Characterisation of lymphocyte subsets in asplenic patients – preliminary report

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Abstract

Introduction: *Acquired asplenia is a common abnormality, following splenectomy due to splenic rupture from trauma, because of tumour or as a treatment for certain diseases, such as idiopathic thrombocytopenic purpura and spherocytosis. Asplenic patients are exposed to the long-term impairment of humoral and cellular immunity. Although the lack of humoral immunity is a well described issue, few studies have addressed the problem of alterations in T cell immunity in splenectomised individuals.*

Aim of study: *Prospectively evaluate the impact of splenectomy on postoperative quantitative changes of circulating lymphocyte subsets as well as assessment of cell activation markers expression in patients after curative resection due to severe spleen injury, hereditary spherocytosis or immune thrombocytopenic purpura.*

Material and methods: *A study group of 26 subjects, with an average age of 31.23 ±28.13 years, was recruited at the Department of Clinical Immunology and Immunotherapy of the Medical University of Lublin. 15 patients (57.70%) were splenectomised because of a blunt abdominal trauma with spleen injury, 7 persons (26.92%) because of hereditary spherocytosis and 4 patients (15.38%) due to thrombocytopenic purpura (ITP). Three-colour immunofluorescence analyses were performed using a FACS Calibur flow cytometer (Becton Dickinson) equipped with 488 nm argon laser. Statistical analysis was performed using Statistica 6.0 (Stat Soft Inc.) software.*

Results and conclusions: *Between splenectomised patients, those who notified more frequent infections after splenectomy and those who did not observe any change in prevalence of upper respiratory tract infections (URTI), statistically significant differences in the percentages of lymphocyte activation markers were observed. Subjects, who did not suffer from common infections had more CD3+/CD69+, CD19+/CD69+, CD3+/CD25+, CD4+/CD69+ and CD8+/CD69+ cells. We did not indicate a statistically significant correlation between lymphadenopathy and frequency of URTI. The reason of splenectomy did not influence lymphocyte subsets ($p > 0.1$). The elapsed time after splenectomy positively correlated with the percentage of CD4+ lymphocyte subset ($r = 0.670$; $p = 0.006$) and negatively with HLA-DR+ T-cells ($r = – 0.736$; $p = 0.002$). In the group of examined patients, who had more frequent URTI, a positive correlation between the elapsed time after surgical treatment and percentage of CD8+/CD25+ cells ($r = 0.613$; $p = 0.045$) and a negative correlation between CD3+/HLA-DR+ lymphocytes ($r = –0.847$; $p = 0.001$) and a mentioned time was observed. Because of interesting preliminary observations, the present research will be expanded on a bigger group and the antigens which may activate lymphocytes in asplenic, otherwise healthy persons are going to be searched for.*

Key words: *asplenic patients, lymphocyte subsets.*

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Introduction

Asplenia refers to the absence of normal spleen function due to various pathologies and may occur in two types: congenital and acquired. Although congenital asplenia is a rare disorder, the acquired one is a common abnormality, following splenectomy due to splenic rupture from trauma, caused by tumour, or as a treatment for certain diseases such as idiopathic thrombocytopenic purpura and spherocytosis. Unfortunatelly, splenectomy is associated with increased postoperative morbidity and mortality and long-term impairment of humoral and cellular immunity [1]. The absence of the phagocytic function of the spleen and the long-term impairment of the humoral response to encapsulated bacteria are the main causes of the overwhelming post-splenectomy infection syndrome (OPSI) [2]. The capsular polysaccharide antigens of these bacteria elicit an immune response that depends primarily on the function of the splenic marginal zone B cells, but is amplified by factors produced by T cells [2, 3]. Although the lack of humoral immunity is a well described issue, few studies have been devoted to the problem of alterations in T cell immunity in splenectomised individuals. According to some authors, splenectomised patients have impaired primary and memory immune responses to antigens that elicit T cell-dependent responses. These observations indicate that T cell-mediated immunity is also defective in these patients [4, 5].

The aim of this study was to prospectively evaluate the impact of splenectomy on postoperative quantitative changes of circulating lymphocyte subsets and cell activation markers in patients after curative resection due to severe spleen injury, hereditary spherocytosis or immune thrombocytopenic purpura. Patients were divided into two groups: those who notified more frequent infections after splenectomy and those who did not observe any change in prevalence of upper respiratory tract infections (URTI).

Materials and methods

Study population

A study group of 26 subjects (19 women and 7 men), with an average age of 31.23 ±28.13 years, was recruited at the Department of Clinical Immunology and Immunotherapy of the Medical University of Lublin. 15 patients (57.70%) were splenectomised because of a blunt abdominal trauma with spleen injury. In 7 persons (26.92%) the reason of splenectomy was hereditary spherocytosis and 4 patients (15.38%) had had immune thrombocytopenic purpura (ITP). The average time from the splenectomy was 7.65 ±7.52 years. 18 persons (69.23%) notified more frequent infections after splenectomy and 8 patients (30.77%) did not observe any change in prevalence of upper respiratory tract infections. Nobody of the splenectomised subjects in the study group complained of ailments characteristic for the current infection. None of them was taking immunosuppresive or immunomodulative treatment within the last 12 months. Local Ethical Committee at Medical University of Lublin approved the research and patients gave their prior written consent.

Cell phenotyping and flow cytometry

Peripheral blood was taken to 10 ml tubes with an EDTA anticoagulant. Three-colour immunofluorescence analyses were performed using a FACS Calibur flow cytometer (Becton Dickinson) equipped with 488 nm argon laser. A minimum of 10 000 events was acquired and analysed using CellQuest Software. Mean fluorescence intensity (MFI) and the percentage of cells expressing surface markers were analysed. The cells were phenotypically characterised by incubation (20 min in the dark at a room temperature) with combination of relevant fluorescein isothiocyanate (FITC) – phycoerythrin (PE) – and CyChrome-labelled monoclonal antibodies (mAbs). Immunofluorescence studies were performed using a combination of the following mAbs: CD3 FITC/CD19 PE, CD8 FITC/CD4 PE, CD25 CyChrome, CD69 CyChrome, CD3 FITC/CD16+CD56 PE, CD3 FITC/HLA-DR PE, purchased from R&D Systems and CD45RA PE, CD45RO PE from Pharmingen. Lymphocyte phenotype count was compared between two groups of patients: those who notified more frequent infections after splenectomy and those who didn't observe any change in prevalence of upper respiratory tract infections.

Statistical analysis

Statistical analysis was performed using Statistica 6.0 (Stat Soft Inc.) software. Mean values in the groups were compared (Student's *t*-test for dependent values). Pearson's linear correlation coefficient (r) was calculated to disclose relationships between variables. The frequencies were compared by χ^2-test. *P*-value less than 0.05 was considered statistically significant. Continuous variables were presented as mean ± standard deviation (SD).

Results

Clinical characteristics of study population is presented in Table 1.

Among patients who observed more frequent occurrence of upper respiratory tract infections after the loss of spleen, 23.08% had enlarged cervical lymph nodes, in 30.77% physical examination revealed enlarged sub-mandibular lymph nodes, 7.65% had enlarged supra-clavicular lymph nodes, axillary lymph nodes were enlarged unilaterally in 11.54% patients and bilaterally in 7.69% cases. No statistically significant correlation between lymphadenopathy and frequency of URTI was indicated. Persons with the highest expression of early activation lymphocyte markers, did not notify more frequent infections (Table 2).

Table 1. Clinical characteristics of study population

Described parameter		Numerical quantity	Mean percentage value (%)
sex	women	19	73.08
	men	7	26.92
the reason of splenectomy	blunt abdominal trauma with spleen injury	15	57.70
	hereditary spherocytosis	7	26.92
	immune thrombocytopenic purpura	4	15.38
persons who notified more frequent infections after splenectomy		18	69.23
persons who did not observe any change in prevalence of upper respiratory tract infections after splenectomy		8	30.77
peripheral lymphadenopathy	enlarged cervical lymph nodes	7	26.92
	enlarged		
	sub-mandibular lymph nodes	10	38.46
	enlarged supraclavicular lymph nodes	2	7.69
	axillary lymph nodes enlarged unilaterally	4	15.38
	axillary lymph nodes enlarged bilaterally	4	15.38

**URTI – upper respiratory tract infections*

Table 2. Mean percentage values of lymphocyte subsets in the study population with more frequent URTI after splenectomy and in the group which did not notice increased incidence of infections

Lymphocyte subsets	Study population with more frequent infections after splenectomy (%)	Study population without increased incidence of infections after splenectomy (%)	*t*-Student test	*p*
T (CD3+)	62.34	58.66	0.73	0.475
B (CD19+)	12.13	15.79	–1.42	0.179
CD3+/ CD69+	1.03	1.89	–2.15	0.050*
CD19+/ CD69+	0.58	1.24	–2.46	0.028*
CD3+/ CD25+	7.74	12.00	–2.43	0.030*
CD19+/ CD25+	1.73	2.76	–1.48	0.161
CD4+	30.33	32.53	–0.55	0.589
CD8+	25.33	18.62	1.66	0.121
CD4+/ CD69+	0.51	1.39	–3.04	0.009*
CD8+/ CD69+	0.29	0.62	–2.56	0.019*
CD4+/ CD25+	6.96	10.53	–1.27	0.22
CD4+/ CD25+ high	0.86	1.03	–0.69	0.500
CD8+/ CD25+	1.65	1.15	0.28	0.780
NK (CD3–/16+56+)	15.04	17.10	–0.43	0.676
NKT (CD3+/16+56+)	3.22	1.78	1.30	0.213
CD3+/ HLA-DR+	7.21	5.64	0.65	0.528
CD5+/ CD19+	2.99	2.93	0.08	0.930
CD45RA+/ CD4+/ CD3+	35.85	38.59	–0.32	0.751
CD45RO+/ CD4+/ CD3+	59.95	64.39	–0.42	0.678

**p < 0.05*

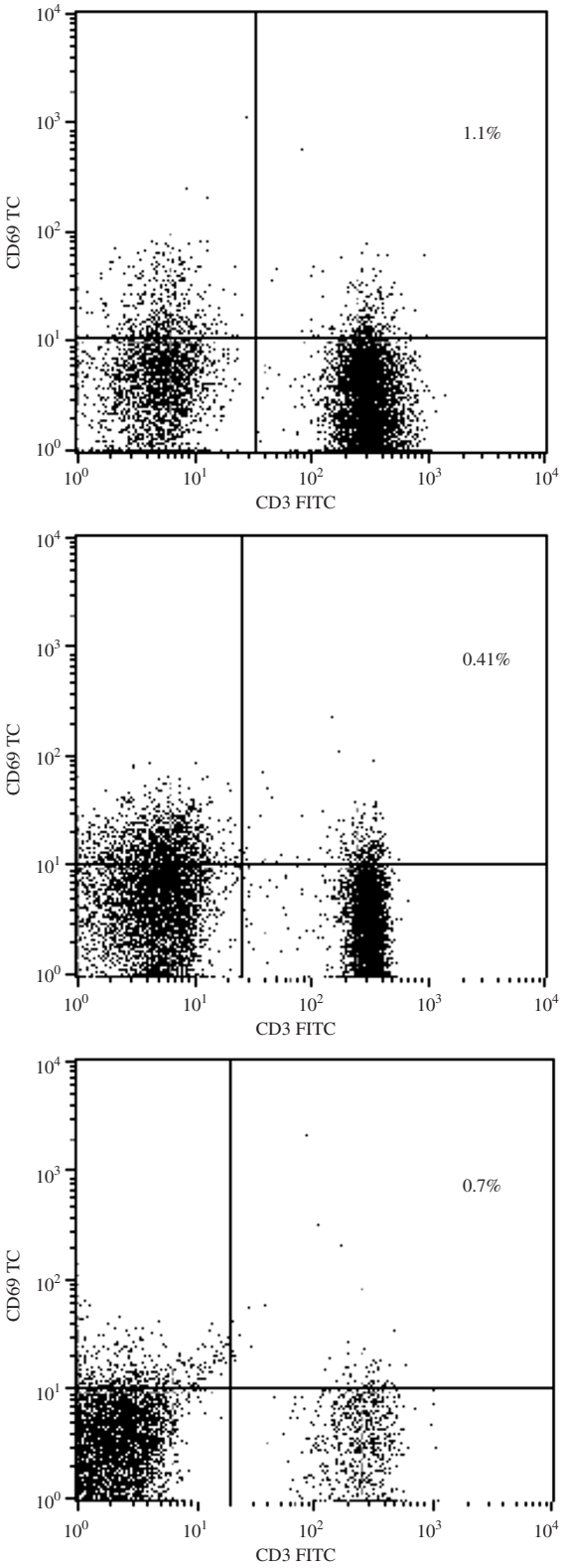

Figure 1A. An example of a three colour flow cytometry analysis of an expression of early activation lymphocyte marker (CD69) on T-cells, CD4+ cells and B-cells in a patient with frequent URTI

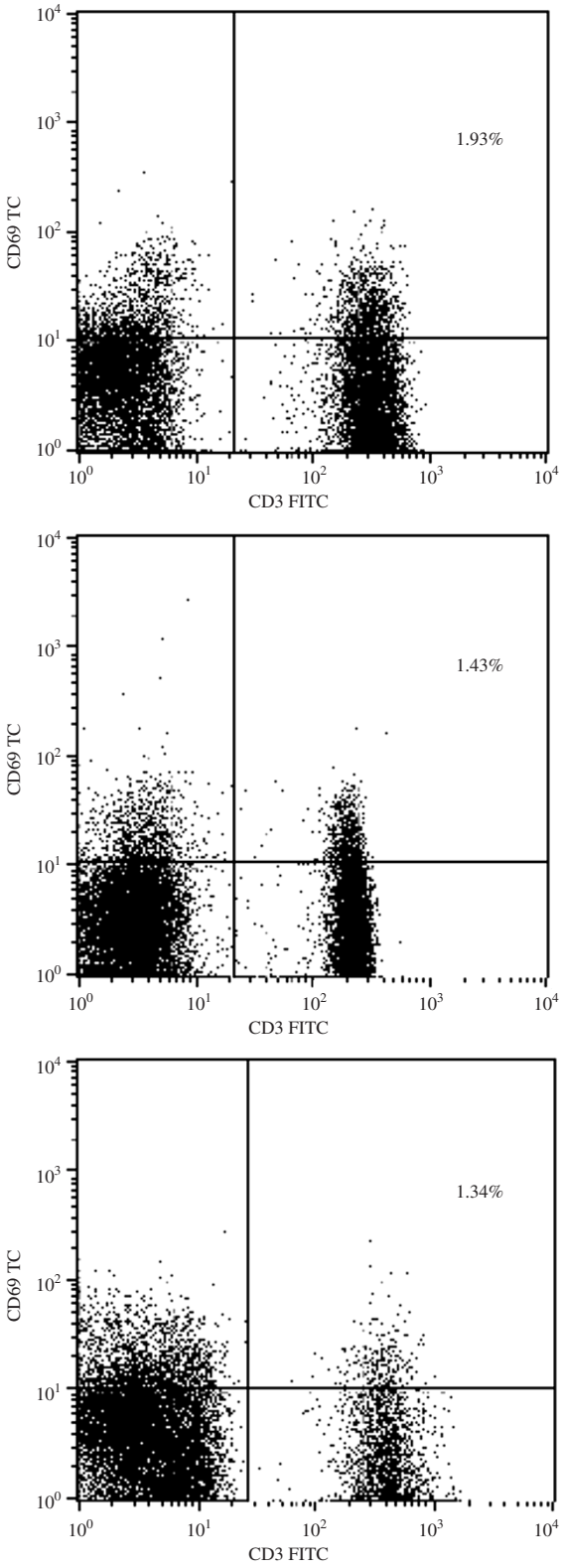

Figure 1B. An example of a three colour flow cytometry analysis of an expression of early activation lymphocyte marker (CD69) on T-cells, CD4+ cells and B-cells in a patient who did not notify frequent infections after the loss of spleen

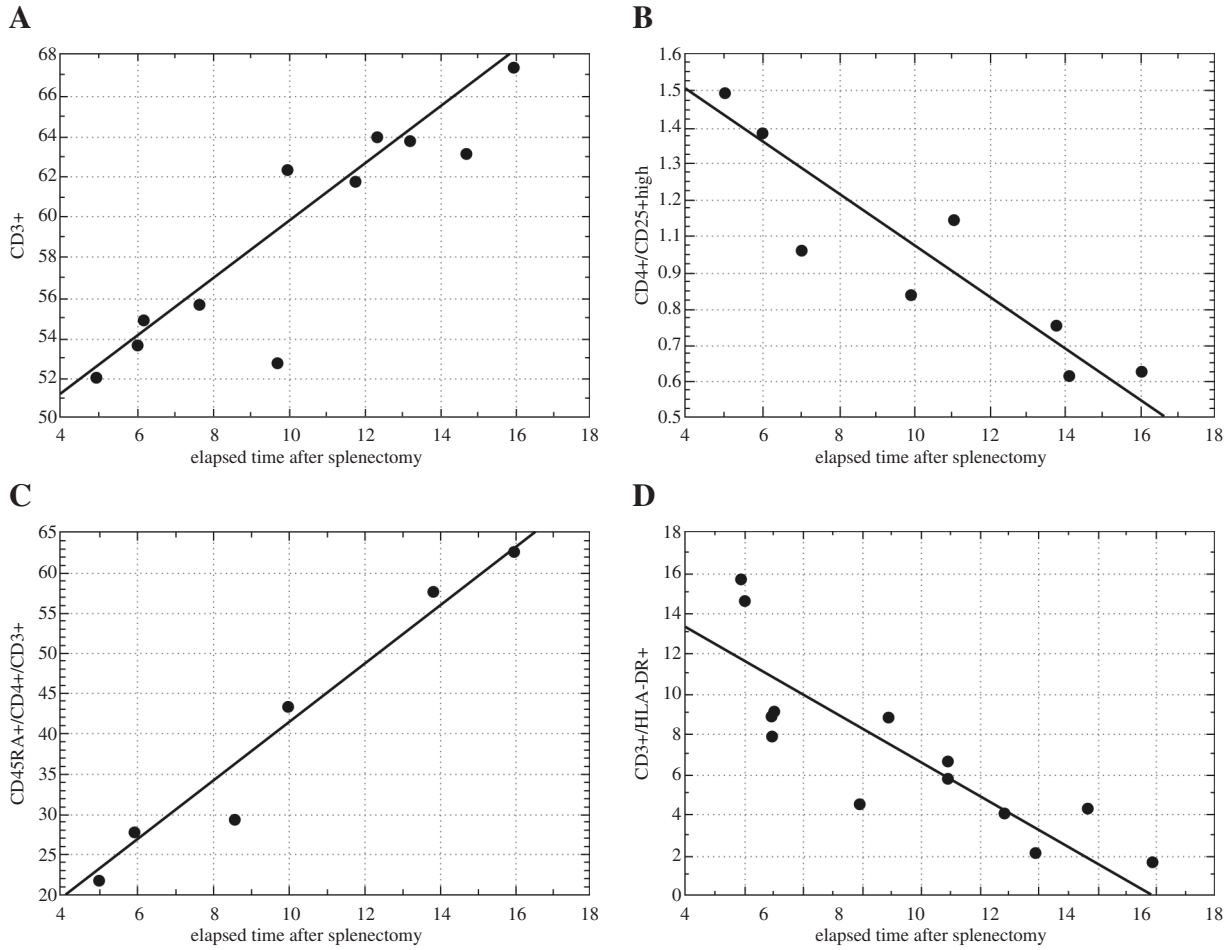

Figure 2. Diagrams presenting: **2A** – correlation between elapsed time after splenectomy and the percentage of T lymphocytes, **2B** – correlation between elapsed time after splenectomy and the percentage of CD4+/CD25+high lymphocytes, **2C** – correlation between elapsed time after splenectomy and the percentage of CD45RA+/CD4+/CD3+ cells, **2D** – correlation between elapsed time after splenectomy and the percentage of CD3+/ HLA-DR+ cells

Table 2 presents mean percentage values of lymphocyte subsets in the study population with more frequent URTI after splenectomy and in the group which did not notice increased incidence of infections.

There were no statistically significant differences in lymphocyte subsets in group of patients splenectomised because of an injury and in persons after surgery treatment of hematologic disorder ($p > 0.1$). Figure 1 presents an example of a three colour flow cytometry analysis of an expression of early activation lymphocyte marker (CD69) on T-cells, CD4+ cells and B-cells in a patient with frequent URTI (1A) and in a patient who did not notify frequent infections after the loss of spleen (1B).

CD4+ : CD8+ ratio was higher in patients without increased incidence of infections than in subjects with more frequent infections after splenectomy (1.15 vs. 1.75).

The discussed study has revealed, that the presence of enlarged axillary lymph nodes correlates with the percentage of CD8+/CD25+ lymphocytes (r = 0.518;

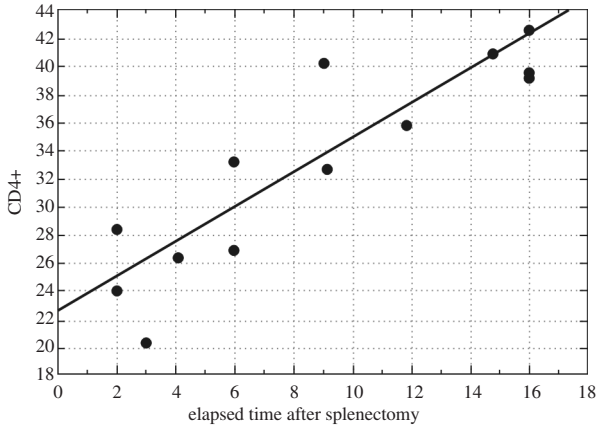

Fig. 3. Scatter diagram presenting a positive correlation between the elapsed time after splenectomy and percentage of peripheral blood CD4+ cells in the group of patients splenectomised because of abdominal trauma. Such correlation did not appear in the study population of asplenic persons due to hematologic diseases

$p = 0.048$) and with CD4+ cells ($r = 0.528$; $p = 0.043$). Moreover, the percentage of NK cells were higher in those subjects, who had enlarged sub-mandibular lymph nodes ($r = 0.636$, $p = 0.011$).

The elapsed time after splenectomy positively correlated with the percentage of CD4+ lymphocyte subset ($r = 0.670$, $p = 0.006$) and negatively with HLA-DR+ T-cells ($r = –0.736$; $p = 0.002$). In the group of examined patients, who had more frequent URTI, there was a positive correlation between the elapsed time after surgery treatment and percentage of CD8+/ CD25+ cells ($r = 0.613$; $p = 0.045$) and a negative correlation between CD3+/ HLA-DR+ lymphocytes ($r = –0.847$; $p = 0.001$) and a mentioned time.

Further observations have shown that in the group of patients without frequent infections, elapsed time after splenectomy had a strongly positive correlation with the percentage of T lymphocytes ($r = 0.976$; $p = 0.023$) and CD45RA+/CD4+/CD3+ cells ($r = 0.996$; $p = 0.003$). There was also a strong negative correlation with CD4+/CD25+high lymphocytes ($r = –0.968$; $p = 0.032$). Figure 2 presents the diagrams of the correlations described above.

Furthermore, there were also a strong positive correlation between the elapsed time after splenectomy and percentage of peripheral blood CD4+ cells in the group of patients splenectomised because the spleen rupture ($r = 0.861$; $p = 0.006$) (Fig. 3).

Discussion

The anatomical structure of the spleen and its central position in the route of the portal vein, highlight its importance both in innate and in adaptive immunity. The role of the spleen in innate immunity is well known, whereas its role in adaptive immunity is still unclear [6]. Several studies have shown that splenectomy affects the distribution of T-lymphocyte subpopulations in the peripheral blood [4, 7-9]. However, these findings are disputed by others [10, 11]. The most frequently reported changes are a decrease in the absolute number or the percentage of CD4+ T cells and a reduction of the CD4+/CD8+ ratio [4, 7-9]. An important clinical issue regarding these patients is whether the immune defects resulting from the splenectomy resolve with time. The present study has shown that the percentage of CD4+ cells increases with the time after splenectomy, especially in cases of post traumatical surgical spleen removal. Similar results were reached by a few other authors [12]. Decreased levels of CD4+CD45RA+ cells were accompanied by an impairment in primary immune responsiveness by measuring antibody responses following primary immunization with a clinically relevant T-dependent antigen, hepatitis A vaccine, in vivo. These findings suggest a possible role of the spleen in the generation, maintenance and/or differentiation of naive, unprimed T cells or their precursors, which might have a possible functional relevance for primary immune responses following splenectomy [4]. The discussed study, however, indicated that in a group with rare URTI, the proportion of the naive T cells increased with the time distance after splenectomy. Unexpectedly, in study population, the majority of patients presented high expression of lymphocyte early activation markers. Surprisingly, the examined patients did not notify more frequent infections. Moreover, subjects in both groups did not take any management at examination time and did not report characteristic for viral infections ailments. There was no correlation between the presence of vaccination against most common pathogens causing severe infections, especially polysaccharide encapsulated bacteria, and the percentage of respective lymphocyte subsets. All the collected data indicate the need to extend the conducted research and examine more patients to discover the reasons of high lymphocyte activation in asplenic population.

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