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FULL PAPER

The association of CMV infection with bacterial and fungal infections in hematopoietic stem cell transplant recipients: a retrospective single-center study

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SUMMARY

This study aims to evaluate the probable association between CMV infection and bacterial or fungal infections in 91 consecutive adult patients who underwent autologous or allogeneic HSCT within a period of two years.

The medical records of the patients were retrospectively reviewed. Blood cultures were evaluated by an automated blood culture system**.** A quantitative real-time polymerase chain reaction was performed to detect CMV DNA.

CMV infection and CMV disease were detected in 42 (46%) and six (6.6%) patients, respectively. Of the 158 microorganisms isolated, 115 (73%) were Gram-positive bacteria. Bacteremia and fungemia developed in 55 (60%) and eight (8%) patients, respectively. Concurrent CMV infection and bacteremia were detected in 17 (18.7%) patients and concurrent CMV infection and fungal infection were detected in five (5.5%) patients. Graft versus host disease (GVHD) developed in 15 (50%) allogeneic HSCT recipients and two (2.2%) autologous HSCT recipients. Twenty-one (23%) patients including 13 (43%) allogeneic and eight (13%) autologous HSCT recipients died.

The most common infection is bacteremia, and it develops concurrently with CMV infection in approximately one-fifth of HSCT recipients. Gram-positive bacteria are more common in bacteremia. Further studies on the follow-up and treatment of infections after HSCT will improve post-HSCT survival rates.

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INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is a well-established technique that offers a potential cure for hematologic cancers including leukemias, lymphomas, myeloma, and other hematologic disorders including primary immunodeficiency, aplastic anemia, myelodysplasia and solid tumors that respond to chemotherapy. Autologous HSCT (auto-HSCT) is performed using the patient's own hematopoietic stem cells, which are harvested before transplantation and reinfused after myeloablation. Allogeneic HSCT (allo-HSCT) uses human leukocyte antigen (HLA)-matched stem cells derived from a donor. Infections are important causes of morbidity and mortality after HSCT (Hatzimichael and Tuthill, 2010). Infectious complications are influenced by time of development of infections after HSCT, prophylaxis strategy against infections, comorbidities, pathogen exposure and the status of immunosuppression (Ghogomu *et al*., 2019). Infectious agents vary according to the post-transplantation period. Bacteria and *Candida* species are the predominant agents in the neutropenic period, while CMV is the major cause of infection between 30-100 days post HSCT. CMV infection occurs in 60% of seropositive allo-HSCT recipients. Without appropriate treatment, CMV infection may progress to CMV disease. Although the incidence of CMV disease has been significantly reduced by prophylaxis and preemptive treatment, this complication develops in 30% of allo-HSCT patients (Lin *et al*., 2017). CMV disease mainly involves the lungs and gastrointestinal system.

Key words:
Bacterial infections, CMV disease, CMV infection, graft-versus-host disease, hematopoietic stem cell transplantation.

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This retrospective study conducted in a population with high CMV seropositivity addresses issues associated with CMV reactivation. We aim to investigate the frequency of CMV infection and CMV disease, bacterial and fungal infections as well as risk factors, the graft-versus-host disease (GVHD) status, mortality rates and overall survivability (OS) in patients who underwent auto- and allo-HSCT in a single center.

MATERIAL AND METHODS

Ljungman's definitions were used to define CMV infection/disease (Ljungman *et al.,* 2017). Accordingly, CMV infection is defined as the detection of CMV nucleic acid in body fluids or tissue in the absence of clinical symptoms. CMV disease is defined as the concomitant presence of CMV nucleic acid in body fluids or tissue and clinical findings characterized by lung, gastrointestinal system, liver, central nervous system, and/or retina involvement. Multiple resistant bacteria are defined as microorganisms resistant to at least one antibacterial agent in each of three or more antimicrobial categories (Magiorakos *et al*., 2012). *Stenotrophomonas maltophilia* strains and methicillin-resistant *staphylococci* are considered multiple resistant bacteria. Neutropenia is defined as severe, moderate and mild neutropenia if absolute neutrophil count is <500 cells/mm^3, 500-1000 cells/mm^3, 1000-1500 cells/mm^3, respectively (Freifeld *et al*., 2011). Lymphopenia is defined as absolute lymphocyte count <1000 cells/mm^3 (Freifeld *et al*., 2011). Acute GVHD grading was performed according to the modified Glucksberg-Seattle criteria (Przepiorka *et al*., 1995). Chronic GVHD patients were classified as mild, moderate, and severe according to the 2014 NIH Consensus criteria (Jagasia *et al*., 2015). Infections occurring 48 hours after hospitalization were defined as hospital infections (Horan *et al*., 2008). Late CMV infection is defined as CMV infection occurring 100 days after HSCT during the late post engraftment period (Asano-Mori *et al*., 2008). Diagnosis of CMV hepatitis required the demonstration of CMV DNA in tissue by PCR in the presence of clinical and laboratory findings (Clarke *et al*., 1979). The cause of death was determined according to the algorithm of Copelen *et al.* (Copelan *et al*., 2007). Time to infection (TTI) was defined as the number of days from time of HSCT to first day of CMV DNA positivity (Sousa *et al*., 2014).

Study design

The medical records of 91 consecutive adult patients (57 males, 34 females) who underwent auto- or allo-HSCT at Istanbul University Istanbul Faculty of Medicine Adult Hematopoietic Stem Cell Transplantation Unit from January 2017 to December 2018 were retrospectively reviewed. This study was carried out following the ethical values specified in the Declaration of Helsinki and was approved by the Istanbul Faculty of Medicine Clinical Research Ethics Committee (2020/883). Informed consent was not required from the participants due to the retrospective nature of the study.

Patients

The mean age of the study cohort was 51.15±14.4 years. The most common diagnosis in the study group was multiple myeloma (54.9%) followed by acute myeloid leukemia (13.2%), non-Hodgkin lymphoma (9.9%), and Hodgkin lymphoma (5.5%) (*Table 1*). Thirty (33%) and 61 (67%) patients underwent allo-HSCT and auto-HSCT, respectively. Serological data for CMV was not available for four allo-HSCT recipients and three allo-HSCT donors. Twenty-five (92.5%) allo-HSCT recipients and 21 (75%) allo-HSCT donors were CMV-seropositive. Of the allo-HSCT donors, 18 (60%) were unrelated donors and 12 (40%) were related. Of the related donors, 11 (91.7%) were HLA full-compatible, and one (8.3%) was a haploidentical donor. Of the unrelated donors, 11 were 10/10 HLA-compatible (61%) and seven (39%) were 9/10 HLA-compatible. For patients who underwent auto- and allo-HSCT, time from diagnosis to HSCT was 33±44 months (5-147) and 37±65 months (3-326), respectively. Pretransplant disease status of the patients who underwent allo-HSCT and auto-HSCT is described in *Table 1*. Fluconazole was our drug of choice for fungal prophylaxis. In allo-HSCT recipients, fluconazole 200 mg/day was started concomitantly with the conditioning regimen and continues until day 75 post-transplant (Marr *et al*., 2000). Our auto-HSCT recipients were given fungal prophylaxis if the anticipated duration of neutropenia after auto-HSCT was long (lymphoma), if a high-intensity conditioning regimen (e.g., BEAM or Bu/Cy/Etoposide for lymphoma, Bu/Cy for leukemia) was administered, or if there is recent exposure to purine analogues. For auto-HSCT recipients, Fluconazole 200 mg/day was initiated on the day of stem cell infusion and continues until day 30 post-transplant. In our center, CMV prophylaxis is not administered to allo- and auto-HSCT recipients. In our routine practice, we administer preemptive treatment for CMV infection. For treatment of CMV infection, intravenous ganciclovir 2x5 mg/kg/day and valganciclovir 900 mg/day were used in 93% and 7% of the patients, respectively. *Table 1* also describes the conditioning regimens used for allo-HSCT and auto-HSCT patients.

Detection of infections and antibiotic susceptibility assays

Blood cultures obtained from febrile patients were incubated for five days in an automated blood culture system (BACTEC FX, Beckton Dickinson-USA) to detect the presence of bacteremia. Identification of

microorganisms isolated from positive signaling bottles were performed by conventional methods and/or with an automated identification system (Phoenix 100, Beckton Dickinson-USA). Skin and soft tissue, respiratory tract, urinary tract, and gastrointestinal system cultures were carried out by conventional methods. Antibiotic susceptibility tests were performed by disc diffusion method and/or gradient test (E-test, bioMerieux, France) and evaluated according to recommendations of the Clinical and Laboratory Standards Institute (CLSI 2017). The first isolate of each bacterial species was included in the study for each patient. Only the susceptibility results of routinely tested antibiotics were evaluated in the study. Pathogens that are intermediate to antibiotics are considered resistant.

Detection of CMV-DNA

The quantitative value of CMV-DNA was determined by real-time polymerase chain reaction (RT-PCR). Following the achievement of hematopoietic engraftment post-HSCT, CMV-DNA follow-up was performed twice a week during hospitalization and once a week during the first 100 days after the patient was discharged.

Plasma samples taken from the patients were stored at -20 °C until analysis. For quantitative CMV DNA detection in plasma samples, extraction with the QIAsymphony DSP Virus/Pathogen Mini Kit (QIAGEN, Germany), amplification and quantitation using the Artus® CMV QS-RGQ Kit (QIAGEN, Germany) on the QIASYMPHONY SP-AS (QIAGEN, Germany) RT-PCR method was studied on the Ro-

Table 1 - *The characteristics of all patients (n=91).*

Patient characteristics	*n*	*%*
Gender		
Male	57	62.6
Female	34	37.4
Stem cell source		
Peripheral stem cell	87	94.4
Bone marrow	3	4.4
Umblical	1	1.1
Donor		
Related	13	41.9
Unrelated	18	58
Underlying disease		
Multiple myeloma	50	54.9
Acute myeloid leukemia	12	13.2
Nonhodgkin lymphoma	9	9.9
Hodgkin lymphoma	5	5.5
Acute lymphoid leukemia	3	3.3
Aplastic anemia	3	3.3
Chronic myeloid leukemia	2	2.2
Myelodysplasia syndrome	2	2.2
Diffuse large B-cell lymphoma	2	2.2
Primary myelofibrosis	1	1.1
Mastocytosis	1	1.1
Mantle cell lymphoma	1	1.1
Remission status at time of allo-HSCT		
First complete remission	13	43.3
Second complete remission	3	10
Third complete remission	1	3.3
Complete remission	2	6.6
Partial response	2	6.6
Refractory disease	4	13.3
Relapse	1	3.3
Relapse refractory	1	3.3
Chemosensitive relapse	1	3.3
Good partial response	1	3.3
Very good response	1	3.3
Remission status at time of auto-HSCT		
First complete remission	6	9.8
Complete remission	1	1.6
Very good partial response	36	59
Complete response	8	13.1
Partial response	9	14.7
Relapse	1	1.6
Conditioning regimen for auto-HSCT		
Melphalan	49	80
BEAM[a]	11	18
Fludarabine + melphalan	1	2
Conditioning regimen for allo-HSCT		
Bisulfan + cyclophosphamide	6	20
Bisulfan + cyclophosphamide	4	13.3
Fludarabine + bisulfan	4	13.3
Fludarabine + melphelan	7	23.3
Bisulfan + cyclophosphamide + ATG[b]	1	3.3
Fludarabine + treosulfan	1	3.3
Fludarabine + cyclophosphamide	2	6.6
Fludarabine + cyclophosphamide + ATG[b]	1	3.3
Fludarabine + melphelan + ATG[b]	1	3.3
Bisulfan+tiotepa	1	3.3
Fludarabine + bisulfan + ATG[b]	1	3.3
Tiotepa + bisulfan + fludarabine + cyclophosphamide	1	3.3
Allogeneic stem cell donor CMV status		
Seropositive	21	75
Seronegative	7	25
Allogeneic stem cell recipient CMV status		
Seropositive	25	92.5
Seronegative	2	7.5
CMV donor/recipient status		
D-R-	2	6.6
D+R-	-	-
D-R+	5	16.6
D+R+	19	63.3

[a]BEAM: BCNU (1,3-Bis2-chloroethyl-1-nitrosourea), etoposid, ARA-C, melphalan; [b]ATG: Anti thymocyte globülin; D: Donor; R: Recipient.

tor-Gene Q (QIAGEN, Germany) device. The analytical sensitivity of the kit is 43 copies/ml, the quantitation range is 80-10^8 copies/ml, and each kit includes an internal control to detect false negatives. Internal control was added to each sample before extraction.

Detection of CMV involvement

CMV pneumonia was diagnosed in the presence of clinical and radiological findings and CMV DNA PCR positivity in bronchoalveolar lavage (BAL) fluid.

In the presence of clinical findings suggestive of CMV involvement in the gastrointestinal tract, immunohistochemical staining in tissue biopsy preparation and/or CMV PCR positivity in fresh tissue was required for confirmation of CMV gastrointestinal system involvement (Durand *et al*., 2013; Mills *et al*., 2013).

CMV cystitis was diagnosed in the presence of inclusion bodies in the tissue sample taken from the bladder and CMV DNA PCR positivity in the tissue (Tutuncuoglu *et al*., 2005).

Statistical analysis

The parameters including clinical and laboratory data were loaded into the SPSS 21.0 program for statistical analysis; values of $p<0.05$ were considered significant. Before making group comparisons for continuous variables and normal distribution compliance tests by Shapiro-Wilk test, a non-parametric test was used for all variables. Comparisons between paired groups were made using the Mann-Whitney U test, since they did not show normal distribution. Chi-square, Fisher, and Freeman-Halton tests were used to examine the distribution of qualitative and categorical variables in groups. The difference in the time to duration between development of CMV disease and CMV infection in patients who underwent allo-HSCT and auto-HSCT was examined by Kaplan-Meier survival analysis.

RESULTS

In this study, a total of 91 patients who underwent auto-HSCT and allo-HSCT were included. Patient characteristics are summarized in *Table 1*. The mean age of patients who underwent auto-HSCT (59; 28-70) and allo-HSCT (37; 20-65) were statistically different ($p<0.001$). The patient groups showed no difference for gender ($p=0.577$). Patients with CMV disease in the allo-HSCT group showed no difference with respect to gender ($p=0.640$). Time to HSCT was 33±44 months (min-max: 5-147) for auto-HSCT patients and 37±65 months (min-max: 3-326) for allo-HSCT patients. All data are shown in *Tables 1, 2, 3,* and *4*.

Sixteen patients (17.5%) developed neutropenia and 27 patients (30%) developed lymphopenia. Forty-two (46%) patients developed CMV infection and six (6.6%) patients developed CMV disease. Bacteremia was detected in fifty-five (60%) patients and fungal infection in eight (8.8%) patients. Concomitant CMV infection and bacteremia developed in 17 (18.7%) patients. Concomitant CMV infection and fungal infection developed in five patients (5.5%). CMV PCR was detected in tissue samples in four (4.4%) patients. In the auto-HSCT group, BEAM and melphalan were the conditioning regimens in 11 patients with lymphoma and in 49 patients with myeloma, respectively. CMV infection and CMV disease were diagnosed in 15 of 49 (30.6%) and one

Table 2 - *The characteristics of auto- and allo-HSCT recipients.*

Characteristics	*Auto-HSCT (n=61)*	*Allo-HSCT (n=30)*	*p value*
Male sex	37, %61	20, %66	0.577
Age of at transplantation (mean-year)	57.2±10.2	38.8±14	*<0.001*
Time of diagnosis up to HSCT (mean-day)	33±44	37±65	0.936
Use of peripheral blood stem cell as stem cell source	60, %98	25, %83	*<0.03*
Presence of CMV infection	22, %36	20, %67	*0.008*
Time to CMV infection (TTI) (mean-day)	370	304	0.158
Duration of neutropenia	62±48	115±161	0.753
Presence of CMV disease	1, %1.6	5, %16.6	*0.014*
Concurrent bacteremia in patients with CMV disease	1, %1.6	5, %16.6	*0.001*
Ground-glass opacity	6, %10	15, %50	*<0.001*
Presence of bacteremia	31, %51	24, %80	*0.007*
Presence of GVHD	2, %3.3	15, %50	*<0.001*
Mortality	8, %13	13, %43	*0.001*
Time to develop bacteremia (mean-day)	29±111	83±140	*0.001*
Duration of antiviral use (mean-day)	22±5,6	40±19	*0.001*
Duration of cotrimoxazole use (mean-day)	38±82	353±65	*<0.01*
Duration of ciprofloxacin use (mean-day)	2,8±6,6	21±47	*<0.01*
Duration of asiviral use (mean-day)	6±47	353±65	*<0.001*
Duration of INH use (mean-day)	31±87	93±128	*0.002*

Table 3 - *Differences between patients with and without CMV disease.*

Parameters	*With CMV disease*	*Without CMV disease*	*p value*
AST values in allo-HSCT (IU/L)	18±7	40±40	*0.014*
AST values in all patients (IU/L)	19±6	34±28	*0.017*
Duration of antiviral use in all patients (mean day)	48±19	26±13	*0.004*

Table 4

	Bacteremia		*Urinary Tr. Inf.*		*Lower Trac Inf.*		*Skin-Soft T. Inf*		*Gastro Int. Inf.*					
Microorganisms	*Auto-HSCT*	*Allo-HSCT*	*Auto-HSCT*	*Allo-HSCT*	*Auto-HSCT*	*Allo-HSCT*	*Auto-HSCT*	*Allo-HSCT*	*Auto-HSCT*	*Allo-HSCT*	*Total Auto-HSCT*	*Total Allo-HSCT*	*Main Total*	*Rate (%)*
GRAM NEGATIVE BACTERIA														
P. aeruginosa		1	1	1				1			1	3	4	2.5
S. maltophilia		1										1	1	0.63
Escherichia coli	2	4	2								4	4	8	5
Enterobacter spp	1	2									1	2	3	1.9
Serratia spp							1				1		1	0.63
K. pneumoniae	5	4	1	3	2	1					8	8	16	10
Acinetobacter spp.	1					3					1	3	4	2.5
H. influenzae					2						2		2	1.2
C. jejuni									1	1	1	1	2	1.2
H. pylori									1		1		1	0.63
Salmonella spp.									1		1		1	0.63
Total	9	12	4	4	4	4	1	1	3	1	21	22	43	26.8
GRAM POSITIVE BACTERIA														
MSSA	3		2								5		5	3.1
MRSA	3	2		1	1						4	3	7	4.4
MRCNS	19	21	4	2	1		1				25	23	48	30
Enterococcus spp.	2	2	1								3	2	5	3.1
VRE		1				1			17	22	17	23	41	25.6
C. difficile									3	1	3	1	4	2.5
S. pneumoniae	1				3						4		4	2.5
Total	27	20	7	3	4	2	1	1	20	27	59	53	114	71.2
FUNGI														
Candida spp.	1				1				1		3		3	1.9
MAIN TOTAL	*37*	*32*	*11*	*7*	*9*	*6*	*2*	*2*	*24*	*28*	*77*	*72*	*160*	*99.92*

of 49 (2%) patients who received melphalan as the conditioning regimen. In the auto-HSCT recipients diagnosed with lymphoma and who received BEAM as the conditioning regimen, CMV infection developed in six (54.5%) patients, while no patients developed CMV disease.

GVHD developed in 15 (50%) patients who underwent allo-HSCT and in two (3.3%) who underwent auto-HSCT (p<0.001). In patients with acute GVHD (n=17), the most common sites of isolated involvement were skin (n=6, 35%) followed by liver (12%), lung (6%) and gastrointestinal system (6%). Skin and liver involvement, skin and mucosa involvement, skin and liver and gastrointestinal system involvement, skin and liver and lung involvement, skin and mucosa and liver involvement were present in 6%, 6% ,12%, 12% and 6% of patients with acute GVHD, respectively.

In Kaplan-Meier survival analysis, no significant difference for overall survival (OS) was shown between patients with CMV infection and patients with no CMV infection (590±34 days, 554±39 days; p=0.695). OS was significantly lower for patients with CMV disease than for those with no CMV disease (425±100 days, 586±26 days; p=0.028) (*Figure 1*). In the allo-HSCT group, OS showed no significant difference for patients with CMV disease and for those with no CMV disease (432±123 days, 413±47 days; p=0.808). OS tended to be shorter in patients with CMV infection than in patients with no CMV infection (511±63 days, 286±58 days; p=0.063) (*Figure 2*).

Bacterial infections

Bacteremia developed in 55 (60%) patients in the whole study group. Time to bacteremia was 29±111 (20-601) days in the auto-HSCT group and 83±140

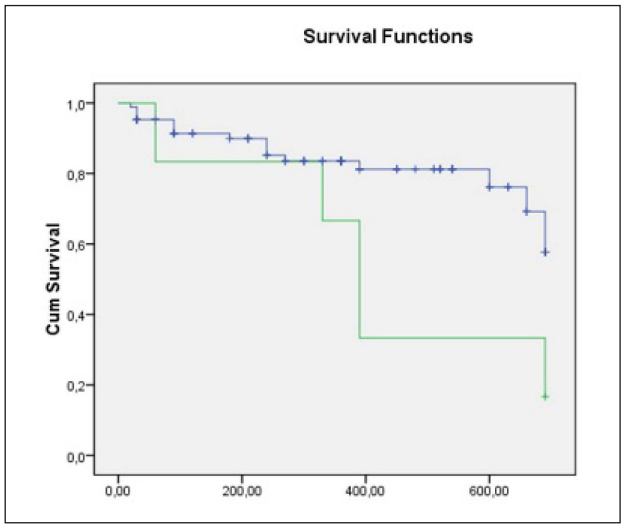

Figure 1 - *Overall survival for patients with CMV disease (in green) and without CMV disease (in blue) (425±100 days, 586±26 days;* ***p=0.028****).*

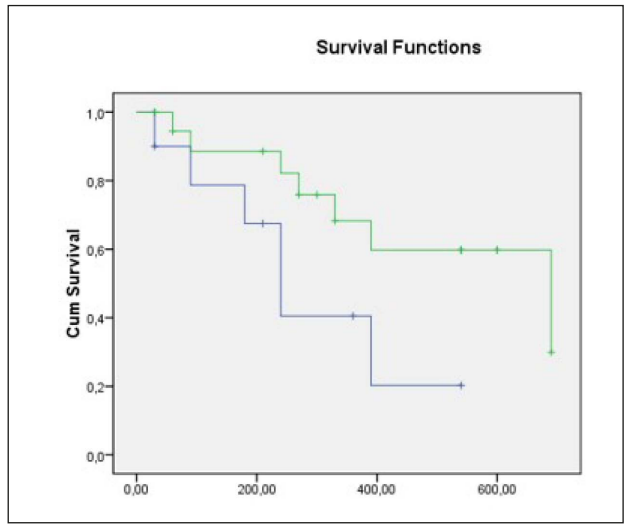

Figure 2 - *Overall survival for allo-HSCT patients with CMV infection (in green) and without CMV infection (in blue) (511±63 days, 286±58 days;* ***p=0,063****).*

(14-639) days in the allo-HSCT group (p=0.001). Of the total of 158 microorganisms isolated, the source was blood in 75 (47.5%) patients, urinary tract in 18 (11.4%) patients, lower respiratory tract in 15 (9.5%) patients, gastrointestinal system in 48 (30.4%) patients, and skin and soft tissue in two patients (1.2%) (*Table 4*). The most frequently (48, 30.4%) isolated bacteria were methicillin-resistant coagulase-negative *staphylococci* (MRCNS) among Gram-positive bacteria and *Klebsiella pneumoniae* (16, 10%) among Gram-negative bacteria, followed by *Escherichia coli* (8, 5%). Of the 37 (41%) patients with inserted catheters, bacteremia was detected in 32 (86.5%) patients. Vancomycin-resistant *Enterococci* (VRE) were isolated from rectal swab samples of 39 (43%) patients. Resistance to fluoroquinolones, third-generation cephalosporins, gentamicin, amikasin, carbapenem, piperacillin-tazobactam, colistin and tigecycline were detected in 39 (43%), 32 (35%), 7 (7.7%), 6 (6.6%), 6 (6.6%), 6 (6.6%), 4 (4.4%), 2 (2.2%) patients, respectively.

Mortality

The mortality rate was 23% (21/91), which consisted of 13 (43%) allo-HSCT patients and eight (13%) auto-HSCT patients. The mortality rate was significantly higher for allo-HSCT compared to auto-HSCT (p=0.001). The rate of GVHD in patients who died was 38% (n: 8; 7 acute GVHD, one chronic GVHD). The most common causes of death were relapse (8/21, 38%), sepsis (7/21, 33%), followed by CMV disease, GVHD, pneumonia, and veno-occlusive disease (VOH). The mortality rate was significantly higher in patients with CMV disease compared to patients with no CMV disease (3/5; 60% and 17/86; 20%, respectively, p=0.002).

DISCUSSION

It is estimated that more than 1.4 million transplants have been carried out worldwide so far and that HSCT treatment is currently being applied to approximately 70,000 patients, half of whom are in Europe (Styczyński *et al*., 2020). HSCT has been performed in our center since 1993 and allo-HSCT has been performed on 433 patients.

In this study, we aimed to study the association between CMV reactivation and bacterial and fungal infections in 91 patients who underwent HSCT at the Istanbul Faculty of Medicine, the oldest tertiary university hospital in our country. The main indications for HSCT are hematological malignancies (Cho *et al*., 2018; Passweg *et al*., 2018), and the most common diagnoses in our study population are multiple myeloma and acute myeloid leukemia (*Table 1*). Our study included a higher rate of males. The source of stem cells was peripheral blood in the majority of patients who underwent allo-HSCT (63%). Sixty percent of allo-HSCT donors were unrelated. Myeloablative regimen (80%) was used as the most frequent conditioning regimen in the allo-HSCT group.

HSCT recipients are at risk for CMV infection both in the early post-transplantation period (<100 days) and later post-transplantation period (>100 days) (Boeckh *et al*., 2003). CMV infection causes significant morbidity and mortality in patients with allo-HSCT (Acar *et al*., 2014). The post-HSCT follow-up period was long (mean 62 months) for the majority of our patients. The data analysis revealed that CMV infection developed in 42 (46%) patients and that 49 had no CMV infection. The majority (78%) of the patients developed CMV infection during the post-transplantation period (late CMV infection).

The median time to infection (TTI) for auto-HSCT was 360 days (range 20-690) and 270 days (range 30-690) for allo-HSCT (p=0.158) (*Table 2*).

The risk of CMV infection varies depending on CMV seropositivity in donors and recipients. CMV seropositivity has been reported to be a risk factor for both CMV infection and CMV-related deaths (Kekik *et al*., 2009). In one study conducted on allo-HSCT recipients in the USA, it was demonstrated that seropositive donor (D+) and seropositive recipient (R+) compared to D+ and R- had higher mortality risk (Nichols *et al*., 2002). In patients who were seropositive before transplantation, the probability of CMV infection after HSCT was reported to be 60-70%; in the D+/R- patient group it was approximately 30% (Kedia *et al.,* 2013). Similarly, it has been reported that CMV infection is present in approximately 50-70% of seropositive patients undergoing allo-HSCT, and that the incidence in the D+/R- patient group is 20-25% (Boeckh 2017). Reported rates of CMV seropositivity are quite high (93-98%) in adults in our country (Ataman *et al.,* 2007; Oruc *et al.,* 2011).

When the patients in allo-HSCT group were classified according to seropositivity as low- (R- / D-), intermediate- (R- / D +) and high-risk (R+ / D-, R + / D +) , 24 (80%) patients were evaluated as high-risk group for CMV infection (*Table 1*).

Similar to the study by Sousa *et al*., our results demonstrated that CMV infection was significantly more common (80%) in CMV D-/R+ and D+/R+ patients (Sousa *et al*., 2014).

Studies regarding CMV reactivation in lymphoma and myeloma patients after auto-HSCT reported the incidence of CMV reactivation as 30%-40% while the reported rate of infection related to mortality ranged from 0% to 100% (Holmberg *et al.,* 1999; Offidani *et al.,* 1999; Fassas *et al*., 2001; Ng *et al*., 2005; Rossini *et al*., 2005; Han 2007; Lin *et al*., 2008). For standard auto-HSCT recipients, the ECIL 7 guideline does not recommend routine monitoring of CMV infection because of the lower CMV disease incidence and frequency (<1%) compared to allo-HSCT recipients (Rossini *et al*., 2005; Ljungman *et al*., 2019). According to the ECIL 7 guideline, high-risk auto-HSCT recipients, including patients with CD34 selection, prior treatment with fludarabine, cladribine or alemtuzumab, or receiving antithymocyte globulin, might benefit from CMV monitoring and pre-emptive therapy because these patients present with profound alteration of T-cell-mediated immunity functional status (Ljungman *et al*., 2019). Moreover, the recent increase in the use of immunotherapeutic drugs for the treatment of lymphomas and the introduction of proteasome inhibitors for myeloma treatment have resulted in an increased rate of CMV infection in auto-HSCT recipients. Since our auto-HSCT recipients have been widely exposed to the above-mentioned agents and have altered T-cell-mediated immunity functional status, CMV infection monitoring is performed for auto-HSCT recipients in our center. In our study, the rate of CMV infection was 36% in the auto-HSCT group and 67% in the allo-HSCT group (p<0.008). The high rate of CMV reactivation following auto-HSCT could be explained by pre-transplant exposure to steroids in multiple myeloma and lymphoma patients and the use of immunotherapeutic drugs for the treatment of lymphomas and proteasome inhibitors for myeloma treatment.

The frequency of CMV infection in allo-HSCT patients in our study was consistent with previous reports (Lindahl *et al*., 2010; Sousa *et al.,* 2014). In the present study, CMV disease was detected in five allo-HSCT recipients (16.6%) and in only one auto-HSCT recipient (1.6%), which corresponded to an overall prevalence of 6.6%. The relatively low prevalence of CMV disease in our study was thought to be attributed to screening for CMV viremia at least once a week with quantitative RT-PCR (qRT-PCR), which is a sensitive method, and to the initiation of appropriate pre-emptive treatment. In fact, the lack of a threshold value in CMV-DNA detection by qRT-PCR often creates difficulty in clinical judgement. The assay for CMV-DNA amplification used in the current study is an ultra-sensitive PCR assay, and our approach is to initiate preemptive therapy for CMV infection when CMV positivity is detected with qRT-PCR.

In the allo-HSCT group, patients with CMV disease had significantly higher AST levels compared to patients with no CMV disease (18 vs 40 IU/L, p=0.014). In all patient groups, patients with CMV disease presented with significantly higher AST levels compared to patients with no CMV disease (19 vs 34 IU/L, p=0.017) (*Table 3*).

CMV infection was not associated with gender, time of diagnosis up to HSCT, duration of neutropenia, type of underlying disease, time to infection (TTI) up to HSCT, but was associated with age at transplantation (p<0.001) and seropositivity of the recipient (p=0.019) (*Table 2*). Donor seropositivity was not significant for CMV infection (p=0.621) and CMV disease (p=0.576).

In the present study, GVHD developed in five (83%) of six patients with CMV disease. The rate of developing GVHD was 50% in the allo-HSCT group and 3.3% in the auto-HSCT group (a remarkable finding), and the difference between them was statistically significant (autologous: 2, allogeneic: 15, p<0.01). GVHD developed in 17 (40%) patients (12 allo-, 5 auto-HSCT) with CMV infection (n=42). The age range of these patients was 23-68 years. Cyclosporine (CSA) was used in 28 (93%) and CSA + methotrexate (MTX) was used in two (7%) allogeneic transplant patients for acute GVHD prophylaxis. Severe chronic GVHD was detected in two (6.7%) allo-HSCT patients.

The use of antiviral treatment was statistically more common in patients with CMV disease compared to

patients without CMV disease (304±149, 107±167; p=0.008) and in patients with CMV infection compared to patients without CMV infection (183±185, 67±143; p=0.002). In our study, the duration of antiviral treatment in CMV infection was statistically longer in the allo-HSCT patients compared to the auto-HSCT patients (40 and 22 days, respectively; p=0.001). In addition, the use of antiviral treatment was significantly more common in allo-HSCT patients than in the auto-HSCT patients (353±65, 6±47; p<0.001).

Recent studies have shown that there may be CMV transmission in the lung in the absence of probable or proven CMV pneumonia. Thus, quantification of CMV DNA in BAL fluids is essential. The general approach is to diagnose CMV pneumonia when CMV DNA is >500 IU/ml (1 copy/ml =1.64 IU/ml) since the positive predictive value of this cut-off value for possible CMV pneumonia is 50% (Durand *et al*., 2013). Validation of the diagnostic viral threshold in BAL is difficult due to the use of different real-time PCR methods among centers for CMV DNA quantification. A recently published article emphasizes that the presence of CMV DNA in BAL fluid should be treated as CMV pneumonia in the presence of relevant clinical and radiological findings (Mills *et al*., 2013). It has been reported that quantification of CMV viral load by qRT-PCR in BAL fluid is more sensitive than in viral culture (Lee *et al.,* 2017). In the present study, CMV positivity in tissue was significantly higher in allo-HSCT patients with CMV disease. It has been found that CMV DNA over 18,900 copies/ml in all patients post-HSCT is associated with CMV pneumonia (Lee *et al*., 2017; Cho *et al*., 2018). In our study population, 21 patients presented with ground-glass opacities in the lungs, 11 patients showed micronodular lung infiltrates, and three showed lobar consolidations in the lungs. CMV-DNA levels of the three patients with lobar consolidations were 17,252 copies/ml, 62,794 copies/ml and 1,676,716 copies/ml respectively. Detection of CMV inclusions in cells obtained from BAL or lung biopsy in transplant recipients supports the diagnosis of CMV pneumonia. Taking into consideration the radiological findings and CMV positivity in BAL, the above-mentioned patients were diagnosed as having CMV pneumonia. Visual impairment developed in one (1.1%) allo-HSCT patient. However, this single patient showed a low viral load, as opposed to previous reports (Kedia *et al.,* 2013), and had been on valganciclovir therapy for two years. Moreover, this patient also presented with fungal infection, methicillin-resistant coagulase negative *staphylococci* (MRCNS) bacteremia, and was colonized with vancomycin-resistant *enterococci* (VRE).

Bacterial infections during the neutropenic period are common in HSCT recipients, and 90% of these infections are of bacterial origin (Collin *et al*., 2001). In this study, coagulase-negative *staphylococci* (CNS) (30%) infections were the most common, followed by *Klebsiella pneumoniae* (10%) and *Escherichia coli* (5%). The rate of VRE colonization in our cohort was 25% (*Table 4*).

The main sources of bacteremia before HSCT are normal gastrointestinal flora and permanent vascular catheters. Common pathogens of gastrointestinal flora origin are Gram-negative bacilli, and indwelling catheter infections are caused by Gram-positive *cocci* (Cho *et al*., 2018). In the present study, 32 of 38 (84.2%) patients with inserted indwelling catheters developed bacteremia, which confirms the role of indwelling catheters as a predisposing risk factor for Gram-positive bacteremia.

Immunodeficiency caused by chronic GVHD increases susceptibility to infections caused by encapsulated bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenza*e, and *Neisseria meningitidis* (Cho *et al.,* 2018). In our study population, the following bacteria were isolated in auto-HSCT patients: four isolates of *S. pneumoniae* (one bacteremia, three from respiratory secretions) and two isolates of *H. influenzae* from respiratory tract secretions.

Among the 55 (60%) patients who developed bacteremia in our study group, a total of 75 microorganisms including 54 (72%) Gram-positive and 21 (28%) Gram-negative bacteria were isolated. In line with published data (Wingard *et al.,* 2011), the rate of bacteremia was statistically higher in allo-HSCT patients than in auto-HSCT patients. In our cohort, CMV infection and CMV disease co-presented with bacteremia in auto-HSCT and allo-HSCT patients, respectively. Singh et al (Singh *et al*., 2017) found that CMV viremia developed in 27.5% (8/29) of patients a median of seven days after the onset of bacteremia. In the present study, the rate of CMV viremia was found to be 15% in patients with bacteremia.

In the whole cohort, a total of 158 microorganisms, including 114 Gram-positive bacteria and 40 Gram-negative bacteria, were isolated. Unlike the results of our study, enteric bacteria were common in the study of Slade et al (Slade *et al*., 2017). The rate of methicillin resistance in our study was 58% in *S. aureus* strains and 92% in all *staphylococci* (*Table 4*). In this study, the rate of VRE colonization was quite high (39/91; 43%). To reduce this rate, precautions such as hand washing, appropriate disinfection, isolation, etc. are implemented by the Hospital Infections Control Committee in our hospital.

Gastrointestinal disease and pneumonia are the most frequent clinical manifestations in allo-HSCT recipients (Boeckh *et al*., 2003; Ljungman *et al*., 2017). In the course of HSCT, *Clostridium difficile* colitis induced by a conditioning regimen and antibiotics is particularly significant. *C. difficile* infection affects approximately 15% of HSCT recipients and is more common in allo-HSCT than in auto-HSCT (Wingard *et al*., 2011; Kedia *et al*., 2013). In our study, *C. difficile* infection developed in four (three auto-HSCT, one al-

lo-HSCT) patients, which is lower than in previous data. VRE was isolated from the rectal swab samples of 39 (25%) patients in our study group (*Table 4*). We perform routine surveillance for vancomycin-resistant *enterococcus* (VRE) colonization at high risk for VRE infection.

In the past decade, the frequency of resistant bacteria has increased worldwide, especially in patients with neutropenic fever and hematological malignancies. Among the *Enterobacteriaceae* isolates, about two-thirds of the resistant bacteria are extended-spectrum beta-lactamase (ESBL) positive *K. pneumoniae* and about one-third are strains of ESBL-positive *E. coli*. The incidence of infection caused by carbapenem-resistant *Enterobacteriaceae* (Averbuch *et al.,* 2014) and ESBL-producing *Enterobacteriaceae* is increasing in patients with hematological malignancies (Cho *et al*., 2018). In a study investigating the colonization of Gram-negative bacteria (CRGNB) resistant to carbapenems in patients who underwent HSCT, it was found that 11% of the patients had CRGNB colonization, the most common CRGNB being *E. coli*, *K. pneumoniae*, and *P. aeruginosa* (Demiraslan *et al*., 2017). In the present study, multiple resistant- (MDR) or pan-drug resistant- (PDR) *K. pneumoniae* (19%) and *A. baumanni* (25%) strains were isolated from lower respiratory tract infections. Unlike Averbuch *et al.* (Averbuch *et al*., 2017), we were pleased to find that carbapenem resistance was around 6% in our study. Although in the literature fluoroquinolone prophylaxis is recommended in absolute neutropenia lasting more than seven days (Averbuch *et al*., 2014), in our study the highest resistance was observed fluoroquinolones (43%).

The pre-engraftment phase (days 0-45 post HCT) increases the risk of bacterial and fungal infections in patients because it is characterized by prolonged neutropenia and breaks in mucocutaneous barriers (Ghogomu *et al*., 2019). The immunomodulatory properties of CMV contribute to the development of bacterial and fungal coinfections (Nichols *et al*., 2002; Boeckh *et al*., 2003). Furthermore, ganciclovir prophylaxis associated with a high incidence of neutropenia was reported to cause invasive bacterial and fungal infections (Nichols *et al*., 2002). In our study group, eight (8.8%) patients had invasive fungal infections, two of which were proven (*Candida* spp), and the others possible or suspicious. Five out of eight (62.5%) patients had CMV infection concurrently with a fungal infection, six (75%) had a bacterial infection concurrently with a fungal infection, and two (25%) had VRE colonization concurrently with a fungal infection. GVHD developed in four (50%) of eight patients with fungal infection.

The overall mortality rate was 23% (43% allogeneic and 13% autologous). The most common causes of mortality were disease recurrence (38%) and sepsis (14%), followed by urosepsis, CMV disease, relapse, GVHD, pneumonia, and veno-occlusive disease (VOD). In our study group, bacteremia/sepsis was present in 16 (76%) of 21 deceased patients. Among the 16 patients with bacteremia, one (6%) had concomitant urinary tract infection, one (6%) concomitant lower respiratory tract infection, and one (6%) had both urinary tract infection and lower respiratory tract infection. However, the rate of bacterial infection-related mortality, excluding relapse and GVHD, was 33.3% (7/21; four from sepsis, three from urosepsis). Therefore, bacterial infection-related mortality was considered to be relatively high. Transplantation centers with over 20 years of experience have reported lower infection-related mortality rates in the early and mid-stage post-transplant period (Styczyński *et al*., 2020), a finding in line with the relatively low mortality rate in our 28-year-old transplantation center. Our results have demonstrated that resistant bacteria emerge as a significant cause of mortality. In our study group, the frequency of resistance to fluoroquinolones and third-generation cephalosporins is high (43% and 35%, respectively). In our study, the frequency of strains resistant to colistin and tigecycline, which are considered life-threatening organisms, is 4.4 and 2.2%, respectively. Nicols *et al*. reported that deaths due to bacteremia or invasive fungal infections were higher in the D+/R- (18.3%) patient group than in the D-/R- (9.7%) group (Nichols *et al*., 2002).

Our study demonstrated a statistically significant shorter post-HSCT survival period for patients with CMV disease than for patients without CMV disease (425 days and 586 days, respectively) (*Figure 1*). In the survival analysis of allo-HSCT patients, the difference in duration of post-HSCT between the group with CMV infection (511 days) and the group without CMV infection (286 days) tends to be statistically significant (p=0.063) (*Figure 2*).

The present retrospective study provides important long-term data in a relatively large group of HSCT patients. However, the study has some limitations, including variable patient follow-up periods, patient selection criteria, and seropositivity status. For allo-HSCT patients, the CMV seropositivity status of three (10%) donors and four (13%) recipients was not available. In addition, it was not possible to make a comparison in statistical analysis due to insufficient data for some variables. Moreover, the data obtained in the Kaplan-Meier survival analysis may not be accurate because the number of patients with CMV disease in the allo-HSCT patient group was too small for statistical analysis.

CONCLUSION

Our results confirm that bacteremia and GVHD are risk factors in allo-HSCT recipients. The most common infection in patients undergoing HSCT is bac-

teremia. Gram-positive bacteria are more common in bacteremia, for which indwelling catheters are an important risk factor. Bacteremia developed concurrently with CMV infection in approximately one-fifth of the patients. The rate of bacteremia was higher in allo-HSCT recipients than in auto-HSCT recipients. CMV infection was associated with age at HSCT and seropositivity of the recipient. Moreover, CMV infection after auto-HSCT and CMV disease after allo-HSCT developed concurrently with bacteremia. CMV infection and/or disease developed concurrently with fungal infections in only 5% of the patients. Time to bacteremia in allo-HSCT recipients was longer than in auto-HSCT recipients. Multi-resistant Gram-negative bacteria remain an obstacle in HSCT recipients. Continuous monitoring of resistance patterns and patient outcome data are required to reduce resistance rates. To improve survival rates in HSCT, more studies reporting data on the rate of post-HSCT infectious diseases and treatment strategies are warranted. A prospective study of bacteremia in HSCT patients would allow the results of this study to be developed further.

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Ethical approval

This study was approved by the Istanbul Faculty of Medicine Clinical Research Ethics Committee (2020/883).

Conflicts of interest

The authors have no conflicts of interest to declare.

References

Acar K., Aki S.Z., Ozkurt Z.N., Bozdayi G., Rota S., et al. (2014). Factors Associated with Cytomegalovirus Reactivation Following Allogeneic Hematopoietic Stem Cell Transplantation: Human Leukocyte Antigens Might Be Among the Risk Factors. *Turkish Journal of Hematology.* **31**, 276-285.

Asano-Mori Y., Kanda Y., Oshima K., Kako S., Shinohara A., et al. (2008). Clinical features of late cytomegalovirus infection after hematopoietic stem cell transplantation. *International Journal of Hematology.* **87**, 310-318.

Ataman S., Colak D., Gunseren F., Senol Y., Colak T., et al. (2007). [Investigation of cytomegalovirus seroepidemiology in Antalya with a population-based cross-sectional study and review of related data in Turkey]. *Mikrobiyol Bul.* **41**, 545-555.

Averbuch D., Orasch C., Cordonnier C., Livermore D.M., Mikulska M., et al. (2014). European guidelines for empirical antibacterial therapy for febrile neutropenic patients in the era of growing resistance: summary of the 2011 4th European Conference on Infections in Leukemia (vol 98, pg 1826, 2013). *Haematologica.* **99**, 400-400.

Averbuch D., Tridello G., Hoek J., Mikulska M., Akan H., et al. (2017). Antimicrobial Resistance in Gram-Negative Rods Causing Bacteremia in Hematopoietic Stem Cell Transplant Recipients: Intercontinental Prospective Study of the Infectious Diseases Working Party of the European Bone Marrow Transplantation Group. *Clinical Infectious Diseases.* **65**, 1819-1828.

Boeckh M. (2017). New Insights Into Cytomegalovirus Infection After Allogeneic Hematopoietic Stem Cell Transplant. *Clinical Advances in Hematology & Oncology.* **15**, 515-517.

Boeckh, M, Leisenring W, Riddell SR, Bowden RA, Huang ML, et al. (2003). Late cytomegalovirus disease and mortality in recipients of allogeneic hematopoietic stem cell transplants: importance of viral load and T-cell immunity. *Blood.* **101**, 407-414.

Cho S.-Y., Lee H.-J., Lee D.-G. (2018). Infectious complications after hematopoietic stem cell transplantation: current status and future perspectives in Korea. *The Korean journal of internal medicine.* **33**, 256.

Clarke J., Craig R.M., Saffro R., Murphy P., Yokoo H. (1979). Cytomegalovirus granulomatous hepatitis. *Am J Med* **66**, 264-269.

CLSI. (2017). Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100.

Collin B.A., Leather H.L., Wingard J.R., Ramphal R. (2001). Evolution, incidence, and susceptibility of bacterial bloodstream isolates from 519 bone marrow transplant patients. *Clinical Infectious Diseases.* **33**, 947-953.

Copelan E., Casper J.T., Carter S.L., van Burik J.A.H., Hurd D., et al. (2007). A scheme for defining cause of death and its application in the T cell depletion trial. *Biology of Blood and Marrow Transplantation.* **13**, 1469-1476.

Demiraslan H., Cevahir F., Berk E., Metan G., Cetin M., et al. (2017). Is surveillance for colonization of carbapenem-resistant gram-negative bacteria important in adult bone marrow transplantation units? *American Journal of Infection Control.* **45**, 735-739.

Durand C.M., Marr K.A., Arnold C.A., Tang L., Durand D.J., et al. (2013). Detection of Cytomegalovirus DNA in Plasma as an Adjunct Diagnostic for Gastrointestinal Tract Disease in Kidney and Liver Transplant Recipients. *Clinical Infectious Diseases.* **57**, 1550-1559.

Fassas A.B.T., Bolanos-Meade J., Buddharaju L.N., Rapoport A., Cottler-Fox M., et al. (2001). Cytomegalovirus infection and non-neutropenic fever after autologous stem cell transplantation: high rates of reactivation in patients with multiple myeloma and lymphoma. *British Journal of Haematology.* **112**, 237-241.

Freifeld A.G., Bow E.J., Sepkowitz K.A., Boeckh M.J., Ito J.I., et al. (2011). Executive summary: clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the Infectious Diseases Society of America. *Clinical Infectious Diseases.* **52**, 427-431.

Ghogomu J.T., Camero Y., Friman K. (2019). Preventing Infection Post Hematopoietic Cell Transplantation. *Us Pharmacist.* **44**, 23-26.

Han X.Y. (2007). Epidemiologic analysis of reactivated cytomegalovirus antigenemia in patients with cancer. *Journal of clinical microbiology.* **45**, 1126-1132.

Hatzimichael E., Tuthill M. (2010). Hematopoietic stem cell transplantation. Stem cells and cloning: advances and applications. **3**, 105.

Holmberg L.A., Boeckh M., Hooper H., Leisenring W., Rowley S., et al. (1999). Increased incidence of cytomegalovirus disease after autologous CD34-selected peripheral blood stem cell transplantation. *Blood.* **94**, 4029-4035.

Horan T.C., Andrus M., Dudeck M.A. (2008). CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *American Journal of Infection Control.* **36**, 309-332.

Jagasia M.H., Greinix H.T., Arora M., Williams K.M., Wolff D., et al. (2015). National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group Report. *Biology of Blood and Marrow Transplantation.* **21**, 389-401.

Kedia S., Acharya P., Mohammad F., Nguyen H., Asti D., et al. (2013). Infectious complications of hematopoietic stem cell transplantation. *J Stem Cell Res Ther.* **3**, 10-4172.

Kekik C., Besisik S.K., Seyhun Y., Oguz F.S., Sargin D., et al. (2009). Relationship Between HLA Tissue Type, CMV Infection, and Acute Graft-vs-Host Disease After Allogeneic Hematopoietic Stem Cell Transplantation: Single-Center Experience. *Transplantation Proceedings*. **41**, 3859-3862.

Lee H.Y., Rhee C.K., Choi J.Y., Lee H.Y., Lee J.W., et al. (2017). Diagnosis of cytomegalovirus pneumonia by quantitative polymerase chain reaction using bronchial washing fluid from patients with hematologic malignancies. *Oncotarget*. **8**, 39736.

Lin H.C., Han S.M., Hwang W.L., Chou C.W., Chang K.H., et al. (2017). Cytomegalovirus Infection and Treatment in Allogeneic Hematopoietic Stem Cell Transplantation: A Retrospective Study from a Single Institution in an Endemic Area. *Turkish Journal of Hematology*. **34**, 159-166.

Lin P.C., Lee M.Y., Lin J.T., Hsiao L.T., Chen P.M., et al. (2008). Virus reactivation in high-risk non-Hodgkin's lymphoma patients after autologous CD34(+)-selected peripheral blood progenitor cell transplantation. *International Journal of Hematology*. **87**, 434-439.

Lindahl J.K., Woxenius S., Brune M., Andersson R. (2010). Cytomegalovirus DNAemia and treatment following allogeneic stem cell transplantation with a focus on long-term outcome. *Scandinavian Journal of Infectious Diseases*. **42**, 691-698.

Ljungman P., Boeckh M., Hirsch H.H., Josephson F., Lundgren J., et al. (2017). Definitions of Cytomegalovirus Infection and Disease in Transplant Patients for Use in Clinical Trials. *Clinical Infectious Diseases*. **64**, 87-91.

Ljungman P., de la Camara R., Robin C., Crocchiolo R., Einsele H., et al. (2019). Guidelines for the management of cytomegalovirus infection in patients with haematological malignancies and after stem cell transplantation from the 2017 European Conference on Infections in Leukaemia (ECIL 7). *Lancet Infectious Diseases*. **19**, E260-E272.

Magiorakos A.P., Srinivasan A., Carey R.B., Carmeli Y., Falagas M.E., et al. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*. **18**, 268-281.

Marr K.A., Seidel K., Slavin M.A., Bowden R.A., Schoch H.G., et al. (2000). Prolonged fluconazole prophylaxis is associated with persistent protection against candidiasis-related death in allogeneic marrow transplant recipients: long-term follow-up of a randomized, placebo-controlled trial. *Blood, The Journal of the American Society of Hematology*. **96**, 2055-2061.

Mills A.M., Guo F.P., Copland A.P., Pai R.K., Pinsky B.A. (2013). A Comparison of CMV Detection in Gastrointestinal Mucosal Biopsies Using Immunohistochemistry and PCR Performed on Formalin-fixed, Paraffin-embedded Tissue. *American Journal of Surgical Pathology*. **37**, 995-1000.

Ng A.P., Worth L., Chen L., Seymour J.F., Prince H.M., et al. (2005). Cytomegalovirus DNAemia and disease: incidence, natural history and management in settings other than allogeneic stem cell transplantation. *Haematologica*. **90**, 1672-1679.

Nichols W.G., Corey L., Gooley T., Davis C., Boeckh M. (2002). High risk of death due to bacterial and fungal infection among cytomegalovirus (CMV)-seronegative recipients of stem cell transplants from seropositive donors: Evidence for indirect effects of primary CMV infection. *Journal of Infectious Diseases*. **185**, 273-282.

Offidani M., Corvatta L., Olivieri A., Rupoli S., Frayfer J., et al. (1999). Infectious complications after autologous peripheral blood progenitor cell transplantation followed by G-CSF. *Bone Marrow Transplantation*. **24**, 1079-1087.

Oruc A.S., Celen S., Citil A., Saygan S., Unlu S., et al. (2011). Screening of cytomegalovirus seroprevalence among pregnant women in Ankara, Turkey: A controversy in prenatal care. *African Journal of Microbiology Research*. **5**, 5304-5307.

Passweg J.R., Baldomero H., Bader P., Basak G.W., Bonini C., et al. (2018). Is the use of unrelated donor transplantation leveling off in Europe? The 2016 European Society for Blood and Marrow Transplant activity survey report. *Bone marrow transplantation*. **53**, 1139-1148.

Przepiorka D., Weisdorf D., Martin P., Klingemann H., Beatty P., et al. (1995). 1994 Consensus conference on acute GVHD grading. *Bone marrow transplantation*. **15**, 825-828.

Rossini F., Terruzzi E., Cammarota S., Morini F., Fumagalli M., et al. (2005). Cytomegalovirus infection after autologous stem cell transplantation: incidence and outcome in a group of patients undergoing a surveillance program. *Transplant Infectious Disease*. **7**, 122-125.

Singh N., Inoue M., Osawa R., Wagener M.M., Shinohara M.L. (2017). Inflammasome expression and cytomegalovirus viremia in critically ill patients with sepsis. *Journal of Clinical Virology*. **93**, 8-14.

Slade M., Goldsmith S., Romee R., DiPersio J.F., Dubberke E.R., et al. (2017). Epidemiology of infections following haploidentical peripheral blood hematopoietic cell transplantation. *Transplant Infectious Disease*. **19**, e12629.

Sousa H., Boutolleau D., Ribeiro J., Teixeira A.L., Vaz C.P., et al. (2014). Cytomegalovirus Infection in Patients Who Underwent Allogeneic Hematopoietic Stem Cell Transplantation in Portugal: A Five-Year Retrospective Review. *Biology of Blood and Marrow Transplantation*. **20**, 1958-1967.

Styczyński J., Tridello G., Koster L., Iacobelli S., van Biezen A., et al. (2020). Death after hematopoietic stem cell transplantation: changes over calendar year time, infections and associated factors. *Bone marrow transplantation*. **55**, 126-136.

Tutuncuoglu S.O., Yanovich S., Ozdemirli M. (2005). CMV-induced hemorrhagic cystitis as a complication of peripheral blood stem cell transplantation: case report. *Bone Marrow Transplantation*. **36**, 265-266.

Wingard J.R., Hsu J., Hiemenz J.W. (2011). Hematopoietic Stem Cell Transplantation: An Overview of Infection Risks and Epidemiology. *Hematology-Oncology Clinics of North America*. **25**, 101.