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Title: Blood Stream Infections during Chemotherapy-Induced Neutropenia in Adult Patients with Acute Myeloid Leukemia: Treatment cycle matters

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Abstract: Purpose: To assess the frequency of bloodstream infections (BSI) during neutropenia in different cycles (C)of intensive chemotherapy treatment in acute myeloid leukemia (AML).
Methods: Register data of 327 consecutive patients aged 16-66 years having de novo AML between September 1992 and December 2001 was prospectively gathered.
Results: There were 956 treatment episodes with 456 (47.7%) positive blood cultures. BSI was monomicrobial in 327 episodes (71.7%) and polymicrobial in 129 (28.3%). The overall incidence rate (per 1000 hospital days) for BSI was 13.2, varying from 6.8 in CI after idarubicin, cytarabine and thioquanine to 15.6 in CII, 15.8 in CIII and 17.6 in CIV. The distribution of monomicrobial gram-positive BSIs was as follows: CI; 71.7%, CII; 62.8%, CIII; 53.3%, CIV; 36.6% and C I-IV together; 43.2%. The most common finding in the four different cycles was coagulase-negative staphylococci (38.3% to 30.6%). Viridans group streptococci were most commonly observed (in 20.4% of positive blood cultures) during CII after high-dose cytarabine and idarubicin treatments. The distribution of monomicrobial gram-negative BSIs was as follows: CI; 21.7%, CII; 36.3%, CIII; 45.7%, CIV; 46.9%, and CI-IV together; 37.9%.
Conclusions: It would be more reasonable to analyze chemotherapy cycle-based BSI results rather than overall results.

Response to Reviewers: Answers for the reviewers

We have changed the text in lines 207 -210 just to improve language.

Reviewer #1:
We agree with the reviewer: due to the register data we could not exclude the possibility, that two or more different micro-organisms found during a chemotherapy cycle did not represent two or more separate episodes of BSI.

"If more than one micro-organism was recovered from blood cultures of a patient during a chemotherapy cycle, the episode was defined as polymicrobial." is added to the method section in lines 115 - 116.

Reviewer #2:
We agree with the reviewer, that the cycle II is a crucial point in the data. As suggested we analyzed the cycle 2 by dividing it in two groups: those with active disease and receiving the 2nd induction (n=80) and those in remission and receiving the 1st consolidation (n=212).

	Active disease (n=80)	Remission (n=212)	p
Neutropenia d	27(22 - 36)	25(19 - 31)	NS
Febrile d	8 (4.5 - 11)	6 (3 - 11)	NS
peak CRP-value	162(123 -215)	159.5(110 - 202)	NS
GI-tox 3&4	37.3 %	29.1 %	0.194
CoNS	25 %	22 %	NS
VGS	11 %	20 %	0.119
Enterococci	9 %	11 %	NS
E.coli	4 %	6 %	NS
Klebsiella	8 %	11 %	NS
Enterobacter	6 %	6 %	NS
Citrobacter	0	3 %	NS
Pseudomonas	4 %	3 %	NS

We could not demonstrate any significant differences between the groups. This may partly be due to the limited number of patients (80 + 212). A more powerful study would probably demonstrate difference in amount of VGS and maybe in amount of gastrointestinal toxicity.
The following sentence has been added into the text in the results section to lines 157 - 159: "On the other hand, no difference in number of febrile or neutropenic days, in amount of grade 3 & 4 gastrointestinal toxicity, peak CRP-values, or recovery of individual microbial strains was found between those patients with active disease and those in remission in the second cycle".

The reviewer asks about the interpretation about the skin contaminants found in blood cultures. Due to the characteristics of the register data we cannot analyze more specifically these blood culture findings. Unfortunately we have to accept decisions of attending physicians.
We have chanced the text about interpretation of blood cultures in the methods section: "Whether blood culture findings were true or contaminant was accepted by attending physicians." is added to the text in the line 114.

The reviewer points to the possible inability of the WHO criteria to detect gastrointestinal toxicity caused by chemotherapy. "Another explanation to our surprising findings may be that measuring toxicity by the WHO method is not sensitive enough for identifying this kind of changes in gastrointestinal tract." is added in the text into the discussion section to lines 212 - 214.

The question, whether the patients who died, did so before acquiring BSI was analyzed in cycles one and two: During the first cycle 13 patients died of whom BSI data of 11 patients were available and five had BSI. During the second cycle, eleven patients with induction treatment died, of whom eight had BSI. Taken together, the interesting idea made by the reviewer, did not seem to explain our results.

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Blood Stream Infections during Chemotherapy-Induced Neutropenia in Adult Patients with Acute Myeloid Leukemia: Treatment cycle matters

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Appendix

The other investigators and centers of the Finnish Leukemia Group collaborated in this study: Liisa Volin and Tapani Ruutu, Helsinki; Timo Siitonen and Eeva-Riitta Savolainen, Oulu; Vesa Lindström, Tampere; Tarja-Terttu Pelliniemi, Turku.

ABSTRACT

Purpose: To assess the frequency of bloodstream infections (BSI) during neutropenia in different cycles of intensive chemotherapy treatment in acute myeloid leukemia (AML).

Methods: Register data of 327 consecutive patients aged 16-66 years having *de novo* AML between September 1992 and December 2001 was prospectively gathered in five Finnish tertiary care leukemia centers. The patients had not received fluroquinolone prophylaxis. Reported BSI rates were compared during neutropenia in four chemotherapy treatment cycles (C).

Results: There were 956 treatment episodes with 456 (47.7%) positive blood cultures. BSI was monomicrobial in 327 episodes (71.7%) and polymicrobial in 129 (28.3%). The overall incidence rate (per 1000 hospital days) for BSI was 13.2, varying from 6.8 in CI after idarubicin, conventional-dose cytarabine and thioquanine to 15.6 in CII, 15.8 in CIII and 17.6 in CIV. The distribution of monomicrobial gram-positive BSIs was as follows: CI; 71.7%, CII; 62.8%, CIII; 53.3%, CIV; 36.6% and CI-IV together; 43.2%. The most common finding in the four different cycles was coagulase-negative staphylococci (38.3% to 30.6%). Viridans group streptococci were most commonly observed (in 20.4% of positive blood cultures) during CII after high-dose cytarabine and idarubicin treatments. The distribution of monomicrobial gram-negative BSIs was as follows: CI; 21.7%, CII; 36.3%, CIII; 45.7%, CIV; 46.9%, and CI-IV together; 37.9%. A great variation of incidence and types of microorganisms between AML chemotherapy cycles was found.

Conclusions: It would be more reasonable to analyze chemotherapy cycle-based BSI results rather than overall results.

Key words: acute myeloid leukemia, adult, chemotherapeutics, neutropenia, bloodstream infection

INTRODUCTION

As early as in the sixties, the importance of severe gram-negative bacterial blood stream infections (BSI) during intensive chemotherapy of acute myeloid leukemia (AML) was identified [1]. In the seventies, empirical treatment protocols were introduced to treat BSIs caused by gram-negative microorganism in neutropenic fever patients [2]. During the next two decades, the causes of BSIs shifted from gram-negative rods to gram-positive cocci [3, 4, 5, 6, 7, 8, 9]. The most important reason for this change has been explained to be due to the use of antibiotic prophylaxis during periods of neutropenia in AML patients. Interestingly, the newest results suggest that the proportion of gram-negative infections is once again increasing [10].

According to the knowledge of the authors, little attention has been paid to BSI rate during different chemotherapy cycles. When the febrile episodes of the so called SIGNIFICANT study [11] were later evaluated, it was found that during the chemotherapy of solid tumors and lymphomas the risk of a febrile episode, as a sign of possible BSI and an indication for empirical antibiotic treatment, was highest during the first treatment cycle, especially in testicular and small-cell lung cancers [12]. It was also noted that if the patient had a febrile episode during the first chemotherapy cycle, he or she would have an increased risk of a febrile episode during the subsequent cycles [12].

In solid tumors and lymphomas patients receive repeated cycles of the same chemotherapeutics, whereas in AML different chemotherapeutics are administrated in consecutive cycles in order to achieve a favorable outcome. As has been seen earlier in a small, one center study, during AML treatment with an idarubicin, conventional-dose cytarabine and thioquanine schedule, the BSI rate was lower than after other regimens [13] whereas high-dose cytarabine treatment was associated with a high BSI rate. The present study was based on the nationwide AML-92 register data collected by the Finnish leukemia group [14], in which 327 AML patients with 956 chemotherapy episodes were included for the evaluation of BSI rates. None of the patients had received any antibiotic prophylaxis during chemotherapy-induced neutropenia, which allowed us to observe the real BSI results in four consecutive chemotherapeutic cycles.

MATERIALS AND METHODS

Background. The study protocol and hematological results of this nationwide study population of 327 consecutive intensive chemotherapy-treated patients, of whom 82% experienced complete remission, has earlier been published elsewhere [14].

Study design. The study protocol is briefly explained as follows (for a more extensive description, see ref. [14]: To be included the patient had to have *de novo* AML diagnosed by standard cytological criteria according to French-American-British (FAB) classification [15], and be between 16 an 65 years old. The study protocol was approved by the ethical committees of all five participating centers. Moreover, the National Agency for Medicines scrutinized the protocol before the beginning of the study. All patients provided informed consent.

During the first induction course the chemotherapeutics used were idarubicin, conventional-dose cytarabine and thioguanine. The second course, which was either first postremission or second induction course, included high-dose cytarabine and idarubicin. The third course included etoposide, high-dose cytarabine and mitoxantrone and the fourth course amsacrine and high-dose cytarabine. The use of supportive treatments (i.e. growth factors, antibiotics, alimentation and blood products) were administered according to the policies of each of the five hematological university centers. All patients had a central venous access. The infections were treated according to generally accepted guidelines [16, 17]. The patients did not receive any antibiotic prophylaxis during chemotherapy-induced neutropenia. For this study the following information was used from the prospectively gathered register data of the AML patients recruited for the original study between September 1992 and December 2001: age at the time of diagnosis, sex, treatment faculty, FAB classification of AML, whether or not the patient had an infection at the time of AML diagnosis and whether or not the patient had a positive blood culture (in ten episodes data was missing). Moreover, the duration of hospital stays, neutropenia (days when absolute neutrophil count was less than 500/µL), febrile days (body temperature $\geq$ 38 °C), peak C-reactive protein level (mg/L) and outcome of the patients were analyzed. Furthermore, gastrointestinal toxicity and the severity of infection categorized according to WHO criteria was recorded in each episode [18].

Microbiological methods. Blood was cultured at participating hospitals using automated continuous-monitoring blood culture systems (CMBCS): in two centers with BacT/Alert ™ (bioMérieux INDUSTRY, Hazelwood, MS) and in three centers with Bactec 9240 ™ (BD Diagnostics**,** Diagnostic Systems, Sparks, MD). The microorganisms were identified by standard microbiological methods based on growth conditions and appearance on solid media, fermentation of sugars and other organic compounds as well as the use of commercial kits. Wheter blood culture findings were true or contaminant was accepted by attending physicians. If more than one micro-organism was recovered from blood cultures of a patient during a chemotherapy cycle, the episode was defined as polymicrobial.

Statistical analysis.

The summary measurements for continuous and ordinal variables are expressed as medians with 25th and 75th percentiles unless otherwise stated. Fisher's exact test was used to analyze simple categorical data. The generalized linear mixed model approach was utilized in comparison of presence of positive BSI and toxicity in different chemotherapy cycles and the linear mixed model approach was used when comparing continuous variables (maximum CRP, duration of neutropenia, length of hospital stay and duration of fever days) between chemotherapy cycles. Both mixed-model approaches take into account that measurements are repeatedly taken from the same subjects and these within-subject measurements are not independent [19]. Furthermore, both methods use all data available, i.e., subjects with missing information are not dropped out from analyses. Incidence rates (per 1000 hospital days or 1000 neutropenic days) with 95% confidence intervals were calculated. If the number of hospital days or neutropenic days was missing it was replaced by the cycle average. Statistical analysis was performed using SPSS (SPSS, version 14.0, SPSS Inc, Chicago, IL) and SAS (version 9.1.3, SAS Institute Inc., Cary, NC) statistical software.

RESULTS

The study population included 327 AML patients and their most common FAB-types were M2 with 32.1%, M1 with 23.9% and M4 with 23.9%. There were 166 men and 161 women. Their median age was 47.7 yrs with a range from 16 to 66 years of age. More detailed hematological results are presented elsewhere [14]. WHO grade IV infections with signs of organ failure, were observed among 22 patients in cycle I (6.7%), 12 patients in cycle II (4.1%), 13 patients in cycle III (6.1%) and 9 patients in cycle IV (7.4%).

During the study period, there were 40 deaths (12.2%), of which 35 (87.5%) were due to infection. 12 patients died due to infection during cycle I. Five of these deaths were due to BSIs. Of the 14 patients who died due to infection in cycle II, 10 were treated with the second induction treatment for active disease. 7 of them had BSI. Only 4 patients treated with consolidation treatment in cycle II died because of infection. 1 of those infections was BSI. In cycle III, five deaths were due to infections, of which three were BSIs. In cycle IV four patients died due to infection, with two BSIs among them.

In total there were 956 treatment episodes with 456 positive blood cultures, constituting a rate of 47.7% of BSI. The overall incidence rate (per 1000 hospital days) for BSI was 13.2 (95% confidence interval 12.0 to 14.5). When the cycles were compared, the median peak C-reactive protein level did not differ between the cycles, but the duration of febrile days was longest during the first cycle (Table 1). The length of hospital stay, the incidence rate for BSI per 1000 patient days or per 1000 neutropenia days were clearly higher in cycles

II, III and IV than in cycle I (Table 1). The odds ratio for BSI (compared to cycle I) was 5.8, 5.5 and 4.8 in cycles II, III and IV, respectively ($P < .001$, in all) (Table 1). Severe gastrointestinal toxicity (WHO grade III and IV) was highest during cycle I and decreased significantly in successive cycles, the odds ratios compared to cycle I being 0.65 at cycle II ($P = .041$), 0. 43 at cycle III ($P < .001$) and 0.33 at cycle IV ($P < .001$) (Table 1, Figure 1).

During the first chemotherapy cycle, the number of BSI was slightly higher, if the patients did not have infections at the time of AML diagnosis (25.6%) than those patients having infections (19.3%, $P = .17$; Figure 2). During the second cycle, the BSI rate was still slightly higher in the patients who had already achieved remission (64.6%) than among those who received the second induction course of chemotherapy (53.6%, $P = .11$; Figure 2). On the other hand, no difference in number of febrile or neutropenic days, in amount of grade 3 & 4 gastrointestinal toxicity, peak CRP-values, or recovery of individual microbial strains was found between those patients with active disease and those in remission in the second cycle.

There was a great inter-cycle variation among positive blood cultures (from 23.3% to 61.6%) as well as for monomicrobial BSIs (from 62.2% to 83.3%) and for polymicrobial BSIs (from 16.7% to 37.2%). In monomicrobial BSIs, the proportion of gram-positive microorganisms decreased from cycle I (71.7%) to cycle IV (36.6%), whereas the change was in opposite direction among gram-negatives; from 21.7% in cycle I to 46.9% in cycle IV (Table 2). The most common gram-positive microorganisms in monomicrobial BSIs were CoNSs and viridans group streptococci; the latter was most frequently detected during cycle II. The most common gram-negative microorganisms were *E. coli* (in cycle III and IV) and *Klebsiella* species (in cycles II and III). Anaerobic microorganisms were seen only in cycle I and some yeasts in cycles I-III (table 2).

Most polymicrobial BSIs contained two microorganisms (Table 3). The most common combination included both gram-positive and gram-negative microorganisms in each cycle (41.8% to 64.3%). Although there was once again great variability between the cycles; the proportion of gram-negative microorganisms was always higher than 50% (from 52.3% to 82.1%).

When the BSI results obtained by two different automated blood systems were compared, there was not any statistical difference between them concerning the cycles or individual microorganisms (data not shown).

DISCUSSION

Our results show that during the chemotherapy courses of adult AML patients, the BSI rate and yielded microorganisms vary greatly from cycle to cycle, probably reflecting the tendency of different chemotherapeutics to predispose patients to infections. Thus, the BSI results in AML should be evaluated based on cycles rather than on the overall results.

Our study has several strengths: It was a prospective multi-center study with a large homogenous study population of 327 adult *de novo* AML patients treated according to the same schedule of chemotherapeutic protocol [14]. The patients had not received any prophylactic antibiotics for neutropenia, which allowed us to study the real influence of different chemotherapeutic cycles on BSI rates during neutropenia. Our statistical approach with linear and generalized linear mixed models took into account that the same subjects were evaluated repeatedly, i.e., the measurements of the same patients are not independent. On the other hand, our register data did not include the detection day of BSI from the onset of neutropenia. The other weakness of this study was that gathered data did not include the resistance patterns of detected microorganisms or systematic information regarding the mucositis of the oral cavity of the study subjects.

Our BSI proportion of 47.7% is higher than has been thus far reported; the figures in other studies have varied from 10% to 38.8% [11, 20, 21, 22, 23, 24, 25]. The crucial questions from the point of view of BSI frequency are study population (only leukemia patients or also patients with solid tumor and lymphomas) and whether the patients had received antibiotic prophylaxis during neutropenia. For example, in a large multi-center study with solid tumors and lymphomas, the BSI rate was 6.2% in the levofloxacin group and 7.9% in the placebo group [11]. In another multi-center study, patients having acute leukemia and a duration of neutropenia of at least 7 days, BSI rate was 20.6% in the levofloxacin group and 38.8% in the placebo group [21]. In our study, the median duration of neutropenia was more than three weeks in each cycle, which is a well-known and real risk of higher BSI rate [1]. In one of our centers, the BSI rate during neutropenia among AML patients increased from 21% to 40% when a manual blood culture system was replaced by a CMBCS [26]. Taken together, we consider that our BSI rate is realistic and moreover, our BSI incidence rate of 13.2 per 1000 patient-days is rather near to the corresponding figure of 10.2 published earlier [27].

When the BSI results were analyzed separately in each cycle, the recovery of micro-organisms during the first chemotherapy-induced neutropenia was clearly lower than in other cycles. This phenomenon was relevant when also incidence rate per 1000 patient-days or per 1000 neutropenia-days was calculated. Our result was rather surprising , when gastrointestinal toxicity (WHO grade III and IV) was taken into account. High-dose cytarabine is well-known for its gastrointestinal toxicity. In the present data, the toxicity was highest in the

first cycle. It should have been lowest, because the dose of cytarabine in the first cycle was conventional rather than high, as in later cycles. As gastrointestinal toxicity was highest in the first cycle, also the recovery of micro-organisms should have been highest, but that was not the case. We can give no exact explanation for these observations, but we can speculate that leukemia per se may have something to do with these findings. In the first cycle all patients had active disease. Altered intestinal permeability and absorption of sugars have been demonstrated in patients with AML even without treatment [28, 29]. Another explanation to our surprising findings may be that measuring toxicity by the WHO method is not sensitive enough for identifying this kind of changes in gastrointestinal tract. On the other hand, in the present data, 63 % of deaths due to infection occurred in patients receiving induction treatment. It seems that outcome of infections is worse among AML patients with active disease than among those who have achieved remission.

During the last few decades there has been a clear shift from gram-negative BSIs to gram-positive ones in neutropenic infections, which has been suggested to be due to the increased use of intravascular devices, fluoroquinolone prophylaxis and chemotherapy-induced mucositis [9]. When all cycles were analyzed together, in monomicrobial BSIs, the proportion of gram-positive microorganisms was 43.2%, and the proportion of gram-negatives was 37.9%. The corresponding figures in a recent study among patients with hematological malignancies and solid neoplasms in hospitals in the USA with neutrophils of less than 1000/µL were 61% and 24.9%, respectively [8]. In our series, from cycle I to cycle IV, the numbers of gram-positives decreased (from 71.7% to 36.6%) and gram-negatives increased (from 21.7% to 46.9%). The great proportion of gram-negatives in cycles III and IV was unexpected, especially because severe gastrointestinal toxicity (WHO III and IV) decreased from cycle to cycle. *E. coli* and *Klebsiella* species were the most commonly yielded gram-negatives, as has been shown in several earlier series.

Of the gram-positive microorganisms, CoNSs were the most common finding also in our series in general or in the four different cycles. During the last few decades, much attention has been paid to the importance of *viridans* group streptococci as a cause of bloodstream infections during neutropenia. In several studies, it has been associated with high-dose cytarabine chemotherapy [30, 31, 32] and quinolone prophylaxis [7]. In our sudy, the incidence of BSIs caused by *viridans* group streptococci was highest during the second cycle with high-dose cytarabine and idarubicin and clearly lower in cycle IV, which also included high-dose cytarabine and amsacrine. Thus, the combination of chemotherapeutics and not only the high-dose cytarabine *per se* seems to be important for BSIs caused by viridans group streptococci.

It has been recently noted that the knowledge of polymicrobial infections among cancer patients is sparse [33]: In trials involving neutropenic patients, the proportion of polymicrobial BSIs has varied from 8% to 32% [34, 35]. Our overall figure of polymicrobial BSIs was 28.3%. However, there was once again substantial inter-cycle variation. In three-fourths of the episodes, polymicrobial BSIs were caused by two microorganisms. The most common combination included both gram-positive and gram-negative microorganisms and the second most common included at least two gram-positive microorganisms together.

When polymicrobial BSIs were also taken into account in our study, which included the series of AML patients recruited between 1992 and 2001, the portion of gram-positive microorganisms in BSIs was even higher (79.6%) than in the earlier-mentioned study from the USA: their highest figure (76%) was from the year 2000 [8]. The result of 50.9%of gram-negative organisms when polymicrobial BSIs were also included, is quite similar to a recent study [36], patients were also treated without antibiotic prophylaxis. In another study the proportion of gram-negatives was 52% without and 25% with antibiotic prophylaxis [23].

In conclusion, our AML patients had a high overall BSi rate during chemotherapy regimen-induced neutropenia. However, there was great variation in BSI findings in four treatment cycles with different chemotherapeutics: BSI rate was lowest in cycle I and proportions of microorganisms and polymicrobial BSIs varied greatly from cycle to cycle. Therefore, in order to obtain more precise information concerning BSIs during the intensive chemotherapeutic treatments of AML, the analysis of BSI results should be focused on different chemotherapy cycles rather than by analyzing the overall results.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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TABLE 1. Length of hospital stay, duration of febrile episodes and neutropenia, the serum peak value of C-reactive protein, incidence rate (IR) of blood stream infections (BSI) and odds ratios (OR) for the risk of BSI and gastrointestinal toxicity in different treatment cycles (cycle I as a reference);

	Cycle I [a]	Cycle II	Cycle III	Cycle IV	*P*[b]
Hospital days [c]	30 (28-36)	37 (31-44)	34 (27-44)	31 (25-38)	<.001
IR of BSIs / 1 000 days (95% CI) [d]	6.8 (5.4 to 8.6)	15.6 (13.4 to 18.1)	15.8 (13.3 to 18.8)	17.6 (13.8 to 22.2)	-
Neutrophils < 500/ µL, days [c]	24 (20-28)	26 (19-32)	25 (17-37)	22 (18-29)	<.001
IR of BSIs/ 1 000 neutropenic days (95% CI) [d]	9.2 (7.2 to 11.6)	23.2 (20.0 to 26.9)	21.3 (17.8 to 25.3)	23.6 (18.4 to 29.7)	-
Temperature ≥ 38°C, days [b]	9 (5-14)	7 (3-11)	6 (3-10)	7 (3-9)	<.001
C-reactive protein (mg/L, peak value) [c]	164 (119-230)	161 (115-206)	158 (113-210)	160 (107-205)	.30
OR for BSI (95% CI) [d]	1.0	5.8 (4.1 to 8.2)	5.5 (3.8 to 8.0)	4.8 (3.1 to 7.4)	
P [e]		<.001	<.001	<.001	
OR for gi-toxicity, grade III and IV (95% CI) [d]	1.0	0.65 (0.43 to 0.98)	0.43 (0.27 to 0.70)	0.33 (0.17 to 62)	
P [e]		.041	<.001	<.001	

1. [a] chemotherapeutics: cycle I; idarubicin, cytarabine and thioguanine, cycle II; high-dose cytarabine and idarubicin, cycle III; etoposide cytarabine and mitoxantrone, Cycle IV; amsacrine and high-dose cytarabin.

[b] *P*-time according to Linear mixed model.

[c] values are medians with 25th -75th percentiles.

[d] values are means with 95% confidence intervals (95%CI).

[e] *P*-time according to the generalized linear mixed model (7).

TABLE 2. Detected microorganisms during different chemotherapy cycles; the number (%) of positive blood cultures per treatment episodes in each cycle.

	Cycle I (n= 323)	Cycle II (n=292)	Cycle III (n=219)	Cycle IV (n=122)	All (n=956)
Blood culture positive, number (%)	**72 (22.3)**	**180 (61.6)**	**133 (60.7)**	**71 (58.2)**	**456 (47.7)**
Monomicrobial	**60 (83.3)**	**113 (62.2)**	**105 (78.9)**	**49 (69.0)**	**327 (71.7)**
Gram-positive	43 (71.7)	71 (62.8)	56 (53.3)	26 (36.6)	197 (43.2)
- CoNS	23 (38.3)	28 (24.8)	34 (32.4)	15 (30.6)	100 (30.6)
- VGS	5 (8.3)	23 (20.4)	12 (11.4)	6 (12.2)	46 (14.1)
- *Enterococci*	7 (11.7)	9 (8.0)	7 (6.7)	4 (8.2)	27 (8.3)
Gram-negative	13 (21.7)	41 (36.3)	48 (45.7)	23 (46.9)	124 (37.9)
- *E. coli*	3 (5.0)	7 (6.2)	15 (14.3)	12 (24.5)	37 (11.3)
- *Klebsiella sp.*	2 (3.3)	16 (14.2)	20 (19.0)	5 (10.2)	43 (13.1)
- *Enterobacter sp.*	1 (1.7)	7 (6.2)	6 (5.7)	2 (4.1)	16 (4.9)
- *Pseudomonas aeruginosa*	3 (5.0)	7 (6.2)	1 (1.0)	3 (6.1)	14 (4.3)
Anaerobic microorganisms	3 (5.0)	-	-	-	3 (0.9)
Yeast	1 (1.7)	1 (0.9)	1 (1.0)	-	3 (0.9)
Polymicrobial	**12 (16.7)**	**67 (37.2)**	**28 (21.1)**	**22 (31.0)**	**129 (28.3)**

NOTE. CoNS, coagulase-negative staphylococci; VGS, viridans group streptococci. Among gram-positives and gram-negatives, only three most common microbes are mentioned, and among gram-negatives also the presence of Pseudomonas.

TABLE 3. Characterization of microorganisms of polymicrobial bloodstream infections in different chemotherapy cycles

Blood stream infections	Cycle I (n=12)	Cycle II (n=67)	Cycle III (n=28)	Cycle IV (n=22)	All (n=129)
Number of microorganisms					
- Two	8 (66.7)	49 (73.1)	24 (85.7)	16 (72.7)	97 (75.2)
- More than two	4 (33.3)	18 (26.9)	4 (14.3)	6 (27.3)	32 (24.8)
Combinations of microorganisms					
- Gram-positives only	5 (41.7)	27 (40.3)	5 (17.9)	3 (13.6)	40 (31.0)
- Gram-positive and anaerobic	-	4 (6.0)	-	-	4 (3.1)
- Gram-positive and yeast	-	1 (1.5)	-	2 (9.1)	3 (2.3)
- Gram-positive and gram-negative	6 (50.0)	28 (41.8)	18 (64.3)	11 (50.0)	63 (48.8)
- Gram-negatives only	1 (8.3)	4 (6.0)	3 (10.7)	5 (22.7)	13 (10.1)
- Gram-negative and yeast	-	3 (4.5)	2 (7.1)	1 (4.5)	6 (4.7)

Figure legends

FIGURE 1. Positive blood culture findings (%) during four chemotherapy cycles among adult acute myeloid leukemia patients. Gram-positive, gram-negative and other micro-organisms (yeast, anaerobes and polymicrobial). Gastrointestinal toxicity grade III and IV according to the WHO. The difference between positive blood cultures in cycle I vs. the other three, $P < 001$ each (multilevel generalized linear model). Cycle I; idarubicin, cytarabine and thioguanine, cycle II; high-dose cytarabine and idarubicin, cycle III; etoposide, cytarabine and mitoxantrone and cycle IV; amsacrine and high-dose cytarabine.

FIGURE 2. Comparison of positive blood culture findings in cycle I between patients having (Inf +) or not having infection (inf -) at the time of diagnosis of AML ($P = .17$), and in cycle II the comparison between those patients having achieved remission (rem +) and those still having an active disease (rem -, $P = .11$, Fisher's Exact Test, two-tailed).

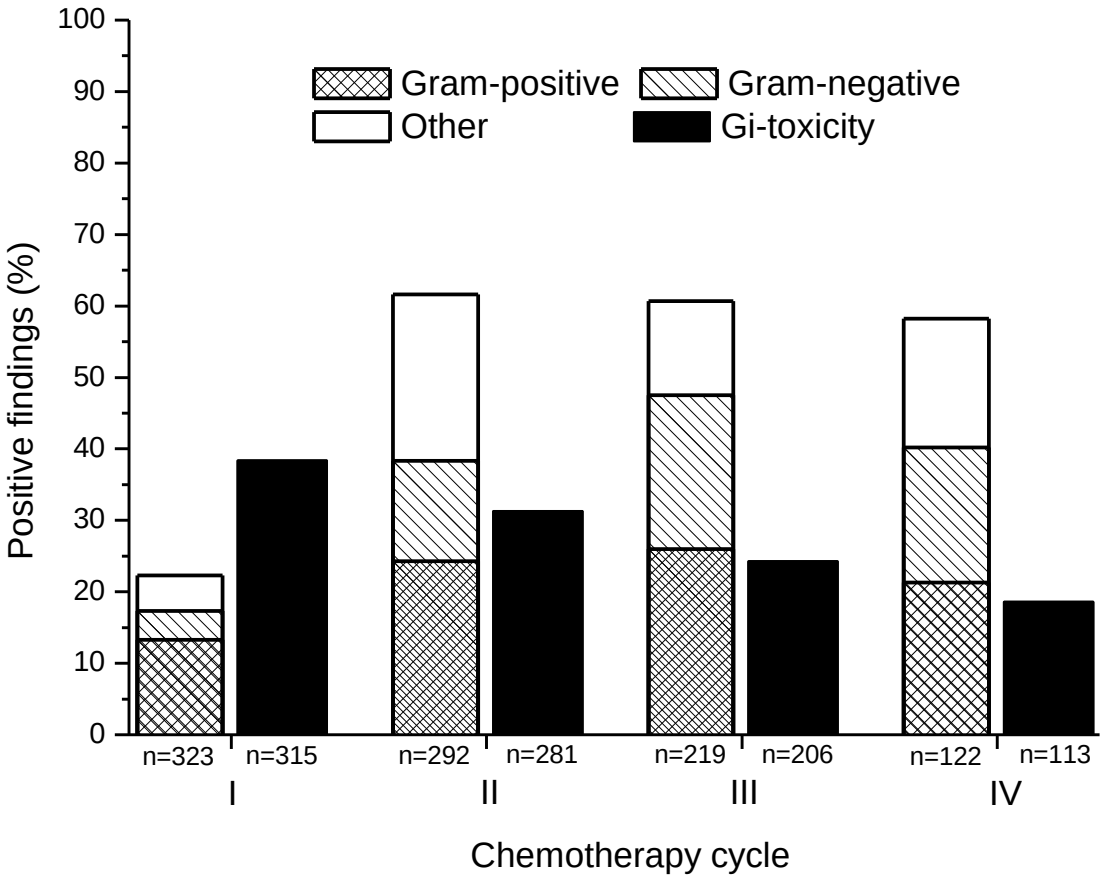
Gram-positive
Gram-negative
Other
Gi-toxicity
Positive findings (%)
100
90
80
70
60
50
40
30
20
10
0
n=323
n=315
n=292
n=281
n=219
n=206
n=122
n=113
I
II
III
IV
Chemotherapy cycle

Figure 1.

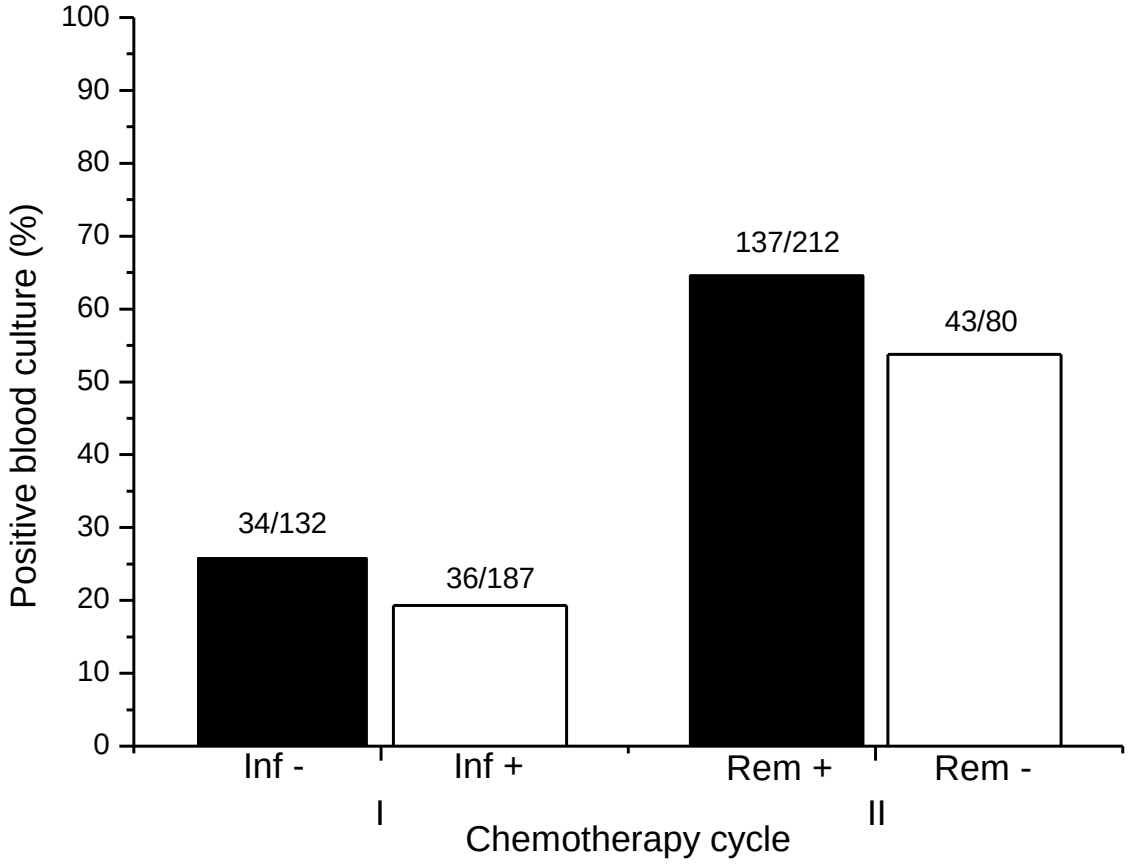
100
90
80
70
60
50
40
30
20
10
0
Positive blood culture (%)
34/132
36/187
137/212
43/80
Inf -
Inf +
Rem +
Rem -
I
II
Chemotherapy cycle

Figure 2.