Ciprofloxacin *versus* colistin prophylaxis during neutropenia in acute myeloid leukemia: two parallel patient cohorts treated in a single center

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Supplemental data

Supplemental materials and methods

Patients

A total of 172 consecutive patients with AML who received in-patient, intensive chemotherapy in the Department of Medicine A of the University Hospital of Muenster, and were at risk of developing chemotherapy-induced neutropenia lasting more than seven days, were included in this retrospective analysis. All patients provided written informed consent prior to the initiation of the anti-leukemic therapy. Approval for this analysis was obtained from the Ethics Board of the Westfalian Wilhelms-University Muenster and the Physicians Chamber of Westfalia-Lippe, Germany (approval file number 2015-695-f-S).

The patients were randomly allocated to receive antibiotic prophylaxis with colistin or ciprofloxacin. An individual patient's characteristics (e.g., disease status, treatment regimen) had no influence on his/her assignment to each treatment group. On the basis of vacancy, all patients were allocated to one of two different attending physician services within the same department; one treatment team used oral colistin, and the other administered ciprofloxacin. All other therapies and supportive care were provided to both groups, according to identical institutional guidelines.

Intensive induction or consolidation chemotherapy and an expected duration of neutropenia over 7 days were prerequisites for eligibility in this study. None of the chemotherapy regimens included lymphocyte-depleting substances. All patients had a confirmed diagnosis of AML according to the World Health Organization (WHO)/ French-American-British (FAB) classification. Patients with AML M3 were included only when they received an intensive protocol with expected neutropenia of more than seven days. Neutropenia was defined as a neutrophil count less than 500 cells/µl, and leukocytopenia was defined as a leukocyte count below 1,000 cells/µl when differential leukocyte counts were not available.

Chemotherapy

Chemotherapy was regarded as induction or consolidation therapy. Intensive induction or relapse therapy predominantly consisted of a combination of cytarabine (100 mg/m²/day, days 1-7) with either daunorubicin (60 mg/m², days 3-5; "3+7") or TAA with thioguanine (100 mg/m², q12 h, days 3-9) and amsacrine (210 mg/m², days 3-5) in a few patients. According to the specific study protocols, a high dose of cytarabine (3 g/m², q12 h, days 1-3; patients ≥60 years received only 1 g/m²) combined with mitoxantrone (10 mg/m², days 3-5) was administered as an alternative induction or reinduction protocol (HAM). Patients <60 years of age routinely received two induction courses, whereas patients aged 60 years and older received a second induction course only when they exhibited persistent bone marrow blasts (> 5%) on day 15 after starting the treatment.

Consolidation therapy consisted of a high-dose cytarabine treatment (<60 years: 3 courses of cytarabine 3 g/m², q12 h, days 1, 3, and 5; 60 years or older: only 2 courses of 1 g/m²).

Prophylactic regimen

Patients received antibiotic prophylaxis simultaneously with the start of chemotherapy. Upon hematological recovery (>500 neutrophils/µl or >1,000 leukocytes/µl), prophylaxis was stopped.

Patients in the colistin group were given 8 million IU daily, divided into four doses. Patients in the ciprofloxacin group were given at 500 mg twice daily. Both drugs were taken orally. In addition, both groups received prophylaxis for *Pneumocystis jirovecii* pneumonia with 960 mg of trimethoprim-sulfamethoxazole twice daily for two days per week. If an oral application was not practical, trimethoprim-sulfamethoxazole was replaced by a monthly inhalation of pentamidine. Additionally, patients in need of induction or relapse treatment received antifungal prophylaxis, primarily based on posaconazole. Unless they were necessary for the specific study protocols, myeloid growth factors, such as granulocyte colony-stimulating factor (G-CSF), and antiviral prophylaxis were not administered on a routine basis.

Infection

In the case of fever or a clinically ascertained infection, antibiotic prophylaxis was withdrawn and switched to an empirical, broad-spectrum antibiotic therapy. In most cases, piperacillin-tazobactam treatment in combination with an aminoglycoside was started and further adapted to the available microbiological findings and specific bacterial manifestations according to in-house recommendations. Simultaneously, radiographic and microbiological analyses were initiated. If indicated, the central venous catheters were removed or replaced.

If the patients did not respond to the first-line empirical antibiotic therapy and if clinically indicated, e.g., because of persistent fever or ongoing signs of infections with negative culture results, antibiotic therapy was further escalated primarily to a carbapenem (meropenem) combined with a glycopeptide (vancomycin). Simultaneously, the growth of multidrug-resistant organisms was monitored according to standard practice. Drug resistance was classified according to the definitions of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the national recommendations of the Robert-Koch Institute.[19] In detail, Gram-negative bacteria, such as *Pseudomonas aeruginosa*, were defined as multidrug-resistant when they were not susceptible to ≥3 of the following antimicrobial classes: penicillin + beta-lactamase inhibitors, extended-spectrum cephalosporins, carbapenems, and fluoroquinolones. Furthermore, treatments with antiviral and/or antifungal agents were initiated or extended according to each patient's risk and microbiology results.

Definitions of variables

The definition of the Charlson Comorbidity Index has been published elsewhere.[20] The mucositis grade was judged daily according to the WHO oral toxicity scale,[21] and only mucositis grades III and IV were considered for analysis. The use of a central venous catheter was included in the analysis if it was present during neutropenia and/or the onset of infection.

Endpoints used in the analysis

The primary endpoint was a clinically documented infection requiring empirical antibacterial therapy, which was defined as the presence of at least two of the following criteria: a) fever during neutropenia (oral temperature above 38.3°C in a single measurement or ≥ 38.0°C in measurements taken over at least one hour), b) clinical signs of infections (e.g., hypotension, tachypnea, or tachycardia), and c) laboratory (e.g., an increase in c-reactive protein or procalcitonin levels) or microbiological findings. Because antibiotic prophylaxis may interfere with the culture results, microbiological findings were not mandatory because this would have resulted in the underreporting of infections.[13]

The secondary endpoints were microbiological findings (positive culture results), an infection-related need for intensive care medicine, and mortality as a result of any type of infection.

Statistical Analysis

Patient baseline characteristics are described by standard methods (absolute and relative frequencies, mean, standard deviation, median, interquartile range). Distributions of patient baseline characteristics in both prophylactic groups were compared using chi-square tests for categorical variables and Mann-Whitney U tests for the continuous variables.

The differences between groups were analyzed through statistical methods capable of modelling repeated measurements. Here, generalized estimation equations (GEEs) were applied. A first-order autoregression model with decreasing correlations between more distant time points was used as a working correlation matrix. Repeated measures were defined for each patient. The median length of hospital stay and median time until the onset of infection were also compared between the two treatment groups, by using the GEE models and applying suitable distribution assumptions. The median length of hospital stay was approximately normally distributed, whereas the length of hospital stay showed a right-tailed distribution. Therefore, for the latter variable, a negative binomial distribution was used for the GEE. Canonical link functions were used.

To assess the effects of colistin *vs.* ciprofloxacin on the time to infection (start of chemotherapy to the onset of infection), we used a shared frailty Cox proportional hazard

model for clustered data. Accordingly, the hospitalization duration was analyzed using a GEE model with a negative binomial link function and the corresponding canonical link.

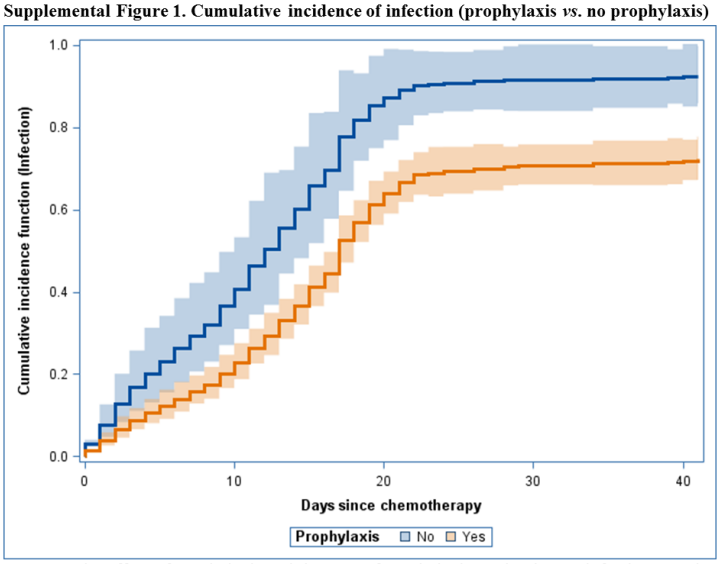
The binary primary outcome, infection, was analyzed, and the infection rates were estimated from the GEE model(s). The following variables were included in the univariate and multivariate analyses: prophylaxis (yes *vs.* no or colistin *vs.* ciprofloxacin), gender, usage of a central venous catheter, incidence of mucositis grade III/IV, therapy stage, age at admission, and Charlson Comorbidity Index. For the GEE analyses, Type-3 tests were interpreted for the models.

The association of prophylaxis type (colistin *vs*. ciprofloxacin) with the type of multidrug-resistant bacteria was assessed using Fisher's exact test. Although the data usually contained repeated measurements, this analysis resulted in a subset of independent measurements (no patients were analyzed more than once), thus enabling this test to be applied.

Unless otherwise stated, the significance level was alpha = 0.05 in all analyses. All statistical analyses were performed with IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA) and SAS software (Version 9.4, for Windows, SAS Institute Inc., Cary, NC, USA).

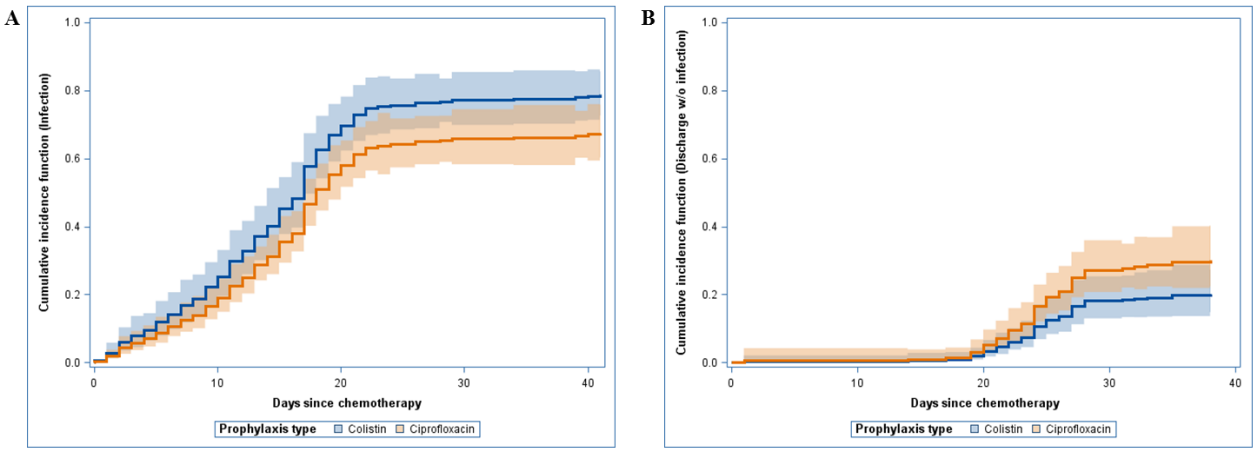
Supplemental figures

Supplemental Figure 1. Cumulative incidence of infection (prophylaxis *vs.* no prophylaxis)



To assess the effect of prophylaxis and the type of prophylaxis on the time to infection, we also applied competing risk analysis. Cumulative incidence functions (i.e., the probability of experiencing an event until a certain time) for the competing events of infection or discharge (w/o infection) were estimated using a proportional subdistribution hazards model. The effect of prophylaxis (yes/no) and the type of prophylaxis (ciprofloxacin vs. colistin) were tested within the model using Type III Wald tests. The cumulative incidence functions are displayed with 95% confidence bands (pointwise).

Supplemental Figure 2. Cumulative incidence of infection (A) and discharge without infection (B) (Colistin *vs.* Ciprofloxacin)



To assess the effect of the type of prophylaxis on the time to infection, we applied competing risk analysis. Cumulative incidence functions (i.e., the probability of experiencing an event until a certain time) for the competing events, infection or discharge (w/o infection), were estimated using a proportional subdistribution hazards model. The effect of the type of prophylaxis (ciprofloxacin vs. colistin) was tested within the model using Type III Wald tests. The cumulative incidence functions are displayed with 95% confidence bands (pointwise).