MEDICAL UNIVERSITY
"PROF. DR. PARASKEV STOYANOV" – VARNA

FACULTY OF MEDICINE
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Role of Lymphocyte Populations Following Allogeneic Hematopoietic Stem Cell Transplantation

THESIS SUMMARY

Of the doctoral dissertation for awarding the educational and scientific degree of

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Scientific supervisor:
Prof. Dr. Ilina Dimitrova Micheva, MD, PhD

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External members to the Medical University – Varna:

- Prof. Veselina Stefanova Goranova–Marinova, MD, PhD
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The final meeting of the Scientific Jury for the defense of the dissertation of doctoral candidate Dr. Yavor Angelov Petrov will be held on **24 September 2025**.

The materials related to the defense of the dissertation are available at the Research Department of the Medical University – Varna and are published on the official website of the Medical University "Prof. Dr. Paraskev Stoyanov" – Varna.

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I. Background

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an established therapeutic approach for a range of malignant and benign hematological disorders. Despite significant advances in the field of transplantation medicine, this method remains associated with a number of complications – graft-versus-host disease (GVHD), infectious complications (viral, bacterial, fungal), non-infectious complications (dysfunction of various organs and systems), and delayed or incomplete recovery of the immune system. Following allo-HSCT, immune system reconstitution is a complex and prolonged process, which has critical importance for patient prognosis. Lymphocyte subpopulations play a key role in the immune regulation of recipients, and imbalance in their number and function can lead to severe consequences – GVHD, relapse of the underlying disease, infectious and non-infectious complications. Understanding the dynamics of these subpopulations in the post-transplant period is essential for improving therapeutic strategies and patient outcomes.

II. Aim of the study

The aim of the study is to analyze the absolute lymphocyte count and lymphocyte subpopulations (CD3+CD4+, CD3+CD8+CD38+, NK cells, CD19+) in patients with malignant hematological diseases following allogeneic hematopoietic stem cell transplantation, and their correlation with various transplant-related factors and complications.

III. Objectives of the study

1. To analyze the absolute lymphocyte count (ALC) on days 30, 100 and 180 of the post-transplant period and its relationship with the following transplant-related factors: donor type, donor gender, conditioning regimen, and transplant-related complications – GVHD, infections, and non-infectious complications.
2. To analyze the number of CD3+CD4+ T-helper subpopulations on days 100, 180, and 270 of the post-transplant period and their relationship with the following transplant-related factors: donor type, donor gender, conditioning regimen, and transplant-related complications – GVHD, infections, and non-infectious complications.
3. To analyze the number of activated cytotoxic T-cell populations (CD3+CD8+CD38+) on days 100, 180, and 270 of the post-transplant period and their relationship with the following transplant-related factors: donor type, donor gender, conditioning regimen,

and transplant-related complications – GVHD, infections, and non-infectious complications.

4. To analyze the number of NK cell populations on days 100, 180, and 270 of the post-transplant period and their relationship with the following transplant-related factors: donor type, donor gender, conditioning regimen, and transplant-related complications – GVHD, infections, and non-infectious complications.
5. To analyze the number of B-lymphocyte populations (CD19+) on days 100, 180, and 270 of the post-transplant period and their relationship with the following transplant-related factors: donor type, donor gender, conditioning regimen, and transplant-related complications – GVHD, infections, and non-infectious complications.

IV. Materials and Methods

1. Patient population

The analysis included 89 patients over the age of 18, with a gender distribution of 47 men (52.8%) and 42 women (47.2%), who underwent allogeneic hematopoietic stem cell transplantation due to malignant hematological disease during the period 2017–2023 at the Hematopoietic Stem Cell Transplantation Unit, Department of Hematology, University Hospital "St. Marina," Varna.

The mean age for male recipients was 48 years (±13.6), and for female recipients – 44.5 years (±13.5). The largest group consisted of patients diagnosed with acute myeloid leukemia – 54 (60.6%), followed by acute lymphoblastic leukemia – 16 (18%), non-Hodgkin lymphomas – 5 (5.6%), Hodgkin lymphoma – 5 (5.4%), aplastic anemia – 6 (6.7%), plasma cell leukemia – 1 (1.1%), blastic plasmacytoid dendritic cell neoplasm (BPDCN) – 2 (2.1%), and chronic myeloid leukemia (CML) – 1 (1.1%).

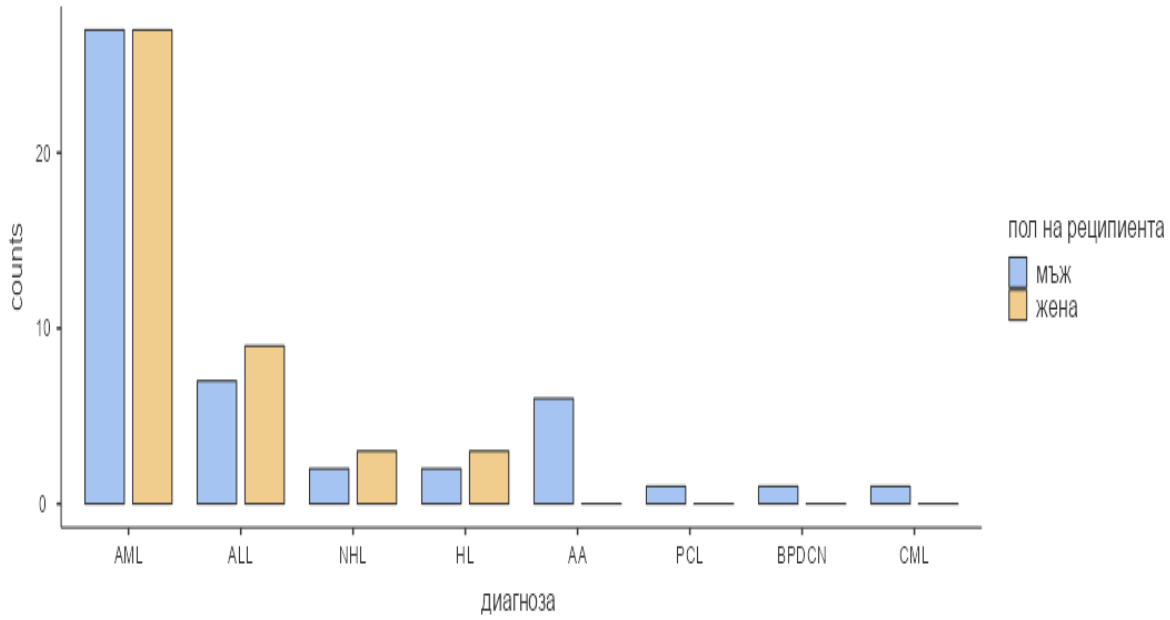


fig.1 Patient distribution by gender and diagnosis

2. Donors and graft type

Patients transplanted from a 10/10 HLA-matched related donor numbered 27 (30.3%), followed by those receiving grafts from a 10/10 HLA fully matched unrelated donor – 29 (32.6%), a 9/10 HLA mismatched unrelated donor – 2 (2.2%), and haploidentical donors – 31 (34.8%).

Most donors were male – 57 (64%), compared to female donors – 32 (36%). Peripheral blood was the predominant source of hematopoietic stem cells (n=87) compared to bone marrow (n=2).

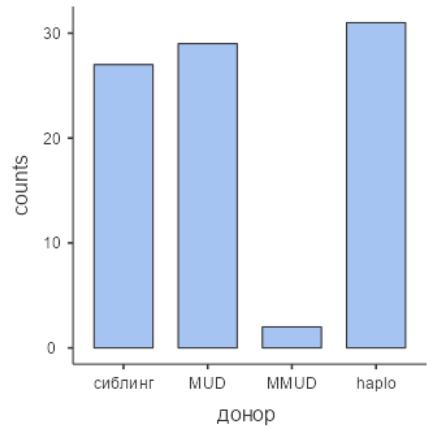


fig. 2. Distribution by donor

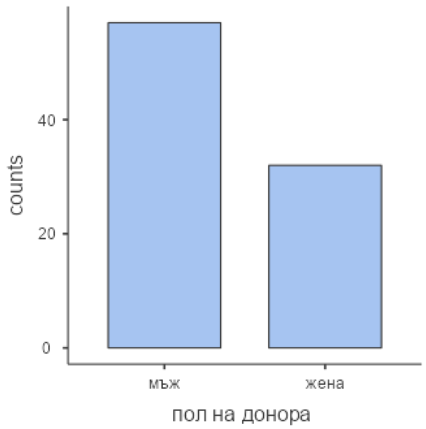


fig. 3. Distribution by gender

3. Conditioning regimens and immunosuppressive therapy

The conditioning regimens used were as follows: myeloablative FluBu – n=25 (28.1%), BuCy – n=27 (30.3%), FluCyATG – n=5 (5.6%), TBF – n=19 (21.3%), FLAMSA-Bu – n=6 (6.7%), CyATG – n=1 (1.1%), FluMelBCNUATG – n=2 (2.2%), and reduced-intensity conditioning (RIC) FluBu – n=4 (4.5%).

Immunosuppressive prophylaxis included the use of calcineurin inhibitors – Cyclosporine A 5 mg/kg/day (target trough concentration 200–250 ng/mL) (n=80) or Tacrolimus 0.01 mg/kg/day (target trough concentration 5–12 ng/mL) (n=9), in combination with Mycophenolate mofetil until day +35 (n=85), ± Methotrexate 20 mg/m² (days +1, +3, +6, +11 for unrelated donor recipients, and a short course on days +1, +3, and +6 for related donor recipients).

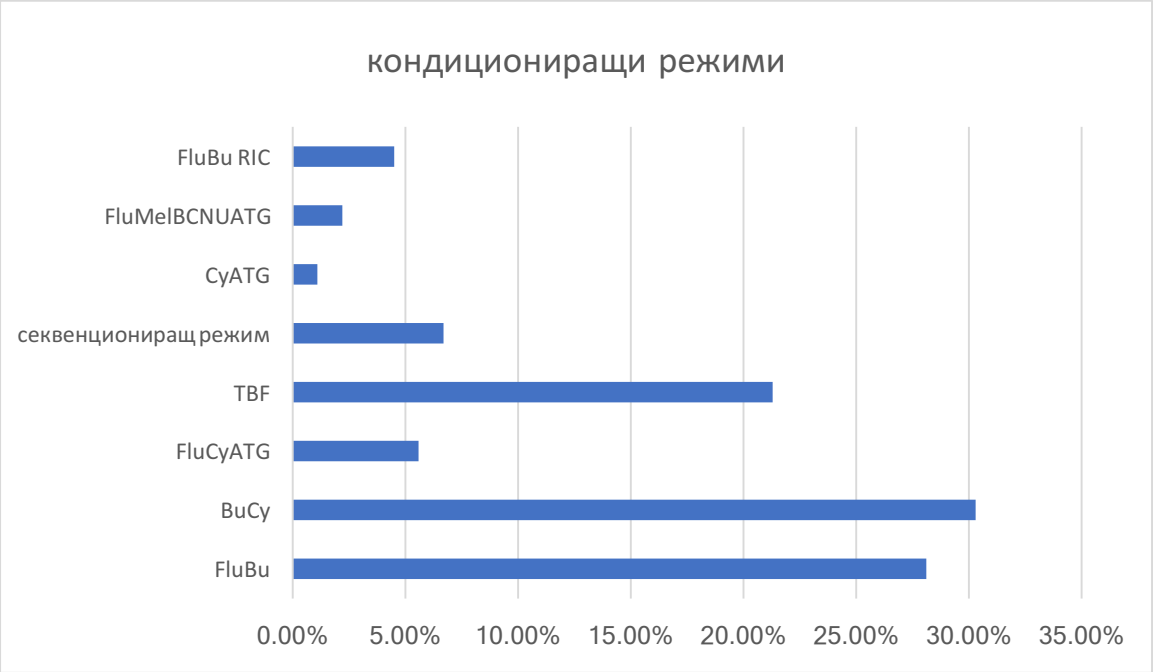


fig. 4. Types of conditioning regimens

4. Flowcytometry of the lymphocyte subpopulations

Monitoring of lymphocyte subpopulations in the post-transplant period was performed by multiparameter flow cytometry of peripheral blood on days 100, 180, and 270. The analyses were carried out at the Clinical Immunology Laboratory of University Hospital "St. Marina" EAD, Varna.

Blood samples were collected via venipuncture using a closed system with EDTA anticoagulant, BD Vacutainer™ K2E 3 ml, catalogue No. 368856 (Becton Dickinson, USA).

Immunophenotyping of lymphocytes and determination of their relative frequency from venous blood was performed by direct immunofluorescence using combinations of monoclonal antibodies conjugated with fluorescein isothiocyanate (FITC), AlexaFluor 488 (AF488), phycoerythrin (PE), peridinin–chlorophyll–protein (PerCP), and allophycocyanin (APC), according to the manufacturer's lyse–wash protocol (Becton Dickinson, USA).

The monoclonal antibodies (Becton Dickinson, USA) used were as follows: CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC, Cat. No. 342417; CD3 FITC/CD16+56 PE/CD45 PerCP, Cat. No. 342411; CD3 FITC/CD19 PE/CD45 PerCP, Cat. No. 342412; CD3 FITC/HLA-DR PE, Cat. No. 337603; CD4 FITC/CD25 PE/CD3 PerCP, Cat. No. 333170; CD4 FITC/CD62L PE, Cat. No. 349513; CD57 FITC/CD8 PE, Cat. No. 333191; CD8 FITC/CD28 PE, Cat. No. 340031; CD8 FITC/CD38 PE, Cat. No. 349527; CD8 PerCP, Cat. No. 345774; CD11b AF488, Cat. No. 557701; CD8 PE, Cat. No. 345773.

Venous blood was incubated for 15 minutes at room temperature with the antibody cocktail, followed by erythrocyte lysis with BD FACS™ Lysing Solution 1×, Cat. No. 349202 (Becton Dickinson, USA), for 10 minutes at room temperature. The cells were then pelleted by centrifugation at 300 × g, washed, and resuspended in isotonic buffer BD CellWASH, Cat. No. 349524 (Becton Dickinson, USA).

Samples were acquired on a FACSCalibur flow cytometer (Becton Dickinson, USA) and analysed using CellQuest Pro software, version 6.1 (Becton Dickinson, USA) (20,000 events per tube). The absolute counts of individual lymphocyte subpopulations were determined using a dual-platform approach: lymphocyte concentrations were obtained with a Sysmex XN-1000 haematology analyser (Sysmex Corporation, Japan). The characterised lymphocyte subpopulations and their phenotypes are shown in **Table 1**.

Lypmhocyte subpopulation	**Phenotype**
Total T lymphocytes	CD3+
Activated T lymphocytes	CD3+HLA-DR+
Helper – inducer T lymphocytes	CD3+ CD4+
Suppressor – inducer T lymphocytes	CD4+CD62L+
Helper T lymphocytes	CD4+CD62L–
Suppressor – cytotoxic T lymphocytes	CD3+ CD8+
Suppressor T lymphocytes	CD8+ CD11b+
Cytotoxic T lymphocytes	CD8+ CD11b–
CD4/CD8 index	CD3+ CD4+ / CD3+ CD8+
B lymphocytes	CD19+

NK cells	CD3– CD16/56+
NKT cells	CD3+ CD16/56+
Terminally differentiated CD8+ T-lymphocytes	CD57+/CD8+
Terminally differentiated CD8- T-lymphocytes	CD57+/CD8–
Activated cytotoxic T lymphocytes	CD8+HLA-DR+
Цитотоксични клетки	CD8+ CD28+
Activated cytotoxic T-lymphocytes	CD8+CD38+
Regulatory T-lymphocytes	CD4+CD25+

Table 1. Phenotype of lymphocyte subpopulations

5. Statistical analysis

Statistical analysis of the data was performed using the IBM SPSS Statistics software package, version 22. All quantitative data are presented as mean ± standard deviation (SD) for normally distributed variables and as median with interquartile range (IQR) for non-normally distributed variables. Categorical variables are expressed as absolute numbers and percentages.

A descriptive statistical analysis was performed for all studied variables in order to characterize the data distribution. The main indicators such as means, medians, minimum and maximum values, standard deviation, and standard error of the mean were reported.

To assess differences between two independent groups, the Mann–Whitney U test was applied, appropriate for non-normally distributed data and small sample sizes. For comparisons among more than two independent groups, one-way analysis of variance (One-Way ANOVA) was used, followed by Tukey's post hoc analysis to identify statistically significant differences between specific groups.

To evaluate the strength and direction of the relationship between two quantitative variables, correlation analysis was performed by calculating Spearman's rank correlation coefficient (Spearman's rho). Associations between categorical variables were tested using the chi-square (χ^2) test.

When analyzing the effect of independent variables (e.g., immunological parameters) on dependent clinical outcomes (e.g., survival), regression analysis—either linear or logistic—was applied, depending on the nature of the dependent variable.

To assess the diagnostic accuracy of the studied markers and to determine optimal cut-off values, Receiver Operating Characteristic (ROC) analysis was used. The area under the curve (AUC), sensitivity, specificity, and corresponding 95% confidence intervals were calculated.

All results were considered statistically significant at a probability level of $p<0.05$, unless otherwise specified.

V. Results

1. Generel reconstitution of immunity after allogeneic stem cell transplantation

Monitoring of the absolute lymphocyte count (ALC) in the post-transplant period demonstrated a positive trend, with mean lymphocyte values as follows:
Day 0 – $0.001x10^9$/L ±0.659; on day +30 - $0.49x10^9$/L ±0.67, on day +100 – $1.07x10^9$/L ±1.10, on day +180 – $1.83x10^9$/L ±1.75.

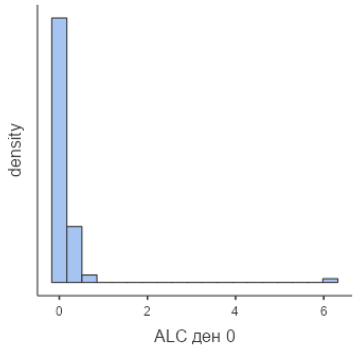


fig. 5 ALC on day 0

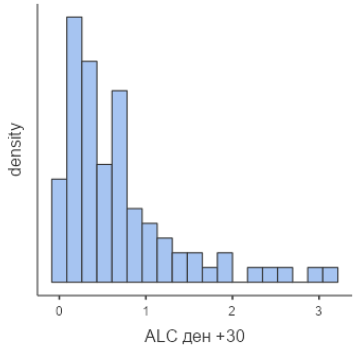


fig. 6 ALC on day 30

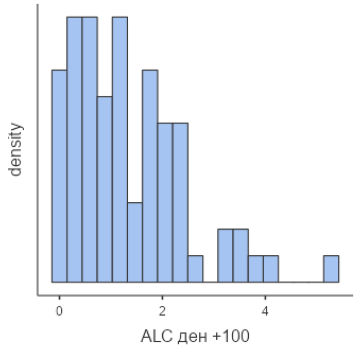


fig. 7 ALC on day 100

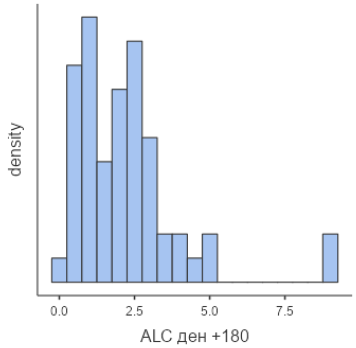
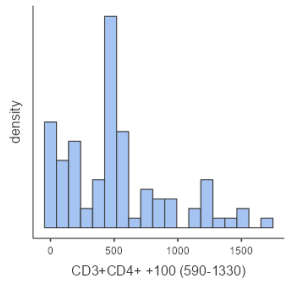
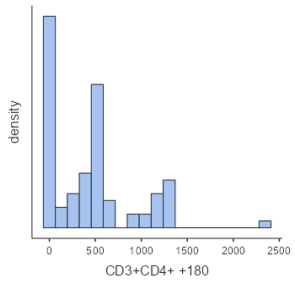


fig. 8 ALC on day 180

The mean values for T-helper cell populations on day +100 were 480 cells/μL (±407), and on day +180 – 429 cells/μL (±474).





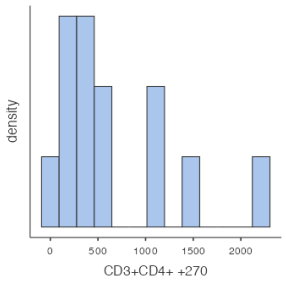
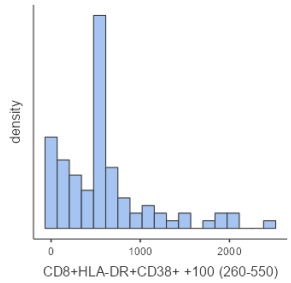
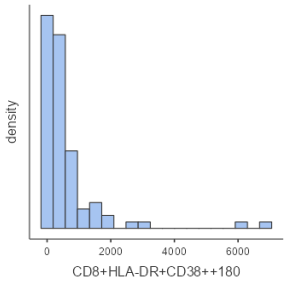


fig. 9 CD3+CD4+ day 100 fig. 10 CD3+CD4+ day 180 fig. 11CD3+CD4+ day 270

For the activated cytotoxic T-cell populations, the mean values were as follows: on day +100 post-transplant – 540 cells/μL (±523), on day +180 – 479 cells/μL (±1070), and on day +270 – 485 cells/μL (±776).





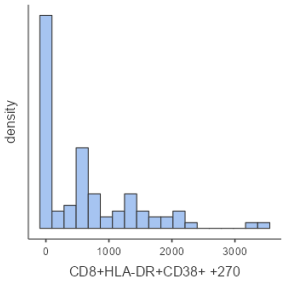
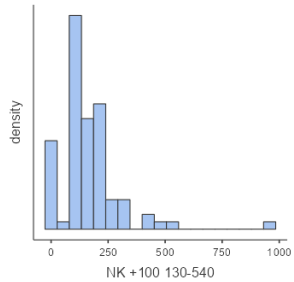
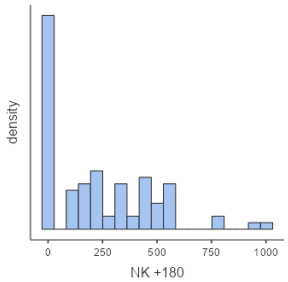


fig.12 CD3+CD8+CD38+ day 100 fig.13 CD3+CD8+CD38+ day 180 fig.14 CD3+CD8+CD38+ day270

For the NK cell populations, the mean values were as follows: on day +100 – 135 cells/μL (±135), on day +180 – 175 cells/μL (±237), and on day +270 – 160 cells/μL (±220).





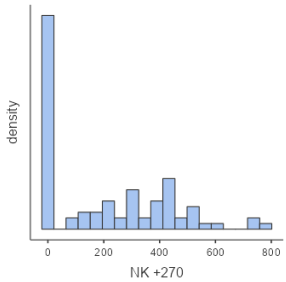
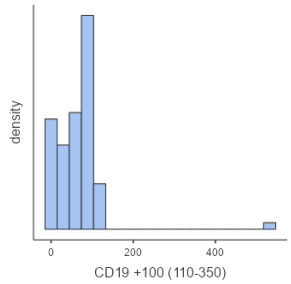
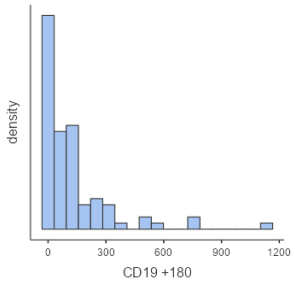


fig. 15 NK cells on day 100 fig. 16 NK cells on day 180 fig. 17 NK cells on day 270

The mean values for CD19+ cells were as follows: on day +100 – 67 cells/μL (±62.5), on day +180 – 80 cells/μL (±194), and on day +270 – 71.5 cells/μL (±282)





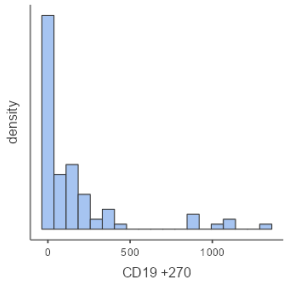
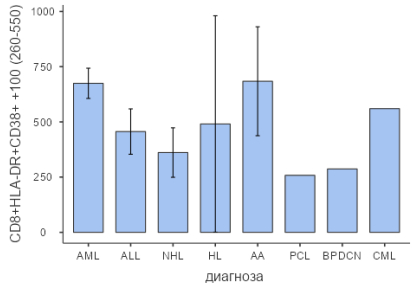


fig. 18 CD19+ cells on day 100 fig. 19 CD19+ cells on day 180 фиг.20 CD19+ cells on day 270

2. Transplant – related factors and immune system recovery

2.1. Type of diagnosis

Correlation analysis demonstrated an association between the diagnosis and the recovery of certain lymphocyte subpopulations. On day +100, the number of activated CD3+CD8+CD38+ lymphocytes were higher in patients with acute myeloid leukemia (median 549 cells/μL ±503), followed by patients with non-Hodgkin lymphoma (median 534 cells/μL ±250), compared to patients with acute lymphoblastic leukemia – 382 cells/μL ±411 (Spearman's rho = –0.233, p = 0.028). On day +180, the number of activated CD3+CD8+CD38+ lymphocytes were higher in patients with acute myeloid leukemia (median 543 cells/μL ±1267) and in patients with non-Hodgkin lymphoma (median 432 cells/μL ±1108), compared to patients with acute lymphoblastic leukemia (median 0 ±350) (Spearman's rho = –0.225, p = 0.035)



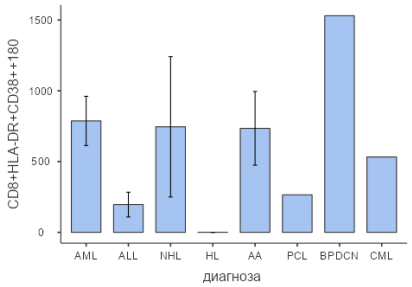


fig. 21 и fig. 22 Recovery of CD3+CD8+CD38+ Cells According to Diagnosis on Day +100 and Day +180

A correlation was also found between CD19+ cell counts on day +100 post-transplant and certain diagnosis types. The number of CD19+ cells was highest in patients with acute myeloid leukemia (median 78 cells/μL ±70) and in patients with non-Hodgkin lymphoma (median 64 cells/μL ±42), compared to patients with acute lymphoblastic leukemia (median 31 cells/μL ±38) (Spearman's rho = –0.245, p = 0.021)

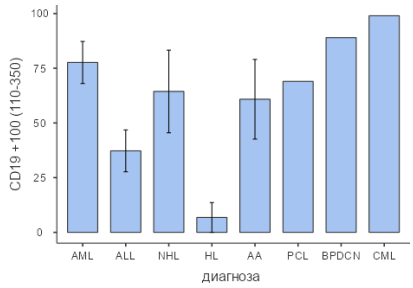


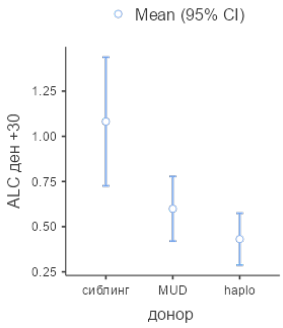
fig.23 Recovery of CD19+ cells on day 100

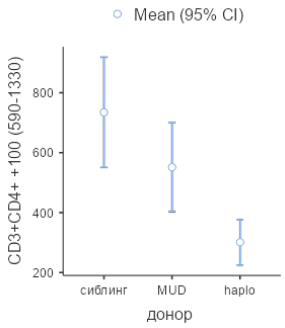
2.2. Donor type

The absolute lymphocyte count (ALC) on day +30 was highest in patients transplanted from a related donor – 1.08 ± 0.9, lower in patients with an unrelated donor – 0.5990 ± 0.479, and lowest in patients transplanted from a haploidentical donor – 0.4311 ± 0.370 (F=6.2371, df=2, p=0.004).

The CD3+CD4+ count on day +100 was highest in patients transplanted from a related donor – 735 cells/μL (±465), lower in those with an unrelated donor – 551 cells/μL (±404), and lowest in haploidentical donor recipients – 301 cells/μL (±207) (F=12.54, df=2, p=0.001).

The CD3+CD4+ count on day +180 was highest in related donor recipients – mean 695 cells/μL (±613), lower in unrelated donor recipients – mean 411 cells/μL (±431), and lowest in haploidentical donor recipients – 274 cells/μL (±240) (F=6.03, df=2, p=0.00)





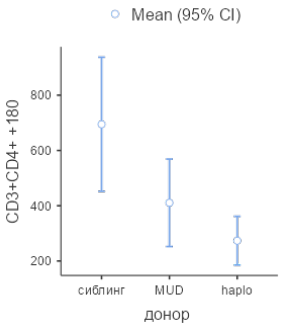
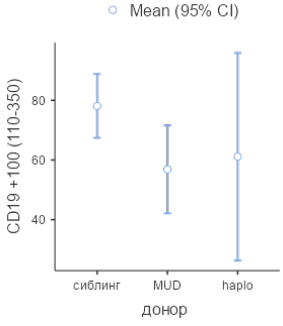
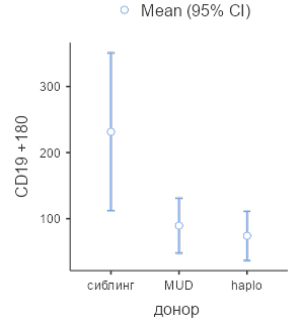


fig. 24 Recovery of ALC fig.25 Recovery of CD3+CD4+ фиг. 26 Recovery of CD3+CD8+CD38+

The highest CD19+ cell count on day +180 was observed in patients transplanted from a related donor – 231.5 cells/μL ± 295.2, lower in patients with an unrelated donor – 89.5 cells/μL ± 112.8, and lowest in haploidentical donor recipients – 74.1 cells/μL ± 100.9 (F=6.139, df=2, p=0.003).





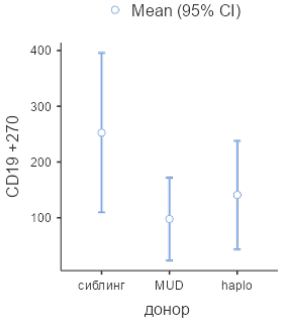


fig. 27 Recovery of CD19+ on day 100 fig. 28 Recovery of CD19+ day 180 fig. 29 Recovery of CD19+ day 270

2.3 Donor sex

It was found that patients transplanted from male donors had better recovery of the absolute lymphocyte count (ALC) on day +30 (Spearman's rho = –0.315, p = 0.003)

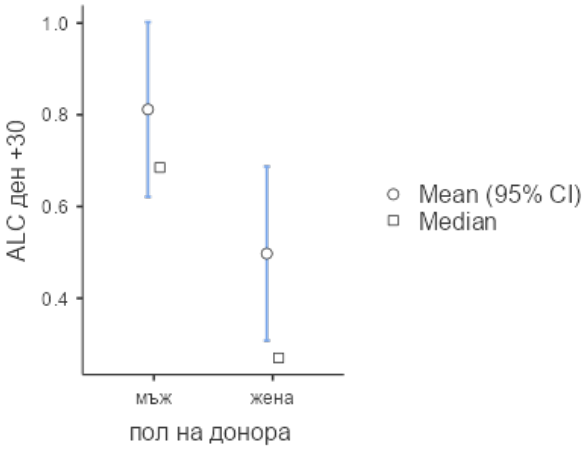


fig. 30 Recovery of ALC regarding the donor sex

Recovery of CD3+CD4+ lymphocyte subpopulations on day +100 was better in patients with a male donor (Spearman's rho = –0.221, p = 0.037).

Recovery of CD3+CD8+CD38+ lymphocyte subpopulations on day +180 was better in patients with a male donor (Spearman's rho = –0.215, p = 0.044).

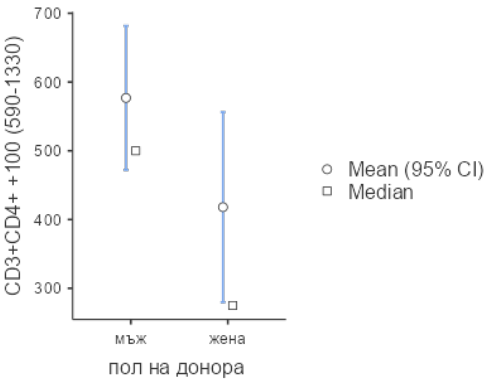


fig. 31 Recovery of CD3+CD4+ on day 100

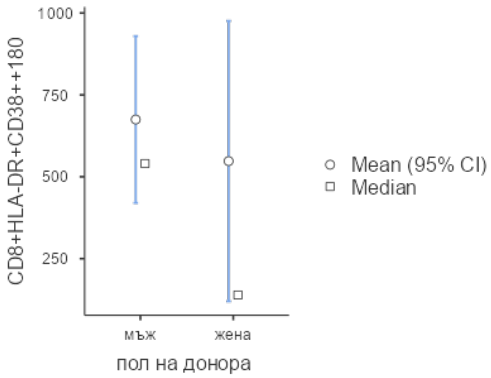


fig.32 Recovery of CD3+CD8+CD38+ on day 180

2.4. Conditioning regimens and serotherapy

The results from the One-Way ANOVA for CD3+CD4+ lymphocytes on day +100 showed statistically significant differences between certain conditioning regimens, supporting the assertion that conditioning regimens influence immune recovery (F = 7.42, p = 0.001). Tukey's post hoc test revealed that patients conditioned with FluBuATG had significantly lower mean

CD3+CD4+ population counts compared to those conditioned with BuCyATG, with a difference of –349 (p = 0.004). In addition, BuCyATG demonstrated a significant difference compared to TBF-ATG, with a difference of 361.1 (p = 0.006).

One – Way ANOVA				
	F	df1	df2	p
CD3+CD4+ day 100	7.42	2	68	0.001
CD3+CD4+ day 180	2.88	2	68	0.063

Table. 2 Differences in conditioning regimens

Tukey Post – Hoc Test CD3+CD4+ ден 100				
Regimen		FluBu	BuCy	TBF
FluBu	Mean difference	-	-349	12.3
	p – value	-	0.004	0.994
BuCy	Mean difference		-	361.1
	p – value			0.006
TBF	Mean difference			-
	p – value			-

Table. 3 Post – Hoc analysis – Impact of Conditioning Regimens on CD3+CD4+ Recovery

Statistically significant negative correlations were identified between patients who received ATG and the recovery of certain lymphocyte subtypes. On day +100 post-transplant, patients in the ATG group demonstrated significantly lower levels of CD3+CD4+ populations (Spearman's rho = –0.301, p = 0.004), with this trend persisting on day +180 (Spearman's rho = –0.266, p = 0.012).

With regard to CD3+CD8+HLA-DR+CD38+ cells on day +100, a correlation approaching statistical significance was observed (p = 0.055), suggesting the need for further in-depth investigation. A negative correlation was also found between ATG use and NK cell counts on day +180 (Spearman's rho = –0.212, p = 0.046) and on day +270 post-transplant (Spearman's rho = –0.220, p = 0.041).

		ATG	CD3+CD4+ +100	CD3+CD4+ +180
ATG	Spearman's rho	—		
	p-value	—		
CD3+CD4+ +100	Spearman's rho	-0.301 **	—	
	p-value	0.004	—	
CD3+CD4+ +180	Spearman's rho	-0.266 *	0.786 ***	—
	p-value	0.012	< .001	—

Note. * p < .05, ** p < .01, *** p < .001

Table. 4 Negative Correlation Between the Use of ATG in the Conditioning Regimen and CD3+CD4+ Cell Recovery on Days +100 and +180

		ATG	NK +100	NK +180	NK +270
ATG	Spearman's rho	—			
	p-value	—			
NK +100	Spearman's rho	-0.145	—		
	p-value	0.179	—		
NK +180	Spearman's rho	-0.212 *	0.726 ***	—	
	p-value	0.046	< .001	—	
NK +270	Spearman's rho	-0.220 *	0.658 ***	0.821 ***	—
	p-value	0.041	< .001	< .001	—

Note. * p < .05, ** p < .01, *** p < .001

Table. 5 Negative Correlation Between the Use of ATG in the Conditioning Regimen and NK Cell Recovery on Days 100, 180, and 270

3. Transplant – related complications and immune recovery

With regard to the absolute lymphocyte count (ALC) on day +30, regression models did not reveal a statistically significant correlation with post-transplant complications. On day +100, however, negative coefficients for GVHD and infections indicated that these conditions were associated with reduced ALC. The mean ALC in patients without complications was 1.61 ×

10^9/L (95% CI 1.3–1.93, p<0.001), compared to –1.19 (p=0.002) in patients with GVHD and –0.76 (p=0.032) in patients with infections.

Predictor	Estimate	SE	95% Confidence Interval		t	p	Stand. Estimate
			Lower	Upper			
Intercept [a]	1.617	0.159	1.300	1.9346	10.170	< .001	
Transplant complications							
GVHD	-1.194	0.363	-1.918	-0.4711	-3.293	0.002	-1.082
infections	-0.765	0.349	-1.461	-0.0684	-2.190	0.032	-0.693
relapse	0.138	0.379	-0.617	0.8933	0.365	0.716	0.125
Non-infectious compl.	-0.681	0.616	-1.909	0.5478	-1.105	0.273	-0.617
GVHD + infections	-1.107	1.043	-3.187	0.9726	-1.062	0.292	-1.004

[a] Represents reference level- живи

Table. 6 Regression model – ALC day 100

The results from the regression model showed that the mean CD3+CD4+ cell count on day +100 post-transplant in patients without complications was 786 cells/μL. The presence of negative coefficients for various transplant-related complications — GVHD (–531, p<0.001), infections (–536, p<0.001), relapse (–348, p=0.003), and non-infectious complications (–612, p<0.001) — indicates that these conditions are associated with lower CD3+CD4+ cell levels and emphasizes that a reduction in their number is linked to clinical deterioration in patients.

Predictor	Estimate	SE	95% Confidence Interval		t	p	Stand. Estimate
			Lower	Upper			
Intercept	786	49.4	688	884	15.907	< .001	
Transplant complications:							
GVHD	-531	101.7	-733	-329	-5.224	< .001	-1.305
infections	-536	98.8	-733	-340	-5.426	< .001	-1.318
relapse	-348	112.7	-572	-123	-3.085	0.003	-0.854
Non-infectious compl	-612	117.6	-846	-378	-5.200	< .001	-1.504
GVHD +infection	-316	324.1	-961	328	-0.976	0.332	-0.777

[a] Represents reference level-пациенти без усложнения

Table. 7 Regression model – CD3+CD4+ day 100

The mean CD3+CD4+ cell count on day +180 post-transplant in patients without complications was 768 cells/μL, with lower levels of these cell populations correlating with transplant-related complications — GVHD (–741, p<0.001), infections (–620, p<0.001), relapse (–450, p<0.001), and non-infectious complications (–583, p<0.001).

Predictor	Estimate	SE	95% Confidence Interval		t	p	Stand. Estimate
			Lower	Upper			
Intercept [a]	768	56.4	656	880	13.635	< .001	
Transplant complications							
GVHD	-741	115.9	-972	-511	-6.395	< .001	-1.565
infection	-620	112.7	-844	-396	-5.504	< .001	-1.310
relapse	-450	128.5	-706	-194	-3.501	< .001	-0.950
Non-infectious compl.	-583	134.1	-850	-316	-4.345	< .001	-1.231
GVHD + infection	-348	369.5	-1083	387	-0.943	0.349	-0.736

[a] Represents reference level- пациенти без усложнения

Table. 8 Regression model – CD3+CD4+ day 180

Of particular importance is the high coefficient for GVHD, indicating that this complication is associated with the lowest CD3+CD4+ cell counts and compromises the favorable outcome of the transplantation.

Regarding CD3+CD8+CD38+ population counts on days +100, +180, and +270, statistically significant correlations with transplant-related complications were also observed. Analysis revealed that the highest mean CD3+CD8+CD38+ cell count on day +100 (900/μL) was found in patients with GVHD (rho = –0.375, p = 0.001), which led to a negative change in the clinical condition of the patients. Similar results were observed for CD3+CD8+CD38+ cells on days +180 and +270.

	Transplant complications	N	SE	Median	St.dev.	Min	Max
CD8+ CD38+ 100	**Patient w/o complications**	42	677.7	548.5	419	71	2100
(rho=-0.375, p=0.001)	**GVHD**	13	877.2	900	768	0	2450

	Transplant complications	N	SE	Median	St.dev.	Min	Max
	infection	14	492.8	346.5	552	0	1958
	relapse	10	338.2	230.0	213	128	700
	Non-infectious compl.	9	168.2	0	313	0	860
	GVHD + infection;	1	1534.0	1534	NaN	1534	1534
CD8+ CD38+180	**Patients w/o complications**	42	942.6	549.0	1122	139	6867
(rho=-0.494, p=0.001)	**GVHD**	13	123.8	0	402	0	1453
	infection	13	271.3	0	367	0	867
	relapse	10	764.5	117.5	1863	0	6030
	Non-infectious compl	9	144.8	0	306	0	865
	GVHD + infection;	1	1645.0	1645	NaN	1645	1645
CD8+ CD38+ 270	**Patients w/o complications**	41	1107.7	870	684	210	3214
(rho=-0.643, p=0.001)	**GVHD**	13	0.0	0	0	0	0
	infection	14	243.3	0.0	512	0	1567
	relapse	10	502.4	85.0	1063	0	3457
	Non-infectious compl.	9	87.3	0	262	0	786
	GVHD + infection;	1	471.0	471	NaN	471	471

Table. 9 Correlation Model for CD3+CD8+ and Transplant-Related Complications on Days +100, +180, and +270 Post-Transplant

The results from the regression model for CD3+CD8+CD38+ populations on day +100 revealed an interesting dynamic depending on the various transplant-related complications. The mean CD3+CD8+CD38+ cell count in patients without complications was 678 cells/μL (95% CI 529–826 cells/μL). Negative coefficients for relapse (–340) and non-infectious complications (–509) were statistically significant (p = 0.049 and p = 0.005, respectively), indicating an association with lower counts of this specific cell population subtype.

			95% Confidence Interval				
Predictor	**Estimate**	**SE**	**Lower**	**Upper**	**t**	**p**	**Stand. Estimate**
Intercept [a]	678	74.6	529	826.13	9.08	< .001	
Transplant complication							
GVHD	199	153.5	-106	504.71	1.30	0.197	0.381
infection	-185	149.2	-482	111.90	-1.24	0.219	-0.354

Predictor	Estimate	SE	95% Confidence Interval		t	p	Stand. Estimate
			Lower	Upper			
relapse	-340	170.2	-678	-1.08	-2.00	0.049	-0.649
Non-infectious compl.	-509	177.6	-863	-156.19	-2.87	0.005	-0.974
GVHD + infection	856	489.3	-117	1829.51	1.75	0.084	1.638

[a] Represents reference level-пациенти без усложнения

Table. 10 Regression model CD3+CD8+ cells on day 100

Regression analysis of CD3+CD8+CD38+ cells on day +180 post-transplant showed a significant inverse relationship between cell levels and various complications. The mean CD3+CD8+CD38+ cell count in patients without complications was 943 cells/μL (95% CI 626–1259). Negative coefficients for GVHD (–819), infection (–671), and non-infectious complications (–798) were statistically significant ($p = 0.014$, $p = 0.043$, and $p = 0.038$, respectively), indicating that these complications are associated with lower levels of CD3+CD8+CD38+ cells.

Predictor	Estimate	SE	95% Confidence Interval		t	p	Stand. Estimate
			Lower	Upper			
Intercept [a]	943	159	626	1259.1	5.925	< .001	
Transplant complications							
GVHD	-819	327	-1470	-167.8	-2.502	0.014	-0.765
infection	-671	327	-1322	-20.3	-2.051	0.043	-0.627
relapse	-178	363	-900	543.6	-0.491	0.625	-0.166
Non-infectious compl.	-798	379	-1551	-44.5	-2.107	0.038	-0.746
GVHD + infection	702	1043	-1373	2777.7	0.673	0.503	0.656

[a] Represents reference level

Table. 11 Regression model CD3+CD8+ cells on day 180

The results from the regression model for CD3+CD8+CD38+ populations on day +270 showed a significant negative association between cell levels and various transplant-related complications. The mean CD3+CD8+CD38+ cell count in patients without complications was 1108 cells/μL (95% CI 911–1304). Negative coefficients for GVHD (–1108), infections (–

864), relapse (–605), and non-infectious complications (–1020) were statistically significant ($p<0.001$, $p<0.001$, $p=0.008$, and $p<0.001$, respectively). These data demonstrate that transplant-related complications contribute to immune dysfunction and deterioration of the immune response.

			95% Confidence Interval				
Predictor	**Estimate**	**SE**	**Lower**	**Upper**	**t**	**p**	**Stand. Estimate**
Intercept [a]	1108	98.9	911	1304	11.202	< .001	
Transplant complications							
GVHD	-1108	201.5	-1509	-707	-5.496	< .001	-1.428
infection	-864	196.0	-1254	-475	-4.410	< .001	-1.115
relapse	-605	223.3	-1050	-161	-2.711	0.008	-0.781
Non-infectious compl.	-1020	233.1	-1484	-557	-4.378	< .001	-1.316
GVHD + infection	-637	640.9	-1912	638	-0.994	0.323	-0.821

[a] Represents reference level

Table. 12 Regression model CD3+CD8+ cells on day 270

A significant negative correlation was also found between NK cell populations on day +100 post-transplant and transplant-related complications. The mean NK cell count in patients without complications was 245 cells/μL (95% CI 209–280.4). Negative coefficients for GVHD (–139), infectious complications (–130), relapse (–124), and non-infectious complications (–204) were statistically significant.

			95% Confidence Interval				
Predictor	**Estimate**	**SE**	**Lower**	**Upper**	**t**	**p**	**Stand. Estimate**
Intercept [a]	245	18.0	209	280.4	13.60	< .001	
Transplant complicatons							
GVHD	-139	36.7	-212	-66.0	-3.79	< .001	-1.027
infections	-130	36.7	-203	-57.0	-3.55	< .001	-0.961
relapse	-124	40.6	-205	-43.2	-3.05	0.003	-0.917
Non-infectious compl.	-204	42.4	-289	-119.8	-4.81	< .001	-1.509
GVHD + infection	-126	116.6	-358	106.4	-1.08	0.285	-0.928

[a] Represents reference leve

Predictor	Estimate	SE	95% Confidence Interval		t	p	Stand. Estimate
			Lower	Upper			

Table. 13 Regression model NK cells on day 100

Statistically significant differences were also observed in the NK cell counts on days +180 and +270 post-transplant, with mean values for patients without complications of 379 cells/μL on day +180 (95% CI 321–436.5) and 355 cells/μL on day +270 (95% CI 304–406.1).

Predictor	Estimate	SE	95% Confidence Interval		t	p	Stand. Estimate
			Lower	Upper			
Intercept [a]	379	29.0	321	436.5	13.082	< .001	
Transplant complications							
GVHD	-337	59.6	-455	-218.2	-5.653	< .001	-1.419
infections	-308	57.9	-423	-192.3	-5.309	< .001	-1.296
relapse	-170	66.0	-301	-38.2	-2.568	0.012	-0.715
Non-infectious compl.	-327	68.9	-464	-189.9	-4.743	< .001	-1.378
GVHD + infection	-169	189.9	-547	208.8	-0.889	0.376	-0.712

[a] Represents reference level- живи

Table. 14 regression model NK cells on day 180

Predictor	Estimate	SE	95% Confidence Interval		t	p	Stand. Estimate
			Lower	Upper			
Intercept [a]	355	25.7	304	406.1	13.814	< .001	
Transplant complications							
GVHD	-355	52.4	-459	-250.8	-6.778	< .001	-1.612
infection	-260	50.9	-361	-158.4	-5.100	< .001	-1.180
relapse	-154	60.6	-275	-33.8	-2.547	0.013	-0.701
Non-infectious compl.	-344	60.6	-464	-223.3	-5.677	< .001	-1.562
GVHD + infection	-141	166.5	-472	190.4	-0.846	0.400	-0.640

[a] Represents reference level- живи

Table. 15 Regression model NK cells on day 270

Regression models for CD19+ cells on days +100, +180, and +270 post-transplant also demonstrated a significant negative association between the levels of this lymphocyte population and complications. The mean CD19+ cell counts in patients without complications were 91 cells/μL (95% CI 8.86–73.9) on day +100, 215 cells/μL (95% CI 161–270.1) on day +180, and 321 cells/μL (95% CI 245–397) on day +270. These data suggest that transplant-related complications also affect the B-cell compartment during the post-transplant period.

			95% Confidence Interval				
Predictor	**Estimate**	**SE**	**Lower**	**Upper**	**t**	**p**	**Stand. Estimate**
Intercept [a]	91.5	8.86	73.9	109.1	10.330	< .001	
Transplant complications							
GVHD	-56.7	18.22	-92.9	-20.4	-3.110	0.003	-0.906
infection	-53.8	17.72	-89.0	-18.6	-3.036	0.003	-0.861
relapse	-28.6	20.20	-68.8	11.6	-1.416	0.161	-0.458
Non-infectious compl.	-69.1	21.09	-111.0	-27.1	-3.275	0.002	-1.105
GVHD + infection	20.5	58.08	-95.0	136.0	0.353	0.725	0.328

Table. 16 Regression model CD19+ cells on day 100

			95% Confidence Interval				
Predictor	**Estimate**	**SE**	**Lower**	**Upper**	**t**	**p**	**Stand. Estimate**
Intercept [a]	215	27.5	161	270.129	7.835	< .001	
Transplant complications							
GVHD	-185	56.6	-298	-72.695	-3.275	0.002	-0.954
infection	-196	56.6	-308	-83.464	-3.465	< .001	-1.009
relapse	-126	62.7	-250	-0.902	-2.004	0.048	-0.647
Non-infectious compl.	-170	65.5	-300	-39.448	-2.592	0.011	-0.874
GVHD + infection	-128	180.3	-487	230.244	-0.712	0.478	-0.662

[a] Represents reference level-живи

Table. 17 Regrression model CD19+ cells on day 180

Predictor	Estimate	SE	95% Confidence Interval		t	p	Stand. Estimate
			Lower	Upper			
Intercept [a]	321	38.3	245	397	8.38	< .001	
Transplant complications							
GVHD	-321	78.1	-476	-166	-4.11	< .001	-1.137
infection	-293	75.9	-444	-141	-3.85	< .001	-1.036
relapse	-280	86.5	-452	-108	-3.24	0.002	-0.992
Non-infectious compl.	-310	90.3	-489	-130	-3.43	< .001	-1.097
GVHD + infection	-306	248.3	-800	188	-1.23	0.221	-1.084

[a] Represents reference level- живи

Table. 18 Regression model CD19+ cells on day 270

4. Relationship between immune recovery and overall survival

Using ROC analysis, cut-off values for lymphocyte subpopulations were established on days +100 and +180. Each value demonstrated 90% sensitivity and 70% specificity.

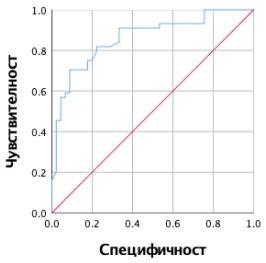


fig. 33 CD3+CD4+ on day 100 AUC 0,86; p= 0,000, 95% CI 0,78 – 0,93

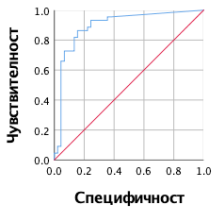


fig.34 CD3+CD4+ on day 180 AUC 0,89; p=0,000, 95% CI 0,82 – 0,96

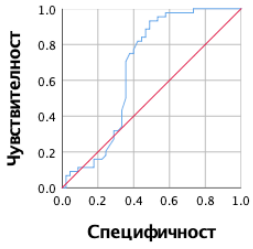


fig.35 CD3+CD8+CD38+ on day 100 AUC 0,67; p= 0,005, 95%CI 0,55 – 0,79

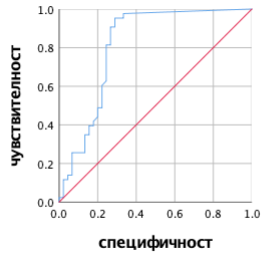


fig.36 CD3+CD8+CD38+ on day 180AUC0,81; p=0,000, 955 CI 0,71 – 0,91

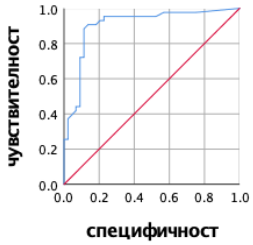


fig.37 NK cells on day 100 AUC 0,90; p=0,000, 95% CI 0,83 – 0,97

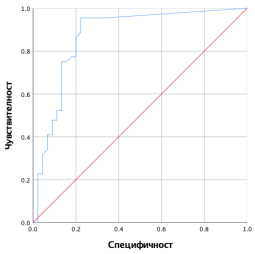


fig.38 NK cells on day 180 AUC 0,87; p=0,000, 95% CI 0,79 – 0,95

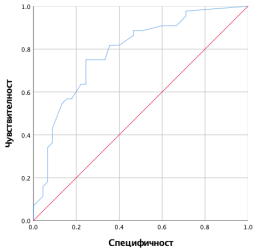


fig. 39 CD19+ cells on day 100 AUC 0,78; p=0,000, 95% CI 0,69 – 0,88

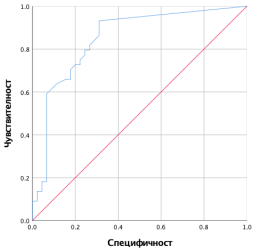


fig.40 CD19+ on day 180 AUC 0,84; p=0,000, 95% CI 0,76 – 0,93

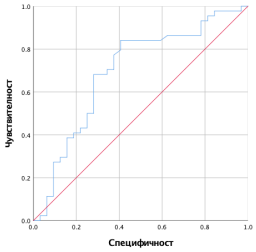


fig. 41 ALC on day 100 AUC 0,69; p=0,004, 95% CI 0,57 – 0,82

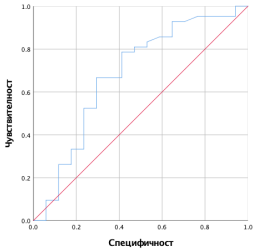


fig. 42 ALC on day 180 AUC 0,68; p=0,031, 95% CI 0,51 – 0,84

Lymphocyte population	Cut – off day 100	Cut – off day 180
CD3+CD4+	375 cells/ µL	128 cells/µL
CD3+CD8+ CD38+	532 cells/µL	284 cells/µL
NK	128 cells/µL	112 cells/µL
CD19+	66 cells/µL	49 cells/µL
ALC	$1.04x10^9$/L	$1.07x10^9$/L

Table. 19 Cut-off Values of Lymphocyte Subpopulations on Days +100 and +180 Following Allo-HSCT

Patients with CD3+CD4+ counts below 375 cells/µL on day +100 had a lower overall survival of 12.9%, compared to patients with counts above 375 cells/µL, who had a survival rate of 69% (p = 0.000).

Patients with CD3+CD4+ counts below 284 cells/µL on day +180 had a lower overall survival of 9.4%, compared to patients with counts above 284 cells/µL, who had a survival rate of 71.9% (p = 0.000).

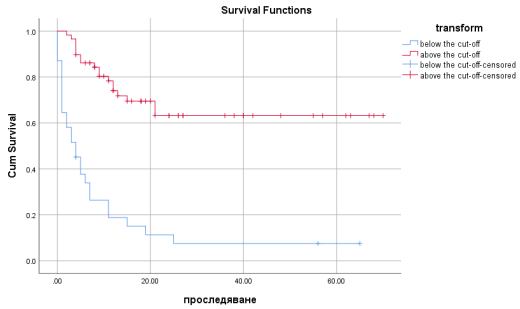


fig.43 OS - CD3+CD4+ day100, cut-off 375 cells/µL

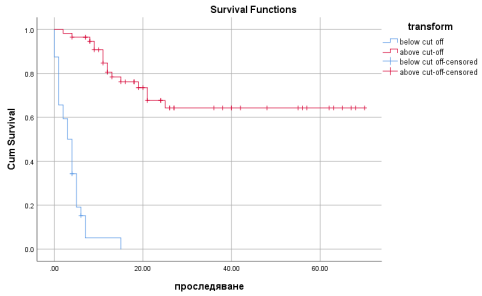


fig. 44 OS - CD3+CD4+ day 180, cut-off 284 cells/µL

Recovery of CD3+CD8+HLA-DR+CD38+ cell populations on day +100 also influences survival. Patients with counts below 532 cells/µL had significantly lower overall survival of 28.2%, compared to patients with counts above 532 cells/µL, who had a survival rate of 66% (p = 0.005).

A similar result was observed on day +180 post-transplant. Patients with counts below 284 cells/µL had lower overall survival of 11.1%, compared to patients with counts above 284 cells/µL, who had a survival rate of 75% (p = 0.000).

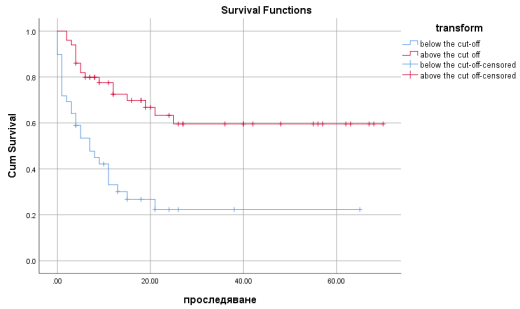
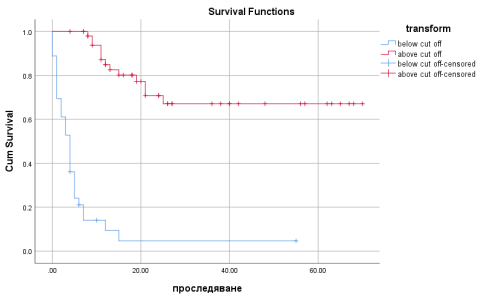


fig. 45 OS - CD3+CD8+CD38+ day 100, cut – off 532 cells/µL



фиг. 46 OS - CD3+CD8+CD38+ day 180, cut – off 284 cells/µL

Regarding NK cell counts, statistically significant differences in patient survival were observed on days +100 and +180 post-transplant. Patients with NK cell counts below 128 cells/µL on day +100 had a lower overall survival of 5.6%, compared to patients with counts above 128 cells/µL, who had a survival rate of 80.4% (p = 0.000). A similar result was observed on day

+180 post-transplant, where patients with NK cell counts below 112 cells/μL had significantly lower survival of 5.9%, compared to patients with counts above 112 cells/μL, who had a survival rate of 76.4% (p = 0.000).

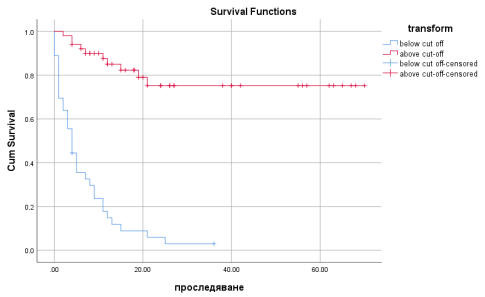


fig. 47 OS - NK cells on day 100, cut – off 128 cells/μL

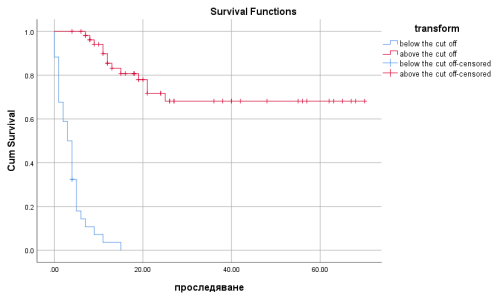


fig. 48 OS - NK cells on day 180, cut – off 112 cells/μL

Analysis showed that patients with CD19+ cell counts below 66 cells/μL on day +100 post-transplant had lower overall survival of 25%, compared to patients with counts above 66 cells/μL, who had a survival rate of 73.3% (p = 0.000). A similarly statistically significant result was found for CD19+ cell counts on day +180, where patients with counts below 49 cells/μL had lower overall survival of 17.9%, compared to patients with counts above 49 cells/μL, who had a survival rate of 74% (p = 0.000).

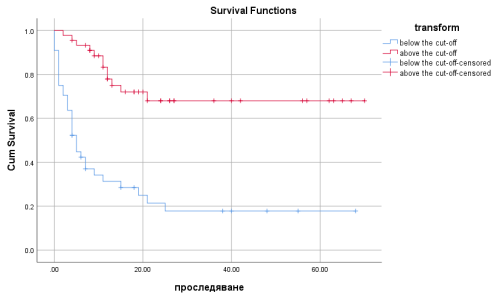


fig. 49 OS - CD19+ cells on day 100, cut – off 66 cells/μL

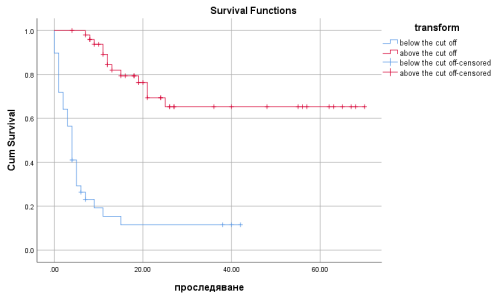


fig. 50 OS - CD19+ cells on day 180, cut – off 49 cells/μL

Analysis of the absolute lymphocyte count (ALC) on days +100 and +180 post-transplant showed that patients with ALC values above 1.05 × 10^9/L on day +100 had higher overall survival compared to patients with lower values, specifically 76.9% versus 37.8% (p = 0.04).

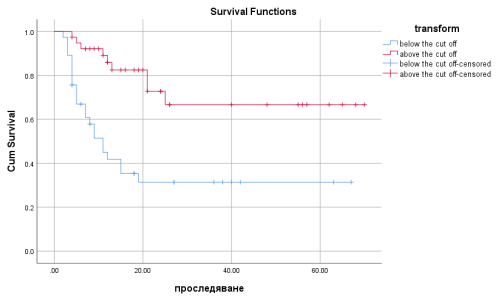


fig. 51 OS - ALC on day 100, cut – off 1.05x10^9/Л

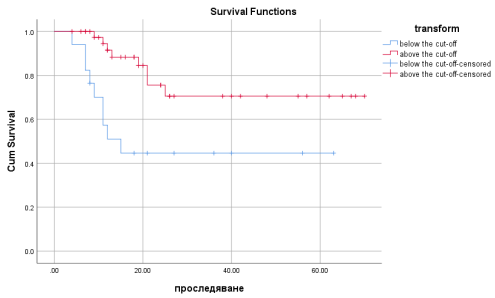


fig. 52 OS - ALC on day180, cut – off 1.08x10^9/Л

Higher overall survival was also observed on day +180 for patients with ALC above 1.08 × 10^9/L compared to those with lower values, namely 81% versus 47.1% (p = 0.031).

VI. Discussion of the results

Immune reconstitution (IR) after allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a dynamic and multifactorial process that depends on numerous factors related to the transplantation itself and on the complications that this therapeutic option may cause in patients. Effective restoration of both innate and adaptive immunity is essential for a favorable transplantation outcome. In the present study, we investigated the dynamics of lymphocyte repopulation in recipients after allo-HSCT and monitored the influence of certain transplantation-related factors and complications on its recovery. Our results complement existing data in the literature and highlight the importance of each individual factor related to IR, demonstrating specific correlations between lymphocyte subsets at different time points. In their study, Zhao et al. found that the dynamics of immune reconstitution, particularly the rapid recovery of CD8+ lymphocytes, are of critical importance for achieving the graft-versus-leukemia (GVL) effect in patients with acute myeloid leukemia (1). Similar to our findings, other authors have also reported faster CD8+ recovery in a population of AML patients, with a proven correlation with improved survival and lower rates of infectious complications (2,3). Donor selection for allo-HSCT plays a crucial role in immune reconstitution, as each donor type (related, unrelated, haploidentical) possesses unique characteristics that affect immune system functionality in the post-transplant period. Due to the inheritance patterns of HLA haplotypes among close relatives, there is a 50% probability of haplotype match. As a result, in patients requiring transplantation, there is more than a 90% chance of identifying a suitable donor (4).

Nevertheless, significant HLA allele mismatches between donor and recipient lead to bidirectional alloreactivity—graft-versus-host (GVH) and host-versus-graft (HVG) (5)—mediated primarily by T lymphocytes. For this reason, various transplantation platforms have been developed to modulate T-cell function with the aim of reducing immunological side effects:

1. High-dose post-transplant cyclophosphamide,
2. *Ex vivo* T-cell depletion combined with megadoses of hematopoietic stem cells (HSC),
3. The GIAC protocol, which includes intensified immunosuppression with a calcineurin inhibitor, mycophenolate mofetil, a short course of methotrexate, the use of anti-

thymocyte globulin (ATG) in the conditioning regimen, and the combined use of peripheral blood and bone marrow HSC from a donor who has undergone prior G-CSF stimulation (6–8).

In our study, we found a statistically significant difference in the recovery of certain lymphocyte subpopulations depending on the donor type. Patients transplanted from an HLA-identical related donor showed better recovery of absolute lymphocyte count (ALC) on day 30, CD3+CD4+ cells on days 100 and 180, and CD19+ cells on day 180 compared to those transplanted from unrelated or haploidentical donors. Similar findings have been reported in other studies (9,10), which emphasize the relationship between donor type, the development of GVHD, and the impact of these factors on the incidence of infectious complications and immune reconstitution.

Donor sex also influences immune system recovery after allo-HSCT. According to most literature sources, the use of female donors for male recipients is associated with an increased incidence of chronic GVHD (cGVHD) and reduced overall survival (11–13). In our study, we found that recipients transplanted from male donors showed better lymphocyte recovery compared to those transplanted from female donors. Recipients from male donors demonstrated better recovery of ALC on day 30 (Spearman's rho −0.315, $p = 0.003$), better recovery of CD4+ lymphocytes on day 100 (Spearman's rho −0.221, $p = 0.037$), and better recovery of CD8+ lymphocytes on day 180 (Spearman's rho −0.215, $p = 0.044$). Servais et al. reported that male donors provide more stable immune reconstitution compared to female donors, which could be attributed to hormonal differences and genetic factors associated with the X chromosome (14). Donor sex has also been shown to influence thymopoiesis after transplantation, which is necessary for generating new T-cell clones. It is well known that the thymus exhibits sex-dependent differences in its function, which can affect the overall process of immune reconstitution (15).

The intensity of conditioning regimens is also an indisputable factor influencing immune reconstitution. These regimens combine different classes of chemotherapeutic agents, with or without total body irradiation (TBI), with the primary aim of providing sufficient immunosuppression to prevent graft rejection while simultaneously eradicating residual disease. In our study, myeloablative, fludarabine-based regimens predominated. Fludarabine is a purine analogue with potent immunosuppressive properties that undoubtedly impact IR in the post-transplant period. Patients conditioned with FluBu had significantly lower mean

CD3+CD4+ lymphocyte counts on days 100 and 180 post-transplant compared to those conditioned with BuCy ($p = 0.004$).

In their study, Mager et al. examined the pharmacokinetic profile of the purine analogue and demonstrated that exposure to fludarabine induces apoptosis in both CD4+ and CD8+ lymphocytes. This effect leads to a reduction in the T-cell compartment and consequently delays IR. This study indicates that, regardless of the immunosuppressive properties of fludarabine, its negative impact on T-cell viability should be carefully considered in the context of IR (16).

Overall, the literature data regarding the use of fludarabine-based regimens are contradictory. The study by Ju et al. supports the finding that the use of fludarabine, particularly in combination with busulfan, results in reduced post-transplant toxicity compared to BuCy. Moreover, this combination has shown significant outcomes with respect to engraftment, GVHD incidence, and immune reconstitution, suggesting that the immunosuppressive properties of the purine analogue can be effectively harnessed to minimize extramedullary toxicity, achieve immunoablation, and simultaneously support the engraftment process (17). On the other hand, in their phase 3 trial, Lee et al. demonstrated a higher relapse rate in patients conditioned with FluBu, but better 2-year overall survival, relapse-free survival, and event-free survival in patients conditioned with BuCy (18).

In clinical practice, peripheral blood is the preferred source of hematopoietic stem cells (HSCs) for transplantation, particularly in adult patients. Cell collection is safe for the donor, and the graft itself enables faster engraftment, reduces relapse rates, and exerts a stronger graft-versus-leukemia (GVL) effect due to the tenfold higher number of T cells compared to bone marrow grafts. A major drawback of using peripheral HSCs is the high incidence of acute and chronic forms of GVHD. As a result, the so-called *in vivo* T-cell depletion has been adopted, aiming to reduce the incidence of this complication. In clinical practice, the most commonly used methods for achieving *in vivo* depletion are anti-thymocyte globulin (ATG) and post-transplant cyclophosphamide (PTCy).

Despite the well-known impact of ATG on immune cells, data on its effect on immune reconstitution following allo-HSCT are scarce (19,20). Our study found that the use of ATG in the conditioning regimen had a negative effect on the recovery of CD3+CD4+ populations on day 100 (Spearman's rho = -0.301, $p = 0.004$), with this trend persisting through day 180 (Spearman's rho = -0.266, $p = 0.012$). The same effect was observed for NK cells on day 180

(Spearman's rho = -0.212, $p = 0.046$) and day 270 (Spearman's rho = -0.220, $p = 0.041$). Other authors have also found that patients receiving ATG during conditioning had lower CD4+ and CD8+ subpopulation counts in the early months post-transplant, with this trend persisting for up to one year (21).

Regarding the use of post-transplant cyclophosphamide (PTCy), our study did not find significant correlations with the recovery of lymphocyte populations. This is most likely due to the small number of patients included in the analysis. Evidence in the literature regarding the influence of cyclophosphamide on IR is limited (22,23). In their study, Espinoza-Gutarra et al. examined the effect of PTCy in 89 HSC recipients from haploidentical or matched unrelated donors (MUDs), comparing their immune reconstitution to that of 48 patients transplanted from fully matched related donors without PTCy (24). The results showed that PTCy use led to delayed recovery of T, B, and NK cells in the post-transplant period, with this trend observed for up to one year post-transplant. These observations suggest that, in addition to the applied immunosuppression, donor type should also be considered.

Other authors have compared the two immunosuppressive platforms — ATG versus PTCy — reporting faster recovery of CD8+ cells and NK cells in patients receiving ATG, and faster recovery of CD4+ and CD19+ populations in those receiving PTCy. These differences resulted in a higher rate of infectious complications in the PTCy arm, without affecting overall survival between the two groups (25).

Despite prophylactic measures, the incidence of acute GVHD remains high, ranging between 35% and 80% (26), while that of chronic GVHD ranges between 30% and 60% (27). On one hand, aggressive GVHD prophylaxis and treatment compromise the recovery of adaptive immunity (28), while on the other, GVHD itself directly suppresses *de novo* T-cell generation (29). The etiology of immune reconstitution (IR) impairment in the context of GVHD is multifactorial and is likely attributable both to a direct effect on thymopoiesis and to alterations in the peripheral T-cell compartment.

In our study, patients with GVHD demonstrated lower levels of CD3+CD4+ cells on days 100 and 180, and of CD3+CD8+ cells on days 180 and 270. Moreover, in the regression model, the GVHD predictor had a very high coefficient, indicating that this complication was associated with the lowest values of these populations compared to other post-transplant complications.

The literature indicates that GVHD leads to dysregulation of T regulatory cell (Treg) differentiation, while the altered cytokine microenvironment contributes to poor recovery of

the lymphocyte compartment (30). Similarly, Lin M. *et al.*reported significantly lower CD4+ and CD8+ cell counts in recipients with GVHD, noting that this phenomenon could be explained by spontaneous apoptosis of allo-activated T cells, induced by cytokine dysregulation (31).

Infectious complications also account for a considerable proportion of morbidity and mortality following allo-HSCT. The results of our study support existing literature indicating that infectious pathogens suppress the repopulation of the lymphocyte compartment (T, B, and NK cells) and contribute to the formation of a so-called "vicious cycle," in which, on the one hand, the immune microenvironment is dysregulated due to the transplantation itself, and on the other, complications such as GVHD further impair the reorganization of post-transplant immunity (32).

Moreover, in their study, Wils *et al.* demonstrated that infectious complications—particularly in the early post-transplant period—also negatively affect thymic regeneration and, consequently, thymus-dependent immune recovery, thereby leading to a significant increase in the incidence of opportunistic infections among transplanted patients (33).

In their study, Ciurea and colleagues demonstrated that lymphocyte recovery is an important determinant of transplantation outcomes and is influenced by donor type. They reported that an absolute lymphocyte count (ALC) greater than 1,000 cells/μL is associated with improved survival in recipients (34). A similar correlation was also observed in our study. Using ROC curve analysis, we identified cut-off values for ALC on days +100 and +180 post-transplant, finding a statistically significant difference in overall survival for patients with values $> 1.05 \times 10^9$/L on day +100 (76.9% vs. 37.8%, $p = 0.04$) and values $> 1.08 \times 10^9$/L on day +180 (81% vs. 47.1%, $p = 0.031$). The literature indicates that early ALC recovery is a powerful predictor of overall survival, non-relapse mortality (NRM), acute GVHD, and relapse incidence (35).

In a study on early ALC recovery, Kostadinova *et al.* reported that, with a median follow-up of 54.7 months, the median overall survival, progression-free survival, cumulative incidence of relapse, and NRM were not reached in patients with early lymphocyte recovery, and that transplantation outcomes at one and three years were significantly better in this group. They identified the disease risk index (DRI), response to allogeneic transplantation, and the use of a haploidentical donor as key risk factors for delayed early ALC recovery (36). Furthermore, Niederwieser *et al.* demonstrated that, in the early post-transplant period, the majority of

peripheral lymphocytes are NK cells, which can mediate cytotoxic effects without prior sensitization and may exert an early graft-versus-leukemia (GVL) effect against residual malignant cells (37).

Studies on the kinetics of immune reconstitution after allo-HSCT have uncovered fundamental aspects of the principles underlying this therapeutic modality. They have contributed to a deeper understanding of the artificial immune ontology post-transplant, the pathophysiological mechanisms of GVHD, viral reactivation, disease relapse, and the GVL effect. In recent years, research in this field has evolved from purely descriptive studies to a highly dynamic and innovative discipline that is shaping the future of allo-HSCT.

New insights have driven the continuous refinement of transplantation platforms, tailored to donor type, recipient comorbidity profile, and underlying disease. Ongoing clinical trials will determine whether adoptive transfer of donor memory lymphocytes, central memory T-cell infusions, or selective graft allodepletion approaches will improve immune recovery and overall transplantation outcomes. Future research will focus on developing more precise models for predicting post-transplant complications, accounting for complex cellular interactions and incorporating additional factors such as graft source, donor type, graft manipulation, and donor–recipient HLA compatibility. Optimizing immune recovery through targeted and well-coordinated interventions will help reduce allo-HSCT-related morbidity and mortality, improving GVHD- and relapse-free survival.

VII. Conclusions from the Results

1. Patients with acute myeloid leukemia demonstrated better recovery of CD3+CD8+CD38+ cells on days +100 and +180 post-transplant, as well as CD19+ cells on day +100.

2. Patients transplanted from fully matched related donors exhibited the best recovery of ALC on day +30, CD3+CD4+ cells on days +100 and +180, and CD19+ cells on day +180. The poorest recovery was observed in patients transplanted from haploidentical donors.

3. Recipients of grafts from male donors had better recovery of ALC on day +30, CD3+CD4+ on day +100, and CD3+CD8+CD38+ on day +180 compared to recipients of grafts from female donors.

5. Patients conditioned with FluBu had significantly lower counts of CD3+CD4+ cells on day +100.
6. The use of ATG in conditioning negatively affected the recovery of CD3+CD4+ cells on day +100 and NK cells on days +180 and +270.
7. Better overall survival was observed in all patients with lymphocyte population values above the determined cut-off.

VIII. Contributions of the Dissertation

Original Contributions

- For the first time in Bulgaria, an evaluation of the role of transplant-related factors in the recovery of lymphocyte subpopulations following allogeneic hematopoietic stem cell transplantation has been performed.
- For the first time in Bulgaria, the relationship between complications associated with allogeneic transplantation and the recovery of immune subpopulations in the post-transplant period has been assessed.
- For the first time in Bulgaria, the correlation between lymphocyte recovery and patient survival after allogeneic hematopoietic stem cell transplantation has been analyzed.

Scientific and Practical Contributions

- Monitoring lymphocyte subpopulations constitutes a clinical approach to assess potential transplantation-related complications.
- Determination of lymphocyte subpopulations in the post-transplant period serves as a method for evaluating immune system functionality.
- Identification of lymphocyte subpopulations after transplantation provides opportunities for therapeutic interventions aimed at improving patient survival.

Confirmatory Contributions

- Diagnosis, donor type, and conditioning regimen influence immune system recovery following transplantation.
- Complications related to allogeneic transplantation (GVHD, infections, non-infectious complications) negatively affect the recovery of lymphocyte subpopulations in the post-transplant period.

- Unsatisfactory recovery of lymphocyte subpopulations adversely impacts long-term patient survival.

IX. Conclusion

This retrospective analysis highlights the pivotal role of lymphocyte subpopulations in the process of immune reconstitution following allogeneic hematopoietic stem cell transplantation and their prognostic value for clinical outcomes in patients.

It was found that patients with acute myeloid leukemia exhibit faster recovery of CD8+ T cells and B cells in the early post-transplant period, suggesting a potential link between the underlying disease and the pace of immune reconstitution. Donor type has a significant impact on the course of immune recovery — the best results are observed with fully matched related donors, whereas haploidentical transplantation is associated with the most pronounced delay in recovery of CD4+ T cells and B cells.

Additional factors such as donor gender, conditioning regimen (particularly the use of FluBu and ATG), as well as the presence of GVHD or infections, demonstrated a clearly negative effect on the rate and completeness of immune recovery regarding CD4+ T cells, activated CD8+ T cells, NK cells, and B cells.

A major contribution of this study is the identification of specific cut-off values for key lymphocyte subpopulations on days +100 and +180 post-transplant, which are associated with significantly improved overall survival. These include absolute lymphocyte count (ALC), CD3+CD4+, CD3+CD8+CD38+, NK, and CD19+ cells, establishing their role as independent prognostic biomarkers.

The results of this dissertation not only confirm the importance of immune monitoring in clinical practice but also highlight opportunities for a personalized approach in post-transplant follow-up and immunosuppressive modulation. Prospective studies on the dynamics and functional status of lymphocyte subpopulations would further contribute to optimizing transplant strategies and improving survival in these high-risk patients.

X. Publications

Impact of Transplant-Related Complications on Lymphocyte Subset Reconstitution Following

Allogeneic Hematopoietic Stem Cell Transplantation. (2025). *Scripta Scientifica Medica*, *57*(2). https://doi.org/10.14748/zy6fb656

Role of transplantation-related factors in the recovery of the lymphocyte compartment following allogeneic hematopoietic stem cell transplantation. (2025). *Scripta Scientifica Medica*, *57*(2). https://doi.org/10.14748/e7gmet41

IMMUNE RECONSTITUTION AND ITS IMPACT ON OUTCOMES AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: A SINGLE- CENTER EXPERIENCE (CIC 1085) **Yavor Petrov, p237, Bone marrow transplantation, EBMT 2025**

XI. Acknowledgements

This doctoral dissertation is the result of many years of focused effort, scientific inquiry, hard work, and unwavering dedication to the chosen topic. Despite my personal contribution, I am deeply aware that its realization would not have been possible without the support, inspiration, professional guidance, and unconditional help of numerous individuals and institutions who, in one way or another, have stood by me throughout this long and challenging journey.

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Special thanks are due to Assoc. Prof. Silvia Nikolova from the Faculty of Public Health at the Medical University – Varna, for her expert help and support in performing and interpreting the statistical analyses, which were key to the scientific validity and reasoning of the results.

I extend my heartfelt appreciation to my colleagues at the Clinic of Hematology and the Transplantation Department, with whom I have shared numerous professional challenges and the joy of our collective achievements. I also express my gratitude to the management of University Hospital “St. Marina” – Varna and the Medical University – Varna for providing the necessary conditions and support for conducting the research.

My sincere thanks go as well to the colleagues from the Clinics of Hematology at University Hospital “St. George” – Plovdiv and University Hospital “St. Ivan Rilski” – Sofia, as well as to the team of the Clinic of Immunology and the Stem Cell Bank at University Hospital “Alexandrovska” – Sofia, for their cooperation, shared experience, and mutual assistance.

I am especially grateful to the patients and their families who, with trust and courage, agreed to be part of this study – their bravery and commitment are a true source of inspiration for every effort I make in the field of medical science.

Last but not least, I extend my heartfelt thanks to my family and friends, who have unfailingly supported me through moments of exhaustion, doubt, and hardship, and who, with love, patience, and understanding, have given me the strength to continue forward.

This work is not only the result of an individual research path but also a reflection of the collective efforts, support, and empathy of all those who believed in me and in the meaning of this endeavor. To each of you, I owe my most sincere “Thank you!”.

XII. Bibliography:

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