintained in patients who underwent reduced intensity (with fludarabine and melphalan) conditioning, whereas no difference was seen in the patients undergoing myeloablative (Cy TBI) conditioning (Fig 2A-B). The median age of patients undergoing MAC transplantation was 35 (range 18 – 52) compared with 59 (range 20 – 70) in the RIC patients ($P<0.0001$). Among those undergoing BMT after reduced intensity conditioning, the majority received transplants from VUD donors and the association between BSI and decreased overall survival was maintained for the VUD cohort, but not the recipients of sibling allografts (Fig 2C-D). It should be noted however that numbers in the latter group were small (34 patients in total).

Cytokine analysis

To assess cytokine dysregulation associated with BSI, we focused on patients who had a confirmed BSI before engraftment, and compared them to those who had sterile blood cultures collected (for any clinical indication). IL-6 and IL-8 were elevated at multiple time points early following transplant and were influenced by conditioning intensity and BSI (Fig

3). Other cytokines analyzed (IFNγ, IL-1, and TNF) were not differentially impacted by early BSI (data not shown).

Discussion

In this cohort study, one-third of patients experienced a confirmed BSI in the first 100 days after BMT. Gram-negative bacilli, enterococci and viridans streptococci were the most commonly isolated organisms and resistance to piperacillin-tazobactam amongst gram-negative isolates was higher than previously reported in Australian cohorts[28] due to both intrinsic and acquired resistance mechanisms. This may reflect changes in patient colonization associated with recurrent exposure to piperacillin-tazobactam during AML induction and consolidation therapy, typically the first-line agent for febrile neutropenia at our center.

When we used the presence or absence of a BSI to divide the patient cohort, BSI was associated with poorer 2-year overall survival while relapse mortality (2 year) and classical severe acute GVHD (i.e. before day 100) were similar. The difference in OS is thus likely due to other transplant-related complications, including chronic GVHD and later opportunistic infection. In stratified analysis, the association remained strongest in the patients undergoing reduced intensity allografts with unrelated donors, though small numbers in the myeloablative subgroup precludes a firm conclusion that the BSI and BSI-free patients are equivalent in this setting. While age was not in itself a risk factor for proven BSI in this study, the group receiving reduced intensity allografts were significantly older than the group receiving myeloablative transplants, which is likely to impact outcome. It is also possible that this older group experienced more pre-transplant BSI (or other significant infections) than the younger patients treated with myeloablative conditioning, but due to the retrospective nature

of data collection for this study we were unable to explore this. The role of pre-transplant BSI as a risk factor should be explored in future prospective studies. It remains unclear whether a BSI reflects additional baseline risk factors not identified in this study, serving as a surrogate for high-risk patients. In contrast, it is possible that BSI itself promotes immune dysfunction and leads to downstream complications. Indeed defects in both antigen presentation and immune dysfunction have been noted in systemic cytokine release syndromes,[29] consistent with this possibility, and now require further exploration.

With larger patient numbers it is likely that early BSI would be associated with higher rates of severe aGVHD than BSI-free status, as this has been reported by others. It is also likely that severe aGVHD (particularly of the GI tract) would predispose to subsequent BSI. [30] There were 5 patients who only experienced BSI after their diagnosis of severe, grade 3-4 aGVHD, and a further four who experienced BSI both prior to and after the onset of GVHD, these small numbers prevented any assessment of the role of GVHD itself as a causal factor of BSI in this cohort.

All patients included in the cytokine analysis had early fevers and exposure to broad spectrum antibiotics, and there were no differences in the empiric antibiotic choice between those who ultimately went on to have proven BSI and those who did not. It is nevertheless possible that duration of antibiotic exposure may contribute to BMT outcome, given the mounting evidence that disruption of the microbiome after BMT is detrimental to survival.[31] However, the lack of available stool samples in this patient cohort meant that we could not assess this possibility and we suggest that this would be a valuable addition to future studies.

IL-6 and IL-8 have been implicated as causative factors in both acute GVHD and the response to sepsis. IL-8 is particularly interesting as a biomarker for the severity of septic episodes in critical illness,[32, 33] despite some contradictory results.[34] IL-8, also referred to as neutrophil chemotactic factor and CXCL8, is involved in neutrophil and T cell chemotaxis during inflammation, and is produced by a variety of cells and tissues, including monocyte/macrophages, neutrophils themselves and epithelial cells within the GI tract.[35, 36] In that context, IL-8 has also been examined as a biomarker for acute GVHD, and has been reported as useful in combination with other cytokines, including IL-2, TNFR1, and hepatocyte growth factor.[37] It is thus biologically plausible that elevated IL-8 in the early post-transplant period can contribute to downstream tissue damage and transplant-related mortality, though this has not yet been explored in mouse or clinical studies of BMT. IL-6, in contrast, has been demonstrated to have a pathogenic role in GVHD in experimental models, and more recently in the clinical setting, with favorable results demonstrated in a phase I/II study of tocilizumab for GVHD prevention.[27, 38-40] IL-6 can be produced by most cell types, and signals via an IL-6 receptor/gp130 heterodimer, of which expression is restricted to T cells, monocytes and hepatocytes. While we have been able to associate IL-6 dysregulation with BSI in this study, a much larger study would be needed to analyze the effects of IL-6 and BSI on acute GVHD in a multivariate fashion.

This is the first study reporting a BSI-specific induction of these cytokines in the setting of the broader cytokine dysregulation after BMT. The contribution of BSI to cytokine dysregulation and transplant-related mortality now needs to be studied in large prospective multi-center studies, ideally in conjunction with microbiome analysis.

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References

1. Shigematsu A, Yamamoto S, Sugita J, et al. Increased risk of bacterial infection after engraftment in patients treated with allogeneic bone marrow transplantation following reduced-intensity conditioning regimen. *Transplant infectious disease : an official journal of the Transplantation Society.* 2010;12:412-420.

2. Orasch C, Weisser M, Mertz D, et al. Comparison of infectious complications during induction/consolidation chemotherapy versus allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant.* 2010;45:521-526.

3. Choeyprasert W, Hongeng S, Anurathapan U, Pakakasama S. Bacteremia during neutropenic episodes in children undergoing hematopoietic stem cell transplantation with ciprofloxacin and penicillin prophylaxis. *Int J Hematol.* 2017;105:213-220.

4. Busca A, Cavecchia I, Locatelli F, et al. Blood stream infections after allogeneic stem cell transplantation: a single-center experience with the use of levofloxacin prophylaxis. *Transplant infectious disease : an official journal of the Transplantation Society.* 2012;14:40-48.

5. El-Ghammaz AMS. Bacteremia During Early Post-allogeneic Hematopoietic Stem Cell Transplantation Period: A Single Center Experience. *Indian J Hematol Blood Transfus.* 2017;33:200-206.

6. Kikuchi M, Akahoshi Y, Nakano H, et al. Risk factors for pre- and post-engraftment bloodstream infections after allogeneic hematopoietic stem cell transplantation. *Transplant infectious disease : an official journal of the Transplantation Society.* 2015;17:56-65.

7. Schuster MG, Cleveland AA, Dubberke ER, et al. Infections in Hematopoietic Cell Transplant Recipients: Results From the Organ Transplant Infection Project, a Multicenter, Prospective, Cohort Study. *Open Forum Infect Dis.* 2017;4:ofx050.

8. Malagola M, Rambaldi B, Ravizzola G, et al. Bacterial Blood Stream Infections Negatively Impact on Outcome of Patients Treated with Allogeneic Stem Cell Transplantation: 6 Years Single-Centre Experience. *Mediterr J Hematol Infect Dis.* 2017;9:e2017036.

9. Mikulska M, Del Bono V, Raiola AM, et al. Blood stream infections in allogeneic hematopoietic stem cell transplant recipients: reemergence of Gram-negative rods and increasing antibiotic resistance. *Biol Blood Marrow Transplant.* 2009;15:47-53.

10. Girmenia C, Bertaina A, Piciocchi A, et al. Incidence, Risk Factors and Outcome of Pre-engraftment Gram-Negative Bacteremia After Allogeneic and Autologous Hematopoietic Stem Cell Transplantation: An Italian Prospective Multicenter Survey. *Clin Infect Dis.* 2017;65:1884-1896.

11. Levinson A, Pinkney K, Jin Z, et al. Acute gastrointestinal graft-vs-host disease is associated with increased enteric bacterial bloodstream infection density in pediatric allogeneic hematopoietic cell transplant recipients. *Clin Infect Dis.* 2015;61:350-357.

12. Liu CY, Lai YC, Huang LJ, et al. Impact of bloodstream infections on outcome and the influence of prophylactic oral antibiotic regimens in allogeneic hematopoietic SCT recipients. *Bone Marrow Transplant.* 2011;46:1231-1239.

13. Bock AM, Cao Q, Ferrieri P, Young JA, Weisdorf DJ. Bacteremia in blood or marrow transplantation patients: clinical risk factors for infection and emerging antibiotic resistance. *Biol Blood Marrow Transplant.* 2013;19:102-108.

14. Blennow O, Mattsson J, Remberger M. Pre-engraftment blood stream infection is a risk factor for acute GVHD grades II-IV. *Bone marrow transplantation.* 2013;48:1583-1584.

15. Yemisen M, Balkan II, Salihoglu A, et al. The Changing Epidemiology of Bloodstream Infections and Resistance in Hematopoietic Stem Cell Transplantation Recipients. *Turk J Haematol.* 2016;33:216-222.

16. Hill GR, Krenger W, Ferrara JL. The role of cytokines in acute graft-versus-host disease. *Cytokines Cell Mol Ther.* 1997;3:257-266.

17. Markey KA, Burman AC, Banovic T, et al. Soluble lymphotoxin is an important effector molecule in GVHD and GVL. *Blood.* 2010;115:122-132.

18. Shlomchik WD. Graft-versus-host disease. *Nature reviews. Immunology.* 2007;7:340-352.

19. Markey KA, MacDonald KPA, Hill GR. Cytokines in Graft-versus-host Disease and Graft-versus-leukemia. *Immune Biology of Allogeneic Hematopoietic Stem Cell Transplantation*. New York, NY, U.S.A: Elsevier Inc.; 2013:357-391.

20. Hill GR, Ferrara JL. The primacy of the gastrointestinal tract as a target organ of acute graft-versus-host disease: rationale for the use of cytokine shields in allogeneic bone marrow transplantation. *Blood.* 2000;95:2754-2759.

21. Johansson JE, Ekman T. Gut toxicity during hemopoietic stem cell transplantation may predict acute graft-versus-host disease severity in patients. *Dig Dis Sci.* 2007;52:2340-2345.

22. CLSI. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-first Informational Supplement. CLSI document M100-S21. . Wayne, PA: Clinical and Laboratory Standards Institute; 2011.

23. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 5.0: http://www.eucast.org 2015.

24. National Healthcare Safety Network. Patient Safety Component Manual. *Bloodstream Infection Event (Central Line-Associated Bloodstream Infection and non-central line-associated Bloodstream Infection)*: Centers for Disease Control and Prevention; 2017:1-38.

25. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant.* 1995;15:825-828.

26. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med.* 2003;31:1250-1256.

27. Kennedy GA, Varelias A, Vuckovic S, et al. Addition of interleukin-6 inhibition with tocilizumab to standard graft-versus-host disease prophylaxis after allogeneic stem-cell transplantation: a phase 1/2 trial. *The Lancet. Oncology.* 2014;15:1451-1459.

28. Conn JR, Catchpoole EM, Runnegar N, Mapp SJ, Markey KA. Low rates of antibiotic resistance and infectious mortality in a cohort of high-risk hematology patients: A single center, retrospective analysis of blood stream infection. *PloS one.* 2017;12:e0178059.

29. Roquilly A, McWilliam HEG, Jacqueline C, et al. Local Modulation of Antigen-Presenting Cell Development after Resolution of Pneumonia Induces Long-Term Susceptibility to Secondary Infections. *Immunity.* 2017;47:135-147 e135.

30. Poutsiaka DD, Munson D, Price LL, Chan GW, Snydman DR. Blood stream infection (BSI) and acute GVHD after hematopoietic SCT (HSCT) are associated. *Bone marrow transplantation.* 2011;46:300-307.

435 **31.** Shono Y, Docampo MD, Peled JU, et al. Increased GVHD-related mortality with
436 broad-spectrum antibiotic use after allogeneic hematopoietic stem cell
437 transplantation in human patients and mice. *Sci Transl Med.* 2016;8:339ra371.
438 **32.** Calfee CS, Thompson BT, Parsons PE, Ware LB, Matthay MA, Wong HR. Plasma
439 interleukin-8 is not an effective risk stratification tool for adults with vasopressor-
440 dependent septic shock. *Crit Care Med.* 2010;38:1436-1441.
441 **33.** Wong HR, Cvijanovich N, Wheeler DS, et al. Interleukin-8 as a stratification tool for
442 interventional trials involving pediatric septic shock. *Am J Respir Crit Care Med.*
443 2008;178:276-282.
444 **34.** Kraft R, Herndon DN, Finnerty CC, Cox RA, Song J, Jeschke MG. Predictive Value of IL-
445 8 for Sepsis and Severe Infections After Burn Injury: A Clinical Study. *Shock.*
446 2015;43:222-227.
447 **35.** Waugh DJ, Wilson C. The interleukin-8 pathway in cancer. *Clinical cancer research :*
448 *an official journal of the American Association for Cancer Research.* 2008;14:6735-
449 6741.
450 **36.** Cotton JA, Bhargava A, Ferraz JG, Yates RM, Beck PL, Buret AG. Giardia duodenalis
451 cathepsin B proteases degrade intestinal epithelial interleukin-8 and attenuate
452 interleukin-8-induced neutrophil chemotaxis. *Infect Immun.* 2014;82:2772-2787.
453 **37.** Paczesny S, Krijanovski OI, Braun TM, et al. A biomarker panel for acute graft-versus-
454 host disease. *Blood.* 2009;113:273-278.
455 **38.** Chen X, Das R, Komorowski R, et al. Blockade of interleukin-6 signaling augments
456 regulatory T-cell reconstitution and attenuates the severity of graft-versus-host
457 disease. *Blood.* 2009;114:891-900.

39. Tawara I, Koyama M, Liu C, et al. Interleukin-6 modulates graft-versus-host responses after experimental allogeneic bone marrow transplantation. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2011;17:77-88.

40. Varelias A, Gartlan KH, Kreijveld E, et al. Lung parenchyma-derived IL-6 promotes IL-17A-dependent acute lung injury after allogeneic stem cell transplantation. *Blood.* 2015;125:2435-2444.

Figure 1: Cumulative incidence of GVHD and 2 year overall survival

A) Overall survival at 2 years amongst patients with and without BSI in the period d-7 to day 100. B) Non-relapse mortality (NRM) at 2 years. C) Cumulative relapse mortality, at 2 years. D) Kaplan-Meier analysis shown of severe acute GVHD in patients with and without proven BSI. n = 19 with acute GVHD in the 69 patient BSI group and n = 12 in the 115 patient BSI-free group.

Figure 2: Stratified OS analysis

A) Kaplan-Meier analysis of OS for the myeloablative (MAC) and B) Reduced intensity conditioning (RIC) subgroups. C-D) Further stratification of OS in the reduced intensity group, by donor type.

Figure 3: Cytokine analysis in patients with and without confirmed BSI

A) IL-6 levels between day -7 and day 120 are shown in patients with pre-engraftment proven BSI and those who had blood cultures collected during the same window but did not return positive results. Individual data points shown, line at mean. Stratified by conditioning. B) As shown in A, IL-8 levels.

490 Table 1: Patient characteristics

Characteristics	**Observational cohort n = 184 (%)**
Age (Median +/- range)	52 (18-70)
Female recipients	76 (41.3%)
Indication for transplant	
Acute myeloid leukemia	65 (35.9%)
Acute lymphoblastic leukemia	34 (18.2%)
Myelodysplasia	31 (16.6%)
Lymphoproliferative disorder	36 (14.4%)
Myeloproliferative disorder	18 (11.0%)
Donor source	
Sibling	63 (34.2%)
Unrelated	121 (65.8%)
Conditioning	
Cy TBI (MAC)	57 (31.0%)
Flu Mel (RIC)	124 (67.4%)
Other	3 (1.6%)

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Table 2: Organisms and susceptibility associated with blood stream infection

Organism	**n**	**%**	**Significant Resistance Phenotypes /Mechanisms**	**n**	**%**
GRAM-NEGATIVE ORGANISMS	54	100	Piperacillin-tazobactam resistant	21	38.9
			Meropenem resistant	6	11.1
Pseudomonas aeruginosa	12	22.2	Piperacillin-tazobactam resistant	2	3.7
			Meropenem resistant	3	5.6
Klebsiella pneumoniae	9	16.7	Piperacillin-tazobactam resistant (ESBL)	1	1.9
Escherichia coli	7	13.0	Piperacillin-tazobactam resistant (ESBL)	3	5.6
Klebsiella oxytoca	5	9.3	-		
Enterobacter spp.	5	9.3	-		
Stenotrophomonas maltophilia	3	5.6	-		
Acinetobacter sp.	2	3.7	-		
Sphingomonas paucimobilis	2	3.7	-		
Ralstonia mannitolilytica	2	3.7	-		
Aeromonas hydrophila	1	1.9	-		
Capnocytophaga sp.	1	1.9	-		
Methylobacterium sp.	1	1.9	-		
Pantoea sp.	1	1.9	-		
Pseudomonas fluorescens	1	1.9	-		
Pseudomonas putida	1	1.9	-		
Serratia marcescens	1	1.9	-		
GRAM-POSITIVE ORGANISMS	35	100	Piperacillin-tazobactam resistant	13	37.4
Streptococcus mitis group	9	25.7	Penicillin-resistant	2	5.7
Enterococcus faecium	7	20.0	VRE vanB	4	11.4
Enterococcus faecalis	5	14.3	-		
Staphylococcus aureus	5	14.3	MRSA	1	2.9
Staphylococcus epidermidis	4	11.4	MRSE	3	8.6
Gemella haemolysans	1	2.9	-		
Granulicatella adiacens	1	2.9	-		
Streptococcus salivarius	1	2.9	-		
Streptococcus sanguinis	1	2.9	-		
Streptococcus gordonii	1	2.9	-		
FUNGI					
Candida glabrata complex	1		Fluconazole SDD	1	
Lomentospora prolificans	1				
ANAEROBIC ORGANISMS					
Clostridium sp.	1				
Veillonella parvula	1				
TOTAL	**9**				

	3				

ESBL: Extended spectrum β-lactamase, MRSA: Methicillin resistant *Staphylococcus aureus*. MRSE: Methicillin resistant *Staphylocccus epidermidis*, VRE: Vancomycin resistant *Enterococcus*, SDD: Susceptible dose dependent. One *Streptococcus mitis* group isolate did not undergo susceptibility testing.

Table 3: Risk factors for confirmed BSI

Patient-specific risk factor	**OR**	**95% CI**	**P value**
Donor			
Related	1		
Unrelated	0.76	0.41 – 1.41	0.42
Conditioning regimen			
Myeloablative	1		
Reduced intensity	0.71	0.37 – 1.35	0.33
Age			
≤ 50 years	1		
> 50 years	0.953	0.53-1.77	>0.99
Episode-specific risk factor			
Fever (T >38)			
No	1		
Yes	1.97	1.21 – 3.23	0.0058
Neutropenia (ANC <0.5)			
No	1		
Yes	1.427	0.95 – 2.15	0.09
Hypotension (<90 SBP)			
No	1		
Yes	1.32	0.39 – 4.5	0.72
Pre-engraftment			
No	1		
Yes	1.096	0.74 – 1.65	0.68
TPN at time of BC collect			
No	1		
Yes	0.73	0.42 – 1.29	0.35