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# Correlation Between Stem and Progenitor Cells Number and Immune Response in Patients After Allogeneic Kidney Transplant

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**Background:** Stem and progenitor cells are of great interest in all medical procedures involving tissue regeneration. There is a consensus that the use of stem cells after solid organ transplantation may play a role in tissue repair and in immunosuppression. The aim of this study was to determine possible relations between stem cell count and the immune response in a group of patients after kidney transplantation.


**Material/Methods:** The study was conducted on a group of 100 patients who underwent kidney transplantation. The following phenotypic markers of the studied cell subpopulations were adopted: T<sub>reg</sub> cells (CD3<sup>+</sup>CD4<sup>+</sup>CD25<sup>high</sup>), circulating hematopoietic cells (CD34<sup>+</sup>CD133<sup>+</sup>CD45<sup>+</sup>CD38<sup>-</sup>), and non-hematopoietic cells (Lin<sup>-</sup>CXCR4<sup>+</sup>CD133<sup>-</sup>CD45<sup>-</sup>). Cell subpopulations were assessed using LSRII flow cytometer (BD Biosciences, San Jose, CA, USA).

**Results:** Positive correlation was observed between non-hematopoietic stem cells percentage and recipient's platelets count ( $P=0.04$ ). Moreover, a higher percentage of non-hematopoietic cells was accompanied by lower numbers of B lymphocytes ( $P=0.03$ ) and T<sub>reg</sub> cells ( $P=0.02$ ).

**Conclusions:** Our study revealed significant associations between the intensity of ongoing immune response processes and tissue damage, and the release of stem and progenitor cells into circulation. These findings suggest their role in the stimulation of protective processes in terms of graft regeneration.

**MeSH Keywords:** Kidney Transplantation • Renal Insufficiency, Chronic • Stem Cells • T-Lymphocytes, Regulatory

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## Background

End-stage renal disease (ESRD) is a life-threatening condition which develops as a consequence of advanced chronic kidney disease. There have been 3 approaches to renal replacement therapy (RRT): peritoneal dialysis, hemodialysis, and allogeneic kidney transplantation [1]. Of these methods, only transplantation allows for the full substitution of the functions of a healthy kidney and significantly increases the quality of life in patients previously undergoing dialysis [2]. In Poland in 2016, 1028 patients received a kidney transplant [3]. Despite the superiority of this method, the issue of acute and chronic rejection of transplants as a consequence of the activity of the recipient's immune system is still not fully understood. Episodes of acute transplant rejection may in turn lead to chronic organ rejection, which decreases allograft half-life. To avoid rejection, immunosuppressive drugs are administered in high doses, which may lead to various complications [4].

The grafting itself and the subsequent immunosuppressive therapy have a significant influence on immune system regulation. Disruption of immune homeostasis may lead to the development of inflammatory changes and promote transplant rejection [5].

Stem and progenitor cells are of great interest in all medical procedures involving tissue regeneration. There is a consensus that the use of stem cells after solid organ transplantation may play a role in tissue repair and in immunosuppression. Multiple studies have investigated the role of donor bone marrow-derived stem cell injections in suppressing the immune processes leading to organ rejection [6,7]. However, the response of the immune system to circulating stem and progenitor cells and the role of these cells in the recipient's body remains unclear.

The objective of this retrospective analysis was to investigate the correlation between concentrations of stem and progenitor cells of both hematopoietic and non-hematopoietic lineages and cells responsible for the regulation of immune response after allogeneic kidney transplantation.

## Material and Methods

The study was conducted on a group of 100 patients who underwent kidney transplantation. The group consisted of 56 female and 46 male patients. All transplanted organs came from deceased donors. Informed consent was obtained from all patients before enrollment. Patients' age ranged from 20 to 78 years, with a mean age of 49.9 years. The kidney was the only transplanted organ in all participants. The average period from transplantation was 1817 days. All patients received immunosuppressive treatment.

## Biochemical tests and morphology

All blood samples obtained from transplant recipients were tested for urea, creatinine, uric acid, glucose and potassium, magnesium and calcium ions. Moreover, a standard morphology was performed to assess the count of red blood cells (RBCs), white blood cells (WBCs), and platelets.

## Flow cytometry

### *B lymphocytes count assessment*

To assess the overall number of B lymphocytes we used the BD Tritest CD3CD19CD45 (BD Biosciences, San Jose, CA, USA). The acquisition was performed using an LSRII flow cytometer (BD Biosciences, San Jose, CA, USA) with FACS Diva 6.2. For each test  $1 \times 10^4$  cells were collected. Total cell count was presented as the number of cells per  $1 \mu\text{L}$  of whole blood.

### *T<sub>reg</sub> lymphocytes percentage assessment*

To investigate the population of circulating T<sub>reg</sub> lymphocytes in whole blood we isolated mononuclear cells using density gradient centrifuging with Ficoll-Histopaque (Sigma, St. Louis, MO, USA). Obtained cells were then incubated with monoclonal antibodies against antigens CD4 (conjugated with FITC), CD 3 (conjugated with PE), and CD 25 (conjugated with APC). The percentage of T<sub>reg</sub> cells representing phenotype CD3<sup>+</sup>CD4<sup>+</sup>CD25<sup>high</sup> was assessed using the LSRII flow cytometer (BD Biosciences, San Jose, CA, USA). In each sample  $5 \times 10^4$  cells were collected.

### *Analysis of circulating stem/progenitor cells*

To assess the amount of circulating hematopoietic (CD34<sup>+</sup>CD133<sup>+</sup>CD45<sup>+</sup>CD38<sup>-</sup>) and non-hematopoietic (Lin<sup>-</sup>CXCR4<sup>+</sup>CD133<sup>-</sup>CD45<sup>-</sup>) stem and progenitor cells the blood sample was lysed to eliminate RBCs using BD Pharm Lyse Ammonium Chloride Lysing Reagent (BD Biosciences, San Jose, CA, USA). Obtained nuclear cells were then incubated with monoclonal antibodies against CD34 (conjugated with FITC), CD45 (conjugated with PE), CD133 (conjugated with APC), and CD38 (conjugated with PE-Cy7) for hematopoietic cells assessment and with antibodies against antigens characteristic for lineage cells (CD2, CD3, CD14, CD16, CD19, CD24, CD56, CD66b, CD235a – conjugated with FITC), CXCR4 (conjugated with APC), CD133 (conjugated with PE), and CD45 (conjugated with PE-Cy7) to assess the percentage of non-hematopoietic cells. Isotype controls were matched to each analysis. To analyze the samples, the LSRII flow cytometer (BD Biosciences, San Jose, CA, USA) was used.

**Table 1.** Biochemical and clinical characteristic of the study group.

Measured parameter	Number of Measurements (n)	Mean value	SD	Median	Min. value	Max. value
Recipient's age (years)	100	49.91	12.67	52	20	78
BMI (kg/m <sup>2</sup> )	98	26.41	4.14	26.1	18	44
Urea (mg/dL)	98	38.91	24.24	34.05	10.5	134.3
Creatinine (mg/dL)	100	1.42	0.66	1.23	0.7	4.02
Glucose (mg/dL)	98	100.72	23.76	95	52	218
Uric acid (mg/dL)	99	6.99	1.68	6.9	2.9	13.5
Na <sup>+</sup> (mmol/L)	100	139.55	2.38	139.9	132	145.6
K <sup>+</sup> (mmol/L)	100	4.32	0.54	4.23	2.61	5.8
Mg <sup>2+</sup> (mmol/L)	98	0.76	0.1	0.75	0.5	1.03
RBC (T/L)	99	4.53	0.61	4.51	3.42	6.14
WBC (G/L)	100	7.66	2.54	7.27	3.27	14.6
PLT (G/L)	99	237.77	75.05	229	105	527

### Statistical analysis

Since most of the results deviated from normal distribution in the statistical analysis, we used the nonparametric U Mann-Whitney test to compare the differences between the tested parameters. As a threshold of statistical significance, we accepted a *P* value <0.05. A *P* value between 0.05 and 0.1 was treated as being on the threshold of statistical significance. All analyses were carried out using Statistica 10.

## Results

### Biochemical blood test and morphology

A summary of morphology and biochemical tests is presented in Table 1.

### Correlation between hematopoietic and non-hematopoietic cells percentage and selected parameters

We observed a significant positive correlation between the percentage of circulating hematopoietic cells and recipient's age (*P*=0.02), and a correlation with body mass index (BMI) on the border of significance (*P*=0.1).

When it comes to non-hematopoietic cells, a positive correlation was observed between cells percentage and recipient's platelets count (*P*=0.04). Moreover, a higher percentage of non-hematopoietic cells was accompanied by lower numbers

**Table 2.** Correlations between HSC, non-HSC and selected parameters of the study group.

Correlated parameters	HSC CD34 <sup>+</sup> CD133 <sup>+</sup> CD38 <sup>-</sup>	Non-hematopoietic stem/progenitor cells (Lin <sup>-</sup> CXCR4 <sup>+</sup> CD133 <sup>-</sup> CD45 <sup>-</sup> )
	p-Value	
Recipient's age	0.02	0.18
Treg lymphocytes	0.12	0.01
B cells	0.30	0.03
Platelets	0.89	0.04
BMI	0.10	0.06

of B lymphocytes (*P*=0.03) and T<sub>reg</sub> cells (*P*=0.02). Correlation analysis is summarized in Table 2.

## Discussion

B cells are responsible mostly for alloimmune antibody-mediated transplant rejection reaction. They take part in presenting antigens to T cells and the production of specific antibodies [8]. It has been proposed that B cells are partially responsible for the pathology of graft loss. A study conducted on mice showed that the adaptive immune system takes part in the thickening

of the intima, and therefore leads to arteriosclerotic changes in graft vessels [9]. On the other hand, interestingly, it has been reported that an increase in transitional B lymphocytes count in a short time period after grafting (7 days) followed by a significant decrease in their number and consequent repopulation during the year after transplantation is associated with lower incidence of graft rejection [10]. Another study, enrolling patients with operational tolerance (normal graft function and immunocompetent immune system after immunosuppressive drugs withdrawal), found that patients who develop chronic transplant rejection are characterized by lower counts of B cells than those with operational tolerance [11]. In our study we observed decreased B cells count in older patients, which may reflect natural impairment of the immune response with age, which in the case of transplant rejection risk might play a protective role. When it comes to correlation between count of hematopoietic stem cells and B cells we did not observe any significant associations. However, the count of non-hematopoietic cells, which may be released to circulation in response to tissue injury, also in cases of chronic endothelial damage in progression of arteriosclerotic changes, was negatively correlated with B cells count. This may support the previous findings [12–14] and suggest that decreased B lymphocytes count in a long-time period after grafting might precede and herald graft changes resulting from chronic rejection.

T regulatory lymphocytes, characterized by CD3<sup>+</sup>CD4<sup>+</sup>CD25<sup>high</sup> phenotype [15], were the first to be described as immunoregulatory cells with activity other than immune response stimulation [16]. They suppress other subpopulations of T cells and therefore prevent various autoimmune diseases. Studies on mice have shown that T<sub>regs</sub> tend to protect them from autoimmunity in a dose-dependent manner [17], and mice lacking the transcription factor specific for T<sub>reg</sub> cells – FoxP3 – developed a fatal lymphoproliferative disorder [18]. This cell population is capable of suppressing both innate and adaptive immune response. T<sub>regs</sub> therapy has been proposed as a potential way to promote allograft survival. Their ability to maintain function during *ex vivo* expansion has made them a promising tool in approaches to stimulate self-tolerance. T<sub>regs</sub> have been

successfully used in preventing graft-versus-host disease in human trials [19–21]. In the presented study we described a negative correlation between the percentage of circulation stem/progenitor cells and T<sub>reg</sub> cells count. This lower concentration of cells inducing immunotolerance has also been reported in other studies in patients after grafting [22]. The same study also suggested that patients with acute rejection after transplantation had lower counts of T<sub>regs</sub> before the procedure in comparison to recipients that did not undergo acute rejection [22].

Increased number of circulating non-hematopoietic stem and progenitor cells was accompanied by increased number of platelets in collected blood samples, which may indicate the role of these cells in the endogenous regeneration of the transplanted organ.

Platelets play an important role in the processes occurring in the human body after organ transplantation. Number of platelets correlates with concentration of cells responsible for immunological reaction, as it was previously described [23]. Chronic kidney disease causes uremic platelets dysfunction, but effective transplantation, resulting in the immediate function of the transplanted kidney, leads to normalization of platelet function [24]. Therapies focused on increasing the number of platelets and modification of their function in the peri-transplantation period, are seriously considered [25]. Until now, cellular therapies supporting organ transplantation, have been used most often after kidney transplantation [26]. Therefore, the relationship between the number of platelets and non-hematopoietic stem cells, described in this paper, is of particular importance.

## Conclusions

Our study revealed significant associations between the intensity of ongoing immune response processes and tissue damage, and the release of stem and progenitor cells into circulation. These findings suggest their role in the stimulation of protective processes in terms of graft regeneration.

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