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STATEMENTS AND DECLARATIONS

The authors do not declare any conflict of interests.

EG and DL contributed to the conceptualization of the protocol, the investigation, the interpretation of the statistical analysis and wrote (original draft) the manuscript.

NG participated in the investigation, the interpretation of the statistical analysis and writing (reviewing and editing) of the final manuscript.

YT performed the formal analyses, participated in their interpretation and wrote (reviewing and editing) the final manuscript.

ML and BK participated in the investigation and writing (reviewing and editing) of the final manuscript.

DS, BS, TC, ML, EB, ALL contributed to the conceptualization of the protocol and wrote (reviewing and editing) the final manuscript.

All authors gave their final approval for the version of the manuscript to be submitted.

ETHICAL DECLARATION

The study was approved by a national expert committee (reference 2019-06-01) and was declared to the CNIL (Comité national de l'informatique et des libertés, reference 2213058 v 0)

SUMMARY

Purpose. Major bleedings have been described with cefazolin. The objective was to determine the frequency of bleeding events in cefazolin-treated patients and to identify risk factors for these complications.

Methods. Monocenter prospective observational study of all consecutive cefazolin-treated patients. Patients benefited from a daily clinical assessment of bleedings and a twice-a-week blood sampling including hemostasis. Bleedings were classified according to the International Society on Thrombosis and Hemostasis classification: major, clinically relevant non-major bleedings (CRNMB) and minor bleedings.

Results. From September 2019 to July 2020, 120 patients were included, with a mean age of 59.4 (± 20.7) years; 70% of them (84/120) were men. At least 1 CRNMB or major bleeding were observed in 10% of the patients (12/120). Compared to patients with no or minor bleeding, patients with CRNMB or major bleeding were, upon start of cefazolin, more frequently hospitalized in an intensive care unit (7/12, 58.3%, vs 12/108, 11.1%, P < 0.001, respectively) and receiving vitamin K antagonists (4/12, 33.3%, vs 8/108, 7.4%, P = 0.019, respectively). After multivariate analysis, patients receiving vitamin K antagonists the day prior bleeding and/or treated for endocarditis were factors associated with an increased risk of CRNMB or major bleeding (Odd ratio 1.36, confidence interval 95%, 1.06–1.76, P=0.020 and 1.30, 1.06–1.61, P= 0.015, respectively).

Conclusion. Bleeding events associated with cefazolin treatment are frequent. Close clinical monitoring should be performed for patients treated for endocarditis and/or receiving vitamin K antagonists. Hemostasis work-up could be restricted to these patients.

1. INTRODUCTION

Cefazolin, a first-generation cephalosporin, was initially used for surgical prophylaxis [1] with a good safety profile [2,3]. Since 2015, American and European guidelines proposed cefazolin as an alternative to penicillinase-resistant penicillins ([Flu]cloxacillin or oxacillin) for the treatment of endocarditis caused by methicillin-susceptible *Staphylococcus* spp. [4,5]. Because of recurring (Flu)cloxacillin or oxacillin stock-outs and rising questions on their safety profile (liver and kidney toxicity), an increasing number of centers positioned cefazolin as a first-line therapy with good efficacy in observational studies [6–8]. An on-going prospective non-inferiority trial is currently enrolling adult patients with methicillin-susceptible *S. aureus* bloodstream infection in order to compare the efficacy of cefazolin and penicillinase-resistant penicillins [9].

Soon after commercialization, reports signaled prolonged prothrombin time (PT), of up to 20%, eventually associated with major bleedings [10,11]. With the increasing number of patients exposed to cefazolin for longer durations, case reports of bleeding in cefazolin-treated patients seemed to increase [12,13]. In 2017, the Summary of Product Characteristics was modified to stipulate monitoring of PT and vitamin K supplementation if required [14]. The suspected pathophysiological mechanism is the inhibition of glutamate carboxylation, a vitamin-K dependent reaction required for the formation of coagulation factors. This inhibition would be caused by thiol heterocyclic metabolites of various cephalosporins, including cefazolin [15,16], unrelated to vitamin-K antagonist anticoagulants.

A recent retrospective monocenter cohort reported 7 major bleedings for 132 included patients (5%) with a significant increase in the activated partial thromboplastin time (aPTT)

[17]. However, the assessment of bleedings was not standardized and hemostasis work-up was not complete (no coagulation factors, D-dimer nor fibrinogen measurement).

Therefore, we performed a prospective study with the primary objective to measure the frequency of major and clinically-relevant non-major bleedings (CRNMB) in cefazolin-treated patients. Our secondary objective was to identify risk factors for CRNMB or major bleeding in this population.

2. MATERIAL AND METHODS

2.1. Study design and inclusion process

This monocenter prospective cohort took place in a 700-bed teaching hospital from September 16th, 2019 to July 8th, 2020. All adult patients (> 18 years-old) were included if they were treated > 48 hours with cefazolin, except for refusal of the patient. Non-inclusion criteria were: *i)* septic shock upon cefazolin initiation, defined by persistent mean arterial pressure < 65 mmHg after fluid resuscitation requiring vasoactive drugs and lactate level > 2 mmol/L [18], *ii)* patients treated > 72 hours before inclusion, *iii)* hospital length of stay < 48 hours and *iv)* estimated life expectancy < 14 days. The sample size was calculated based on the expected 5% prevalence for major bleedings [17] according to the formula : $n = (Z^2 \times p \times q) / d^2$, where n = sample size; Z = 1.96, Z statistic for a level of confidence; p = expected prevalence; q = 1 – p, 0.95 ; d = precision, 0.05 [19]. The minimal number of patients to be included in the study was 73 patients. To avoid loss of data and enable comparison, we decided to include 120 patients, based on the capacity of recruitment evaluated in our hospital. To ensure inclusion of every consecutive patient, daily information was communicated to the principal investigator (EG) by the microbiologist (all *S. aureus* positive blood culture and/or bone and joint biopsy), by the pharmacist (every cefazolin initiation)

and by physicians of the antimicrobial stewardship program (every intervention about a cefazolin-treated patient).

As part of the daily routine of the infectious disease team, clinical rounds were performed each day in the three intensive care units of the hospital, the orthopedics unit devoted to bone and joint infections and the cardiology and cardiovascular surgery departments. These rounds enabled further identification of patients. Patients could be included several times if they were treated with cefazolin more than once, with ≥7 days of cefazolin-free interval between each inclusion.

2.2 Routine management

In our institution, cefazolin was established in 2016 as the first-line treatment of severe infections caused by methicillin-susceptible *Staphylococcus* spp. (endocarditis, bloodstream infection, bone and joint infection). Usual cefazolin dosage was prescribed, with adaptation to the estimated glomerular filtration rate (eGFR, using the Modification of Diet in Renal Disease (MDRD) equation, cut-off 30 mL/min/1.73m^2): 2 grams IV bolus followed by a continuous infusion of 80 mg/kg/day dose for patients with an eGFR >30 mL/min/1.73m^2 and 20 mg/kg twice daily for eGFR <30 mL/min/1.73m^2. For patients with uncomplicated *S. aureus* bloodstream infection, an oral step-down was proposed after 5-7 days of intravenous therapy.

2.3 Biological work-up

A formatted work-up was implemented for this study in order to facilitate prescription and limit missing data. The twice-weekly biological work-up included complete blood count, PT, aPTT, coagulation factors II (FII), V (FV), VII (FVII) and X (FX), fibrinogen using STAR-Max coagulometers (Diagnostica Stago, France). D-dimer were measured using the Vidas D-

Dimer® assay (Biomérieux, France). Alanine amino-transferase (ALT), aspartate amino-transferase (AST), Gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), conjugated and free bilirubin and serum creatinine levels were measured using UniCel DxI 800 Access Immunoassay System (Beckman-Coulter, USA). Plasmatic cefazolin concentration was performed using Liquid Chromatography coupled to tandem Mass Spectrometry (LC-MS/MS). Sampling was taken at any time of the day for continuous cefazolin recipients and minutes before next administration for intermittent cefazolin recipients. Plasma cefazolin target concentration at steady state was 40-80 mg/L [20].

2.4 Clinical evaluation

After inclusion, patients benefited from a daily clinical assessment (bleeding, quick Sepsis-related Organ Failure Assessment (qSOFA), edema). All data were prospectively compiled on an electronic Case Report Form hosted on the REDCap platform of our center [21] (**Supplementary method A**).

2.5 Bleeding classification

The International Society on Thrombosis and Hemostasis (ISTH) classification was used to describe bleeding events. The ISTH defines a major bleeding by a fatal bleeding, and/or symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intra-articular or pericardial, or intramuscular with compartment syndrome, and/or bleeding causing a fall in hemoglobin levels of 20 g/L or more, or leading to a transfusion of 2 units or more of whole blood or red cells [22]. A CRNMB is defined as a bleeding not falling under the definition of major bleeding but either requiring medical intervention by a health care professional or leading to increased level of care. “Prompting a face to face evaluation” was not retained as a part of the definition of CRNMB in our study

since every patient benefited from a daily clinical assessment, regardless of their bleeding status [23]. A minor bleeding corresponded to any bleeding not classified as major or CRNMB.

2.6 Pharmacovigilance

When a major bleeding or a suspected adverse event was diagnosed in a cefazolin-treated patient, the case was declared to our local pharmacovigilance department, as part of the usual care by the physician in charge of the patient.

2.7 Statistical analysis

For each patient, the first occurrence of the most severe bleeding was considered for group comparison. We compared two groups: "no bleeding or non-clinically relevant bleeding" and "major or CRNMB". Mean (standard deviations, SD) were used for continuous variables. Categorical variables were expressed as number and percentages. An independent analyst performed a univariate analysis using the Fisher or Chi-squared tests for categorical variables and the t-test Student for continuous variables or the Wilcoxon-Mann-Whitney test when a non-parametric test was required. Then, he performed a multivariate analysis based on the minimization of Akaike's Information Criterion. Using multivariate logistic regression tables, we calculated odd-ratios (ORs) with 95% confidence interval (95% IC). All statistical tests were performed with R language on R Studio Software (R Core Team 2021, v4.0.4).
The STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement was used to report this observational study (**Supplementary method B**) [24].

2.8 Ethics

The study was approved by a national expert committee (reference 2019-06-01) and was declared to the CNIL (Comité national de l'informatique et des libertés, reference 2213058 v

0). Patients were informed of the present study and could refuse to participate at any time. Patient confidentiality was ensured with anonymization of their clinical record.

3. RESULTS

3.1 Description of the population and characteristics of cefazolin-treated infections

From September 16th, 2019 to July 8th, 2020, among 179 consecutive patients screened, 120 were included in the study, with a mean age of 59.4 (± 20.7) years and 70% of them (84/120) were men (**Figure, Table 1**). Three patients were included twice during the study period, with cefazolin-free intervals of 7, 7 and 194 days, respectively. Patients were mainly hospitalized in surgical wards (58/120, 48.3%), and 15.8% (19/120) were hospitalized in intensive care units (ICU). Mean cefazolin duration was 8.2 (SD ± 5.1) days.

Cefazolin-treated infections were mainly bone and joint infections (50/120, 41.7%), followed by catheter-related infections and endocarditis (18/120, 15%, and 11/120, 9.2%, respectively). Sixty-three of the 120 (52.5%) patients had positive blood cultures.

3.2 Description of bleedings

Twelve patients (10.0%) experienced major or CRNMB, with a median number of bleedings of 0.65 (±1.45). Overall, 16 major and 3 CRNMB occurred. Major bleedings mostly involved deep organs (upper gastrointestinal tract, hematuria and visceral hematoma in 3, 2 and 2 patients, respectively) and bone and joints (hemarthrosis and bleeding of a leg amputation wound in 1 patient each) (**Supplementary Table A**). Of note, two intracranial bleedings occurred. Three CRNMB occurred: 1 catheter-related bleeding, 1 epistaxis and 1 bleeding of a leg amputation. Minor bleedings are described in **Supplementary Table A**. Major and CRNMB bleedings resulted in decreased hemoglobin count for 7 patients, including 2 patients with ≥ 30 g/L decrease. Red blood cells transfusion was performed in 4 patients, and platelets transfusion for 2 patients. Four patients were transferred to the ICU at the time of bleeding diagnosis (**Supplementary Table B**).

3.3 Risk factors associated with CRNMB or major bleedings

Compared to patients with no or minor bleeding, patients with CRNMB or major bleeding were, upon start of cefazolin, more frequently treated for endocarditis (4/12, 33.3%, vs 7/108, 6.5%, P=0.013), had more frequently a qSOFA score of 2 or more (4/12, 33.3%, vs 5/107, 4.7%, P = 0.006), were more frequently hospitalized in an ICU (7/12, 58.3%, vs 12/108, 11.1%, P < 0.001, respectively) and receiving vitamin K antagonists (4/12, 33.3%, vs 8/108, 7.4%, P = 0.019) (**Table 1**). As opposed to that, no significant difference was observed in patients receiving direct oral anticoagulants (1/12, 9.1%, vs 10/108, 9.3%, P = 1) (**Table 1**). In addition, they had more frequently positive blood cultures (11/12, 91.7%, vs 52/108, 48.6%, P = 0.005) (**Table 1**). Cefazolin duration did not impact on occurrence of bleeding (10 (SD ± 4.8) vs. 8.0 (SD ± 5.1) days, P = 0.19).

Patients with CRNMB or major bleedings were more frequently receiving vitamin K antagonists prior to bleeding (3/12, 25% vs 6/108, 5.6%, P= 0.046) compared to patients with no or minor bleeding (**Table 2**). No difference was noted in patients receiving direct oral anticoagulants (0/12, 0%, vs 5/108, 4.6%, P = 1) (**Table 2**). Regarding last known biological results prior to bleeding, patients with CRNMB or major bleedings had lower PT, FII and FV levels (67.8% ± 16.5 vs 81.8% ± 16.9, P = 0.015 (n=109), 66.2% ± 21.6 vs 105.5% ± 30.5, P = 0.005 (n=80), and 102% ± 17.3 vs 126.9% ± 37.6, P = 0.014 (n=80), respectively) compared to patients with no or minor bleedings. In contrast, there was no modification for fibrinogen nor D-dimer levels (**Table 2**). Furthermore, cefazolin concentration was more frequently supra-therapeutic (> 80 mg/L) in these patients (n=72, 2/4, 50% vs 8/68, 6.4%) (**Table 2**). Nonetheless, on the day of bleeding, we did not observe differences regarding the proportion of patients with supra-therapeutic cefazolin concentration (>80 mg/L) among the

different groups (no bleeding, minor, CRNMB and major bleeding, **Supplementary Figure**). When comparing biological data of patients upon inclusion and the last known results before bleeding, no statistical difference was found to predict bleeding occurrence (**Table 3**).

In the multivariate analysis, the factors significantly associated with an increased risk of CRNMB or major bleeding were vitamin K antagonists intake the day prior bleeding and/or cefazolin administration for endocarditis (OR, IC 95%, 1.36 (1.06–1.76), P=0.020 and 1.30 (1.06–1.61), P= 0.015, respectively) (**Table 4**). Peripheral edema upon inclusion and the day prior bleeding was a protective factor (OR, IC 95%, 0.71 (0.56–0.90), P=0.006 and 0.84 (0.74–0.96), P=0.012, respectively.

4. DISCUSSION

In this prospective monocenter study, we found that 10% of the cefazolin-treated patients had at least one bleeding event classified as CRNMB or major bleeding during their follow-up, based on daily standardized clinical assessment. Risk factors for the occurrence of CRNMB or major bleeding were patients treated for endocarditis and patients receiving vitamin K antagonists the day prior bleeding.

To our knowledge, no other prospective study has described the occurrence of bleedings in cefazolin-treated patients with systematic biological exploration. Published clinical trials on cefazolin do not report major bleedings as an adverse event [25,26]. Our result of 10% bleedings contrasts with the 5% of the only retrospective study conducted by Stratzulla *et al.* in 2018 (e.g. 7/132, 5%) [17]. This difference may be explained, along with data loss inherent to the retrospective design (with data retrieved from a software for daily clinical practice), by a less severe profile of patients in this study (no hospitalization in the ICU), who were less frequently receiving vitamin K antagonists (5/132, 4%, vs 12/120, 10%) and no patient treated for endocarditis. Furthermore, our study used the ISTH classification whereas Strazulla *et al.* defined severe bleeding as any bleeding with clinical instability requiring care in ICU. There was a non-significantly higher incidence of greater severity of bleeding in univariate analysis: patients treated for endocarditis, with bloodstream infection upon inclusion and/or hospitalized in the ICU. Other confounding factors for bleeding might be present in this population. In our study, bleedings were not the consequence of coagulation intravascular disorder, as suggested by D-dimer and fibrinogen in normal ranges.

In our study, no biological feature was predictive of bleeding, unlike suggested by Strazulla *et al* [17]. In the multivariate analysis, the last available PT level prior bleeding was not

298 statistically significant. In the univariate analysis, even though last available PT and FII prior
299 bleeding were lower in patients with CRNMB or major bleeding than in patients with no or
300 minor bleeding, mean percentage remained more than 60%, making these results difficult to
301 use in daily clinical practice. Delta between biological data upon inclusion and last available
302 prior hemorrhage was not statistically significant either. These findings challenge the
303 recommendation to monitor PT under cefazolin therapy [14].

304 Other ß-lactams have been reported to either increase the risk of bleeding or the occurrence
305 of coagulation disorders. A risk scoring system was developed for cephamycin-associated
306 bleeding by Chien *et al*. based on history of bleeding, bleeding tendency, age and chronic
307 hepatic disease [27]. Wang *et al.* reported in a retrospective cohort study of 23 242 patients
308 with propensity-score matching analyses that cefoperazone-sulbactam, compared to
309 ceftazidime, increased the risk of PT prolongation (adjusted OR (aOR), IC 95%, 2.26 (1.61–
310 3.18)), coagulation disorders (aOR, IC 95%, 1.81 (1.43–2.30)), and decreased platelet count
311 (aOR, IC 95%, 1.46 (1.25–1.72)), but did not increase risk of bleeding (aOR, IC 95%, 1.05 (
312 0.79–1.40)) [28]. This led to the mentioning of “bleeding potentially fatal in unknown
313 frequency” on the cefoperazone product information in 2016, mandated by the European
314 Medicine Agency [29].

315 Treatment at therapeutic dose by oral anticoagulation the day prior bleeding was a risk
316 factor after multivariate analysis in our study. Most of patients with major or CRNMB with
317 oral anticoagulation were treated by vitamin K antagonist. An alternative anticoagulant
318 therapy could be discussed in these patients such as heparin (low molecular weight heparin
319 or unfractionated heparin for ICU patient) but further studies are needed to corroborate or
320 refute this finding. Abbas *et al.* reported the risk of bleeding with concomitant antibiotic and

phenprocoumon (coumarin-derived vitamin K antagonist) administration [30]. Strongest associations were found for cotrimoxazole and fluroquinolones (OR, IC 95%, 3.96 (3.20–4.91), P < 0.01, and OR, IC 95%, 3.41 (2.98–3.89), P < 0.01, respectively). Third-generation cephalosporins had the highest risk among ß-lactams (OR, IC 95%, 2.37 (1.61–3.49), P < 0.01). Only 4 cases and 12 controls were receiving first generation cephalosporin, making the absence of risk of bleeding difficult to interpret (OR, IC 95%, 1.39 (0.45–4.32), P < 0.01).

Drug interactions are frequent causes of adverse events with vitamin K antagonist. Particularly, medications that interfere with the endogenous synthesis of vitamin K could lead to increased anticoagulation: for instance, antibiotics eliminate bacterial flora and worsen vitamin K deficiency and are a risk factor for bleeding [31].

Our multivariate analysis revealed that peripheral edema was a protective factor. No clear explanation could be proposed at the light of the literature, but this might be of interest for future pathophysiology research.

Our study has several limitations. Given the monocenter design and the absence of other prospective studies, generalizability is difficult. Furthermore, our study design was not made to compare cefazolin over another antibiotic (such as (cl)oxacillin) to compare the incidence of bleeding events. Our study aimed to determine cefazolin-treated patients' phenotypes for which increased clinical surveillance and hemostasis work-up would be useful. Despite a robust design with a daily clinical assessment, some biological data are missing.

5. CONCLUSION

Close clinical monitoring should be performed for patients treated for endocarditis and/or receiving vitamin K antagonists while treated with cefazolin. Given the absence of predictive biological tests, restraining hemostasis work-up to these patients might be sufficient.

6. STATEMENTS AND DECLARATIONS

The authors de not declare any conflict of interests.

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Table 1. Patient characteristics upon inclusion and comparison by univariate analysis of the patients with "major or clinically relevant non-major bleeding (CRNMB)" and those without bleeding or minor bleeding based the ISTH classification in a cohort of 120 cefazolin-treated patients.

Patients characteristics[a]	**Total population** n = 120	**Major or CRNMB** n = 12	**No or minor bleeding** n = 108	P-value
Demography				
Age	59.4 (20.7)	66.4 (18.1)	58.6 (20.9)	0.18
Male	84 (70.0)	11 (91.7)	73 (67.6)	0.11
BMI	24.5 (4.9)	26.1 (5.5)	24.3 (4.8)	0.31
Hospitalization ward				< 0.001
Surgery	58 (48.3)	0 (0.0)	58 (53.7)	
Medicine	43 (35.8)	5 (41.7)	38 (35.2)	
Intensive care unit	19 (15.8)	7 (58.3)	12 (11.1)	
Comorbidities				
Diabetes	23 (19.2)	1 (8.3)	22 (20.4)	0.46
Solid tumor	13 (10.9)	1 (8.3)	12 (11.2)	1.0
Liver disease	4 (3.3)	0 (0.0)	4 (3.7)	1.0
Daily medication				
Heparin	37 (30.8)	3 (25.0)	34 (31.5)	0.75
Platelet antiaggregation	31 (25.8)	4 (33.3)	27 (25.0)	0.51
Vitamin K antagonists	12 (10.0)	4 (33.3)	8 (7.4)	0.019
Direct oral anticoagulants	11 (9.2)	1 (9.1)	10 (9.3)	1
Anticoagulation type (n = 37)				1.0
Prophylactic	25 (67.6)	2 (66.7)	23 (67.6)	
Curative	12 (32.4)	1 (33.3)	11 (32.3)	
Antidepressant	13 (10.8)	0 (0.0)	13 (12)	0.36
Immunosuppressant	7 (5.8)	0 (0.0)	7 (6.48)	1.0
Enteral feeding	4 (3.4)	0 (0.0)	4 (3.7)	1.0
Parenteral feeding	1 (0.8)	0 (0.0)	1 (0.9)	1.0
Cefazolin-treated infection				
qSOFA (n = 119)				0.006
0-1	110 (92.4)	8 (66.7)	102 (95.3)	
2-3	9 (7.6)	4 (33.3)	5 (4.7)	
Location of infection				
Bone and joint infection	50 (41.7)	3 (25)	47 (43.5)	0.36
Catheter-related infection	18 (15)	0 (0.0)	18 (16.7)	0.21
Endocarditis	11 (9.2)	4 (33.3)	7 (6.5)	0.013
Pneumonia	6 (5)	0 (0.0)	6 (5.6)	1.0
Urinary infection	4 (3.3)	1 (8.3)	3 (2.8)	0.35
Cutaneous infection	4 (3.3)	1 (8.3)	3 (2.8)	0.35
Intra-abdominal infection	1 (0.8)	1 (0.9)	0 (0.0)	1.0
Other[b]	31 (25.8)	4 (33.3)	27 (25)	0.51
Bloodstream infection	63 (52.5)	11 (91.7)	52 (48.1)	0.005
Blood culture positivity (days)	2.9 (2.6)	4.2 (4.8)	2.7 (1.8)	0.33

Blood culture positivity (pair)	3.5 (3.9)	5.1 (7.9)	3.2 (2.4)	0.45
Biology				
Hemoglobin (n=118)	10.5 (1.9)	9.6 (1.5)	10.6 (1.9)	0.058
Platelet (n=118)	309 (157)	261 (217)	315 (150)	0.42
Prothrombin time (n=113)	72.2 (18.7)	58.5 (22.8)	79.4 (16.9)	0.009
aPTT (n=51)	1.0 (0.1)	1.1 (0.2)	1.0 (0.1)	0.21
INR (n=113)	1.3 (0.5)	1.9 (1.4)	1.2 (0.3)	0.11
Factor II (n=41)	101.4 (34.6)	54.0 (21.7)	108.0 (30.7)	0.002
Factor V (n=43)	112.6 (37.1)	82.4 (46.1)	116.6 (34.5)	0.18
Factors VII + X (n=41)	86.8 (29.8)	45.0 (29.3)	92.0 (25.2)	0.019
D-dimer (n=30)	1452 (1535)	1650 (NA)	1445 (1562)	---
Fibrinogen (n=65)	5.7 (1.6)	5.0 (2.0)	5.8 (1.5)	0.35
AST (n=75)	61.0 (224.8)	467.2 (706.5)	29.5 (17.2)	0.25
ALT (n=78)	49.9 (155.4)	262.1 (490.1)	28.9 (32.0)	0.26
Gamma-GT (n=72)	98.8 (160.2)	111.6 (82.5)	98.6 (166.7)	0.73
Alkaline phosphatase (n=77)	141.5 (157.5)	170.7 (157.3)	139.1 (158.4)	0.65
Total bilirubin (n=74)	15.2 (18.1)	37.9 (37.8)	12.8 (18.1)	0.13
eGFR (n=113)	92.6 (51.0)	62.3 (46.7)	96.2 (50.5)	0.033

NOTE. [a]Continuous variables are presented as the mean ± standard deviation and categorical variables are presented as numbers and frequencies, unless otherwise indicated.

Abbreviations: ALT, alanine aminotransferase; aPTT, activated partial thromboplastin clotting time; AST, aspartate aminotransferase; BMI, body mass index (kg/m^2); CRNMB, clinically relevant non major bleeding; eGFR: estimated Glomerular Filtration Rate; Gamma-GT, Gamma-glutamyl transferase; INR, index normalized ratio; qSOFA, quick Sepsis-related Organ Failure Assessment. Units: Age, years; hemoglobin, g/dL; platelet, G/L; prothrombin time, Factors II, V, VII+X, %; APTT, seconds; D-dimer, ng/mL; fibrinogen, g/L; AST, ALT, Gamma-GT, alkaline phosphatase, UI/L; total bilirubin, µmol/L; eGFR, $mL/min/1.73^2$

[b]Other: bloodstream infection (n=10), heart-device infection (left ventricular assist device or pacemaker, n=7), endovascular infection (n=6), mediastinal infection (n=4), parotitis (n=2), pleural infection (n=1), post-hepatectomy (n=1)

The univariate analysis was performed using the Fischer or Chi-2 tests for categorical variables and the t-test Student for continuous variables or the Wilcoxon-Mann-Whitney test when a non-parametric test was required.

Table 2. Last-known medication and biological results prior hemorrhage of the total population and comparison by univariate analysis of the patients with "major or clinically relevant non-major bleeding (CRNMB)" with patients without bleeding or minor bleeding using the ISTH classification in a cohort of 120 cefazolin-treated patients.

Patients characteristics[a]	**Total population** n = 120	**Major or CRNMB** n = 12	**No or minor bleeding** n = 108	P-value
Daily medication				
Platelet antiaggregation	29 (24.1)	4 (33.3)	25 (23.1)	0.48
Vitamin K antagonists	9 (7.5)	3 (25)	6 (5.6)	0.046
Direct oral anticoagulants	5 (4.2)	0 (0)	5 (4.6)	1
Heparin	82 (68.9)	8 (66.7)	74 (69.1)	1.0
Anticoaagulation type (n=82)				0.43
Prophylactic dose	55 (67.1)	4 (50)	51 (68.9)	
Therapeutic dose	27 (32.9)	4 (50)	23 (31.1)	
Antidepressant	13 (10.8)	0 (0)	13 (12)	0.36
Immunosuppressant[b]	5 (4.2)	0 (0)	5 (4.6)	1.0
Enteral feeding	4 (3.3)	0 (0)	4 (3.7)	1.0
Parenteral feeding	1 (0.8)	0 (0)	1 (0.9)	1.0
Biology				
Cefazolin concentration (quantitative, n=72)	56.3 (32.5)	72.6 (33.8)	55.4 (32.4)	0.39
Cefazolin concentration (qualitative, n=72)				0.09
Supra-therapeutic[c]	10 (13.9)	2 (50)	8 (11.8)	
Normal range	62 (86.1)	2 (50)	60 (88.2)	
Cefazolin administration (n=116)				0.37
Continuous infusion	77 (66.4)	6 (50)	71 (68.3)	
Intermittent administration	14 (12.1)	2 (16.7)	12 (11.5)	
None	25 (21.5)	4 (33.3)	21 (20.2)	
Hemoglobin (n=117)	10.1 (1.8)	9.2 (1.8)	10.2 (1.8)	0.08
Platelet (n=117)	317 (152)	241 (214)	326 (142)	0.2
Prothrombin time (n=109)	80.3 (17.3)	67.8 (16.5)	81.8 (16.9)	0.015
aPTT (n=11)	1.1 (0.1)	1.2 (0.2)	1.1 (0.1)	0.21
INR (n=109)	1.2 (0.3)	1.4 (0.5)	1.2 (0.3)	0.15
Factor II (n=80)	102.6 (31.6)	66.2 (21.6)	105.5 (30.5)	0.005
Factor V (n=80)	125.0 (37.0)	102.0 (17.3)	126.9 (37.6)	0.014
Factors VII + X (n=80)	88.0 (25.3)	66.5 (26.6)	89.7 (24.5)	0.087
D-dimer (n=74)	1836 (1508)	2473 (1126)	1809 (1522)	0.42
Fibrinogen (n=89)	5.2 (1.5)	5.1 (1.7)	5.3 (1.5)	0.80
AST (n=98)	32.0	29.8 (16.5)	65.0 (30.9)	0.038
ALT (n=99)	23.4 (26.3)	58.8 (52.9)	21.1 (22.3)	0.14
Gamma-GT (n=97)	111.4 (162.5)	140.2 (71.0)	109.5 (166.8)	0.39

Alkaline phosphatase (n=98)	149.8 (151.2)	206.6 (161.2)	146.7 (151.0)	0.46
Total bilirubin (n=98)	12.4 (17.9)	27.7 (20.6)	11.4 (17.4)	0.11
Serum creatinine clearance (n=113)	102.3 (52.0)	74.9 (45.1)	105.6 (52.0)	0.045

NOTE. [a]Continuous variables are presented as the mean ± standard deviation and categorical variables are presented as numbers and frequencies, unless otherwise indicated.

Biological data are the most recent one prior hemorrhage.

[b]immunosuppressants: corticosteroids (n=3) and chemotherapy (n=2) in the "no or minor bleeding" group

[c]cefazolin concentration was considered supra-therapeutic if > 80mg/L, depending on the laboratory range

Abbreviations: ALT, alanine aminotransferase; aPTT, activated partial thromboplastin clotting time; AST, aspartate aminotransferase; CRNMB, clinically relevant non major bleeding; eGFR: estimated Glomerular Filtration Rate; Gamma-GT, Gamma-glutamyl transferase; INR, index normalized ratio.

Units: hemoglobin, g/dL; platelet, G/L; prothrombin time, Factors II, V, VII+X, %; APTT, seconds; D-dimer, ng/mL; fibrinogen, g/L; AST, ALT, Gamma-GT, alkaline phosphatase, UI/L; total bilirubin, µmol/L; eGFR, mL/min/1.73^2

The univariate analysis was performed using the Fischer or Chi-2 tests for categorical variables and the t-test Student for continuous variables or the Wilcoxon-Mann-Whitney test when a non-parametric test was required.

Table 3. Delta of main biological tests between inclusion value and last available result (in the absence of bleeding) or before hemorrhage (in case of bleeding) in a cohort of 120 cefazolin-treated patients

	Total population n = 120	**Major or CRNMB** n = 12	**No or minor bleeding** n = 108	P-value
Delta between inclusion and prior hemorrhage values				
Hemoglobin (n=115)	- 0.41 (1.15)	- 0.49 (0.91)	-0.41 (1.18)	0.77
Platelet (n=115)	6.92 (71.98)	- 20 (91.51)	10.06 (69.23)	0.29
Prothrombin time (n=105)	2.92 (12.69)	9.33 (16.34)	2.1 (12)	0.16
aPTT (n=20)	-0.01 (0.05)	0 (0)	-0.01 (0.06)	0.61
INR (n=105)	-0.08 (0.48)	-0.48 (1.28)	-0.03 (0.21)	0.25
Factor II (n=36)	2.67 (11.18)	8.75 (10.75)	1.91 (11.16)	0.3
Factor V (n=834)	2.21 (21.66)	24 (29.13)	-0.35 (19.61)	0.19
Factors VII + X (n=36)	2.31 (13.04)	19.75 (24.36)	0.12 (9.51)	0.21
AST (n=71)	-30.86 (220.28)	-359.17 (731.11)	-0.55 (16.57)	0.28
ALT (n=75)	-25.96 (140.87)	-243.83 (476.28)	-7.01 (19.05)	0.28
Gamma-GT (n=74)	7.1 (42.28)	13.33 (20.88)	6.51 (43.83)	0.52
Alkaline phosphatase (n=69)	4.59 (34.02)	17.4 (31.07)	3.67 (34.25)	0.39
Total bilirubin (n=70)	-2.09 (10.64)	-15.33 (18.65)	-0.84 (8.83)	0.12
Serum creatinine clearance (n=111)	9.21 (23.1)	12.58 (25.03)	8.8 (22.96)	0.63

Abbreviations: ALT, alanine aminotransferase; aPTT, activated partial thromboplastin clotting time; AST, aspartate aminotransferase; CRNMB, clinically relevant non major bleeding; Gamma-GT, Gamma-glutamyl transferase; INR, index normalized ratio.

Units: hemoglobin, g/dL; platelet, G/L; prothrombin time, Factors II, V, VII+X, %; APTT, seconds; AST, ALT, Gamma-GT, alkaline phosphatase, UI/L; total bilirubin, µmol/L.

The univariate analysis was performed using the t-test Student for continuous variables or the Wilcoxon-Mann-Whitney test when a non-parametric test was required.

Table 4. ISTH-based multivariate analysis of the patients with "major and clinically relevant non-major bleeding (CRNMB)" compared to patients with "no or minor bleeding" based on the minimization of Akaike's Information Criterion

Variable	OR (95%)	P-value
Vitamin K antagonists the day before bleeding	1.36 (1.06–1.76)	0.020
Endocarditis	1.30 (1.06-1.61)	0.015
Peripheral edema upon inclusion	0.71 (0.56–0.90)	0.006
Peripheral edema the day before hemorrhage	0.84 (0.74–0.96)	0.012
qSOFA 2-3 upon inclusion	1.22 (0.99–1.51)	0.068
Cause of cefazolin stop: end of treatment of infection	1.22 (0.99–1.50)	0.071
Cause of cefazolin stop: other	1.18 (0.95–1.46)	0.14
Cefazolin intermittent administration	1.00 (0.99–1.01)	0.23
No cefazolin administration upon inclusion	1.06 (0.88–1.27)	0.56
Last available prothrombin time before hemorrhage	0.96 (0.68–1.35)	0.80

NOTE. Abbreviations: ISTH, International Society on Thrombosis and Hemostasis; qSOFA, quick Sepsis-related Organ Failure Assessment.
Population: 99; no bleeding : 87 ; major + CRNMB bleeding : 7

The multivariate analysis was performed based on the minimization of Akaike's Information Criterion. Using multivariate logistic regression tables, odd-ratios (ORs) were calculated.

Figure. Flow chart of patients included in the prospective evaluation of the frequency of and risk factors for bleeding complications in patients treated with cefazolin.