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Original Article

Effect of Fresh versus Cryopreserved Grafts on Febrile Neutropenia and Infections in Allogeneic Hematopoietic Cell Transplantation: Factors Determining Mortality

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Abstract. ***Background*: The study aimed to compare the incidence and course of febrile neutropenia (FN) and factors affecting mortality in hematologic patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT) with either fresh or cryopreserved grafts.**
***Methods*: The clinical data of 155 patients who underwent allo-HSCT at our hematology clinic between 2010 and 2023 were retrospectively analyzed. The incidence of bloodstream infection (BSI) and FN-related mortality was analyzed in these patients. Factors affecting FN-related mortality were examined using a logistic regression model.**
***Results*: A total of 143 patients who developed FN were included in the study. Ninety-eight patients underwent transplantation with fresh stem cells, and 45 patients with cryopreserved stem cells. The duration of FN episodes was similar between groups ($p = 0.077$); however, the duration of deep neutropenia (neutrophils < 100/mm³) was significantly longer in the cryopreserved group (11.56 ± 4.84 vs. 7.78 ± 3.03; $p < 0.001$). GNB infections and invasive fungal infections were more frequent in the cryopreserved group ($p = 0.009$ and $p < 0.001$, respectively). In the logistic regression model, the most important determinants of FN-related mortality were duration of the FN episode (OR 1.18; 95% CI 0.99–1.41; $p = 0.046$) and higher hematopoietic cell transplantation comorbidity index (HCT-CI) score (score 1; $p = 0.014$ and score 2; $p = 0.039$).**
***Conclusions*: This study demonstrated that, regardless of graft type, prolonged FN duration and a high HCT-CI score are the primary determinants of mortality. Therefore, clinical management of patients should also address these risk factors.**

Keywords: Febrile neutropenia; Allogeneic stem cell transplantation; Cryopreserved; Fresh.

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Introduction. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potentially curative treatment option for selected patients with hematologic disorders despite being associated with high morbidity and mortality rates.[1,2] Until the COVID-19 pandemic, allo-HSCT was performed predominantly using fresh grafts.[3] The COVID-19 pandemic altered this paradigm and necessitated cryopreservation of grafts before the start of conditioning regimens in many centers.[4] Comparative studies of allo-HSCT performed with fresh versus cryopreserved grafts have reported delayed platelet engraftment and increased acute graft-versus-host disease (GVHD) with cryopreserved grafts; however, non-relapse mortality (NRM), relapse, progression-free survival, and overall survival (OS) were statistically similar between groups.[5-7]

During the transplantation process, the risk of febrile neutropenia (FN) increases substantially due to intensive chemoradiotherapy-induced immunosuppression, neutropenic failure, prolonged retention of central venous catheters, and mucositis.[8] In patients who develop FN, bacterial, fungal, or viral infections may follow a severe course, be rapidly progressive, and be fatal.[9] In particular, bloodstream infection (BSI) is a common infectious complication in the early period following allo-HSCT, with reported incidences ranging from 13.6% to 38.9%.[10-12] In addition, laboratory findings, such as elevated procalcitonin and high C-reactive protein (CRP) levels, are associated with poor prognosis.[13-14]

The aim of this study was to compare the incidence of FN, infection characteristics, and the effect of FN on mortality in patients with hematologic malignancies who received either fresh or cryopreserved stem cell infusions. Additionally, the independent effects of variables, such as patients' demographic and clinicopathological data, duration of FN episodes, depth of neutropenia, length of hospital stay, infectious agents, and patients' comorbidity scores on FN-related mortality were investigated.

Materials and Methods

Study design and population. In this study, the data of 155 patients who underwent allo-HSCT at our hematology clinic between 2010 and 2023 were retrospectively reviewed. A total of 143 patients aged ≥18 years with FN and a hematological disease were included in the study. Twelve patients who did not develop FN during allo-HSCT were excluded. Patient data were obtained from the hospital information system and patient files.

Mobilization and apheresis procedures for allo-HSCT were performed according to international standards. All donors were mobilized with G-CSF alone (10 μg/kg/day for 4–5 days); plerixafor was not used in healthy donors in this cohort. Apheresis was performed using the Spectra Optia (Terumo BCT, Lakewood, CO, USA) cell separators, and $CD34^+$ cell counts were monitored by flow cytometry. No collection failures occurred during the study period. Peripheral blood stem cells collected from donors after mobilization were obtained using an apheresis device. The collected fresh cell products were preserved under appropriate conditions (+4°C) on the same day and infused into the recipient within 24 h at the latest. No freezing or cryoprotectant agents were used for fresh products.[15] For cryopreserved products, cells were frozen at a controlled rate with a cryoprotectant solution containing 10% DMSO and stored long-term at –80°C.[16] According to the ASTCT guidelines, the minimum acceptable $CD34^+$ cell dose for transplantation is $\geq 2 \times 10^6$ $CD34^+$ cells/kg, while the ideal collection target is $2–5 \times 10^6$ $CD34^+$ cells/kg.

A febrile episode during FN was defined as a single oral or axillary temperature measurement>38.3°C or a sustained temperature>38°C for at least 1 h. Neutropenia was defined as either an expected neutrophil count of <500/μL or a neutrophil level between 500–1000/μL anticipated to fall to <500/μL within 48 h.[17] All patients received ciprofloxacin prophylaxis.

We performed computed tomography scans and serum galactomannan measurements to identify patients with invasive pulmonary aspergillosis (IPA). Starting on the first day of the FN episode, serum samples were collected twice weekly for galactomannan testing. According to the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) revised definitions of invasive fungal disease, IPA was categorized as "possible," "probable," or "proven".[18]

The Hematopoietic Cell Transplantation–Comorbidity Index (HCT-CI) was used to evaluate the patients' pre-transplant comorbidity burden. According to the original definition by Sorror et al., patients were evaluated in three risk groups: a score of 0 was classified as low risk, 1–2 as intermediate risk, and ≥3 as high risk.[19]

Bacterial identification was performed by matrix-assisted laser desorption ionization–time-of-flight–mass spectrometry (MALDI–TOF–MS) using a Bruker Daltonics 5 system from Germany. Antimicrobial susceptibility testing encompassed the PhoenixTM 100 System Kirby–Bauer Disk Diffusion test (Oxoid, UK) and gradient diffusion methods (bioMérieux, France). The recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were applied, and, according to CDC recommendations, carbapenem resistance was defined as resistance to at least one carbapenem.[20,21]

The primary outcome was to compare the incidence and duration of FN between recipients of fresh and cryopreserved grafts. Secondary outcomes included evaluating BSI and IPA and identifying independent risk

factors for FN-related mortality.

Statistical Analysis. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.0. Descriptive statistics were presented as n (%) for categorical variables and as median for continuous variables. Continuous variables were compared using the Mann–Whitney U test. Categorical variables were compared using the chi-square test. Factors affecting FN-related mortality were first analyzed by univariate logistic regression. Variables with p-values of <0.05 in univariate analysis were included in the multivariate logistic regression model. A p-value of <0.05 was considered statistically significant in all analyses.

Ethical Approval. The ethics committee of the Faculty of Medicine, Bursa Uludag University, approved this study (decision number: 2023-7/9).

Results. A total of 143 patients were included in the study; 98 underwent allo-HSCT with fresh stem cells and 45 with cryopreserved stem cells. Of the patients, 88 had acute myeloid leukemia (AML), and 46 had acute lymphoblastic leukemia (ALL). Additionally, 98 transplants were performed prior to the COVID-19 pandemic. No significant differences were found between the two groups regarding age, sex distribution, type of diagnosis, rate of relapsed/refractory disease, HLA compatibility, conditioning regimens used, or the application of total body irradiation (TBI) (p > 0.05). The CD34+ cell count was significantly higher in the cryopreserved group (7.58 ± 0.88 × 10^6/kg vs. 5.45 ± 1.27 × 10^6/kg; p <0.001). The duration of the FN episode was similar between the groups (11.40 ± 5.59 vs. 12.65 ± 4.79 days; p = 0.077). However, the duration of deep neutropenia (ANC <100/mm^3) was significantly longer in the cryopreserved group (11.56 ± 4.84 vs. 7.78 ± 3.03 days; p <0.001). There was no significant difference between the groups in the number of days with neutrophils <500/mm^3 or in length of hospital stay (p > 0.05).

No significant differences were observed between groups regarding the day of onset of the FN episode, etiology of FN (Fever of unknown, clinical, microbiological), initial treatment regimens, or treatment response. Gram-positive bacterial (GPB) infections were similar between the groups (p = 0.176). Gram-negative bacteria (GNB) were more frequent in the cryopreserved group (p <0.001). The probability of invasive fungal infection (IFA) was also higher in the cryopreserved group (p = 0.009). Although 30-day mortality (fresh 1% vs. cryopreserved 6.8%; p = 0.092) and FN-related mortality (fresh 2% vs. cryopreserved 6.8%; p = 0.078) were higher in the cryopreserved group, these differences did not reach statistical significance **(Table 1).**

GNB accounted for 64% (41/63) of BSI episodes, and GPB for 36% (22/63). The most frequently isolated GPBs were *S. epidermidis* (8%) and *S. hominis* (3%). The most frequently isolated GNB were *E. coli* (n = 27, 19%) and *K. pneumoniae* (n = 14, 8%). Among *E. coli* isolates, 96% were carbapenem-sensitive, and 4% were carbapenem-resistant. Among *K. pneumoniae* isolates, 75% were carbapenem-sensitive, and 25% were carbapenem-resistant. Thirty-seven percent of *E. coli* isolates were ESBL-positive, and 63% were ESBL-negative. Sixty-seven percent of *K. pneumoniae* isolates were ESBL-positive, and 33% were ESBL-negative **(Table 2).**

Univariate logistic regression analysis for FN-related mortality revealed that the significant risk factors were duration of FN episode (16.67±7.06 vs. 12.07 ± 4.91 days; p = 0.038), higher procalcitonin level (2.30 ± 2.31 vs. 0.77 ± 1.40; p = 0.046), and higher HCT-CI score (p = 0.037) **(Table 3**). In the multivariable logistic regression analysis, prolonged FN episode duration (OR 1.18; 95% CI 0.99–1.41; p = 0.046) and higher HCT-CI scores (Score 1: OR 20.80; p = 0.014; Score 2: OR 22.88; p = 0.039) were identified as independent risk factors for FN-related mortality. No significant correlation was found between procalcitonin level and risk of FN-related mortality **(Table 4).**

Discussion. This study provides significant data on the incidence, course, and mortality of FN in patients undergoing allo-HSCT with fresh and cryopreserved grafts. Although cryopreserved stem cell grafts prolonged the duration of deep neutropenia and increased the risk of GNB and fungal infections, they did not translate into increased FN-related mortality. This study demonstrated that, irrespective of graft type, prolonged FN duration and high HCT-CI comorbidity score are the primary determinants of mortality.

Nevertheless, most studies reported no effect of cryopreservation on engraftment and hematopoietic recovery.[22,23] There was also biological evidence that the different cell types comprising the graft exhibit varying degrees of sensitivity to cryopreservation and storage. In recent years, knowledge in this field has expanded significantly, indicating that cryopreservation, as well as the various variables associated with it, may differentially affect not only the viability but also the function of various graft components.[24]

Prolonged and deep neutropenia is a strong factor that increases mortality risk in patients with hematological malignancies and those undergoing allo-HSCT. Moreover, prolonged neutropenia increases the risk of gram-negative bacteremia and sepsis by facilitating bacterial translocation, which significantly elevates FN-related mortality.[25,26] Islas-Muñoz et al. reported that prolonged neutropenia significantly increases the risk of BSI and 30-day mortality in patients with hematological

Table 1. Comparison of sociodemographic and clinicopathological characteristics of fresh versus cryopreserved stem cell transplants (n = 143).

Variable	Fresh, n = 98 Mean ± SD	Cryopreserved, n = 45 Mean ± SD	p-value
Age	40,85±10,59	43,31±13,86	0,293
CD34+ stem cells (106)	5,45±1,27	7,58±0,88	**<0,001**
Duration of FN episode (days)	12,65±4,79	11,40±5,59	0,077
Initial day of FN episode	6,52±2,39	6,47±2,48	0,949
Neutrophil < 500/mm3 (days)	14,55±4,75	15,53±5,63	0,427
Neutrophil < 100/mm3 (days)	7,78±3,03	11,56±4,84	**<0,001**
Length of hospital stay (days)	34,36±7,24	35,47±8,65	0,502
CRP	116,74±71,19	122,40±68,35	0,576
Procalcitonin	0,84±1,46	0,82±1,51	0,138
Sex *Male* *Female*	 63 35	 22 23	0,082
Diagnosis *AML* *ALL* *Other*	 62 32 4	 26 14 5	0,140
Relapsed/Refractory *No* *Yes*	 79 19	 31 14	0,122
HLA mismatch *10/10* *9/10*	 88 10	 44 1	0,172
Conditioning regimens *BuCy* *VP/Cy/TBI* *Flu/Mel* *Flu/Cy/ATG* *Cy/TBI* *BEAM* *RIC-NMA*	 81 12 1 1 1 1 1	 33 7 3 2 0 0 0	0,302
TBI *No* *Yes*	 85 13	 38 7	0,714
FN episodes FUO Clinical documented Microbiological evidence	 55 30 13	 16 22 7	0,621
Prior FN episode *No* *Yes*	 15 83	 7 38	0,172
FN initial treatment PIP/TAZ+glycopeptide PIP/TAZ Cefepime Meropenem PIP/TAZ+clarithromycin	 38 50 6 2 2	 23 20 0 0 2	0,224
Initial treatment response *No* *Yes*	 52 46	 17 28	0,089
Gram-negative bacteria No *E. coli* *K. pneumoniae* *A. baumanni*	 81 13 3 0	 21 14 9 1	**<0,001**

P. aeruginosa	1	0	
Gram-positive bacteria No *S. epidermidis* *S. hominis* *E. faecium* *S. hemoliticus* *C. jeikeium* *E. species*	 79 11 3 1 2 1 1	 41 0 1 2 0 1 0	0,176
IPA *No* *Possible* *Probable*	 92 4 2	 35 9 1	0,009
Pitt Score *0* *1* *2* *3*	 87 11 1 0	 39 3 2 1	0,193
HCT-CI Score *0* *1* *2* *3*	 84 8 5 1	 35 4 4 2	0,444
CMV reactivation *No* *Yes*	 77 21	 31 14	0,211
30-day mortality *No* *Yes*	 97 1	 42 3	0,092
FN-related mortality *No* *Yes*	 96 2	 41 4	0,078

BEAM: carmustine/etoposide/cytarabine/melphalan, BuCy: busulfan/cyclophosphamide, CMV:cytomegalovirus, CRP: C-reactive protein, Flu/Mel: fludarabine/melphalan, FN: febrile neutropenia, FUO: fever unknown origin, HCT-CI: hematopoietic cell transplantation–comorbidity index, IPA: invasive pulmonary aspergillosis, PIP/TAZ: piperacillin and tazobactam, RIC-NMA: reduced intensity conditioning-nonmyeloablative, VP/Cy/TBI: etoposide/cyclophosphamide/total body irradiation

Table 2. Carbapenem resistance and ESBL positivity in gram-negative bacteria isolated in blood culture.

Gram-negative bacteria	**Total n = 41**	**Carbapenem**	**Carbapenem**	**ESBL**	**ESBL**
Gram-negative bacteria	Total n = 41	Sensitive n, %	Resistant n, %	Positive n, %	Negative n, %
E. coli	27	26 (96)	1 (4)	10 (37)	17 (63)
K. pneumoniae	12	9 (75)	3 (25)	8 (67)	4 (33)
A. baumannii	1	1 (100)	0	0	1 (100)
P. aeruginosa	1	1 (100)	0	0	1 (100)

ESBL: Extended-spectrum beta-lactamases

Table 3. Univariate logistic regression analysis of variables affecting FN-related mortality.

Variable	**Alive Mean ± SD**	**Exitus Mean ± SD**	p-value
Age	41,80±11,66	37,67±13,69	0,402
CD34+ stem cells (106)	6,07±1,48	7,37±2,12	0,123
Duration of FN episode (days)	12,07±4,91	16,67±7,06	0,038
Initial day of FN episode	6,58±2,36	4,67±3,01	0,185
Neutrophil < 500/mm3 (days)	14,85±5,14	15,00±2,01	0,845
Neutrophil < 100/mm3 (days)	9,01±4,13	8,00±2,44	0,552
Length of hospital stay (days)	34,61±7,71	36,17±7,83	0,636

CRP	117,14±69,14	150,17±91,35	0,257
Procalcitonin	0,77±1,40	2,30±2,31	0,046
Sex			
Female	*55*	3	0,687
Male	*82*	3	
Diagnosis			
AML	85	3	0,546
ALL	44	2	
Other	8	1	
Relapsed/Refractory			
No	106	4	0,622
Yes	31	2	
HLA mismatch			
10/10	127	5	0,415
9/10	10	1	
Conditioning regimens			
BuCy	109	5	0,989
VP/Cy/TBI	18	1	
Flu/Mel	4	0	
Flu/Cy/ATG	3	0	
Cy/TBI	1	0	
BEAM	1	0	
RIC-NMA	1	0	
TBI			
No	118	5	0,847
Yes	19	1	
FN episodes			
FUO	70	0	0,799
Clinical documented	20	1	
Microbiological evidence	47	5	
Prior FN episode			
No	22	0	0,590
Yes	115	6	
FN initial treatment			
PIP/TAZ+glycopeptide	69	1	0,368
PIP/TAZ	56	5	
Cefepime	6	0	
Meropenem	2	0	
PIP/TAZ+clarithromycin	4	0	
Initial treatment response			
No	69	5	0,150
Yes	68	1	
Gram-negative bacteria			
No	101	1	0,315
E. coli	24	3	
K. pneumoniae	11	1	
A. baumanni	0	1	
P. aeruginosa	1	0	
Gram-positive bacteria			
No	114	6	0,977
S. epidermidis	11	0	
S. hominis	4	0	
E. faecium	3	0	
S. hemoliticus	2	0	
C. jeikeium	2	0	
E. species	1	0	
IPA			
No	122	5	0,803
Possible	12	1	
Probable	3	0	
Pitt Score			
0	125	0	0,559
1	11	3	
2	1	2	

3	0	1	
HCT-CI Score			
0	117	2	
1	10	2	
2	8	1	0,037
3	2	1	
CMV reactivation			
No	105	3	0,158
Yes	32	3	

BEAM: carmustine/etoposide/cytarabine/melphalan, BuCy: busulfan/cyclophosphamide, CMV:cytomegalovirus, CRP: C-reactive protein, Flu/Mel: fludarabine/melphalan, FN: febrile neutropenia, FUO: fever unknown origin, HCT-CI: hematopoietic cell transplantation–comorbidity index, IPA: invasive pulmonary aspergillosis, PIP/TAZ: piperacillin and tazobactam, RIC-NMA: reduced intensity conditioning-nonmyeloablative, VP/Cy/TBI: etoposide/cyclophosphamide/total body irradiation

Table 4. Multivariate logistic regression analysis of factors affecting FN-related mortality.

Variables	OR (95% CI)	p-value
Duration of FN episode (days)	1,18 (0,99-1,41)	0,046
Procalcitonin	1,23 (0,86-1,76)	0,252
HCT-CI score (ref: 0)		
1	20,80 (1,83-235,86)	**0,014**
2	22,88 (1,17-444,05)	**0,039**
3	21,27 (0,54-903,87)	0,100

$p < 0.001$; −2 log likelihood = 34.615; Nagelkerke R2: 0.241. FN: febrile neutropenia, HCT-CI: hematopoietic cell transplantation–comorbidity index.

malignancies.[27] Cao et al. showed that prolonged neutropenia (>21 days) is an independent risk factor for BSI in patients with allo-HSCT.[28] Our findings are consistent with these reports and indicate that prolonged neutropenia is associated with increased FN-related mortality during allo-HSCT.

The rate of infection, particularly bacterial infections, is high in patients with FN and is frequently observed in the early post-transplant period.[29] Zhang et al. showed that 70.8% of BSIs during FN were caused by GNB, among which high rates of ESBL and carbapenem-resistant strains were noted, and mortality was higher in the presence of resistant GNB.[30] A multicenter study in Argentina involving patients with hematological malignancies and HSCT recipients demonstrated that carbapenem-resistant strains were strongly associated with mortality when GNB were isolated.[31] In the present study, similar to published reports, GNB-related BSIs were significantly more frequent in the cryopreserved graft cohort.[32-34] However, no statistically significant difference in FN-related mortality was observed between the groups.

Among the six patients who died, GNB were isolated in five cases, whereas no GPB were isolated in any of these patients. Of the isolates, three had *E. coli* (2 ESBL+, 1 carbapenem-resistant), one was *K. pneunomiae* (ESBL+ and carbapenem-resistant), and one was *A. baumannii* (ESBL- *and* carbapenem-sensitive). In one patient, no bacteria were isolated in the culture. Our findings indicate that GNB in BSIs were more closely associated with mortality than GPB.

Pagano et al. and his group showed that invasive fungal infections significantly reduced 1-year survival in patients undergoing allo-HSCT and that Aspergillus infections were particularly associated with high mortality.[35,36] Neofytos et al. reported a 12-week mortality rate of 46.7% among HSCT recipients who developed invasive aspergillosis.[37] A meta-analysis including 51 studies found that prolonged neutropenia after HSCT is associated with an increased risk of invasive fungal infections.[38] In the present study, the incidence of IPA was higher among recipients of cryopreserved grafts; however, IPA was not significantly associated with FN-related mortality. This suggests that although IPA may occur more frequently in cryopreserved graft recipients, it does not alone determine early FN-related mortality.

Another finding of the present study is that patients with a high HCT-CI score had a markedly increased risk of FN-related mortality. HCT-CI is an index developed by Sorror et al. to quantify the burden of pretransplant comorbidities in allo-HCT recipients and to predict non-relapse mortality (NRM) and survival.[19] Acosta-Medina et al. demonstrated that a high HCT-CI score was associated with reduced long-term OS in 87 patients with myelofibrosis who underwent allo-HSCT.[39] Bayrakter et al. reported that in-hospital mortality was significantly higher among patients with HCT-CI ≥ 2 who were admitted to the intensive care unit within the first 100 days after allo-HSCT.[40] Our results suggest that HCT-CI may be useful for predicting long-term mortality and estimating the risk of early FN-related mortality.

Unlike previous studies focused on engraftment and GVHD, our research examines infection-related outcomes and FN-related mortality in allo-HSCT. We provide novel insights by identifying that prolonged FN duration and comorbidity burden (HCT-CI), rather than graft cryopreservation, are the primary drivers of FN-related mortality. This distinction shifts the focus from graft processing to patient-specific risk factors in clinical management.

Limitations of the present study include its retrospective, single-center design, limited sample size, and temporal changes in transplant care standards. One limitation of our study is the relatively small number of mortality events, which may have led to high and less stable Odds Ratio estimates for certain predictors, such as the HCT-CI score. While these results underscore the clinical importance of pre-transplant comorbidities and FN duration, the exact magnitude of their impact should be interpreted with caution and validated in larger, prospective multicenter cohorts. Although the optimal storage condition for hematopoietic stem cells is widely considered to be -163°C, another limitation of our study is the use of mechanical freezers at -80°C for graft preservation. However, recent evidence and clinical reviews suggest that storage at -80°C is a safe and effective alternative for periods of up to several years, with no significant compromise in cell viability or engraftment kinetics.[41,42]

Conclusions. The results of the present study showed that infections were more frequent in cases that received cryopreserved grafts, although this did not have a direct effect on FN-related mortality. In contrast, multivariable analysis suggested that prolonged neutropenia duration and high HCT-CI scores may be more critical risk factors for FN-related mortality. However, due to the limited number of deaths, the high odds ratio estimates should be interpreted with caution. Although the associations were statistically significant, the exact effect sizes may reflect the high-risk nature of the cohort and require confirmation in larger multicenter studies. Overall, patient-related factors, including comorbid conditions and the duration of neutropenia, may be more relevant to FN-related outcomes than graft source.

Data availability statement. The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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