Coagulase negative *Staphylococcus* bacteremia in hematopoietic stem cell transplant recipients: Clinical features and molecular characterization

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ABSTRACT

The purpose of our study was to investigate the epidemiology of coagulase negative staphylococci (CoNS) responsible for bacteremia in hematopoietic stem cell transplant (HSCT) recipients and to determine the prevalence and the genetic background of methicillin resistance. The prevalence of CoNS bacteremia was 7.4% (54/728), higher in allograft (10.7%) than in autograft (4.7%) recipients. A sepsis or a septic shock were observed in 9% of cases. No deaths were attributable to CoNS bacteremia. The methicillin resistance rate was 81%. All MR-CoNS, harbored *mecA* gene and 90% were typeable with *SCCmec* typing using PCR amplification. The *SCCmec* type IV was the most frequent (44%). Clonal dissemination of MR- *Staphylococcus epidermidis* strains was limited. Our study showed a low prevalence and favorable outcome of CoNS bacteremia in HSCT recipients with limited clonal diffusion. However, they were associated with a significant rate of severe infections and a high rate of methicillin resistance, mediated by *SCCmec* IV element in most cases.

KEYWORDS

INTRODUCTION

Coagulase negative staphylococci (CoNS) are currently the most common pathogens implicated in bacteremia in hematopoietic stem cell transplant (HSCT) recipients [1]. The vulnerability of these patients to these commensal bacteria is increased by immunosuppression induced by myeloablative chemotherapy as well as by the use of central venous catheters (CVC), essential tool for many treatments prescribed in these patients. CoNS isolates are characterized by their multi-resistance to antibiotics and its persistence in biomaterials by the formation of biofilm [2], thus complicating the management of HSCT recipients. They are responsible for an additional morbidity and an increase of the length and the cost of hospital stay [3]. Resistance to methicillin, and therefore resistance to all the β-lactams except for 5th generation cephalosporins, is frequent in CoNS [4]. This resistance is mainly encoded by the *mecA* gene, carried by the Staphylococcal Chromosomal Cassette mec (*SCCmec*) [4]. This structure, composed of highly mobile genetic elements, includes, in addition to the *mecA* gene, insertion segments, transposons and plasmids coding for other

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resistance genes, thus explaining the multi-resistance of methicillin resistant CoNS strains (MR-CoNS) [4]. We conducted this study to describe the epidemiology of CoNS bacteremia in HSCT recipients in the National Bone Marrow Transplant Center of Tunisia and to investigate the prevalence and the genetic background of methicillin resistance in these strains.

MATERIAL AND METHODS

Patients

Our retrospective study was conducted between January 2012 and December 2018 in the Hematology ward of the National Bone Marrow Transplant Center, an university hospital center referral for allografts in Tunisia. The hematology ward was composed of a transplant unit including nine laminar flow cabins and an hematology unit including 10 conventional rooms. In this study, we included all patients hospitalized in the hematology and transplant units and presented at least one episode of CoNS bacteremia during the pre-transplant conditioning phase or after HSC transplantation. Day of receiving of HSCT was considered day 0. Patients with incomplete medical records were excluded from the study. Empirical antibiotic therapy in case of febrile neutropenic episodes was started after having done central venous catheter and peripheral blood cultures. It was based, in the absence of clinical or microbiological orientation, on the combination of a β-lactam (piperacillin-tazobactam) and an aminoglycoside (amikacin). In the absence of apyrexia after 48–72 hours and in the presence of grade 4 mucositis, a catheter inflammation or a digestive colonization with an ampicillin resistant *Enterococcus*, a glycopeptide (vancomycin or teicoplanin) was added. For each patient, data concerning demographics, underlying disease, transplant procedures, BSI episode, and survival were collected from medical records.

Bacteriological study

Non-duplicated strains of CoNS responsible for bacteremia in HSCT recipients were included in this study. CoNS bacteremia was retained if at least two pairs of blood culture collected at two different collection sites or at different times were positive for the same CoNS (same antibiotype). Peripheral and central blood cultures were analyzed according to the « Référentiel en Microbiologie Médicale » guidelines [5]. The isolates were identified using conventional methods and API ID 32 STAPH (bioMérieux®). Antimicrobial susceptibility testing was determined by the disk diffusion method according to the CA-SFM standards annually revised [6]. The minimal inhibitory concentrations of teicoplanin and vancomycin were determined by the E-test method (bioMérieux®) for MR strains isolated before 2017 and by broth dilution method (UMIC Biocenric®) for MR strains isolated from January 2017 [6].

Molecular study

MR-CoNS strains that lost resistance to methicillin during storage were excluded from the molecular study.

The DNA extraction was performed by thermal lysis method using the InstaGene Matrix® kit (Bio-Rad®). The search of the *mecA* gene and the typing of *SCCmec* cassettes were performed by polymerase chain reaction (PCR) amplification as previously described [7–9]. Molecular typing concerned only *Staphylococcus epidermidis* strains, given the small number of strains belonging to other species. It was carried out by pulsed-field gel electrophoresis (PFGE) after digestion with *SmaI* enzyme to search a possible genetic relatedness between these strains [10]. The analysis of the dendrogram was carried out by the software Gelcompar II for Windows version 6.6 (Applied Math®). Obtained patterns were compared by using the Dice coefficient, according to the instructions of the Gelcompar manufacturer. Strains with at most three difference bands on the PFGE profile were considered to be of the same clone [3].

Statistical analyzes

The results were analyzed by SPSS software version 19.0. The study of patient characteristics was carried out in number of patients. The study of bacteremia variables was made in bacteremia episodes. Changes in the prevalence of bacteremia over the study period were studied by the Spearman rank correlation coefficient (rs). The significance level (p) was set at 0.05 for all statistical tests.

RESULTS

Prevalence of CoNS bacteremia

During the study period, 52/246 patients with blood cultures positive for CoNS were classified as having CoNS bacteremia. Also, 52 patients among a total of 728 HSCT recipients (7.1%) developed 54 CoNS bacteremia episodes (7.4%). Fifty patients presented a single bacteremia episode and two patients developed two bacteremia episodes to two different CoNS species. Forty-four episodes (6%) were due to MR-CoNS strains. The prevalence of CoNS bacteremia had a non-significant downward trend over time (rs = −0.32; P = 0.49).

Patients' characteristics

Patients with CoNS bacteremia had a median age of 35 years (7–63 years) and a sex ratio man/woman equal to 1. The prevalence of CoNS bacteremia was higher in patients with acute myeloblastic leukemia (13.7%) and Hodgkin lymphoma (10.3%); and in patients receiving allograft (10.7%) than in those receiving autograft of HSC (4.7%). Median time of CoNS bacteremia was 13 days post-graft, ranging from −2–3,344 days. Forty-seven CoNs bacteremia (87%) occurred within the first 100 days post graft. All patients carried CVC with a median time of pre-bacteremia

catheterization of 17 days [1–80 days]. A deep neutropenia was found in 59% of cases with a median duration of 12 days, ranging from 0 to 79 days (Table 1). Among the 44 MR-CoNS bacteremia, 24 (55%) had been preceded by hospitalization in an onco-hematology department. Prior antibiotic therapy within one month was noticed in 75% (33/44) of cases. Penicillins (45%), aminoglycosides (43%) and glycopeptides (39%) were the most prescribed antibiotics (Table 1). These antibiotics were used for treatment of febrile neutropenia in most of cases. No colonization and/or prior infection with a MR *Staphylococcus spp.* was documented in patients who developed MR-CoNS bacteremia.

Clinical presentation, treatment and outcome

Clinically, isolated fever was the common infectious symptom (67% of cases). A sepsis or a septic shock were observed in three and two cases, respectively. The infection source was identified in 34% of cases. It was the CVC in 15 cases (28%) and the autologous graft in three cases (6%). In eight episodes, patients were asymptomatic and non-neutropenic. They were not then received antibiotics, but their outcome was favorable. Among the 46 patients who received first-line antibiotic therapy, 21 (46%) were already on antibiotic therapy at the time of the occurrence of bacteremia, for an average of eight days [1–29 days]. Twenty-five patients (54%) received antibiotic therapy after the onset of bacteremia with an average delay of 1 day [0–6 days]. Teicoplanin (54%) and piperacillin-tazobactam (52%) were the most prescribed drugs. This initial antibiotic therapy was

Table 1. Patient's characteristics

Clinical features	Number (percentage)
Total of patients	52
Number of bacteremic episodes	54
Hematological disease	
Acute myeloblastic leukemia	14/52 (27%)
Acute lymphoblastic leukemia	6/52 (11.5%)
Biphenotypic acute leukemia	1/52 (2%)
Hodgkin lymphoma	6/52 (11.5%)
Non hodgkin lymphoma	6/52 (11.5%)
Myeloma	12/52 (23%)
Myelodysplastic syndrome	2/52 (4%)
Aplastic anemia	5/52 (9.5%)
Treatment	
Allograft	32/52 (62%)
Autograft	20/52 (38%)
Neutropenia	32/54 (59%)
Presence of venous central catheter	54/54 (100%)
Mucositis	16/54 (30%)
Acute GVH	12/54 (22%)
Prior hospital stay	24/54 (44%)
Prior antibiotic therapy	33/54 (61%)
Penicillins	20/54 (45%)
Cephalosporins	13/54 (30%)
Carbapenems	14/54 (32%)
Glycopeptides	17/54 (39%)
Aminoglycosides	19/54 (43%)
Fluoroquinolones	11/54 (25%)

appropriate in 59% of cases (27/46). The use of second-line antibiotic treatment was indicated in 41% of cases ($n = 19$) either because of the resistance of the CoNS ($n = 17$), or because of the persistence of symptoms despite appropriate first-line antibiotic therapy ($n = 2$). Teicoplanin ($n = 14$) was the most prescribed second-line antibiotic, followed by vancomycin ($n = 5$) and linezolid ($n = 1$). The overall mortality rate was 4% (2/52). No deaths were attributed to CoNS bacteremia.

Bacteriological results

Among 257 bacteremia identified during the study period, 54 (21%) were due to CoNS. *S. epidermidis* was the most frequently isolated species (65%) followed by *Staphylococcus haemolyticus* (26%), *Staphylococcus hominis* (7%) and *Staphylococcus warneri* (2%). The methicillin resistance rate was 81% (44/54). Among the 10 strains susceptible to methicillin (MS), nine (90%) produced penicillinase. CoNS strains had high rates of antibiotic resistances except for quinipristin-dalfopristin (2%), vancomycin (2%), teicoplanin (2%), linezolid (0%) and tigecycline (0%). MR-CoNS had higher antibiotic resistance rates than MS-CoNS (Table 2). This difference was statistically significant ($P < 0.05$) for kanamycin (89% vs 40%), tobramycin (84 vs 20%), gentamicin (77 vs 0%), ciprofloxacin (60 vs 0%), ofloxacin (68 vs 10%), erythromycin (84 vs 50%), cotrimoxazole (64 vs 0%), fusidic acid (80 vs 30%) and rifampicin (43 vs 0%). Among the 44 strains resistant to methicillin, only 30 (68%) were included in the molecular study. They all carried the *mecA* gene. Of which, 27 (90%) were typable. *SCCmec* type IV was the predominant type (44%) followed by type II (24%), type III (13%) and types I, VI and VIII (3% each) (Table 3). Four strains (13%) carried, in addition to their classic *SCCmec*, an additional ccr complex, thus forming a combined *SCCmec*. Among the 15 MR- *S. epidermidis* strains, there were 10 pulsotypes. Eight isolates were assigned to three minor clusters: two clusters of three isolates and one cluster of two isolates. The strains of the same clone were isolated from different patients, in different hospital units and/or in different years (Fig. 1)

DISCUSSION

The CoNS, commensal bacteria of the cutaneous and mucous flora, considered for long time as contaminants, have shown their pathogenic power, especially in bacteremia in immunocompromised patients. HSCT recipients with CVC are particularly at high risk for CoNS bacteremia. In the present study, the prevalence of these bacteremia was 7.4%, lower than rates in other studies [11]. The decrease in the prevalence of CoNS bacteremia during the study period (rs = 0.32; $P = 0.49$) has been reported by other authors [12, 13]. This could be explained by the improvement of hygiene measures, the reduction in the duration of neutropenia due to the use of spinal growth factors and the use of peripheral stem cells as a preferential source of HSC [12].

Table 2. Antibiotic susceptibility of coagulase negative staphylococci

Antibiotic resistance profiles (n/% antibiotic resistant)	All CoNS strains (*n* = 54)	*S. epidermidis* (*n* = 35)	*S. haemolyticus* (*n* = 14)	Other species (*n* = 5)
Cefoxitin	44 (81%)	26 (74%)	14 (100%)	4 (80%)
Kanamycin	43 (80%)	27 (77%)	12 (86%)	4 (80%)
Tobramycin	39 (72%)	25 (71%)	11 (78%)	3 (60%)
Gentamicin	34 (63%)	22 (63%)	10 (71%)	2 (40%)
Erythromicin	41 (76%)	16 (46%)	12 (86%)	3 (60%)
Quinupristin-dalfopristin	1 (2%)	0 (0%)	1 (7%)	0 (0%)
Ciprofloxacin	26 (48%)	15 (43%)	8 (57%)	3 (60%)
Ofloxacin	31 (57%)	18 (51%)	10 (71%)	3 (60%)
Cotrimoxazol	28 (52%)	16 (46%)	9 (64%)	3 (60%)
Rifampicin	19 (35%)	7 (20%)	9 (64%)	3 (60%)
Fusidic acid	38 (70%)	21 (60%)	13 (93%)	4 (80%)
Linezolid	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Tigecyclin	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Teicoplanin	1 (2%)	0 (0%)	1 (7%)	0 (0%)
Vancomycin	1 (2%)	0 (0%)	1 (7%)	0 (0%)

CoNS: coagulase negative staphylococci, Other species: *S. hominis, S. warneri.*

Table 3. SCCmec types of coagulase negative staphylococci

SCCmec type	All CoNS strains (*N* = 30) (n, %)	*S. epidermidis* (*N* = 20) (n)	*S. haemolyticus* (*N* = 8) (n)	Other species (*N* = 2) (n)
Type I	1 (3%)	1	0	0
Type II	7 (24%)	3	4	0
Type III	4 (13%)	3	1	0
Type IV	13 (44%)	11	2	0
Type VI	1 (3%)	0	0	1
Type VIII	1 (3%)	0	1	0
Untypable	3 (10%)	2	0	1

CoNS: coagulase negative staphylococci, Other species: *S. hominis, S. warneri.*

Acute myeloid leukemia followed by Hodgkin's Lymphoma were associated with the highest rates of CoNS bacteremia (13.7% and 10.3%, respectively). Indeed, it has been reported for years that acute leukemia and Hodgkin's Lymphoma are associated factors of staphylococcal bacteremia [14]. CoNS bacteremia was more frequent in patients receiving allogenic HSC. Balletto et al., as well as Frère et al., reported that bacteremia, was more common after allograft than after autograft [12, 15]. The common risk factors associated with CoNS bacteremia that had been reported in the literature in HSCT recipients were the CVC and the neutropenia [16, 17]. They were found in 100 and 59% of the bacteremic episodes, respectively. Concerning MR-CoNS bacteremia, risk factors, described in many studies, were mainly prior hospitalization and prior antibiotic therapy [16]. They were
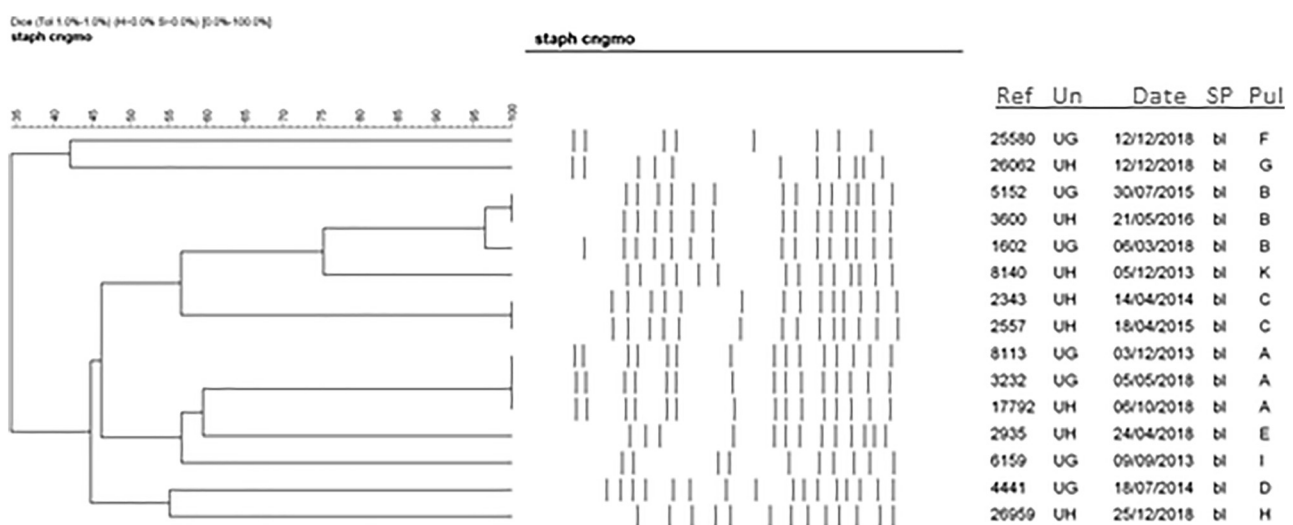

Fig. 1. Dendrogram of pulsed-field gel electrophoresis finger printing of methicillin resistant *S. epidermidis* after diestion with SmaI enzyme
*Ref: reference, Un: unit, SP: spicemen, Pul: pulsotype, UG: transplant unit, UH: hematology unit, bl: blood.
Legend: Among the 15 methicillin resistant *S. epidermidis* strains, there were 10 pulsotypes (A-I, K). Eight isolates were assigned to three minor clusters: two clusters (A and B) of three isolates and one cluster (C) of two isolates

noticed in 55 and 75% of episodes, respectively. Colonization and/or previous infection with MR-*S. spp* had been also reported as a risk factor of MR-CoNS bacteremia [16]. However, in the present study, no colonization and/or infection with a MR-*S. spp.* was documented in patients who developed MR-CoNS bacteremia. CoNS bacteremia was classified as CVC related bacteremia in 28%. According to Bertrand et al., the risk of CVC related bacteremia is higher with CoNS than with other bacteria (RR = 5.89) [18]. This risk increases with the duration of catheterization, the frequency of handling and the number of lumens and decreases with the use of catheters made of silicone elastomers or polyurethane, which reduce the risk of bacterial adhesion [19]. Severe clinical signs were observed in 9% of episodes. In previous studies, a severe clinical presentation (SOFA score ≥5) was found in approximately 28% of CoNS bacteremia [20, 21]. No deaths were attributed to CoNS bacteremia. According to the literature, the mortality attributable to CoNS bacteremia can reach up to 14% [16]. In 15% of bacteremic episodes, no antibiotic treatment was administered but the outcome was favorable. This prompts us to review the diagnostic criteria for CoNS bacteremia. Indeed, having two positive blood cultures with two phenotypically identical CoNs would not be sufficient to retain the diagnosis of bacteremia. In a study carried out in our center, the PFGE analysis of two phenotypically identical strains of *S. epidermidis* (same biotype and same antibiotype) isolated in two blood cultures (one on peripheral blood and the other on CVC) in the same patient, showed that they belonged to two different pulsotypes and were therefore genotypically distinct [22]. This suggests that phenotypical identity of two CoNS isolates is not always sufficient to decide whether they are responsible for true bacteriaemia or for contamination. Should genomic typing of CoNS strains be introduced for a better interpretation of the results, which would be restrictive for a routine medical laboratory? Many studies investigated the criteria used to distinguish CoNS bacteremia from contamination. García et al. found that time to positive culture, species identification, antimicrobial susceptibility pattern, slime production, and PFGE pattern were the most useful parameters for the diagnosis of true CoNS bacteremia [23]. In a more recent study, Karakullukçu et al. suggested that combination of the laboratory-confirmed bloodstream infection criteria and critically clinical signs is associated with higher diagnostic accuracy than either alone [24]. In patients with onco-haematological diseases and febrile neutropenia, P. Puerta-Alcalde et al. reported that the vast majority of bloodstream infection had a time to positivity of blood cultures <24h [25]. Similarly, in cancer patients, Morioka et al. found, that a time to positivity of ≤16 h was associated with CoNS bacteraemia, while that of >20 h was associated with CoNS contamination [26]. Teicoplanin (54%) and piperacillin-tazobactam (52%) were the most prescribed drugs in 1st line antibiotic therapy. First line antibiotic treatment was appropriate in 59% of cases only, given the predominance of MR-strains. The choice of antibiotic therapy for febrile neutropenia is not always easy. Glycopeptides are the

treatment of choice for MR-*Staphylococcus* infections [27, 28]. The preferential prescription of teicoplanin compared to vancomycin is mainly due to pharmacokinetic and biological reasons. New anti-staphylococcal antibiotics such as linezolid and daptomycin offer an excellent solution for infections with strains resistant to glycopeptides [29]. During the study period, CoNS were responsible for 21% (54/257) of bacteremia in HSCT recipients. In previous studies, the rate of CoNS responsible for bacteremia varies from 15 to 27% in these patients. These pathogens often occupy the 1st or 2nd rank of the agents responsible for this type of infection [1]. The rate of methicillin resistance was 81% (44/54). This rate was also high, exceeding 70%, in several studies [16, 17, 30]. It ranged from 73 to 85% and from 79 to 90% in *S. epidermidis* and *S. haemolyticus* strains, respectively [4]. Repetitive exposure to antibiotics as well as long-term recurrent hospitalizations, a frequent situation in our patients, could explain this high rate of methicillin resistance [16]. MR-CoNS had significantly higher rates of antibiotic resistance than MS-CoNS. This difference was reported in several studies [31, 32]. This would be explained genetically by the diversity of the antibiotic resistance genes carried by the *SCCmec*. All the MR-strains carried the *mecA* gene. The *mecA* gene, coding for the PBP2a, is the main genetic background for methicillin resistance in *S. spp.* [20, 30]. There are other methicillin resistance genes reported in the literature, which are *mecB* and *mecC*, but which have been found mainly in strains of animal origin [4]. In our study, 90% of the strains carrying the *mecA* gene were typable by PCR amplification. *SCCmec* type IV was predominant (44%) followed by type II (24%) and type III (13%). These results are consistent with literature data. In fact, the predominance of *SCCmec* type IV in MR-CoNS has been proven worldwide [4, 20, 30]. Given the scarcity of studies carried out in Tunisia, the local molecular epidemiology of MR-CoNS has not been established. On the other hand, several studies carried out in *Staphylococcus aureus* have shown the predominance of SCCmec types I, II and III in nosocomial strains [33, 34] and SCCmec type IV in community strains [35]. Four (13%) strains carried, in addition to their classic *SCCmec*, an additional ccr complex, thus forming a "combined *SCCmec*" containing two ccr complexes. This phenomenon has been reported by other authors [20]. It could be explained by the great genetic diversity which characterizes this chromosomal cassette. Indeed, *SCCmec*, which is a very mobile genetic element, risks during its transfer, to undergo several recombinant events, giving a mosaic-like structure [30]. PFGE molecular typing revealed three minor clones among MR-*S. epidermidis* strains. This shows a great genetic diversity and a limited clonal diffusion of these strains and demonstrates the effectiveness of hygiene measures applied in our center. In the literature, several local or inter-centric CoNS outbreaks have been reported [36, 37]. Clonal dissemination is mainly due to cross-transmission through the nursing staff [38]. The present study had some limitations such as the small number of patients included, making it difficult to exploit the results. Furthermore, the retrospective nature has caused the lack of certain clinical

data, and the alteration of some strains during storage making impossible the study of genetic background of methicillin resistance in these strains.

In conclusion, CoNS bacteremia had a low prevalence rate and a favorable outcome in our patients. However, there was a significant rate of severe infections. Also, methicillin resistance, of limited clonal diffusion, was high in isolated CoNS strains requiring the appropriate use of antibiotics and the maintaining of hygiene measures.

Ethical approval: Not required. Clinical data was collected anonymously and confidentially. As the bacterial strains were analyzed anonymously the study was exempted from Human Research Commitee approval according to the regulations of the Local Medical Ethical Commitee of Charles Nicolle Hospital, Tunis, Tunisia. Specifically, the Medical Ethical Commitee of Charles Nicolle Hospital waived the need for written informed consent.

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