ДИНАМИКА СУБПОПУЛЯЦИЙ Т-ХЕЛПЕРОВ В КРИТИЧЕСКОМ ПЕРИОДЕ ТЯЖЕЛОЙ МЕХАНИЧЕСКОЙ ТРАВМЫ У ДЕТЕЙ

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Резюме. Тяжелая механическая травма является одной из основных причин детской инвалидизации и смертности. Развивающийся дисбаланс гипервоспаления и иммуносупрессии в критическом периоде тяжелой травмы повышает риск развития инфекционных осложнений и/или полиорганной недостаточности. Целью работы было определение информативных иммунологических критериев тяжести повреждения, оценки риска развития осложнений и прогноза исхода травматической болезни у детей при тяжелой механической травме (ТМТ, ISS ≥ 16, n = 87) в группах с благоприятным (n = 47) и неблагоприятным исходом (n = 40), а также в зависимости от развития гнойно-септических осложнений (ГСО, n = 16) и синдрома полиорганной недостаточности (СПОН, n = 11). Методом проточной цитометрии проведена оценка содержания Т-хелперов (Th) и их субпопуляций: регуляторные Т-лимфоциты – $CD4^+CD127^{low}CD25^{high}$ (Treg), Th17-го типа – $CD4^+CD161^+$ и $CD4^+CD127^{higt}CD25^{high}$Т-клетки (T127hi) в 1-е, 3-и, 5-е, 7-е, 14-е сутки с момента получения травмы. Группу сравнения составили 34 ребенка с травмой легкой и средней степени тяжести (ЛТ, ISS < 16). Контрольную группу в исследовании составили 80 условно здоровых детей, сопоставимых по возрасту и полу. В исследовании выявлена обратная зависимость тяжести травмы, степени кровопотери и исхода с абсолютным числом всех субпопуляций, однако для Th и Treg отмечена наиболее сильная связь (R Спирмана ≤ -0,70, p < 0,00001). Для группы ТМТ обнаружено выраженное снижение абсолютного количества клеток Th, Treg, T127hi и Th17 в раннем посттравматическом периоде с тенденцией к повышению к 14-м суткам. Значения в первые сутки для показателей пациентов с ЛТ соответствовали значениям контрольной группы и значимо отличались от группы ТМТ. Были выявлены различия в динамике процентного содержания субпопуляций Th в остром посттравматическом периоде. Процентное содержание Th17 и T127hi достоверно повышено в 1-3-й и 3-7-й дни после травмы соответственно в сравнении с кон-

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трольной группой и ЛТ. Для процентного содержания Treg достоверных различий в первую неделю после травмы выявлено не было. Низкий уровень абсолютного количества клеток Treg у пациентов с ТМТ на первые сутки после травмы в значительной степени связан с развитием ГСО и неблагоприятным исходом. Динамика абсолютного количества клеток Th17 в остром посттравматическом периоде значимо отличалась у детей с ТМТ при развитии СПОН. Абсолютное количество Th17 достоверно снижено в 3-7-й дни после травмы в группе ТМТ с СПОН. Результаты исследования свидетельствуют о том, что у детей уровни Treg, T127hi и Th17 достоверно связаны с тяжестью травмы и могут быть использованы для прогнозирования исхода травматического заболевания и оценки риска развития ГСО и СПОН.

Ключевые слова: дети, тяжелая травма, политравма, субпопуляции Т-хелперов, синдром полиорганной недостаточности, инфекционные осложнения

DYNAMICS OF T HELPER SUBPOPULATIONS IN THE CRITICAL PERIOD OF SEVERE INJURY IN CHILDREN

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Abstract. Severe mechanical injury is one of the main reasons behind children's disability and mortality. Severe injure induces a complex host immune response to tissue injury, a parallel pro- and anti-inflammatory state, bearing an elevated risk for infectious complications (IC) and/or multiple organ failure (MOF). This study aimed to determine the informative immunological criteria of traumatic injury severity and prognosis outcome in children (severe injury group (SInj, ISS ≥ 16), n = 87; mild/moderate injury group (MInj, ISS < 16), n = 34) based on the assessment of absolute cell count (abs) and percentage of such T helper subpopulations as regulatory T lymphocytes – $CD4^+CD127^{low}CD25^{high}$(Treg), Th17 lymphocytes – $CD4^+CD161^+$ and $CD4^+CD127^{higt}CD25^{high}$ T cells(T127hi) in severe injury cases grouped by the outcome (favorable, n = 47; unfavorable, n = 40) and depending on IC (n = 16) and the development of MOF (n = 11) on the 1^{st}, 3^d, 5^{th}, 7^{th}, 14^{th} day after injury. The control group was comprised of 80 apparently healthy children comparable in age and sex. An inverse relationship between severity of injury, degree of blood loss and outcome of injury was revealed with the abs of all Th populations, but for Th abs and Treg abs the most significant correlation was found (Spearman's R ≤ -0,70, p < 0.00001). For SInj group, a pronounced decrease of Th abs, Treg abs, T127hi abs and Th17 abs, in the acute post-traumatic period with an increase to 14 days was revealed. The values of in the first day for indicators of patients with MInj group correspond to the values of control group and significantly differ from SInj group. There are different kinetics of percentage Th subpopulations in peripheral blood of children with severe injuries. The Th17%$CD4^+$ and T127hi%$CD4^+$ significant increase in 1^{st}-3^d and 3^d-7^{th} days after injury respectively in comparation with control and MInj groups. There were no differences between groups in terms of Treg%$CD4^+$. The lower-level Treg abs in trauma patients admitted to the ICU is significantly associated with develop the infectious complications and outcome of trauma. The Th17 abs is significantly reduced in 3-7^{th} days after the injury in the SInj group with MOF. The results of the study indicate that in children levels of Treg, T127hi and Th17 is significantly associated with severity of injury and may be used to predict outcome of trauma and assess the risk of IC and MOF.

Keywords: children, severe injury, polytrauma, T helper subpopulations, multiple organ failure, infectious complications

Introduction

Severe mechanical injury is one of the main reasons behind children's disability and mortality [3]. Severe injury induces a complex host immune response to tissue injury, a parallel pro- and anti-inflammatory state, bearing an elevated risk for infectious complications (IC) and/or multiple organ failure (MOF) [1, 5]. There is usually a period of prominent immunosuppression the pathogenesis of which is largely shaped by the decreasing level of

T lymphocytes (Th) in severe injury [7]. Activity, absolute cell number and frequency of such T helper subpopulations as regulatory T lymphocytes (Treg) – $CD4^+CD127^{low}CD25^{high}$ and Th17 lymphocytes (Th17) can be a significant marker in determining the severity of the pathological process and predicting its outcome. Regulatory T cells play an important role in the orchestration of self-tolerance and immune reaction. It has been demonstrated that Tregs were activated in response to injury which driven trauma-induced suppression of Th1 responses and T cell anergy [4, 8]. Moreover, they have an important but not yet profoundly characterized role during the post-traumatic inflammation; and may, therefore, be an important determinant of the extent and/or severity of the post-traumatic immunosuppression as well [9]. Th17 cell is a novel subset of $CD4^+$T cells characterized by the production of IL-17 and other abundant cytokines such as IL-22, and IL-26 and TNFα with a week and proinflammatory response [12]. Trauma patients admitted to the intensive care unit (ICU) ward have identified increased Th17 cells during the first week after admission, which are correlated with development of early poor outcomes [2]. Therefore, we have characterized and analyzed the dynamics of some subsets of Th, which may play an important role in the post-traumatic immunosuppression, and thereby the recovery from trauma in children.

The purpose of this study was to identify informative immunological criteria for the traumatic disease severity and as applicable to children. The identification relies on the assessment of absolute and relative number of T helper subpopulations – Th17, Treg and $CD4^+CD127^{higt}CD25^{high}$ lymphocytes.

Materials and methods

The study involved 87 patients (58 boys (66.6%), 35 girls (33.4%); 331 observation sessions) with severe injury (SInj), treated at the Department of Anesthesiology and Resuscitation of the Research Institute of Emergency Pediatric Surgery and Traumatology. We used the laboratory of the National Medical Research Center for Children's Health for laboratory studies, which were prescribed 1 to 5 times, depending on the length of stay of a given child at the ICU. The median age of the children was 12.0 (5.75-15.0) years (Me ($Q_{0.25}$-$Q_{0.75}$)). The time options for laboratory studies were the first, third, fifth, seventh and 14th days from the day of injury. The comparison group was comprised of 34 patients (15 boys (44.1%), 19 girls (55.9%); 34 observation sessions) with mild/moderate injury (MInj) treated at the Department of Neurotrauma. The control group was comprised of 80 apparently healthy children, all of them underwent medical examination at the National Medical Research Center for Children's Health. The children were comparable in age and sex: age – 12.41 (4.4-16.2) years; 47 boys (58.7%), 33 girls (41.3%).

Assessing the injury, we relied on the Injury Severity Score (ISS) and the Glasgow Coma Scale (GCS). The outcome of an SInj was assessed with the help of the Glasgow Outcome Scale (GOS) and the Severe Injury Outcomes Scale (OISS) [11]. These scales were applied to assess the condition of the patient at discharge from the hospital.

The patients in our study met the following criteria: severe injury (ISS ≥ 16), aged 1-18 years, admittance to the ICU within 72 hours. Concomitant acute inflammatory and chronic diseases were a reason for exclusion.

At the first stage, we analyzed the results from the control group, the MInj (ISS 4.0 (4.0-9.0)) and the SInj group (ISS 26.0 (21.0-29.0)). At the second stage, we analyzed the two groups from SInj formed with the help of GOS and OISS, the favorable outcome group (SInjfav, n = 47) and the unfavorable outcome group (SInjunfav, n = 40). The distribution into these groups was based on the scores: patients were allocated to the SInjfav group if they scored 4-5 points on the GOS scale and 1-2 points on the OISS scale, and to the SInjunfav group if they scored 1-3 points on the GOS scale and 3-5 points on the OISS scale.

Clinical and laboratory indicators of systemic inflammatory response syndrome and organ failure were evaluated in all patients with severe injury. Organ functioning was assessed daily after admission to the ICU using MODS (Multiple Organ Dysfunction Score) [6]. Patients with severe injury were divided into groups depending on infectious complications (IC n = 16) and the development of MOF (MOF n = 11).

We assessed the quantity of Th – $CD4^+$ cells, T127hi – $CD4^+CD127^{higt}CD25^{high}$, Th17 – $CD4^+CD161^+$, Treg – $CD4^+CD127^{low}CD25^{high}$ in the patients. Two-platform technology enabled assessment of the quantitative indicators of the subpopulation composition of peripheral blood T helpers. The absolute number of lymphocytes was calculated with the help of a Sysmex XT-2000i hematology analyzer (Sysmex Corporation; Japan). The preparation of samples for cytofluorimetric analysis included incubation of 100 μL of whole blood with 10 μL of monoclonal antibodies tagged with fluorochromes for 20 min in a dark place. The erythrocytes were lysed with BD FACS™ Lysing Solution (BD Biosciences; USA); the duration of incubation therewith in the dark at room temperature did not exceed 10-12 minutes. The resulting samples were analyzed in a Novocyte flow cytometer (ACEA Biosciences; USA). The surface markers used to determine lymphocyte subpopulations were as follows: CD45, IgG1, IgG2a, CD3, CD4, CD25, CD127, CD161 (Beckman

Coulter, USA; BD Biosciences, USA; SONY corp., Japan).

Analysis of quantitative indicators of the population composition of blood lymphocytes in children is particularly difficult due to the existence of age characteristics. To unify the indicators, we previously carried out a transformation according to the formula, considering the normative values for different age groups of the parameters of the main and small populations of peripheral blood lymphocytes [10]:

Xn = (Xmin-X) / 0,01*(Xmax – Xmin)

X – the value of the studied indicator, Xn – the value of the indicator normalized by the age norm, Xmax is the upper limit of the age norm, Xmin is the lower limit of the age norm.

After the transformation, an array of data was obtained on all the main indicators of the population composition of peripheral blood lymphocytes in children, in which the values of the studied indicators are presented in uniform conventional units. If the obtained value of the studied indicator falls into the range from 0 to 100, then it corresponds to the age norm. Thus, this allows you to analyze the data without considering the age characteristics of the dynamics of the quantitative indicators of the population composition of blood lymphocytes in children.

We used MS Excel 365 (Microsoft corp.; USA), Statistica 10 (StatSoft, Inc.; USA) to process the data obtained. The results are presented as a median (Me) and quartiles ($Q_{0.25}$-$Q_{0.75}$). Mann–Whitney U test and Kruskal–Wallis test with Bonferroni correction enabled comparison of differences in the attributes. Spearman's rank correlation coefficient (R) was used to assess relations between the attributes. The conclusions were considered significant at $p < 0.05$ (*).

Results and discussion

The absolut cell count and relative amount Th, T127hi, Treg and Th17 obtained in children on the 1st day of injury, underwent a correlation analysis with clinical parameters (Table 1). An inverse relationship between the severity of the injury, the degree of blood loss and the outcome of the injury according to OISS was revealed with the absolute number of all analyzed lymphocyte populations, but for Th and Treg absolut cell count the most significant correlation was found (Spearman's $R \leq -0.70$, $p < 0.00001$) (Table 1, Figure 1B, C). The similar data have been obtained

TABLE 1. SPEARMAN'S R CORRELATION COEFFICIENT FOR Th, T127hi, Treg AND Th17 ABSOLUTE CELL COUNT AND PERCENTAGE OF T127hi, Treg AND Th17 AND CLINICAL INDICATORS IN THE 1ST DAY OF INJURY IN CHILDREN

Index	Factor	N	Spearman R	t(N-2)	p-level	p-level
ISS	Th abs	102	-0.70	-9.76	0.00000	< 0.00001
	Treg abs	102	-0.71	-10.08	0.00000	< 0.00001
	Treg %CD4	102	-0.05	-0.52	0.60616	
	T127hi abs	102	-0.50	-5.80	0.00000	< 0.00001
	T127hi %CD4	102	0.27	2.85	0.00524	< 0.01
	Th17 abs	102	-0.59	-7.25	0.00000	< 0.00001
	Th17 %CD4	102	0.34	3.57	0.00055	< 0.01
OISS	Th abs	102	-0.52	-6.12	0.00000	< 0.00001
	Treg abs	102	-0.52	-6.06	0.00000	< 0.00001
	Treg %CD4	102	-0.03	-0.30	0.76363	
	T127hi abs	102	-0.47	-5.34	0.00000	< 0.00001
	T127hi %CD4	102	0.07	0.73	0.47008	
	Th17 abs	102	-0.51	-5.92	0.00000	< 0.00001
	Th17 %CD4	102	0.18	1.86	0.06590	
Blood loss degree	Th abs	102	-0.54	-6.47	0.00000	< 0.00001
	Treg abs	102	-0.52	-6.16	0.00000	< 0.00001
	Treg %CD4	102	0.09	0.94	0.35031	
	T127hi abs	102	-0.39	-4.30	0.00004	< 0.00001
	T127hi %CD4	102	0.25	2.56	0.01186	< 0.05
	Th17 abs	102	-0.48	-5.46	0.00000	< 0.00001
	Th17 %CD4	102	0.26	2.66	0.00914	< 0.01

in other studies. It has been shown that persistence of lymphopenia shaped by the decreasing level of Th in severe injury following trauma is correlated with severity and is associated with poorer prognosis [7]. The lymphocyte counts decrease immediately after trauma in patients compared to the control group [5].

Using the nonparametric Kruscal-Wallis test with Bonferroni correction, we compared the differences in Th, T127hi, Treg and Th17 absolut cell count and the frequency of T127hi, Treg and Th17 in children with injury over time in different groups: Control, MInj and SInj groups (Table 2, Figure 1A, D-I).

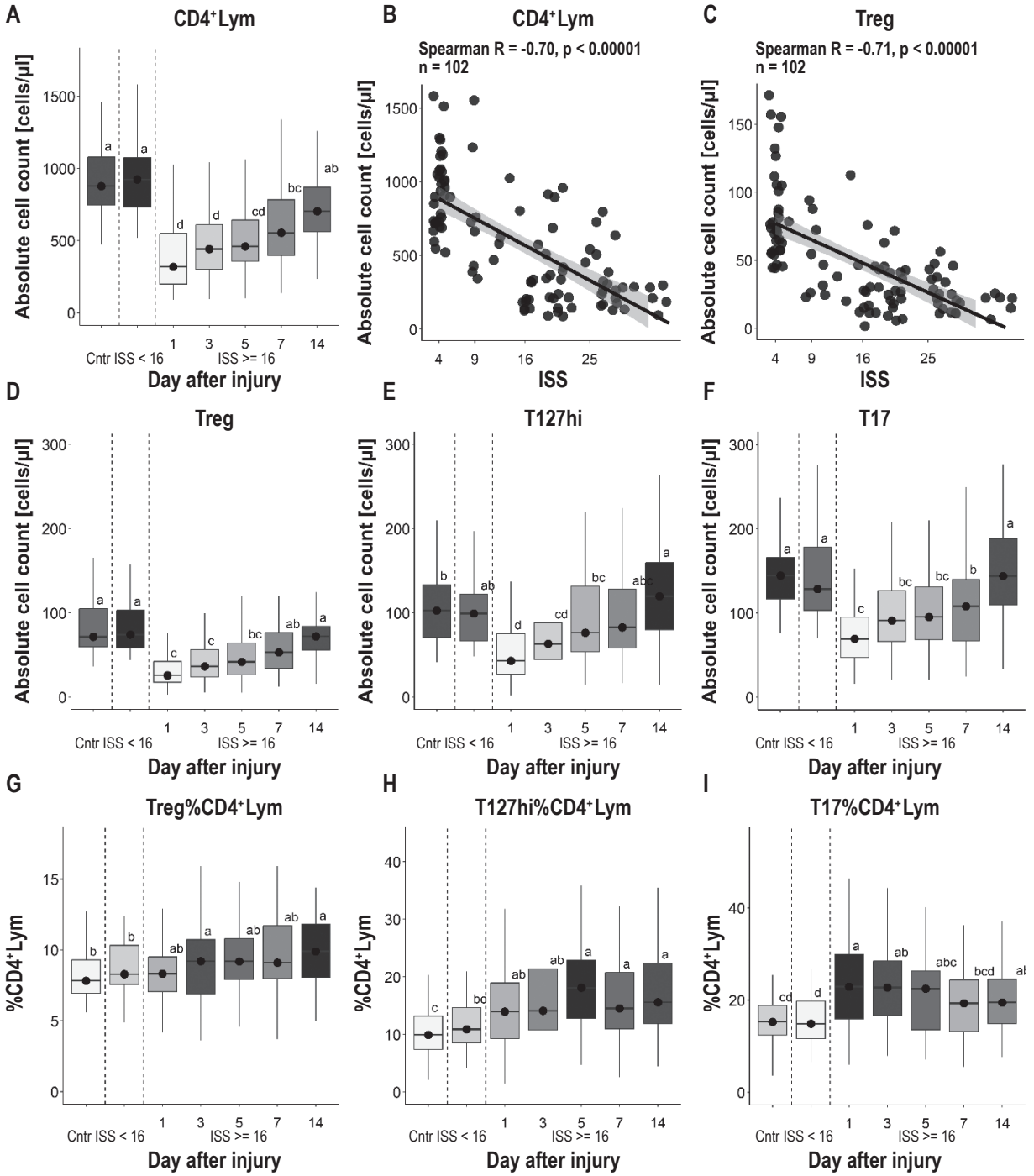

Figure 1. Dynamics of Th absolute cell count in the critical period of injury in children (A). Relationship between the Th and Treg absolute cell count and Injury Severity Score in the 1st day of injury in children (B, C). Absolute cell count dynamics of T127hi, Treg and Th17 cells in the critical period of injury in children (D-F). The frequency dynamics of T127hi, Treg and Th17 cells in the critical period of injury in children (G-I)

Note. Me ($Q_{0.25}$-$Q_{0.75}$) Min-Max; the significance is represented by letters according to pairwise comparation through the Kruskal–Wallis test adjusted with Bonferroni correction; comparison groups: Cntr, control group; ISS < 16, MInj group; SInj group by day after severe injury.

TABLE 2. DYNAMICS OF Th, T127hi, Treg AND Th17 ABSOLUTE CELL COUNT, PERCENTAGE AND NORMALIZED ABSOLUTE CELL COUNT IN THE CRITICAL PERIOD OF SEVERE INJURY IN CHILDREN IN COMPARATION WITH CONTROL GROUP AND MINJ GROUP AND ALSO IN SINJ GROUP DEPENDING DEVELOPMENT OF INFECTIOUS COMPLICATION, MULTIPLE ORGAN FAILURE DEVELOPMENT AND OUTCOME OF SEVERE INJURY, Me ($Q_{0.25}$-$Q_{0.75}$)

Factor		Control	MInj	SInj, day after injury				
				1	3	5	7	14
n		80	34	68	87	35	74	67
Th	abs	886.57 (752.75-1094.20)	923.50 (729.6-1076.2)	319.22 (199.63-550.65)	446.18 (302.98-615.83)	460.40 (356.36-642.75)	565.28 (398.96-804.30)	720.83 (562.76-942.50)
Treg	abs	71.67 (59.37-105.07)	74.02 (58.06-103.31)	25.90 (17.49-42.23)	36.50 (24.08-56.48)	41.76 (26.30-64.30)	53.18 (34.01-76.50)	72.00 (55.90-84.00)
	% Th	7.83 (6.95-9.29)	8.30 (7.58-10.33)	8.33 (7.06-9.50)	9.20 (6.88-10.72)	9.20 (7.92-10.80)	9.10 (7.98-11.70)	9.90 (8.05-11.80)
	abs n, c. u.		39.50 (11.21-101.87)	-112.64 (-139.44--78.08)	-75.26 (-118.09--29.15)	-60.96 (-106.17--3.86)	-28.33 (-76.38-56.26)	39.04 (-25.23-89.81)
MOF Treg abs n, c. u.	N			-111.55 (-148.04--80.69)	-73.33 (-117.56--24.42)	-60.03 (-93.94--10.00)	-23.33 (-69.52-58.58)	40.98 (-25.23-89.81)
	Y			-125.74 (-131.87--111.79)	-98.83 (-132.77--66.92)	-136.17 (-173.36-45.49)	-73.33 (-114.58-29.48)	25.82 (-15.69-67.30)
IC Treg abs n, c. u.	N			-102.25 (-133.34--72.87)**	-72.42 (-118.62--22.39)	-49.03 (-93.94-22.38)*	-28.33 (-76.38-56.26)	50.00 (-33.37-89.82)
	Y			-144.00 (-154.27--130.84)**	-90.15 (-119.48--74.18)	-125.58 (-156.43--90.00)*	-26.59 (-73.52-32.33)	13.77 (-10.80-53.54)
Outcome Treg abs n, c. u.	F			-96.74 (-134.04--60.20)*	-72.42 (-117.56--20.00)	-58.35 (-92.95--23.53)	-11.11 (-64.96-61.14)	44.52 (-35.56-93.02)
	UF			-128.24 (-141.98--102.64)*	-83.03 (-119.19--48.81)	-78.19 (-128.23-48.82)	-47.30 (-90.79-38.83)	36.67 (-8.69-89.51)
T127hi	abs	102.65 (70.62-133.63)	99.23 (66.79-122.26)	43.04 (27.03-75.31)	63.18 (44.72-88.23)	76.31 (53.92-131.67)	82.85 (58.26-127.99)	120.74 (81.00-161.54)
	% Th	9.91 (7.38-13.16)	10.90 (8.53-14.62)	13.95 (9.25-18.95)	14.10 (10.78-21.42)	18.10 (12.75-22.90)	14.50 (10.95-20.75)	15.60 (11.85-22.40)
	abs n, c. u.		50.94 (9.10-110.44)	-48.26 (-82.90-0.61)	-17.18 (-50.30-32.94)	19.50 (-17.53-99.38)	26.33 (-31.68-110.29)	104.30 (3.77-168.58)
MOF T127hi abs n, c. u.	N			-50.91 (-87.06--0.40)	-13.60 (-49.51-50.94)	21.07 (-10.94-96.17)	33.14 (-14.01-113.31)**	109.43 (3.77-168.58)
	Y			-62.91 (-76.57--41.56)	-29.20 (-71.00--1.14)	-68.42 (-81.58-99.39)	-32.92 (-84.10--24.51)**	88.55 (2.59-174.04)

Таблица 2 (окончание)
Table 2 (continued)

Factor		Control	MInj	SInj, day after injury				
				1	3	5	7	14
n		80	34	68	87	35	74	67
IC T127hi abs n, c. u.	N			-51.42 (-81.05- -0.96)	-12.46 (-48.45- 35.73)	24.21 (-7.08- 99.39)*	32.94 (-17.76- 120.00)	109.43 (2.69- 168.58)
	Y			-70.16 (-111.08- -25.12)	-32.68 (-84.91- 0.45)	-49.77 (-96.77- -10.33)*	-26.75 (-40.80- 11.57)	41.75 (22.13- 195.53)
Outcome T127hi abs n, c. u.	F			-37.16 (-85.65- 26.00)	-24.67 (-58.74- 15.63)	16.54 (-10.94- 80.44)	33.14 (-0.81- 131.02)*	114.22 (17.55- 168.66)
	UF			-60.70 (-87.85- -24.76)	-1.89 (-47.12- 43.47)	19.50 (-68.42- 99.39)	-4.26 (-44.96- 58.67)*	82.76 (-10.91- 166.05)
Th17	abs	144.36 (116.90- 166.04)	128.61 (103.20- 178.35)	69.31 (47.03- 95.12)	91.15 (66.29- 126.83)	95.62 (70.50- 133.57)	109.37 (67.36- 141.61)	144.92 (111.13- 196.72)
	% Th	15.30 (12.40- 18.80)	14.90 (11.65- 19.78)	22.90 (15.93- 29.90)	22.75 (16.68- 28.52)	22.50 (13.60- 26.35)	19.30 (13.20- 24.38)	19.50 (14.90- 24.55)
	abs n, c. u.		17.20 (-60.85- 99.92)	-160.14 (-211.16- -101.13)	-93.31 (-149.53- -23.86)	-88.23 (-152.06- -11.65)	-53.08 (-146.56- 21.43)	28.32 (-53.72- 128.90)
MOF Th17 abs n, c. u.	N			-160.38 (-211.41- -101.89)	-81.08 (-144.59- -18.00)*	-60.26 (-121.19- -4.21)**	-45.45 (-139.56- 22.21)*	38.74 (-43.19- 147.54)
	Y			-156.14 (-181.26- -136.85)	-170.82 (-232.67- -101.43)*	-277.79 (-325.00- -215.66)**	-137.74 (-237.73- -67.58)*	-6.65 (-58.13- 44.37)
IC Th17 abs n, c. u.	N			-153.46 (-197.67- -99.62)	-90.47 (-148.16- -18.62)	-64.95 (-121.43- -1.95)*	-46.31 (-132.29- 21.43)	19.50 (-60.09- 126.42)
	Y			-202.25 (-259.88- -167.77)	-128.30 (-158.49- -38.85)	-152.06 (-204.08- -124.06)*	-141.99 (-153.13- -66.07)	43.14 (-16.14- 137.67)
Outcome Th17 abs n, c. u.	F			-163.97 (-213.25- -80.74)	-96.94 (-149.53- -25.18)	-45.71 (-119.09- 6.48)	-24.06 (-113.70- 23.74)	24.61 (-45.15- 119.70)
	UF			-157.40 (-204.81- -139.29)	-81.08 (-148.16- -22.73)	-116.70 (-219.45- -45.28)	-112.23 (-153.72- -7.99)	28.32 (-56.18- 147.17)

Note. Mann–Whitney U test; *, $p < 0,05$; **, $p < 0,01$; comparison groups: MOF group (N – no, Y – yes); IC, infectious complication (N – no, Y – yes); Outcome (F – favorable outcome, UF – unfavorable outcome); c. u., conventional units.

For SInj group, a pronounced decrease of Th, Treg, T127hi and Th17 absolute cell count, in the acute post-traumatic period with an increase to 14 days was revealed. The values of in the first day for indicators of patients with MInj correspond to the values of control group and significantly differ from patients with SInj in the 1-7th day for Th17 and Treg, for T127hi in the 1st-3d day after the injury (Figure 1A, D-F).

There are different kinetics of the percentage Th subpopulations in peripheral blood of children with severe injuries. The percentage of Th17 and T127hi significant increse in 1st-3d and 3d-7th days after injury respectively in comparation with Control and MInj group (Figure 1H, I). Zhang et al. demonstrated that the level of Th17 showed increased initially and then decreased in patients with thoracic trauma.

The frequency of Th17 was significantly increased in traumatic patients compared to healthy controls on the day after admission [12]. At the same time, in our study there were no differences between groups in terms of the percentage of Treg cells (Figure 1G). In contrast, it has been demonsrated the percentage of Treg cells in the peripheral blood was higher in patients with thoracic trauma compared with that of healthy group [12].

Using the nonparametric Mann–Whitney test, we compared the differences in normalized absolut cell count Treg (Tregn), T127hi (T127hin) and Th17 (Th17n) in children with severe injury over time in different groups: with and without MOF, with and without IC and outcome groups (Table 2). Patients with MOF had significantly lower median values of analised parmetrs within 1st-7th days after admission to the ICU than patients without MOF. Significant differences in Th17n and T127hin level were found in MOF groups from 3rd to 7th day and in 7th day respectively. Only one previous study has examined significant decreases in the number of lymphocytes between MOF and non-MOF groups appear after day 2 [5].

Despite improvements in prognosis and survival, there is a lack of validated diagnostic tools for post-traumatic complications. In a retrospective study involving 188 patients after severely injured trauma the immunoregulatory index, in the polytrauma patients with purulent-inflammatory complications, was lower than in the patients without purulent-inflammatory complications due to the reduced values of Th [7]. In our study the levels of Tregn in 1st and 5th day and Th17n, T127hin in 5th day after injury differed significantly in groups without and with IC: patients from the latter group had it considerably lower. Study on trauma patients admitted to the ICU ward have identified increased Th17 cells and serum IL-17 levels during the first week after admission, which are correlated with development of early poor outcomes [2].

A comparative analysis of the post-traumatic period data from SInjfav and SInjunfav groups has shown a significant increase Tregn in 1st day and T127hin in 7th day after injury in the SInjunfav group. At the same time, there were no differences between groups in terms of Th17n. It has been observed that the decrease and increase of Treg cells in the early and late phases of the disease, respectively, are associated with poor prognosis of trauma [2, 9, 12].

Conclusions

The results of the study indicate that in children the levels of Treg, T127hi and Th17 is significantly associated with the severity of injury and may be used to predict outcome of the traumatic disease and assess the risk of infectious complications and multiple organ dysfunction syndrome. The lower-level absolute cell count Treg in trauma patients admitted to the ICU is significantly associated with develop the infectious complications and outcome of the traumatic disease. The dynamics of absolute cell count Th17 in the post-injury period are important for development multiple organ dysfunction in children with severe trauma.

Compliance with ethical standards

The study was conducted in accordance with the Declaration of Helsinki and approved by the Committee on Biomedical Ethics of Institute of Urgent Children Surgery and Traumatology (Protocol No. 2 of 26.05.2020). All study participants signed an informed consent.

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