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Article type : Original

Inflammatory events during Food Protein-Induced Enterocolitis Syndrome reactions.

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Running title: FPIES reaction: inflammatory events.

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/pai.12723

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Food Protein-Induced Enterocolitis Syndrome (FPIES) is not an “allergic disease”.

Pediatr Allergy Immunol.

Background: An increasing number of infants are diagnosed with food protein induced enterocolitis syndrome (FPIES), a non-IgE-mediated food allergy. Until now, T-cell, food-specific mechanisms have been hypothesized.

Methods: 16 children (11M, 5F), affected by FPIES from cow's milk, wheat, fruit, rice and others, experienced 25 acute episodes managed at our Emergency Department (ED) and eight FPIES reactions during oral food challenges (OFC). We compared the laboratory data in resting conditions, in absence of infectious diseases, with data collected during the 25 acute ED episodes (blood samples drawn at 2 to 12 hours) and the eight positive OFCs (three samples at 2, 6 and 12 hours). The onset of symptoms was used as a reference time-point.

Results: In basal conditions, total IgE, WBC, neutrophil and eosinophil count, CRP, and SGPT were found normal. LDH and SGOT values were high (627.81 ± 97.88 and 45.75 ± 10.26 UI/L, respectively). During ED reactions LDH and SGOT increased to 794.21 ± 247.28 ($p=0.028$) and 51.08 ± 16.99 UI/L ($p=0.14$), neutrophils count and CRP to $8.44 \pm 3.82 \times 10^3/\mu\text{L}$ ($p=0.0009$) and 3.27 ± 5.73 mg/dL ($p=0.0014$) respectively. During positive OFC, LDH and SGOT did not vary significantly; CRP increased from 0.14 ± 0.18 to 2.49 ± 3.65 mg/dL ($p=0.00189$) and neutrophil count from 2.79 ± 1.42 to $7.10 \pm 3.98 \times 10^3/\mu\text{L}$ ($p=0.00096$).

Conclusions: FPIES reactions are characterized by neutrophilia and by a time-dependent, significant increase of CRP, indicating that inflammatory mechanisms are in place. This suggests new directions for research on FPIES pathogenesis.

Key words: C-reactive protein, food protein induced enterocolitis syndrome, inflammatory mechanisms, T-cell food-specific mechanisms.

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INTRODUCTION

Food Protein-Induced Enterocolitis Syndrome (FPIES) is increasingly diagnosed among infants¹, despite the problematic differential diagnosis² and the limited awareness among pediatricians³. This food-allergic disorder is characterized by profuse, repetitive emesis, lethargy and pallor accompanied or followed by watery/bloody diarrhea after the ingestion of specific foods. In severe cases, hypothermia, acidemia and hypotension/hypovolemic shock may ensue, showing several similarities to a septic state. Although triggered by a specific food, in FPIES food-specific IgE could be rarely found. No cutaneous or respiratory involvement is described.

The immune mechanism remains elusive. FPIES may shift to IgE-mediated food allergy (FA) and vice versa⁴. Eosinophils do not seem to play a central role in this disease, so the involvement of T cells, in particular Th1, has been investigated⁵. Indeed, white blood counts with neutrophilia, thrombocytosis, metabolic acidosis, and methemoglobinemia characterize a severe acute FPIES reaction⁶. TNF- α increases and higher expression of HLA-DR in dendritic cells have been observed⁷. A comprehensive analysis of T-cell cytokine

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responses in children with non-IgE-mediated gastrointestinal allergies to milk (FPIES, enteropathy and proctocolitis) found an increased expression of inflammatory (TNF- α), monocyte (IL-10), eosinophil (IL-5), T-cells (IL-2, IL-6), and Th2 (IL-13) cytokines. In FPIES triggered by cow's milk, CD4⁺ T cell-proliferative response and Th2 cytokines production after casein stimulation were found to be similar to those of control subjects, while after positive oral food challenges (OFCs) higher levels of IL-10 and of IL-8 have been recently reported⁸. A profound activation of cells of the innate immune system after food challenge, including NK-cells, has been found⁹. We investigated inflammatory markers in a series of well-characterized acute FPIES reactions, hypothesizing that FPIES may be associated with features typical of acute inflammatory reactions.

METHODS

Patients and institutional protocol.

From January 2013 to June 2016, infants diagnosed with acute FPIES at our center were followed according to the institutional protocol. Standardized criteria¹⁰ were used and the food reactions were classified in mild-moderate, severe e very severe (Table 1) according to the FPIES guidelines¹¹.

After an allergy, dietary, nutritional and growth evaluation, the families of FPIES children were instructed to avoid the offending food(s). Breastfeeding was encouraged. Educational material regarding allowed and prohibited food(s), challenge procedures, and management of accidental exposure were widely explained. The parents living near Rome were urged to bring their infants to our Emergency Room in case of acute FPIES reactions resulting from a fortuitous ingestion. Follow-up visits were planned on three-monthly basis (± 14 days). OFCs with the offending food were scheduled at yearly intervals and anticipated only if no symptoms were evoked by an accidental consumption. Considering the chance that FPIES may be determined by multiple food allergens, solid food associated to FPIES have

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been hired for the first time under medical supervision during an oral challenge. The patients who experienced acute Emergency Department (ED) reactions were managed and monitored for 24 hours. Blood samples were drawn in resting conditions, during acute ED reactions and positive OFCs (Table 2). . Written informed consent was obtained from all procedures performed.

Allergy evaluation

Skin prick tests (SPTs) for the triggering food allergen/s were performed by standard technique¹² during the follow-up visits and shortly before starting the OFC. Serum specific IgE to the trigger food allergen(s) were determined using a fluorescent enzyme immunoassay (ImmunoCAP System - Thermo Scientific, Uppsala, Sweden) according to the manufacturer's instructions with a positive cut-off point set at 0.1 kU_A/L.

Oral food challenge (OFC)

The challenge procedure was performed according to international guidelines¹³ and an intravenous access was always secured. Fresh foods were administered at a dose of 0.3 grams of the food protein per kilogram of body weight in three equal doses over 30 minutes, generally not to exceed a total of 3 grams of protein or 10 grams of total food (100 mL of liquid for milk) for an initial feeding. Only reactions to OFC occurring within six hours are reported.

Statistical analysis

Descriptive data are shown as means (SD) or number of observations, with 95% confidence intervals. Since the blood values in each group were normally distributed, comparisons of continuous variables among groups were performed by one-way ANOVA. The non-parametric Wilcoxon test was used to compare continuous variables within single groups. The non-parametric randomized block analysis of variance (Friedman's test) was used to compare repeated variables during challenge-induced reactions. Comparisons of

discrete variables were performed by the chi square test or Fisher's exact test, as appropriate. P values ≤ 0.05 were considered to indicate statistical significance (2-tailed tests). The SPSS 14.0 Package for Windows (SPSS Inc., Chicago, IL) was used for the statistical analysis.

RESULTS

16 children (11M, 5F) diagnosed with FPIES entered in our institutional follow-up protocol. Before and during the follow-up, 66 reactions were reported to different offending foods including cow's milk, wheat, fruit, rice and others. Of these 66 reactions, 25 episodes were documented at our Pediatric Emergency Unit and eight developed during a diagnostic OFC. The clinical characteristics of the 16 patients and the type of reactions are reported in Table 3.

Laboratory features in resting conditions

In resting conditions, the mean values of white blood cells (WBC), neutrophil and platelets count, CRP, and SGPT fell in the normal range for our lab. LDH and SGOT values were on average high (627.81 ± 97.88 and 45.75 ± 10.26 UI/L, as reported in table 4), exceeding the normal range in 68.7% and 75.0% of subjects enrolled, respectively. Positive SPT and sIgE determination to beta-lactoglobulin were observed in two patients without suggestive clinical history. Two patients showed an IgE-mediated allergy to different trigger foods.

Laboratory features during ED reactions

Fourteen patients experienced 25 acute reactions managed in our Pediatric Emergency Unit (Table 3). Six of them had one reaction, five were observed during two reactions and three on three occasions. Their intensity was variable: mild in one case, mild-moderate in 14 cases from 11 patients, severe in 10 reactions from six patients. Three patients displayed reactions of various severity (mild-moderate end severe). Blood samples were drawn at a median of 5 hours (range: 2-12) after the onset of symptoms.

The mean values of LDH and SGOT exceeded the normal range in 73.6% and 76% respectively. Compared to resting condition, LDH values significantly increased ($p=0.0280$), while SGOT levels improved without a statistical significance ($p=0.1401$) (Table 4). The mean number of neutrophils and platelets, within normal range in baseline conditions, also exceeded the upper limit in more than half of reactions. Peculiarly, over four-fifths (82.6%) of acute episodes showed an abnormal CRP mean level, with normal values observed only in four mild reactions (blood samples collected at 2-3 hours from the onset of symptoms). Statistically significant increases in absolute neutrophil count ($p=0.0009$) and CRP levels ($p=0.0014$) compared to baseline were observed.

Reactions during follow-up challenges

Six children experienced eight reactions during the follow-up challenges (three during a re-challenge with the original offending food, five during a challenge with a new food). Four reactions were mild-to-moderate, two severe and two very severe. Symptoms began 0.8-4 hours after the completion of the challenge procedure. Blood samples were collected before starting the OFC and at 2, 6 and 12 hours from the onset of clinical manifestations. The time-trend showed no rise in tissue enzymes. WBC increased significantly (Fig. 1; Tab. 4). A very significant rise of neutrophils (ANOVA Chi Square=16.35, $p=0.00096$) and particularly of CRP (ANOVA Chi Square=14.92, $p=0.00189$) was observed, peaking at 6 and 12 hours respectively (Fig. 1, Tab. 4). The rise in CRP became evident 6 hours after the onset of symptoms and this is in line with its kinetics¹⁴.

Parallel to these findings, a modest, yet significant, decrease in red blood cell number (ANOVA Chi Square= 10.5, $p=0.01476$) was observed.

Severity of the reactions and laboratory data

Altogether, thirty-three FPIES reactions were observed, seven of which mild-moderate, 14 severe and 12 very severe. The laboratory characteristics of reactions of different severity using the 12-hours' time-point for the follow-up reactions were compared (Table 5). CRP levels significantly correlated with the degree of reaction severity ($p=0.007$).

DISCUSSION

The increasing frequency of diagnosis, with many new case series reported worldwide¹⁵, makes the pathogenetic interpretation of FPIES of importance for clinicians³. FPIES is the only non-IgE-mediated food allergy classified as “immediate”^{16,17}. The time-trend of its reactions is hardly compatible with a T-cell-mediated event. To date, the only food reactions that typically take place 3-6 hours after the food ingestion are the delayed anaphylactic reactions to red meat related to serum IgE antibodies to the oligosaccharide galactose-alpha-1,3-galactose (alpha-gal). These reactions are clearly different from FPIES for clinical characteristics, age of patients, outcomes and eliciting allergens¹⁸, and a different mechanism should be involved.

Although an increase in neutrophils after food exposure is included in the diagnostic criteria of FPIES, the increase in serum CRP level has not been noted in any of the case series published since 1960. Only recently, some reports from Japan suggested that CRP may increase after oral food challenge in some patients with ‘sepsis-like’ milk-induced FPIES¹⁹. The authors hypothesize that CRP is witness of the FPIES reaction, drawing a comparison with contact dermatitis. More precisely, “a systemic absorption of antigens, which customarily acts only at organ level causing T cell-mediated hypersensitivity, may systemically activate CRP as part of an immunologic reaction”²⁰. The execution timing of the blood sample for CRP determination is decisive and influences its assessment. In fact, Hwang

JB and colleagues²¹ did not find a statistically significant difference in CRP levels after positive OFCs in patients affected by cow's milk protein-induced enterocolitis, but in this study the blood sample was drawn 6 hours after the end of challenge and not after the onset of symptoms.

Our data for the first time provide evidence that CRP systematically increases in the first 6 hours after an FPIES reaction, in parallel with neutrophilia. Concurrently, we also observed increased levels of LDH and SGOT in almost all patients in resting conditions, suggesting the presence of some preexisting intestinal cellular damage^{22,23}. More strikingly, FPIES acute food reactions are characterized by neutrophilia and by a time-dependent, significant increase of CRP not only in severe cases, but – to a lesser extent – in all cases.

The dynamics of these changes during the FPIES reactions confirm that the food-related event happens in a limited time, soon after the transit of foods through pylorus. This time-course mirrors what occurs in food-borne infectious diseases. Indeed, also in these cases initial symptoms including nausea, abdominal cramps, vomiting and non-bloody watery diarrhea, appear within some hours after ingestion of the contaminated food or water²⁴. These data suggest that the FPIES food reaction could resemble an innate response to a bacterial infection, allowing the hypothesis that abnormalities of the host innate immune response could induce a misrecognition of specific foods in FPIES children⁹.

The acute decrease in red blood cell number, despite the dehydration, may in turn be interpreted as a typical consequence of an acute inflammatory response. Indeed, inflammatory cytokines, in particular IL-6, recently reported elevated following positive OFC in FPIES⁸, are well known to increase the release of ADH and to decrease the hematocrit, for hemodilution secondary to increased plasma volume.

This study has clear limitations. Since this is a case series including acute ED reactions, it was not possible to obtain the time-course of the relevant inflammatory parameters in all cases, but only in those following hospital challenges. We also have chosen to pool all the reactions, including those taking place in the same children. Nevertheless, our results appear to have some implications.

First, we show here that severe reactions may occur at the first introduction of specific solid foods. Although children with FPIES tolerate the bulk of food, the risk of reactions at home - with possible severe hypotension - justifies a cautious approach to their introduction.

Second, the gradual administration of food doses²⁵ during the OFC, what happens in IgE-mediated food allergy, does not seem justified in this case. In fact, the FPIES reaction always develops after the last dose administered, therefore the OFC can be performed by one or two doses.

Third, in clinical practice the timing of sampling is critical to interpret the reaction. CRP becomes abnormal only after a few hours, indicating that a FPIES reaction to a food ingested two hours ago may not be so evident at hematologic evaluation.

Fourth, our data confirm that there is a range of severity in FPIES reactions, some more severe, other milder. This finding, already widely described in the literature, raises several questions. For example, do milder reactions imply a better prognosis as to the duration of the disease, as is the case in the IgE-mediated allergy²⁶? Do they carry a lower risk of developing reactions at the introduction of new foods? Most importantly, have they the same biological substrate? In other words, is FPIES a unique disease or it is a syndrome with different causes/mechanisms?

CONCLUSIONS

Inflammatory events play an important role in the pathogenesis of FPIES. Although food-specific, a possible role emerges for basic mechanisms of innate immunity. To clarify the nature of the process, studies should focus on the early events after food ingestion, with particular regard to cytokines and chemokines typically induced at early times during an innate immune response that may allow unraveling a spectrum of different genetic-epigenetic-immunologic abnormalities.

Table 1 – Diagnostic criteria and classification of FPIES reactions experienced by our patients.

<i>Diagnostic criteria</i>	
Major criterion	vomiting one to four hours after ingestion of a specific food in an infant without IgE-mediated allergic skin or respiratory symptoms.
Minor criteria	- other episodes of repetitive vomiting after eating the same suspect food - similar episodes after eating a different food - extreme lethargy with any suspected reaction - remarkable pallor with any suspected reaction - emergency room visit with at least one suspected reaction - need for intravenous fluid support with any suspected episode - diarrhea in the following 24 hours
<i>Classification of FPIES reactions</i>	
Mild-moderate	- only vomiting - repetitive vomiting with less than three episodes of emesis, pallor and/or decreased activity level and/or water diarrhea
Severe	- repetitive vomiting with more than three episodes of emesis, pallor, lethargy, dehydration
Very severe	- repetitive vomiting with more than three episodes of emesis, pallor, lethargy, dehydration, cyanotic appearance and water/bloody diarrhea

Table 2 – Blood sampling in resting conditions and during acute Emergency Department reactions or positive oral food challenges.

<i>Acute Emergency Department reactions</i>	<i>Positive Oral Food Challenges</i>
Resting condition Baseline blood samples collected from one to a maximum of 30 days before the acute episode during a follow-up visit.* - Complete blood count (CBC) with differential - C-reactive protein (CRP) - Serum glutamic oxaloacetic transaminase (SGOT) - Serum glutamic pyruvic transaminase (SGPT) - Lactate dehydrogenase (LDH) - IgE antibodies specific for trigger food allergen(s)	Resting condition Baseline blood samples collected 1 hour before starting the challenge test. - Complete blood count (CBC) with differential - C-reactive protein (CRP) - Serum glutamic oxaloacetic transaminase (SGOT) - Serum glutamic pyruvic transaminase (SGPT) - Lactate dehydrogenase (LDH) - IgE antibodies specific for trigger food allergen(s)
Acute FPIES reaction Depending on arrival at the Emergency Room 1 or 2 blood samples were collected. - Arriving within 6 hours from the onset of symptoms, two blood samples were drawn (the first upon arrival at the Emergency Room and the second after 12 hours from the onset of symptoms); - Arriving after 6 hours from the onset of symptoms, a single blood sample was drawn (at 12 hours from the onset of symptoms).	Acute FPIES reaction During the acute reaction 3 blood samples were collected at 2, 6 and 12 hours from the onset of clinical manifestations.
- Complete blood count (CBC) with differential - C-reactive protein (CRP) - Serum glutamic oxaloacetic transaminase (SGOT) - Serum glutamic pyruvic transaminase (SGPT) - Lactate dehydrogenase (LDH)	- Complete blood count (CBC) with differential - C-reactive protein (CRP) - Serum glutamic oxaloacetic transaminase (SGOT) - Serum glutamic pyruvic transaminase (SGPT) - Lactate dehydrogenase (LDH)

* During the follow up visit infectious diseases were excluded.

Table 3 – Demographics and clinical characteristics of FPIES patients.

No. of patients	16	
Sex (%)		
Male	11	(68.8%)
Female	5	(31.2%)
Route of delivery (%)		
Vaginal delivery	6	(37.5%)
Dystocic delivery	2	(12.5%)
Cesarean section	8	(50%)
Age (months) at first reaction		
Mean (SD)/median	4.1	(2.9)/4
Range	0,5-12	
Age (months) at diagnosis		
Mean (SD)/median	13	(13.6)/7.5
Range	2-48	
Delay (months) between first reaction and diagnosis		
Mean (SD)/median	8.9	(12.3)/4.25
Range	1-38	
No. of episodes before diagnosis for each patient		
Mean (SD)	2.85	(1.68)
Range	1-10	
Offending foods, n (%)	All reactions (n. 66)	
Cow's milk	24	(36.4%) 2 after OFC
Wheat	13	(19.7%) 3 after OFC
Fruit	10	(15.1%)
Rice	8	(12.2%)
Fish	4	(6,1%)
Beef	3	(4.5%) 1 after OFC
Chicken	1	(1.5%)
Turkey	1	(1.5%) after OFC
Soy	1	(1.5%)
Flounder	1	(1.5%) after OFC
No. of patients with single or multiple incriminated foods (%)		
Single food	10	(62.5%)
Multiple foods	6	(37.5%)
Additional atopic conditions (No of patients)		
Atopic dermatitis	6	
Allergic rhinitis/asthma	1	
IgE-mediated food allergies	2	
Time (hours) to onset of symptoms in the 66 reactions		
Mean (SD)/median	1.88	(0.55)/2
Range	0.8-4	
Type of food reactions (%)		
Mild-moderate	18	(27.3%)
Severe	28	(42,4)
Very severe	20	(30.3%)
Characteristics of the 25 reactions observed at our ER		
Time (hours) to onset of symptoms		
Mean (SD)/median	1.74	(0.48)/2
Range	1-3	
Type of food reaction (%)		
Mild-moderate	3	(12%)
Severe	12	(48%)
Very severe	10	(40%)
Characteristics of the 8 reactions observed during OFC		
Time (hours) to onset of symptoms		
Median	1.5	
Range	0.8-4	
Type of food reaction (%)		
Mild-moderate	4	(50%)
Severe	2	(25%)
Very severe	2	(25%)

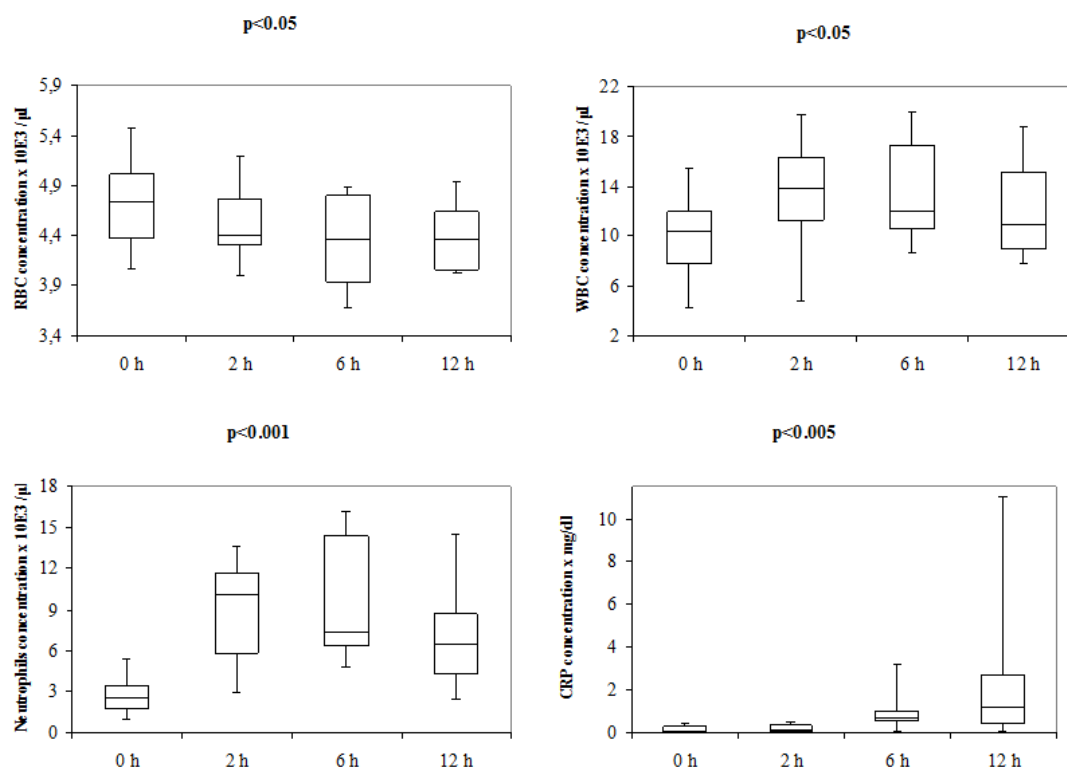
Table 4 – Blood values (mean $\pm$ SD) in basal conditions and during a spontaneous (column 4) or challenge-induced (columns 7-9) acute FPIES reaction.

Parameter	Normal range	FPIES basal condition (16 patients) mean $\pm$ SD	FPIES during ED reaction (25 cases) mean $\pm$ SD	Wilcoxon test p-value	FPIES oral food challenge (8 cases)				
					Baseline mean $\pm$ SD	2 hours mean $\pm$ SD	6 hours mean $\pm$ SD	12 hours mean $\pm$ SD	Friedman's test p-value
RBC	3.2 - 4.8 $\times 10^3$ / μ L	4.74 $\pm$ 0.59	4.04 $\pm$ 0.49	0.0035	4.71 $\pm$ 0.47	4.52 $\pm$ 0.38	4.34 $\pm$ 0.49	4.39 $\pm$ 0.35	0.01476
WBC	6 - 17.5 $\times 10^3$ / μ L	10.95 $\pm$ 0.56	15.38 $\pm$ 6.24	0.0691	10.09 $\pm$ 3.59	13.56 $\pm$ 4.84	13.56 $\pm$ 4.06	12.04 $\pm$ 4.03	0.0180
Neutrophils	1.08 - 8.4 $\times 10^3$ / μ L	3.67 $\pm$ 1.51	8.44 $\pm$ 3.82	0.0009	2.79 $\pm$ 1.42	9.01 $\pm$ 3.95	9.65 $\pm$ 4.66	7.10 $\pm$ 3.98	0.00096
Platelets	150–450 $\times 10^3$ / μ L	340.43 $\pm$ 137.64	447.79 $\pm$ 177.76	0.2329	330.75 $\pm$ 34.63	318.0 $\pm$ 65.43	296.12 $\pm$ 66.99	310.37 $\pm$ 44.38	0.56446
CRP	0 - .5 mg/dL	0.083 $\pm$ 0.073	3.27 $\pm$ 5.73	0.0014	0.14 $\pm$ 0.18	0.21 $\pm$ 0.19	1.02 $\pm$ 0.98	2.49 $\pm$ 3.65	0.00189
SGPT	5 – 40 UI/L	25.62 $\pm$ 8.26	30.12 $\pm$ 16.22	0.7064	25.25 $\pm$ 9.73	24.0 $\pm$ 7.07	24.37 $\pm$ 7.13	22.75 $\pm$ 6.60	0.8075
SGOT	5 – 40 UI/L	45.75 $\pm$ 10.26	51.08 $\pm$ 16.99	0.1401	45.25 $\pm$ 18.06	45.0 $\pm$ 12.91	48.5 $\pm$ 17.06	47.7 $\pm$ 19.3	0.9846
LDH	100 – 600 UI/L	627.81 $\pm$ 97.88	794.21 $\pm$ 247.28	0.0280	686.57 $\pm$ 218.02	698.43 $\pm$ 280.51	605.00 $\pm$ 138.56	641.14 $\pm$ 197.36	0.773

Table 5 – Blood values (mean $\pm$ SD) during reactions at the 12-hours' time-point according to the degree of severity.

<i>Parameter</i>	<i>Mild-moderate</i> <i>mean $\pm$ SD</i>	<i>Severe</i> <i>mean $\pm$ SD</i>	<i>Very severe</i> <i>mean $\pm$ SD</i>	<i>F</i>	<i>p</i>
<i>RBC</i>	4.30 $\pm$ 0.27	4.06 $\pm$ 0.49	3.99 $\pm$ 0.51	1.036	.367
<i>WBC</i>	14.88 $\pm$ 7.10	13.91 $\pm$ 5.78	14.87 $\pm$ 4.39	0,116	.891
<i>Neutrophils</i>	7.94 $\pm$ 5.18	8.17 $\pm$ 3.50	8.49 $\pm$ 3.07	0.051	.951
<i>Platelets</i>	348.84 $\pm$ 101.11	392.52 $\pm$ 218.44	427.93 $\pm$ 180.68	0.403	.672
<i>CRP</i>	0.76 $\pm$ 0.47	1.18 $\pm$ 1.86	4.02 $\pm$ 3.39	5.97	.007

Fig 1. Box and whisker plot showing blood values concentrations prior to the initiation of the challenge procedure (0 h) and at 2, 6 and 12 hours from the onset of clinical manifestations. The bars, box and whiskers indicate the medians, 25th and 75th centiles and the ranges, respectively.



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