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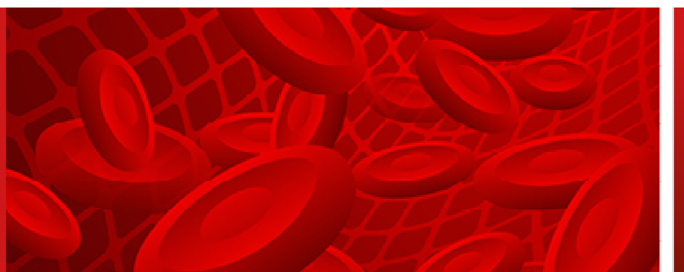

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Authors: Michał Cezary Czarnogórski, Mateusz Maziewski, Katarzyna Ruckemann-Dziurdzińska, Justyna Sakowska, Maciej Zieliński, Jacek M. Witkowski, Piotr Trzonkowski, Magdalena Dutka, Agnieszka Piekarska, Igor Obuchowski, Mikołaj Młyński, Alicja Sadowska-Klasa, Ewa Zarzycka, Maria Bieniaszewska, Andrzej Hellmann, Jan M. Zaucha

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Long-term allogeneic hematopoietic cells transplantation survivors' proinflammatory cytokine profiles compared to their respective donors and immunophenotype differences depending on GvHD history and infection status

Michał C. Czarnogórski[1*], Mateusz Maziewski[2], Katarzyna Ruckemann-Dziurdzińska[2], Justyna Sakowska[3], Maciej Zieliński[3], Jacek M. Witkowski[2], Piotr Trzonkowski[3], Magdalena Dutka[1], Agnieszka Piekarska[1], Igor Obuchowski[4], Mikołaj Młyński[1], Alicja Sadowska-Klasa[1], Ewa Zarzycka[1], Maria Bieniaszewska[1], Andrzej Hellmann[1], Jan M. Zaucha[1]

[1]Department of Hematology and Transplantology, Medical University of Gdańsk, Gdańsk, Poland

[2]Department of Physiopathology, Medical University of Gdańsk, Gdańsk, Poland

[3]Department of Medical Immunology, Medical University of Gdańsk, Gdańsk, Poland

[4]Intercollegiate Faculty of Biotechnology, Medical University of Gdańsk, University of Gdańsk, Poland

***Address for correspondence:** Michał C. Czarnogórski, Department of Hematology and Transplantology, Medical University of Gdańsk, Skłodowskiej-Curie 3a, 80–210 Gdańsk, Poland, e-mail: mcczarnogorski@gmail.com

Abstract

Introduction: In the course of allogeneic hematopoietic cell transplantation (allo-HCT) the donor's hematopoietic progenitor cells are exposed to immense proliferative stress to reconstitute in the recipient the functional hematopoiesis. Moreover, recipients who develop infections or chronic graft-versus-host disease (cGvHD) are subjected to further proliferative stress, especially in the lymphocyte subset. We hypothesized that allo-HCT may induce changes in proinflammatory cytokines profile and immunophenotype in the allo-HCT recipients, especially in patients with a cGvHD history.

Material and methods: We compared the cytokine profile [interleukin (IL)-6, IL-10, and tumor necrosis factor α (TNF-α)] between long-term allo-HCT recipients and their respective donors and we analyzed cytokine profiles and the immunophenotype of lymphocytes in long-term recipients grouped according to their infection and GvHD history.

Results: We found no differences in the proinflammatory cytokines between allo-HCT recipients and their respective donors, or between recipients grouped according to their infectious risk status. Immunophenotyping of recipients grouped according to their GvHD status revealed an increased percentage of B-cell presenting programmed death-1 in recipients without a history of GvHD.
Conclusions: A lack of differences in proinflammatory cytokines concentrations between recipients and donors of allo-HCT would suggest that allo-HCT does not induce acceleration of the 'inflammaging'-resembling phenomenon. No differences in the cytokine profile and immunophenotype between recipients grouped according to infectious risk status suggest that infectious risk is not reflected by the immunophenotype and cytokine profile. Furthermore, the lack of significant differences in immunophenotype of the recipients grouped according to a history of GvHD may suggest that in long-term survivors the immune system tends to stabilize with time.
Key words: GvHD, cytokines, allo-HCT, immunophenotype

Introduction

The introduction of allogeneic hematopoietic cell transplantation (allo-HCT) as a standard method of treatment for several malignant and non-malignant hematological diseases has created an excellent platform upon which to study human immunology and cell senescence. Since only a small percentage of the donor stem cells pool is collected and infused into the donor to engraft and reconstitute hematopoiesis, the cells are exposed to immense proliferative stress.

However, successful allo-HCT requires also that two important immunological barriers be overcome: host versus graft and graft versus host. Graft-versus-host reaction results from the exposure of lymphoid donor cells to the recipient antigens which induce donor lymphocyte activation and proliferation. Partially in patients with malignant diseases, this reaction is responsible for HCT's success in eradicating the residual malignant cells (graft-versus-leukemia reaction). However, it may also lead to undesirable complications such as graft-versus-host disease (GvHD) resembling autoimmune diseases affecting several host organs. To prevent and control symptoms of graft versus host reaction, immunosuppressive agents disrupting lymphocyte proliferation (such as methotrexate and calcineurin inhibitors) are routinely administered after transplantation. A key role in GvHD is played by donor T cell

lymphocytes but also B-lymphocytes [1, 2]. Involved donor lymphocytes undergo an additional intensive proliferation which may contribute to the accelerated telomere shortening in donor lymphocytes.

All of the above lead to the immense proliferative activity of the cells, including lymphocytes in allo-HCT recipients. We hypothesized that this could lead not only to accelerated telomeric shortening but also to immunophenotypic changes characteristic of natural aging. Healthy human ageing process includes in its characteristics the phenomenon of 'inflammaging'. It may be defined as chronic, low-grade inflammation, without the presence of infection. In biochemical evaluation it presents with increased concentrations of proinflammatory cytokines due to antigenic stimulation over a lifespan of an individual [2]. It is also well known that the concentration of some proinflammatory cytokines [such as tumor necrosis factor α (TNF-α), interleukin (IL)-6] increases, whereas others decrease (such as IL-10) during the course of chronic GvHD [3–5].

We reported recently our observations regarding the changes in immunophenotype and shortened telomeres in CD8+ lymphocyte subpopulation in long-term allo-HCT recipients compared to their respective donors [6]. Here, we present data on the proinflammatory cytokine profile of the same population of patients, i.e. long-term recipients of allo-HCT and their respective donors, to determine whether allo-HCT led to the changes in the proinflammatory cytokines. Moreover, we compared the immunophenotype of the recipients grouped according to their infection and cGvHD status.

Material and methods

The content of the materials and methods section were adapted from Czarnogórski et al. 2022 [6].

Patients

The study consist of 20 allo-HCT recipient-donor pairs. The span from the transplantation was more than 12 years ago. The study was conducted at University Clinical Center, Medical University of Gdansk. From all participants full venous blood sample was collected (50 mL).

GvHD and infectious status assessment

Patients were stratified according to their history of chronic GvHD status (yes vs. no) and infectious complications according to an infection risk status score developed for the purpose of this study [6].

Peripheral blood mononuclear cells and lymphocyte isolation

Perpheral blood mononuclear cells collection was performed from full venous blood with Ficoll-Hypaque centrifugation technique. Following lymphocyte isolation was performed by immunomagnetic separation. The lymphocyte subpopulations were TCD4+, TCD8+, B-lymphocytes and natural killers (NK) cells. The quality of collected material was assessed according to validated protocols [7, 8].

Proinflammatory cytokine concentrations

Proinflammatory cytokines concentrations (IL-1B, IL-2, IL-4, IL-6, IL-10, TNF-α and IL-17F) were assessed with flow cytometry. The results which did not reach the reference were excluded from the study.

Immunophenotyping

Immunophenotyping was performed according to protocol used by Czarnogórski et al. [6].

Statistical analysis

The statistical analysis was performed by STATISTICA 12.0 and with Microsoft Exel, detailed analysis was described according to Czarnogórski et al. [6]. The W Shapiro-Wilk test, and the Leven’s (Brown-Forsythe) test were used. The significance of differences between the two groups (independent samples model) was tested by Student’s *t*-test or by U Mann-Whitney. The significance of differences between more than two groups was verified using the Kruskal-Wallis test. In the case of receiving statistically significant differences between groups, the Dunn test was performed. A p value <0.05 was considered significant.

Results

Patient characteristics

The time from Tx to full venous blood cytometric analysis was at least 12 years with range 12–25 years (median 17.4 years). The population studied consisted of 12 males and 8 females. The prevalence of chronic graft versus host disease among recipients was 40%. Infection risk

status was assessed according to Czarnogórski et al. [6]: 12 low risk recipients and 8 high risk recipients.

Proinflammatory cytokine concentrations

Surprisingly, we have found no statistically significant differences in the concentrations of the cytokines: TNF-α, IL-6, IL-10 (Table I). The results of assessment of IL-17F, IL-1β, IL-4, IL-2 concentrations were out of range, therefore they could not be included into analysis.
Neither we have found any differences between recipients when grouped according to infection risk status (Table II).

Table I. Recipients and donors of hematopoietic cell transplantation — cytokines concentrations

Parameter	**R** **N = 20**	**D** **N = 20**	***p* value**
IL-6 [ng/L]:	N = 20	N = 20	0.5792*
• avr (standard deviation)	0.99 (1.17)	1.61 (2.37)	
• range	0.38–5.42	0.07–9.53	
• median	0.58	0.72	
• 95% CI	0.44–1.54	0.50–2.72	
IL-10 [ng/L]:	N = 19	N = 18	0.5333*
• avr (standard deviation)	0.58 (0.69)	0.72 (0.71)	
• range	0.01–3.20	0.15–3.04	
• median	0.42	0.52	
• 95% CI	0.25–0.91	0.36–1.07	
TNF-α [ng/L]:	**N = 18**	**N = 19**	0.3234*
• avr (standard deviation)	0.77 (1.53)	0.83 (1.91)	
• range	0.01–6.78	0.02–8.51	
• median	0.33	0.22	
• 95% CI	0.01–1.54	–0.09–1.75	

*U Mann-Whitney test; IL — interleukin; CI — confidence interval; TNF-α — tumor necrosis factor α

Table II. Recipients grouped according to infection risk status — cytokines concentrations

Parameter	**Low risk** **N = 12**	**Intermediate/high risk** **N = 8**	***p* value**
IL-6 [ng/L]:	N = 12	N = 8	0.3159

			*
• avr (standard deviation)	1.19 (1.49)	0.69 (0.20)	
• range	0.38–5.42	0.48–1.10	
• median	0.52	0.61	
• 95% CI	0.24–2.14	0.52–0.86	
IL-10 [ng/L]	N = 11	N = 8	0.9671 *
• avr (standard deviation)	0.68 (0.88)	0.45 (0.27)	
• range	0.01–3.20	0.11–0.87	
• median	0.48	0.39	
• 95% CI	0.09–1.27	0.23–0.67	
TNF-α [ng/L]:	N = 12	N = 6	0.1898 *
• avr (standard deviation)	1.02 (1.85)	0.28 (0.20)	
• range	0.09–6.78	0.01–0.62	
• median	0.37	0.28	
• 95% CI	–0.15–2.19	0.07–0.49	

*U Mann-Whitney test; IL — interleukin; CI — confidence interval; TNF-α — tumor necrosis factor α

Immunophenotype of allo-HCT recpients grouped according to chronic GvHD history

The analysis of immunophenotype of the allo-HCT recipients grouped according to cGvHD history showed no significant differences (*see* Supplementary Table 1), with the exception of a few parameters such as Treg Helios-Eomes+, B1 PD1+, B2 PD1+ and C19 PD1+. Lymphocytes B in recipients of allo-HCT who did not develop cGvHD had greater expression of PD-1 (Table III).

Table III. Recipients grouped according to chronic graft-versus-host disease (cGvHD) status — immunophenotype

Parameter	**cGvHD**	**Without cGvHD**	***p* value**
Treg Helios–Eomes:			**0.0227**
• avr (standard deviation)	4.1 (1.3)	8.7 (4.8)	

• range	2.4–5.4	4.2–19.1	
• median	4.6	7.2	
• 95% CI	2.7–5.5	5.2–12.1	
B1 PD1:			**0.0147**
• avr (standard deviation)	4.0 (2.7)	10.4 (5.5)	
• range	0.2–8.7	3.6–18.7	
• median	3.7	9.7	
• 95% CI	1.2–6.9	6.4–14.3	
B2 PD1			**0.0448**
• avr (standard deviation)	0.7 (0.7)	1.8 (1.8)	
• range	0.1–2.1	0.6–6.2	
• median	0.5	1.1	
CD19 PD1			**0.0147**
• avr (standard deviation)	1.2 (0.9)	3.3 (2.3)	
• range	0.2–2.9	1.2–8.9	
• median	0.9	3.0	
• 95% CI	0.2–2.2	1.6–4.9	

SD — standard deviation; CI — confidence interval

Discussion

In this study, we tried to answer the question of whether allo-HCT accelerates the aging of the hematopoietic system by determining the differences in cytokine profile between long-term allo-HCT survivors and their respective donors of allo-HCT.

Studying donor-recipient pairs creates a unique model in which donor cells remaining in the donor could be compared to the donor cells infused into respective recipients. We were particularly interested in the features of postulated ‘inflammaging’. We also compared the same cytokine profile of the recipients when grouped according to infectious status (low vs intermediate/high) (*see* Czarnogórski et al. [6]). We hypothesized that allo-HCT recipients should have higher concentrations of proinflammatory cytokines as a robust indicator of aging. We also hypothesized that low-risk recipients according to their infection status would have increased concentrations of the same cytokines as an adaptation for fighting the infections.

Physiologically, the proinflammatory cytokine profile of older people is characterized by increased concentrations of the aforementioned cytokines (IL-1B, IL-2, IL-4, TNF-α, IL-6, IL-10, IL-15, IL-17, IL-18). These concentrations however do not exceed the upper reference

range. Hence, inflammaging is defined as the process of chronic, sterile, low-grade inflammation.

There is no data on inflammaging in a population of allo-HCT survivors compared to their respective recipients serving as controls. We did not find any statistically significant differences in IL-6, IL-10 and TNF-α concentrations, either between main groups (recipients vs. donors) nor between recipients grouped according to infection risk status. Our data did not confirm our initial hypothesis that allo-HCT accelerates the inflammaging-resembling process.

We also did not find any differences between low and intermediate/high risk recipients stratified by their infection status, which could imply that infectious risk is not directly connected to the efficacy of one's innate immune response. It would imply that allogeneic hematopoietic cells transplantation by itself does not impact the inflammaging [9]. However, the issue remains controversial since chronic low-grade inflammation (inflammaging) is a well-established risk factor for developing neoplasia [10, 11] which could be debatable in the population of our allo-HCT survivors since they were diagnosed with hematological malignancies in their 20 s and 30 s. On the other hand, there is ample data on the reduction of relapse risk after allo-HCT in patients who developed chronic GvHD that is in fact a chronic inflammation [12]. Moreover, it is difficult to differentiate if heightened concentrations of proinflammatory cytokines after allo-HCT result from chronic GvHD [13] or possibly are an adaptation for fighting the infection. There is some data correlating the occurrence of inflammaging and immune exhaustion in some hematological malignancies, such as plasmocytic myeloma [14]. Thus, it is challenging to determine whether the inflammaging features are due to older age or to the neoplasia itself.

Surprisingly, the incidence of chronic GvHD also did not impact any studied parameters, especially immunophenotype with the exception of B-cells expressing PD-1 which serves as the programmed death ligand-1 (PD-L1) receptor and plays a role in modulating immune response [15]. We also found no differences in T-cells expressing PD-1. An increased percentage of B-cells presenting PD-1 in recipients without chronic GvHD in anamnesis is difficult to interpret. Those differences in receptor expression in antigen-presenting cells (APCs) such as B-cells seem to be insignificant or accidental. The lack of differences in long-term (12 years+ from Tx) recipients of allo-HCT when grouped according to cGvHD history may suggest that the immune system tends to stabilize in the years following Tx. Many factors might explain such notion, that is immune suppression used, history of chronic degenerative diseases, GvHD resolution and small number of participants.

Our study has several limitations. Firstly, it was performed in long-term survivors who were able to fight infections successfully and whose cGvHD status became stable. Secondly, the results are affected by the small population (20 pairs) and unfortunately the results of some cytokines assays were out of range, which might be related to laboratory errors. Unfortunately, we were unable to repeat tests with out-of-range results due to sample destruction during an electricity outage. Nevertheless, our results may suggest that allo-HCT does not accelerate the aging of the hematopoietic system despite a clear reduction of telomere shortening in specific cell populations and some immunophenotypic differences reported by us [6].

Authors' contributions

All authors revised manuscript and read and approved final manuscript. MCC and JMZ wrote manuscript. MCC, PT, JMW, MD, JMZ were responsible for study design. MCC, AP, AS, JMZ, EZ, MB, MD, AH took part in patient recruitment and clinical data acquisition. MCC, IO, JMW, JMZ, MM and KRD performed laboratory and clinical data analysis. MC, JS, MM, MZ, JMW and PT performed laboratory work.

Conflict of interest

The authors declare no conflict of interest.

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Ethics

This study was approved by the Ethics Committee at the Medical University of Gdańsk — NKBBN/394-594/2019 and NKBBN/394-45/2020. Each participant signed an informed consent form.

Supplementary Table 1. Comparative characteristics of allogeneic hematopoietic cell transplantation recipients immunophenotype when grouped according to graft-versus-host disease status

Parameter	*p* value	Parameter	*p* value	Parameter	*p* value
B1	0.1193	NK CD39	0.5508	B1 PD1	0.0147
B2	0.0973	NK CD56 dim	0.2548	B2	0.2123
CD19	0.6511	NK CD56 high	0.2548	B2 Fas	0.9567
CD3	0.9599	NK Eomes	0.9567	B2 PD1	0.0448
DNT	0.4808	NK Perforin	0.7042	CD19	0.9567
NK	0.7595	NKT like	1.00	CD19 Fas	0.9567
NK CD56 dim	0.9512	Q1	0.5508	CD19 PD1	0.0147
NK CD56 high	0.4624	Q1 CD39	0.8708	CD4 CD27+CD28–	0.3290
NKT like	0.0662	Q1 Eomes	0.3566	CD4 CD27+CD28+	0.2123
T CD4	0.7250	Q1 IL10	0.5508	CD4 CD27– CD28–	0.4808
T CD8	0.9567	Q1 Perforin	0.0827	CD4 CD27– CD28+	0.7042
B1	0.0927	Q2	0.9567	CD4 CD28	0.3566
B1 CD39	0.4159	Q2 CD39	0.3028	CD4 CD57	0.3028
B1 Eomes	0.3566	Q2 Eomes	0.7863	CD4 FasL	0.8283
B1 IL10	0.1752	Q2 IL10	0.1585	CD4 PD-1	0.6255
B2	0.0927	Q2 Perforin	0.1752	CD8 CD27+CD28–	0.7683
B2 CD39	0.7449	Q3	0.3290	CD8 CD27+CD28+	0.1949
B2 Eomes	0.0577	Q3 CD39	0.7042	CD8 CD27– CD28–	0.6800
B2 IL10	0.6255	Q3 Eomes	0.2123	CD8 CD27– CD28+	0.5959
CD19	0.4808	Q3 IL10	0.1931	CD8 CD28	0.7683
CD19 CD39	0.4159	Q3 Perforin	0.8708	CD8 CD57	0.6800
CD19 Eomes	0.0448	RTE	0.1158	CD8 PD-1	0.3165
CD19 IL10	0.4477	T CD4	0.7863	DNT	0.3566
CD3	0.6255	T CD8	0.7863	Memory B	0.0735
CD4 CD39	0.4808	Treg FoxP3	0.9567	NK	0.2123
CD4 CM	0.3028	Treg FoxP3 CD39	0.6255	NK CD27	0.7449
CD4 EM	0.7042	Treg FoxP3	0.9136	NK CD28	0.8708

		Eomes			
CD4 Eomes	1.00	Treg FoxP3 IL10	0.0735	NK CD56 dim	0.2123
CD4 IL10	0.5508	Treg FoxP3 Perforin	0.2548	NK CD56 high	0.5508
CD4 Naive	0.9567	Treg Helios–	0.6255	NK CD57	0.3566
CD4 Perforin	0.0577	Treg Helios– CD39	0.3566	NK PD-1	0.6255
CD4 Temra	0.7863	Treg Helios– Eomes	0.0227	NKT like	0.9567
CD8 CD39	0.8137	Treg Helios– IL10	0.1752	Q1	0.8708
CD8 CM	0.3768	Treg Helios– Perforin	0.2548	Q1 CD27	0.9567
CD8 EM	0.0875	Treg Helios+	0.7042	Q1 CD28	0.1431
CD8 Eomes	0.2159	Treg Helios+ CD39	0.7042	Q1 CD57	0.2123
CD8 Naive	0.2629	Treg Helios+ Eomes	0.0577	Q1 FasL	0.7863
CD8 Perforin	0.3768	Treg Helios+ IL10	0.0927	Q1 PD-1	0.3566
CD8 Temra	0.953	Treg Helios+ Perforin	0.6255	Q2	0.9567
DNT	0.4159	B1	0.2123	Q2 CD27	0.7449
NK	0.2123	B1 Fas	0.8708	Q2 CD28	0.5508
Q2 CD57	0.0735	CD3	0.9567	Treg FoxP3 CXCR5	0.1431
Q2 FasL	0.4159	CD4 CD152	0.6255	Treg FoxP3 TIGIT	0.7863
Q2 PD-1	0.9567	CD4 CXCR4	0.7042	Treg Helios–	0.4808
Q3	0.1158	CD4 CXCR5	0.1431	Treg Helios– CCR5	0.5508
Q3 CD27	0.3566	CD4 TIGIT	0.4477	Treg Helios– CD152	0.7042
Q3 CD28	0.7449	CD8 CXCR4	0.7683	Treg Helios– CXCR4	0.4159
Q3 CD57	0.0057	CD8 CXCR5	0.1116	Treg Helios– CXCR5	0.6255
Q3 FasL	0.1431	CD8 TIGIT	0.5169	Treg Helios– TIGIT	0.7042
Q3 PD-1	0.0577	DNT	0.4159	Treg Helios+	0.5508

T CD4	0.7042	NK	0.2123	Treg Helios+ CCR5	0.8708
T CD8	0.6255	NK CCR5	0.4477	Treg Helios+ CD152	0.7042
Treg FoxP3	0.9567	NK CD56 dim	0.2123	Treg Helios+ CXCR4	0.6255
Treg FoxP3 CD27	0.1037	NK CD56 high	0.4159	Treg Helios+ CXCR5	0.4808
Treg FoxP3 CD28	0.7042	NK CXCR4	0.2548	Treg Helios+ TIGIT	0.8708
Treg FoxP3 CD57	0.0735	NK CXCR5	0.3566		
Treg FoxP3 FasL	0.1931	NK TIGIT	0.4808		
Treg FoxP3 PD-1	0.4808	NKT like	0.9567		
Treg Helios–	0.0735	Q1	0.5508		
Treg Helios– CD27	0.9567	Q1 CCR5	0.4159		
Treg Helios– CD28	0.3028	Q1 CD152	0.5876		
Treg Helios– CD57	0.0577	Q1 CXCR4	0.8708		
Treg Helios– FasL	0.2781	Q1 CXCR5	0.1431		
Treg Helios– PD-1	0.4808	Q1 TIGIT	0.3028		
Treg Helios+	0.7863	Q2	0.8708		
Treg Helios+ CD27	0.1585	Q2 CCR5	0.9567		
Treg Helios+ CD28	0.8708	Q2 CD152	0.3028		
Treg Helios+ CD57	0.0079	Q2 CXCR4	0.4477		
Treg Helios+ FasL	0.3028	Q2 CXCR5	0.9567		
Treg Helios+ PD-1	0.7863	Q2 TIGIT	0.2548		
B1	0.0927	Q3	0.4159		
B1 CCR5	0.3566	Q3 CCR5	0.7863		
B1 CD152	0.0735	Q3 CD152	0.6255		
B1 CXCR5	0.2548	Q3 CXCR4	0.7042		
B2	0.0577	Q3 CXCR5	0.4808		
B2 CCR5	0.7449	Q3 TIGIT	0.6644		
B2 CD152	0.3028	T CD4	0.8708		
B2 CXCR5	0.4477	T CD8	0.8708		
CD19	0.4159	Treg FoxP3	0.9567		
CD19 CCR5	0.4159	Treg FoxP3 CCR5	0.7042		
CD19 CD152	0.1752	Treg FoxP3 CD152	0.7042		
CD19 CXCR5	0.7042	Treg FoxP3 CXCR4	1.00		

*U Mann-Whitney

References

1. Choi SW, Levine JE, Ferrara JLM. Pathogenesis and management of graft-versus-host disease. Immunol Allergy Clin North Am. 2010; 30(1): 75–101, doi: 10.1016/j.iac.2009.10.001, indexed in Pubmed: 20113888.
2. Magenau J, Runaas L, Reddy P. Advances in understanding the pathogenesis of graft-versus-host disease. Br J Haematol. 2016; 173(2): 190–205, doi: 10.1111/bjh.13959, indexed in Pubmed: 27019012.
3. Barak V, Levi-Schaffer F, Nisman B, et al. Cytokine dysregulation in chronic graft versus host disease. Leuk Lymphoma. 1995; 17(1-2): 169–173, doi: 10.3109/10428199509051718, indexed in Pubmed: 7773155.
4. Skert C, Damiani D, Michelutti A, et al. Kinetics of Th1/Th2 cytokines and lymphocyte subsets to predict chronic GVHD after allo-SCT: results of a prospective study. Bone Marrow Transplant. 2009; 44(11): 729–737, doi: 10.1038/bmt.2009.80, indexed in Pubmed: 19398965.
5. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. 1999; 340(6): 448–454, doi: 10.1056/NEJM199902113400607, indexed in Pubmed: 9971870.
6. Czarnogórski MC, Sakowska J, Maziewski M, et al. Ageing-resembling phenotype of long-term allogeneic hematopoietic cells recipients compared to their donors. Immun Ageing. 2022; 19(1): 51, doi: 10.1186/s12979-022-00308-6, indexed in Pubmed: 36324179.
7. Zielinski M, Tarasewicz A, Zielinska H, et al. Impact of donor and recipient human cytomegalovirus status on kidney transplantation. Int Immunol. 2017; 29(12): 541–549, doi: 10.1093/intimm/dxx062, indexed in Pubmed: 29121254.
8. Trzonkowski P, Debska-Slizień A, Jankowska M, et al. Immunosenescence increases the rate of acceptance of kidney allotransplants in elderly recipients through exhaustion of CD4+ T-cells. Mech Ageing Dev. 2010; 131(2): 96–104, doi: 10.1016/j.mad.2009.12.006, indexed in Pubmed: 20060852.
9. Fülöp T, Larbi A, Witkowski JM. Human inflammaging. Gerontology. 2019; 65(5): 495–504, doi: 10.1159/000497375, indexed in Pubmed: 31055573.
10. Aunan JR, Cho WC, Søreide K. The biology of aging and cancer: a brief vverview of shared and divergent molecular hallmarks. Aging Dis. 2017; 8(5): 628–642, doi: 10.14336/AD.2017.0103, indexed in Pubmed: 28966806.

11. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144(5): 646–674, doi: 10.1016/j.cell.2011.02.013, indexed in Pubmed: 21376230.
12. Cutler CS, Koreth J, Ritz J. Mechanistic approaches for the prevention and treatment of chronic GVHD. Blood. 2017; 129(1): 22–29, doi: 10.1182/blood-2016-08-686659, indexed in Pubmed: 27821505.
13. MacDonald KPa, Blazar BR, Hill GR. Cytokine mediators of chronic graft-versus-host disease. J Clin Invest. 2017; 127(7): 2452–2463, doi: 10.1172/JCI90593, indexed in Pubmed: 28665299.
14. Zelle-Rieser C, Thangavadivel S, Biedermann R, et al. T cells in multiple myeloma display features of exhaustion and senescence at the tumor site. J Hematol Oncol. 2016; 9(1): 116, doi: 10.1186/s13045-016-0345-3, indexed in Pubmed: 27809856.
15. Cassady K, Martin PJ, Zeng D. Regulation of GVHD and GVL activity via PD-L1 interaction with PD-1 and CD80. Front Immunol. 2018; 9: 3061, doi: 10.3389/fimmu.2018.03061, indexed in Pubmed: 30622541.