ACTA MEDICA SALINIANA

ORIGINAL PAPERS

THE IMPACT OF ANTI-INFLAMMATORY IMMUNE RESPONSE ON FATAL OUTCOME IN THE EARLY STAGE OF A COMPLICATED INTRA-ABDOMINAL INFECTION

Evgeni Dimitrov[1,2], Krasimira Halacheva[3], Georgi Minkov[1,2], Emil Enchev[1,2], Yovcho Yovtchev[1,2]

ISSN 0350-364X

DOI: 10.5457/822

Evgeni Dimitrov
Krasimira Halacheva
Georgi Minkov
Emil Enchev
Yovcho Yovtchev

ABSTRACT

Background: There is still no study investigating the role of anti-inflammatory immune response in the early stage of a complicated intra-abdominal infection (cIAIs).
Aim: To evaluate its impact on unfavorable outcome using the already established anti-inflammatory biomarkers $CD14^{+}HLA\text{-}DR^{+}$ (mHLA-DR) and $CD4^{+}CD25^{+}CD127^{low/neg}$ (Tregs).
Methods: We conducted a single-center prospective study at University Hospital "Prof. Dr. Stoyan Kirkovich" Stara Zagora between November 2018 and August 2021 on 62 patients with cIAIs. We measured the perioperative expression levels of mHLA-DR and Tregs by flow cytometry.
Results: The mortality rate during hospital stay was 14.5%. We found no significant differences between survivors and non-survivors in the values of mHLA-DR (p = 0.061 and p = 0.13 pre- and postoperatively) and Tregs (p = 0.992 and p = 0.109 pre- and postoperatively). ROC Curve analysis revealed that neither mHLA-DR (AUROC = 0.682 and 0.72 pre- and postoperatively), nor Tregs (AUROC = 0.501 and 0.689) levels were able to prognosticate the outcome.
Conclusion: By 3rd POD the anti-inflammatory reaction is not directly responsible for the fatal outcome in patients with cIAIs.

Keywords: mHLA-DR, Tregs, intra-abdominal infections, mortality, anti-inflammatory immune response

Affiliations:
[1]Clinic of Surgical Diseases, University Hospital "Prof. Dr. Stoyan Kirkovich" Stara Zagora, Bulgaria
[2]Department of Surgical Diseases and Anesthesiology, Faculty of Medicine, Trakia University Stara Zagora, Bulgaria
[3]Laboratory of Clinical Immunology, University Hospital "Prof. Dr. Stoyan Kirkovich" Stara Zagora, Bulgari

Received:
10.09.2024.

Accepted:
06.11.2024.

Corresponding author:
Evgeni N. Dimitrov, PhD
e-mail: evgeni_d1984@yahoo.com
tel: +359 887 609 943;

Funding: none

Competing interests: none

INTRODUCTION

The complicated intra-abdominal infections (cIAIs) represent the second most common cause of sepsis after pneumonia and are still associated with high levels of morbimortality [1]. An early prognostic assessment of cIAIs allows selection of the patients facing a higher risk, which further enables the adoption of a timely and suitable correction in the therapeutic strategy, reducing the development of sepsis and sepsis-related conditions as well as improving survival rates.
The current concept is that both pro- and anti-inflammatory cytokines are elevated early in sepsis, causing both a systemic pro-inflammatory response and a systemic anti-inflammatory response, creating the so-called mixed antagonist response syndrome (MARS). After an early pro-inflammatory response, usually the anti-inflammatory response becomes dominant, especially as the severity of sepsis increases [2]. The initiation of an anti-inflammatory response in sepsis aims to counteract and limit the activated pro-inflammatory process and stimulate tissue recovery [3]. As a result of persistent immunosuppression, secondary infections with low-virulence pathogens may occur [4]. A strong correlation between high anti-inflammatory response and adverse outcome was observed in patients with sepsis [5].
According to Monneret and Venet [6], the most reliable biomarker for monitoring immune changes in the critically ill is low expression of HLA-DR on monocytes (mHLA-DR). The main role of HLA-DR in infection is expressed in the antigen presentation of T-helper cells, which leads to the release of pro-inflammatory cytokines. In septic patients, however, this pathway can be disrupted, leading to immunosuppression [7]. In patients with sepsis, the reduction of monocyte antigen-presenting capacity by lowering mHLA-DR can lead to a fatal outcome [8]. Decreased expression of HLA-DR on monocytes is generally accepted criteria for immunosuppression, as it leads to cellular deactivation, in which monocytes lose their antigen-presenting function and the ability for intercellular signaling, this in turn leads to impaired cytokine se-

cretion and maturation to a phagocytic cell [9]. Monocyte HLA-DR expression ≤ 30% on monocytes strongly correlates with unfavorable outcome [10].
Regulatory T cells (Tregs) are a component of the immune system that suppresses the immune response of other cells, which is an important „self-check" aimed at preventing hyperacute reactions [11]. Tregs are distinguished from other T lymphocyte populations based on high surface expression of CD25, high intracellular expression of the transcription factor „forkhead box P3 (Foxp3)" [12] and low surface expression of CD127 (IL-7Rα) [13]. Tregs are critical for maintaining immune balance and limiting abnormal inflammation, with subsequent risk of developing autoimmune diseases [14]. The percentage of T-regs in peripheral blood reflects the immune status in sepsis by maintaining immunological homeostasis and tolerance [15]. A high percentage of Tregs in peripheral blood represents an effective biomarker of immunosuppression in septic patients and is associated with death [16].
The aim of this study was to evaluate the association between anti-inflammatory response and mortality in the early stage of cIAI using the anti-inflammatory biomarkers mHLA-DR and Tregs.

MATERIAL AND METHODS

This single-center prospective study was conducted at a University hospital Stara Zagora over a period of 34 months (November 2018 – August 2021). Sixty-two patients were admitted as emergency cases and operated on for cIAIs in the Clinic of Surgical Diseases. The exclusion criteria were age under 18 years, use of immunosuppressive drugs or pregnancy, however no patient met them and at the end 62 participants were involved. The study protocol was approved by the ethics committee of the hospital (№ РД-10-275/05.04.2018). All procedures performed in the study involving human participants were in accordance with the ethical standards of the 1964 WMA Helsinki Declaration and its later amendments or comparable ethical standards.
We defined cIAIs as intra-abdominal infections spreading beyond the source organ and cause local or diffuse peritonitis [17].

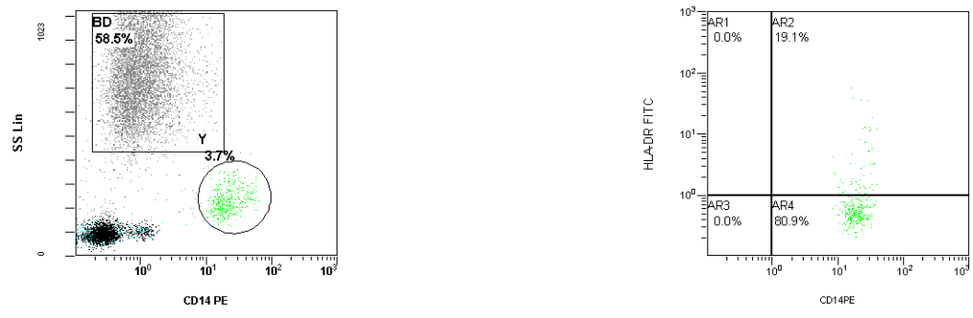
Demographic, laboratory and clinical data were obtained from patients medical records before surgery (day 0) and postoperatively (day 3). The primary endpoint of the study was to evaluate the association between anti-inflammatory response and final outcome using mHLA-DR and T-regs. In-hospital mortality was considered for the study.
The study group was compared with 31 healthy controls who had a similar sex and age distribution to the patients.

FLOW CYTOMETRY

In fresh peripheral blood samples, we evaluate the expression of proliferative cell markers collected in a tube with EDTA anticoagulant. Within one to three hours after sampling, the flow cytometry was performed. We incubated aliquots of 100 µl of blood for 15 min in the dark with monoclonal antibodies (protocol 1: anti-HLA-DR FITC, anti-CD14 PE, anti-CD64 PC5; protocol 2: anti-CD25 FITC, anti-CD127 PE, anti-CD3 PC5, anti-CD4 PC7) indicated by the manufacturer (Beckman Coulter, USA). Lyse/no wash procedure was applied on an automatic TQ-Prep Workstation & Immunoprep Reagent system (Beckman Coulter, USA). A minimum of 100 000 events for each sample were counted on a Cytomics FC500 flow cytometer and analyzed using CXP software (Beckman Coulter, USA)

Determination of HLA-DR expression on monocytes

Initially, monocytes were gated from the remaining cell populations based on the expression of a specific surface marker CD14, then an examination of HLA-DR expression (mono-parametric histogram) as a percentage of HLA-DR positive monocytes was also performed (Figure 1-A,B). An appropriate isotype control was used to identify positive and negative populations. HLA-DR expression on monocytes was determined in 60 patients on day 0, because in 2 the peripheral blood sample was clotted, and on the 3rd postoperative day (POD) in 59 patients - in 1 peripheral blood sample was clotted and 2 died before the day of sampling.



A. B.

Figure 1. Monocyte HLA-DR expression determined by flow cytometry in peripheral blood of patients with cIAIs.

(A) CD14 vs side scatter (SS) dot-plot histogram allowing gating for $CD14^+$ monocytes – 3.7% (Y);
(B) CD14 vs HLA-DR double histogram, gated on $CD14^+$ monocytes, allowing gating on $CD14^+HLA\text{-}DR^+$ monocytes – 19.1% (AR2)

Determination of $CD4^+CD25^+CD127l^{low/neg}$ T-regs

A 3-color flow cytometric assay (CD4-PC7/CD25-FITC/CD127-PE) was used to identify the $CD4^+CD25^+CD127^{low/neg}$ T cell population.
1). Labeling of lymphocytes based on size (FS) and granularity (SS) (Figure 2A)
2). Labeling of $CD4^+$ T lymphocytes based on SS/CD4 dot-plots analysis (Figure 2B)
3). After gating of $CD4^+$ T lymphocytes, $CD4^+CD25^+CD127^{low/neg}$ cells were selected based on CD25/CD127 dot-plots analysis and results expressed as percentage of $CD4^+CD25^+CD127^{low/neg}$ cells out of total $CD4^+$ cells (Figure 2C). An appropriate isotype control was used to identify positive and negative populations. Preoperatively, we investigated the $CD4^+CD25^+CD127^{low/neg}$ expression in 61 patients, because in 1 the peripheral blood sample was clotted, and on day 3 in 55 patients – in 5 the peripheral blood sample was clotted and 2 died before the day of sampling.

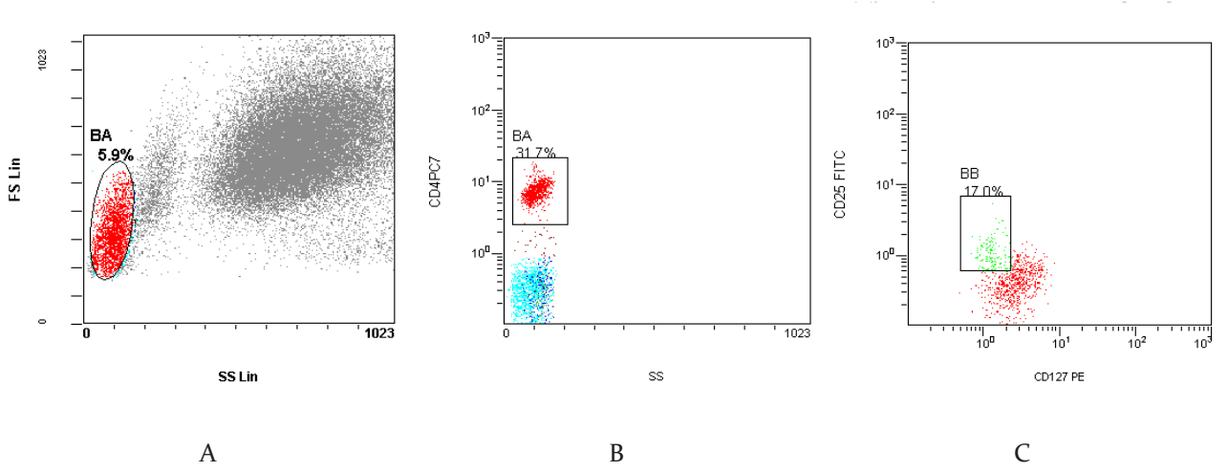


Figure 2. Flow cytometric determination of regulatory T lymphocytes in peripheral blood of patients with cIAIs

(A) forward scatter (FS) vs side scatter (SS) dot-plot histogram allowing gating for lymphocytes – 5.9% (BA);
(B) CD4 vs side scatter (SS) dot-plot histogram allowing gating for $CD4^+$ T helper lymphocytes – 31.7% (BA);
(C) T-regs are identified as $CD25^+CD127^{low/neg}$ cells among $CD4^+$ lymphocytes on the basis of CD25/CD127 dot plot – 17 % (BB).

Statistical analysis

Statistical analysis Sensitivity, specificity, and area under the receiver op[1]erating characteristics (AUROC) for predicting out[1]come were calculated for mHLA-DR and Tregs. Qualitative variables were presented as frequency (%) and were analyzed by Pearson $\chi 2$ test or Fisher exact test. Quantitative variables were presented as mean (SD) or median (IQR) and compared by Mann-Whit[1] U-test or Student t-test. P-values <0.05 were considered statistically significant. For statistical analysis, we used statistical software SPSS version 19 (IBM, Chicago, Illinois, USA).

RESULTS

Basic characteristics

Of the 62 patients included, nine (14.5%) died during the hospital stay. Their median age was higher than those with favorable outcome (79 vs 65, p = 0.032). We found no significant differences between non-survivors and survivors according to gender (p = 1.00), exudate (p = 0.59) and comorbidity (p = 0.423). In contrast, patients with local peritonitis had lower risk of death (Table 1).

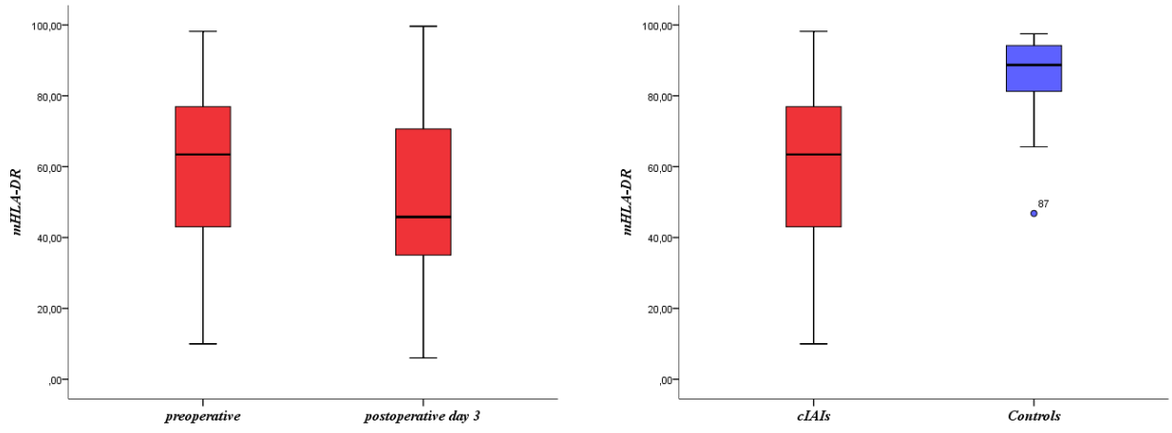
Table 1. Basic characteristics, biomarkers and prognostic scores

Variable	Total population	Survivors	Non-Survivors	p value
Age, years (IQR)	65 (49.5-76.25)	65 (47.5-75)	79 (61-86)	0.032
Sex, n (%)				1.000
male/female	35(56.5)/27(43.5)	30(85.7)/23(85.2)	5(14.3)/4(14.8)	

Variable	Total population	Survivors	Non-Survivors	p value
Exudate, n (%)				
Clear	8 (12.9)	8 (15.1)	0 (0)	0.59
Purulent	54 (87.1)	45 (84.9)	9 (100)	
Feculent	0 (0)	0 (0)	0 (0)	
Peritonitis, n (%)				
Local	37 (59.7)	35 (66)	2 (22.2)	0.024
Diffuse	25 (40.3)	18 (34)	7 (77.8)	
Comorbidity,n(%)	45 (72.6)	37 (69.8%)	8 (88.9%)	0.423
Cardiovascular	38 (61.3)	31 (58.5)	7 (77.8)	0.462
Endocrine	8 (12.9)	8 (15.1)	0 (0)	0.59
Neurologic	7 (11.3)	5 (9.4)	2 (22.2)	0.266
Excretory	5 (8.1)	3 (5.7)	2 (22.2)	0.149
Oncologic	3 (4.8)	3 (5.7)	0 (0)	1.000
Neu^{o}, % ±SD	79.7±8.7	79.7±8.4	79.8±11.1	0.99
Neu^{3}, % ±SD	74.9±10.2	73.3±9.7	84.3±9.7	0.007
CRP^{o}, mg/L ±SD	177.7±103.9	168.3±106.4	232.8±68.5	0.085
CRP^{3}, mg/L ±SD	143.9±70.6	138.1±69.9	187.3±64.4	0.083
mHLA-DR^{o}, % ±SD	59.91± 23.26	61.78± 24.01	49.34±15.4	0.14
mHLA-DR^{3}, % ±SD	50.41± 23.1	52.48± 23.08	35.07± 17.86	0.061
Tregs^{o}, % (IQR)	7.3 (5.35-10.75)	7.05 (5.28-10.95)	8 (5.9-8.85)	0.992
Tregs^{3}, % (IQR)	6.6 (5.2-9.3)	6.75 (5.53-9.68)	5.2 (3.4-7)	0.109
SIRS^{o}, n (%)	36 (58.1)	30 (56.6)	6 (66.7)	0.722
SIRS^{3}, n (%)	5 (8.3)	1 (1.9)	4 (57.1)	< 0.0001
APACHE II^{o}, ±SD	7.6 ± 4.9	6.8 ± 4.6	12.2 ± 4.4	0.001
APACHE II^{3}, (IQR)	7 (3.25-10.75)	7 (3-8)	20 (16-26)	< 0.0001

CD14^{+}HLA-DR^{+} (mHLA-DR)

Preoperatively measured mean values of mHLA-DR (mHLA-DR^{o}) were higher compared to their levels determined on the 3rd POD (mHLA-DR^{3}) - 59.92% vs 50.41%, p = 0.027 (Figure 3A). The expression of mHLA-DR before surgery was significantly lower in patients with cIAIs compared to healthy controls – 59.92% vs. 88.7%, p < 0.0001 (Figure 3B).



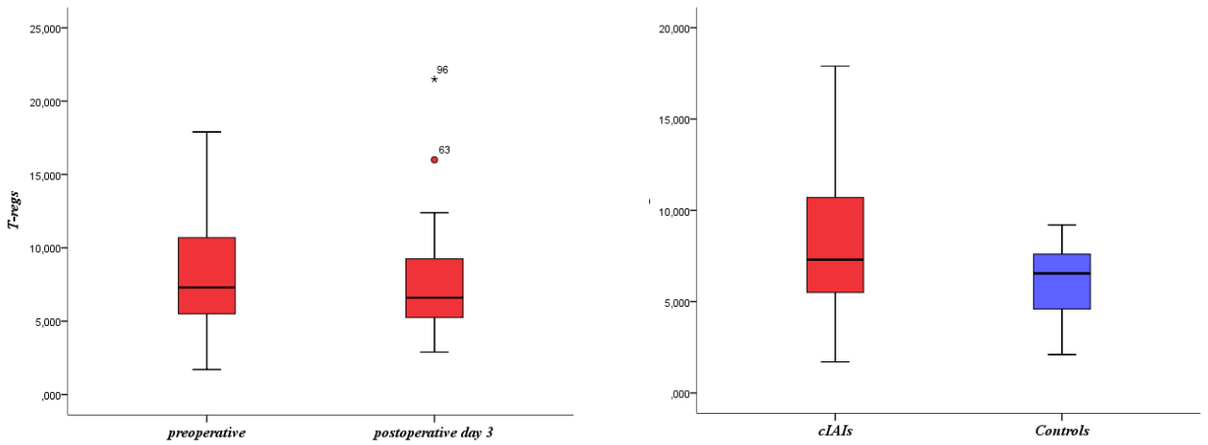
A B

Figure 3. Mean levels of mHLA-DR: (A) perioperatively and (B) in patients with cIAIs and healthy controls

Despite the established differences in mHLA-DR between the study group and healthy controls, no such observation was found according to the outcome (mHLA-DR^{0} survivors vs non-survivors = 61.78% vs 49.34%, p = 0.14 and mHLA-DR^{3} survivors vs non-survivors = 52.48% vs 35.07%, p = 0.061) (Table 1). The ROC-Curve analysis confirmed the lack of prognostic performance both before surgery (AUROC = 0.682, p = 0.084) and on the 3rd POD (AUROC = 0.72, p = 0.061) (Table 2) (Figure 5- A,B).

$CD4^{+}CD25^{+}CD127^{low/neg}$ regulatory T lymphocytes (Tregs)

Percentages in peripheral blood of Tregs before surgery (Tregs^{0}) and postoperatively (Tregs^{3}) showed comparable median values (7.3% vs 6.6%, p = 0.215) (Figure 4A). A significant difference was found between the preoperative Tregs in patients with cIAIs and control group (7.3% vs 6.55%, p = 0.023) (Figure 4B).



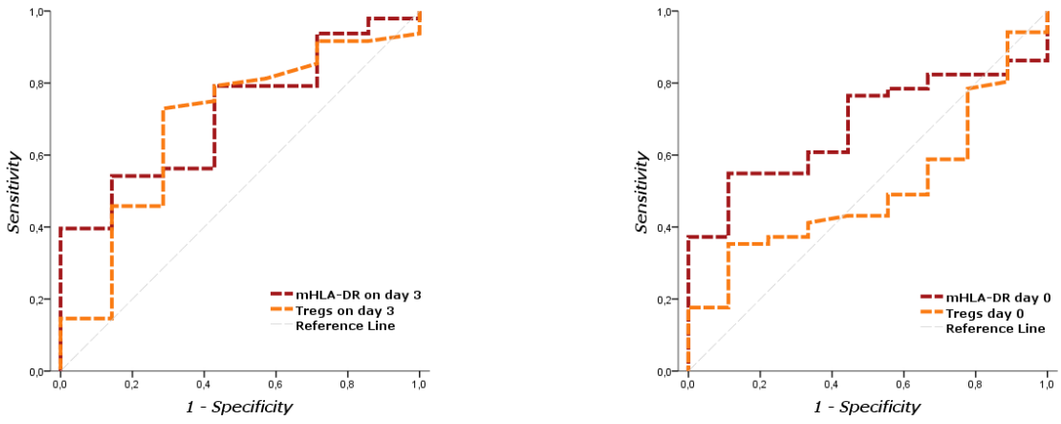
A B

Figure 4. Median levels of Tregs: (A) perioperatively and (B) in patients with cIAIs and healthy controls

Preoperative levels of Tregs failed to discriminate survivors from non-survivors (7.05 % vs 8%, p = 0.992) and the same finding was recorded on the 3rd POD (S = 6.75 % vs NS = 5.2%, p = 0.109) (Table 1). In prediction of survival both pre- and postoperative percentages of Tregs showed no prognostic ability (AUROC = 0.501, p = 0.992 and AUROC = 0.689, p = 0.109) (Table 2) (Figure 5-A,B).

Table 2. Sensitivity, Specificity and AUROCs of perioperative values of mHLA-DR and Tregs

Variable	Cut-off, %	Sensitivity, %	Specificity, %	AUROC	95% CI	
					LB	**UB**
mHLA-DR^{0}	58.15	60.8	66.7	0.682	0.537	0.827
mHLA-DR^{3}	33.65	80.8	57.1	0.720	0.545	0.895
Tregs^{0}	8.2	40.4	66.7	0.501	0.324	0.678
Tregs^{3}	5.7	72.9	71.4	0.689	0.475	0.903



A B

Figure 5. ROC Curves of: (A) preoperative mHLA-DR (n=60) and Tregs (n=61) in prediction of survival; (B) postoperative mHLA-DR (n=59) and Tregs (n=55) in prediction of survival

DISCUSSION

The complicated intra-abdominal infections are still a leading factor in non-traumatic mortality worldwide, and any delay in their treatment usually leads to the development of sepsis and septic shock. In the United States, they occur on average in 9.3 per 1000 hospitalized patients [18] and about 15-20% of surgical patients with acute abdomen are hospitalized with signs of cIAIs. [19] Even nowadays, regardless of the modern surgical techniques, the intensive care and the antimicrobial medications, cIAIs are associated with a high morbidity and mortality [20]. They often lead to sepsis which might evolve into a septic shock with a subsequent multi organ failure and exitus.

The early prognostic assessment of the cIAIs is of vital importance for determining the patient outcome. Prognostic markers in cIAIs can be divided into pro-inflammatory and anti-inflammatory depending on which phase of the immune response to infection they reflect. In the last decades, there was evidence that at first the pro-inflammatory response dominates, which is subsequently replaced by a predominant anti-inflammatory response. Increasingly, there is evidence that such a concept may not be entirely accurate. Both a systemic pro-inflammatory response and a systemic anti-inflammatory response are thought to be present initially, creating the so-called mixed antagonistic response syndrome [21]. The mixed antagonistic response may be a temporary state in the transition from a pro-inflammatory response to an anti-inflammatory response, or a coexistence of predominant inflammation and suppression of innate and adaptive immunity [2]. This is confirmed by the fact that in case of a pronounced pro-inflammatory reaction with high serum concentrations of IL-6 and IL-8, a simultaneous decrease in monocyte HLA-DR expression is observed [22], as and that a positive correlation was found between IL-10 and TNF in patients with septic shock [23].

Decreased expression of HLA-DR on monocytes is a commonly accepted criterion for immunosuppression, as it leads to cellular deactivation, in which monocytes lose their antigen-presenting function and ability for intercellular signaling, this in turn leads to impaired cytokine secretion and impaired maturation to phagocytic cell [9]. Functional defects of circulating monocytes are associated with outcome in patients with septic shock. The expression of HLA-DR on monocytes is currently considered the best biomarker for assessing post-septic immunosuppression, associated with an increased risk of nosocomial infections and increased mortality [24]. Despite the conclusion of our 2019 review article [25] that mHLA-DR could be a reliable and independent prognostic biomarker in patients with cIAIs, we did not find prognostic significance for this marker in the present study. Neither its preoperative values ($p = 0.14$) nor those on the 3rd POD ($p = 0.061$) were able to differentiate deceased from survivors. In contrast to the observed association between decreased expression of mHLA-DR and death in patients with sepsis [26-28], we found that up to the 3rd POD in cIAIs, this biomarker has no ability to predict fatal outcome. However, we found that the presence of cIAIs leads to a significantly decreased expression of HLA-DR, which we established when comparing the values between patients and healthy controls (59.92% vs. 88.7%, $p < 0.0001$). The postoperative expression of mHLA-DR was significantly lower than that preoperatively (59.92% vs. 50.41%, $p = 0.027$), which could be considered as the initial manifestation of the starting dominant anti-inflammatory reaction on postoperative day 3.

Like us, Perry et al. [29] in sepsis ($p = 0.85$) and Skirecki et al. [30] in septic shock ($p = 1.00$) found no predictive power of monocyte HLA-DR expression. A significant prognostic ability of mHLA-DR in patients with sepsis

was reported by Hirsh et al. [31] - died - 21.3% ± 4.7% vs. survived 42.5% ± 7.2% (p = 0.026), Hynninen et al. [8] – 62% in deceased versus 84% in survivors (p = 0.025), Lekkou et al. [32] - died - 23.4% vs survived - 43.1% (p < 0.05), Abe et al. [26] - 44.3% in deceased versus 72.2% in survivors (p < 0.05), Tian et al. [27] - 31.3% in deceased versus 48.6% in survivors (p < 0.01), Wang et al. [28] - 3.41% in deceased versus 43.05% in survivors (p = 0.011) and in patients with septic shock Monneret et al. [24] on days 3 and 4 - 18% in those who died vs. 43% in those who survived (p < 0.001). Only Fu et al. [33] investigated the prognostic performance of mHLA-DR in patients with cIAIs. The authors divided the patients according to their APACHE II score into four groups: mild, moderate, severe and most severe group. The expression of mHLA-DR on the first day in the severe and the most severe groups was 33.45%±3.52% and 27.84%±3.81%, respectively, and the deaths in these two groups were significantly higher than the other groups (p < 0.05).

Regulatory T cells perform a negative immunomodulatory function essential for maintaining peripheral immune tolerance, preventing autoimmune reactions, and limiting inflammatory diseases [34-36]. They show potent anti-inflammatory activity and mediate specialized functions in tissue remodeling [37]. Tregs can affect the pathophysiological mechanisms in sepsis by affecting the innate and acquired immune response by weakening the immune response, causing immunoparalysis and ultimately leading to multiple organ failure and death [38]. In 2009, Venet et al. [16] reported that increased levels of Tregs in peripheral blood are an effective biomarker of immunosuppression in patients with sepsis and associated it with a high risk of adverse outcome. In sepsis, Chen et al. [39] found that decedents had higher levels of peripheral blood Tregs on day 7 of admission than did survivors (5.7% vs. 2.8%, respectively, p = 0.004).

Conversely, Liu et al. [40] in patients with sepsis and septic shock reported that the absolute number of Tregs was higher in survivors than in those who died (31 cells/μL vs. 22 cells/μL, respectively, p = 0.001). Yin et al. [41] found no prognostic value of Tregs in patients with sepsis and septic shock (2.29% in survivors vs. 2.02% in deaths, p = 0.157). In patients with cIAIs, the prognostic qualities of Tregs have not been studied at the moment. Perioperative values of Tregs in the present study demonstrated comparable values (7.3% preoperatively vs. 6.6% at the 3rd POD, p = 0.215), while preoperative levels were significantly higher than those in healthy controls (7.3% vs. 6.55, respectively, p = 0.023), which showed, however, that activation of this type of cells is present in cIAIs. Tregs failed to distinguish patients at high risk of death both preoperatively (8% in deceased vs. 7.05% in survivors, p = 0.992) and postoperatively (5.2% in non-survivors vs. 6.75% in survivors, p = 0.109). These cells are not related to mortality in cIAIs up to 3 days, confirming the observations of several authors that Treg levels up to the third day of sepsis are not associated with fatal outcome [34-36]. The lack of ability to predict death was also demonstrated by the resulting areas under the curves (preoperative AUROC = 0.501, p = 0.992 and postoperative AUROC = 0.689, p = 0.109).

We performed a clinical evaluation of the pro-inflammatory reaction by comparing the frequency of SIRS according to the final outcome. Preoperatively, we recorded SIRS in a similar number of survivors and deaths (56.6% vs. 66.7%, respectively, p = 0.722). The lack of preoperative prognostic ability can be explained by the adequate treatment approach and the successful removal of the infectious source. The correct therapeutic strategy can also be judged by a reduction in the frequency of the systemic inflammatory reaction postoperatively. Before the operative intervention, SIRS was reported in more than half of the patients (58.1%), while on the 3rd POD was found in only five (8.3%). The excellent ability (p < 0.0001) of the postoperative SIRS to differentiate patients according to the outcome (its presence was found in 57.1% of non-survivors and only in 1.9% of the survivors) confirmed our observations that the persistence of a systemic pro-inflammatory response postoperatively is the main cause of occurrence of the lethal outcome in the study population.

As limitations of this study we can highlight the small sample size, the single center experience and the short postoperative period for measuring the investigated biomarkers, respectively the short follow-up of the influence of the immune response on the final outcome.

CONCLUSION

Even if the anti-inflammatory reaction occurs together with the pro-inflammatory reaction early in the systemic immune response (according to current concepts), we established that it is not responsible for mortality in early phase of complicated intra-abdominal infection.

Funding: The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Conflict of interest/Competing interests: The authors declare no conflicts of interest or competing interests.

REFERENCES

1. Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, Moreno R, Lipman J, Gomersall C, Sakr Y, Reinhart K; EPIC II Group of Investigators. International study of the prevalence and outcomes of infection in intensive care units. JAMA. 2009 Dec 2;302(21):2323-9. doi: 10.1001/jama.2009.1754. PMID: 19952319.
2. Novotny AR, Reim D, Assfalg V, Altmayr F, Friess HM, Emmanuel K, Holzmann B. Mixed antagonist response and sepsis severity-dependent dysbalance of pro- and anti-inflammatory responses at the on-

set of postoperative sepsis. Immunobiology. 2012 Jun;217(6):616-21. doi: 10.1016/j.imbio.2011.10.019. Epub 2011 Nov 3. PMID: 22204813.
3. van der Poll T, van de Veerdonk FL, Scicluna BP, Netea MG. The immunopathology of sepsis and potential therapeutic targets. Nat Rev Immunol. 2017 Jul;17(7):407-420. doi: 10.1038/nri.2017.36. Epub 2017 Apr 24. PMID: 28436424.
4. Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. Nat Rev Immunol. 2013 Dec;13(12):862-74. doi: 10.1038/nri3552. Epub 2013 Nov 15. PMID: 24232462; PMCID: PMC4077177.
5. Frencken JF, van Vught LA, Ong DS, Klein Klouwenberg PMC, Horn J, Bonten MJM, van der Poll T, Cremer OL. A high anti-inflammatory response is associated with intermediate-term mortality in patients with sepsis. Intensive Care Med Exp. 2015 Oct 1;3(Suppl 1):A79. doi: 10.1186/2197-425X-3-S1-A79. PMCID: PMC4798235.
6. Monneret G, Venet F. Monocyte HLA-DR in sepsis: shall we stop following the flow? Crit Care. 2014 Jan 6;18(1):102. doi: 10.1186/cc13179. PMID: 24393356; PMCID: PMC4056426.
7. Döcke WD, Randow F, Syrbe U, Krausch D, Asadullah K, Reinke P, Volk HD, Kox W. Monocyte deactivation in septic patients: restoration by IFN-gamma treatment. Nat Med. 1997 Jun;3(6):678-81. doi: 10.1038/nm0697-678. PMID: 9176497.
8. Hynninen M, Pettilä V, Takkunen O, Orko R, Jansson SE, Kuusela P, Renkonen R, Valtonen M. Predictive value of monocyte histocompatibility leukocyte antigen-DR expression and plasma interleukin-4 and -10 levels in critically ill patients with sepsis. Shock. 2003 Jul;20(1):1-4. doi: 10.1097/01.shk.0000068322.08268.b4. PMID: 12813360.
9. Venet F, Lukaszewicz AC, Payen D, Hotchkiss R, Monneret G. Monitoring the immune response in sepsis: a rational approach to administration of immunoadjuvant therapies. Curr Opin Immunol. 2013 Aug;25(4):477-83. doi: 10.1016/j.coi.2013.05.006. Epub 2013 May 28. PMID: 23725873; PMCID: PMC3931134.
10. Genel F, Atlihan F, Ozsu E, Ozbek E. Monocyte HLA-DR expression as predictor of poor outcome in neonates with late onset neonatal sepsis. J Infect. 2010 Mar;60(3):224-8. doi: 10.1016/j.jinf.2009.12.004. Epub 2009 Dec 16. PMID: 20025903.
11. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature. 2006 May 11;441(7090):235-8. doi: 10.1038/nature04753. Epub 2006 Apr 30. PMID: 16648838.
12. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol. 2003 Apr;4(4):330-6. doi: 10.1038/ni904. Epub 2003 Mar 3. PMID: 12612578.
13. Seddiki N, Santner-Nanan B, Martinson J, Zaunders J, Sasson S, Landay A, Solomon M, Selby W, Alexander SI, Nanan R, Kelleher A, Fazekas de St Groth B. Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. J Exp Med. 2006 Jul 10;203(7):1693-700. doi: 10.1084/jem.20060468. Epub 2006 Jul 3. PMID: 16818676; PMCID: PMC2118333.
14. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell. 2008 May 30;133(5):775-87. doi: 10.1016/j.cell.2008.05.009. PMID: 18510923.
15. Cao C, Ma T, Chai YF, Shou ST. The role of regulatory T cells in immune dysfunction during sepsis. World J Emerg Med. 2015;6(1):5-9. doi: 10.5847/wjem.j.1920-8642.2015.01.001. PMID: 25802559; PMCID: PMC4369532.
16. Venet F, Chung CS, Kherouf H, Geeraert A, Malcus C, Poitevin F, Bohé J, Lepape A, Ayala A, Monneret G. Increased circulating regulatory T cells (CD4(+) CD25 (+)CD127 (-)) contribute to lymphocyte anergy in septic shock patients. Intensive Care Med. 2009 Apr;35(4):678-86. doi: 10.1007/s00134-008-1337-8. Epub 2008 Oct 23. PMID: 18946659; PMCID: PMC2789433.
17. Sartelli M. A focus on intra-abdominal infections. World J Emerg Surg. 2010 Mar 19;5:9. doi: 10.1186/1749-7922-5-9. PMID: 20302628; PMCID: PMC2848006.
18. Anaya DA, Nathens AB. Risk factors for severe sepsis in secondary peritonitis. Surg Infect (Larchmt). 2003 Winter;4(4):355-62. doi: 10.1089/109629603322761418. PMID: 15012862.
19. Saveliev VS, Gelfand BR. Abdominal surgical infection. National recommendations. Moscow; „Borges“, 2011: 98 p., ;
20. Sartelli M, Viale P, Catena F, Ansaloni L, Moore E, Malangoni M, Moore FA, Velmahos G, Coimbra R, Ivatury R, Peitzman A, Koike K, Leppaniemi A, Biffl W, Burlew CC, Balogh ZJ, Boffard K, Bendinelli C, Gupta S, Kluger Y, Agresta F, Di Saverio S, Wani I, Escalona A, Ordonez C, Fraga GP, Junior GA, Bala M, Cui Y, Marwah S, Sakakushev B, Kong V, Naidoo N, Ahmed A, Abbas A, Guercioni G, Vettoretto N, Díaz-Nieto R, Gerych I, Tranà C, Faro MP, Yuan KC, Kok KY, Mefire AC, Lee JG, Hong SK, Ghnnam W, Siribumrungwong B, Sato N, Murata K, Irahara T, Coccolini F, Segovia Lohse HA, Verni A, Shoko T. 2013 WSES guidelines for management of intra-abdominal infections. World J Emerg Surg. 2013 Jan 8;8(1):3. doi: 10.1186/1749-7922-8-3. PMID: 23294512; PMCID: PMC3545734.
21. Sikora JP, Karawani J, Sobczak J. Neutrophils and the Systemic Inflammatory Response Syndrome (SIRS). Int J Mol Sci. 2023 Aug 30;24(17):13469. doi: 10.3390/ijms241713469. PMID: 37686271; PMCID: PMC10488036.
22. Tschaikowsky K, Hedwig-Geissing M, Schiele A, Bremer F, Schywalsky M, Schüttler J. Coincidence of pro- and anti-inflammatory responses in the early phase of severe sepsis: Longitudinal study of mononuclear histocompatibility leukocyte antigen-DR expression, procalcitonin, C-reactive protein, and changes in T-cell subsets in septic and postoperative patients. Crit Care Med. 2002 May;30(5):1015-23. doi: 10.1097/00003246-200205000-00010. PMID: 12006796.

23. Marchant A, Alegre ML, Hakim A, Piérard G, Maréchaux G, Friedman G, De Groote D, Kahn RJ, Vincent JL, Goldman M. Clinical and biological significance of interleukin-10 plasma levels in patients with septic shock. J Clin Immunol. 1995 Sep;15(5):266-73. doi: 10.1007/BF01540884. PMID: 8537471.
24. Monneret G, Lepape A, Voirin N, Bohé J, Venet F, Debard AL, Thizy H, Bienvenu J, Gueyffier F, Vanhems P. Persisting low monocyte human leukocyte antigen-DR expression predicts mortality in septic shock. Intensive Care Med. 2006 Aug;32(8):1175-83. doi: 10.1007/s00134-006-0204-8. Epub 2006 Jun 2. PMID: 16741700.
25. Dimitrov E, Enchev E, Minkov G, Halacheva K, Yovtchev Y. Poor Outcome Could Be Predicted by Lower Monocyte Human Leukocyte Antigen-DR Expression in Patients with Complicated Intra-Abdominal Infections: A Review. Surg Infect (Larchmt). 2020 Mar;21(2):77-80. doi: 10.1089/sur.2019.050. Epub 2019 Sep 4. PMID: 31483200.
26. Abe R, Hirasawa H, Oda S, Sadahiro T, Nakamura M, Watanabe E, Nakada TA, Hatano M, Tokuhisa T. Up-regulation of interleukin-10 mRNA expression in peripheral leukocytes predicts poor outcome and diminished human leukocyte antigen-DR expression on monocytes in septic patients. J Surg Res. 2008 Jun 1;147(1):1-8. doi: 10.1016/j.jss.2007.07.009. Epub 2007 Aug 20. PMID: 17720196.
27. Tian Y, Tao T, Zhu J, Zou Y, Wang J, Li J, Bo L, Deng X. Soluble tumor necrosis factor related apoptosis inducing ligand level as a predictor of severity of sepsis and the risk of mortality in septic patients. PL0S One. 2013 Dec 12;8(12):e82204. doi: 10.1371/journal.pone.0082204. PMID: 24349222; PMCID: PMC3861352.
28. Wang JF, Li L, Shi S. Expression of monocyte HLADR and blood lactic acid level in patients with sepsis and correlation with prognosis. Int J Clin Exp Med 2018;11:4905–4911.334.
29. Perry SE, Mostafa SM, Wenstone R, Shenkin A, McLaughlin PJ. Is low monocyte HLA-DR expression helpful to predict outcome in severe sepsis? Intensive Care Med. 2003 Aug;29(8):1245-52. doi: 10.1007/s00134-003-1686-2. Epub 2003 May 28. PMID: 12774155.
30. Skirecki T, Mikaszewska-Sokolewicz M, Hoser G, Zielińska-Borkowska U. The Early Expression of HLA-DR and CD64 Myeloid Markers Is Specifically Compartmentalized in the Blood and Lungs of Patients with Septic Shock. Mediators Inflamm. 2016;2016:3074902. doi: 10.1155/2016/3074902. Epub 2016 Jun 19. PMID: 27413252; PMCID: PMC4930815.
31. Hirsh M, Mahamid E, Bashenko Y, Hirsh I, Krausz MM. Overexpression of the high-affinity Fcgamma receptor (CD64) is associated with leukocyte dysfunction in sepsis. Shock. 2001 Aug;16(2):102-8. doi: 10.1097/00024382-200116020-00003. PMID: 11508860.
32. Lekkou A, Karakantza M, Mouzaki A, Kalfarentzos F, Gogos CA. Cytokine production and monocyte HLA-DR expression as predictors of outcome for patients with community-acquired severe infections. Clin Diagn Lab Immunol. 2004 Jan;11(1):161-7. doi: 10.1128/cdli.11.1.161-167.2004. PMID: 14715564; PMCID: PMC321326.
33. Fu Q, Cui NQ, Yu WL. [The relationship between immune imbalance and intestinal barrier dysfunction in patients with severe intra-abdominal infections]. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue. 2009 Jul;21(7):429-33. Chinese. PMID: 19615138.
34. Cortez JT, Montauti E, Shifrut E, Gatchalian J, Zhang Y, Shaked O, Xu Y, Roth TL, Simeonov DR, Zhang Y, Chen S, Li Z, Woo JM, Ho J, Vogel IA, Prator GY, Zhang B, Lee Y, Sun Z, Ifergan I, Van Gool F, Hargreaves DC, Bluestone JA, Marson A, Fang D. CRISPR screen in regulatory T cells reveals modulators of Foxp3. Nature. 2020 Jun;582(7812):416-420. doi: 10.1038/s41586-020-2246-4. Epub 2020 Apr 29. PMID: 32499641; PMCID: PMC7305989.
35. Gao Y, Wang C, Wang Z, Li W, Liu Y, Shou S, Chai Y. Semaphorin 3A contributes to sepsisinduced immunosuppression by impairing CD4+ T cell anergy. Mol Med Rep. 2021 Apr;23(4):302. doi: 10.3892/mmr.2021.11941. Epub 2021 Mar 2. PMID: 33649856; PMCID: PMC7930987.
36. von Knethen A, Heinicke U, Weigert A, Zacharowski K, Brüne B. Histone Deacetylation Inhibitors as Modulators of Regulatory T Cells. Int J Mol Sci. 2020 Mar 29;21(7):2356. doi: 10.3390/ijms21072356. PMID: 32235291; PMCID: PMC7177531.
37. Panduro M, Benoist C, Mathis D. Tissue Tregs. Annu Rev Immunol. 2016 May 20;34:609-33. doi: 10.1146/annurev-immunol-032712-095948. PMID: 27168246; PMCID: PMC4942112.
38. Yao RQ, Ren C, Wang JN, Wu GS, Zhu XM, Xia ZF, Yao YM. Publication Trends of Research on Sepsis and Host Immune Response during 1999-2019: A 20-year Bibliometric Analysis. Int J Biol Sci. 2020 Jan 1;16(1):27-37. doi: 10.7150/ijbs.37496. PMID: 31892843; PMCID: PMC6930382.
39. Chen K, Zhou QX, Shan HW, Li WF, Lin ZF. Prognostic value of CD4(+)CD25(+) Tregs as a valuable biomarker for patients with sepsis in ICU. World J Emerg Med. 2015;6(1):40-3. doi: 10.5847/wjem.j.1920-8642.2015.01.007. PMID: 25802565; PMCID: PMC4369529.
40. Liu Q, An L, Qi Z, Zhao Y, Li C. Increased Expression of Programmed Cell Death-1 in Regulatory T Cells of Patients with Severe Sepsis and Septic Shock: An Observational Clinical Study. Scand J Immunol. 2017 Nov;86(5):408-417. doi: 10.1111/sji.12612. Epub 2017 Oct 5. PMID: 28888058.
41. Yin F, Xi YL, Wang Y, Li BR, Qian J, Ren H, Zhang J, Tang HZ, Ning BT. The clinical outcomes and biomarker features of severe sepsis/septic shock with severe neutropenia: a retrospective cohort study. Transl Pediatr. 2021 Mar;10(3):464-473. doi: 10.21037/tp-20-230. PMID: 33850805; PMCID: PMC8039791.

Scan this QR code with your mobile device for instant access to the current Issue of Acta Medica Saliniana