

LEUKOCYTE AND OTHER HEMATOLOGY PARAMETERS IN CROATIAN VETERANS WITH POSTTRAUMATIC STRESS DISORDER

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SUMMARY – The aim of the study was to assess differences in white blood cell (WBC), neutrophil, monocyte, basophil, eosinophil and lymphocyte counts between Croatian veterans with combat-related posttraumatic stress disorder (PTSD) and those with combat-related PTSD comorbid with major depressive disorder (MDD). PTSD and/or MDD were diagnosed according to a structured clinical interview based on DSM-IV criteria. Additional criteria were Clinician Administered PTSD Scale (CAPS) for PTSD and Montgomery-Asberg Depression Rating Scale (MADRAS) for MDD. WBC was measured on an automatic blood counter. Results showed no statistically significant difference in WBC, neutrophil, lymphocyte, monocyte, eosinophil, basophil and red blood cell counts (RBC), hemoglobin, hematocrit, MCV, MCH, MCHC and platelet count between the veterans with combat-related PTSD, veterans with combat-related PTSD + MDD comorbidity, and patients with MDD only. In conclusion, there were no differences in WBC, neutrophil, lymphocyte, monocyte, eosinophil, basophil and RBC counts, hemoglobin, hematocrit, MCV, MCH, MCHC and platelet count among veterans with combat-related PTSD, veterans with combat-related PTSD comorbid with MDD, and patients with MDD only.

Key words: *Stress disorders, post-traumatic – immunology; Anxiety disorders – immunology; Hypersensitivity – immunology; Veterans – psychology; Leukocyte count; Croatia; War*

Introduction

Many studies have confirmed connection between neuroendocrine and immune systems¹. This link is provided by neural impulses and humoral factors. Primary and secondary lymphoid organs are innervated by the sympathetic and parasympathetic nervous system. The lymphoid cells (lymphocytes and macrophages), apart from producing hormones (ACTH and b-endorphins) also have receptors (b-adrenergic receptors) and can bind neurotransmitters (norepinephrine). Moreover, cytokines, apart from regulating immune responses, also represent an important communication between the

immune and nervous systems. Interleukin 1 (IL-1), IL-2, IL-6, interferon-g (IFN-g) and tumor necrosis factor (TNF) activate hypothalamic-pituitary-adrenal (HPA) axis, and their production is under control of glucocorticoid hormones. That shows how the links between the nervous and immune systems as well as between the endocrine and immune systems are two-way¹.

Consequently, there are studies which report alterations in the immune function that lead to elevation in leukocyte activity upon exposure to severe stress²⁻⁴. Also, stress activates HPA axis, leading to an intensified production of glucocorticoid hormones that significantly change immune response. This means that they provoke changes in the number, activity and function of immune cells¹. On the other hand, stress causes many somatic, psychosomatic and psychiatric disturbances. Of these, posttraumatic stress disorder (PTSD) is of special interest as a clinical-psychiatric entity that devel-

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Received January 26, 2006, accepted April 25, 2006

ops strictly as a consequence of experiencing an extreme stress situation^{5,6}. Previous studies on PTSD have shown changes in many biochemical parameters in PTSD patients, among others changes in cortisol and catecholamine levels⁷⁻¹². Veterans with PTSD have lower circulating cortisol and/or higher circulating catecholamine levels. Cortisol and catecholamines can influence leukocytes and their subpopulations, as cortisol is known to act as an immunosuppressor in some circumstances¹. So far, studies have confirmed changes in WBC, which were significantly elevated in patients with PTSD.

To our knowledge, there have been only a few studies in which WBC was analyzed in chronic combat-related PTSD^{2,4,12-16}. To date, one study has confirmed that, although PTSD victims had reduced natural killer (NK) cytotoxicity, they had significantly increased white blood cell (WBC) counts³. Another study showed chronic combat-related PTSD to be associated with clinically elevated leukocyte and total T-cell counts². Furthermore, a recent meta-analysis of five studies of the immune system also suggested a consistent increase in leukocyte counts in humans after exposure to different acute and chronic stressors⁴. Moreover, it has been suggested that constant HPA axis activation, such as seems to occur in chronic PTSD victims^{12,13}, alters delta-5 adrenal androgens, which in turn may result in a predominance of type 2 helper T-cells¹⁴⁻¹⁶. That is why in this study we assessed whether veterans with chronic PTSD showed

elevation in WBC counts, because there is some evidence suggesting it being possible.

However, epidemiological as well as clinical studies have shown that PTSD often occurs in association with other psychiatric disorders¹⁷. These studies indicated that approximately 80% of individuals with PTSD met the criteria for at least one additional psychiatric diagnosis. Major depressive disorder (MDD) is one of the most common PTSD comorbidities, and the prevalence of MDD in patients with PTSD is up to 70%¹⁸.

The aim of this study was to analyze the possible differences in total leukocyte count as well as in WBC subpopulation (neutrophil, lymphocyte, monocyte, eosinophil and basophil) counts and other hematology parameters (red blood cells (RBC), hemoglobin, hematocrit, MCV, MCH, MCHC and platelets) between patients with PTSD, PTSD + MDD comorbidity and MDD alone.

Subjects and Methods

Subjects

Group 1 consisted of 22 male veterans with combat related PTSD, aged 25-59; group 2 of 66 male veterans with chronic combat-related PTSD + MDD, aged 29-67; and group 3 of 15 men with MDD only, aged 22-68. After being drafted for active military service, all study subjects underwent rigorous entry testing to ensure they

Table 1. Sociodemographic characteristics of veterans with posttraumatic stress disorder (PTSD), PTSD comorbid with major depressive disorder (MDD), and patients with MDD only

	PTSD (n=22)	PTSD + MDD (n=66)	MDD (n=38)
Age (yrs), (mean \pm SD)*	43.7 \pm 9.4	44.5 \pm 8.8	49.1 \pm 12.5
Marital status, n (%) [†]			
Married	6 (27.3)	11 (16.7)	0 (0.0)
Unmarried	13 (59.1)	45 (68.2)	10 (66.7)
Divorced	3 (13.6)	10 (15.2)	5 (33.3)
Education, n (%) [‡]			
Elementary school	2 (9.1)	14 (21.2)	3 (20.0)
High school	16 (72.7)	45 (68.2)	9 (60.0)
University	4 (18.2)	7 (10.6)	3 (20.0)
Place of residence, n (%) [§]			
Urban	14 (63.6)	41 (62.1)	12 (80.0)
Rural	8 (36.4)	25 (37.9)	3 (20.0)

*F=1.670, $p=0.193$; [†] $\chi^2=2.778$, $p=0.596$; [‡] $\chi^2=6.778$, $p=0.148$; [§] $\chi^2=1.743$, $p=0.418$

had not had any previous major psychiatric or somatic disorders. None of study patients had any other psychiatric comorbid disorders or medical problems, and had a history of taking different medications prior to the study, mostly antidepressants (fluoxetine 8.9%, fluvoxamine 5.2%, paroxetine 25.4%, sertraline 27%, clomipramine 18.7%, trazadone 1.4%, and maprotiline 13.4%) and anxiolytics (alprazolam 18.7%, oxazepam 8.6%, diazepam 57.6% and clonazepam 15.1%). None of these drugs influences WBC count¹⁹. They all had negative history of alcohol or other substance of dependence abuse. An informed consent was obtained from all study patients after complete and extensive description of the study protocol.

Since WBC counts are sensitive to even relatively subtle psychosocial influences, our experience indicated it was important to describe these factors in some detail. Descriptive parameters of all study subjects are presented in Table 1.

Almost half of the subjects were married (59.1% in PTSD, 68.2% in PTSD + MDD, and 66.7% in MDD group). Most subjects had high school education (72.7% in PTSD, 68.2% in PTSD + MDD, and 60.0% in MDD group). Most of them lived in urban areas (63.6% in PTSD, 62.1% in PTSD + MDD, and 80.0% in MDD group). Both PTSD and PTSD + MDD patients were

treated as inpatients. During hospitalization, they had an intensive schedule of 30 h of individual and group therapy *per* week, as well as other sociotherapeutic activities. Treatment protocol was based on therapeutic community²⁰.

Medical examination and study design

From the pool of all male patients (n=103) who were receiving treatment at University Department of Psychiatry, Sestre milosrdnice University Hospital from January 2001 till July 2005, we selected three study groups: patients with combat-related PTSD only (n=66), patients with combat-related PTSD + MDD (n=22) and patients with MDD only (n=15).

A psychiatrist performed a structured clinical interview and made the diagnosis based on DSM-IV criteria for PTSD or/and MDD²¹. A clinical psychologist applied Clinician Administered PTSD Scale (CAPS) on PTSD interview based on DSM-IV criteria to measure post-traumatic stress reaction²². Definitive diagnosis of PTSD was only made in cases where both sets of criteria were fulfilled. Diagnostic agreement between psychiatric and psychological criteria was high ($\kappa=0.96$). The alpha coefficient for the CAPS questionnaire in our study was 0.91. The diagnosis of MDD was made when patients

Table 2. White blood cell (WBC), neutrophil, lymphocyte, monocyte, eosinophil, basophil and red blood cell (RBC) counts, hemoglobin, hematocrit, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), and platelets in veterans with posttraumatic stress disorder (PTSD), PTSD comorbid with major depressive disorder (MDD) and patients with MDD only

Hematology variable [†]	PTSD (n=22)	PTSD + MDD (n=66)	MDD (n=15)	F-value*	p-value
WBC (x10 ⁹ /L)	7.96 ± 1.42	7.24 ± 1.98	8.06 ± 3.01	1.608	0.205
Neutrophils (x10 ⁹ /L)	4.52 ± 1.26	4.01 ± 1.50	4.55 ± 2.30	1.297	0.278
Lymphocytes (x10 ⁹ /L)	2.55 ± 0.48	2.43 ± 0.79	2.69 ± 0.91	0.799	0.453
Monocytes (x10 ⁹ /L)	0.59 ± 0.16	0.57 ± 0.20	0.58 ± 0.24	0.023	0.977
Eosinophils (x10 ⁹ /L)	0.17 ± 0.08	0.21 ± 0.12	0.20 ± 0.09	1.319	0.272
Basophils (x10 ⁹ /L)	0.08 ± 0.03	0.08 ± 0.02	0.07 ± 0.03	0.866	0.424
RBC (x10 ¹² /L)	4.82 ± 0.35	4.91 ± 0.39	4.80 ± 0.26	0.657	0.521
Hemoglobin (g/L)	151.45 ± 8.88	148.68 ± 21.20	144.40 ± 14.45	4.188	0.018
Hematocrit (l/L)	0.44 ± 0.02	0.44 ± 0.03	0.41 ± 0.03	3.765	0.027
MCV (fL)	91.13 ± 4.55	90.06 ± 4.39	86.86 ± 6.57	1.595	0.207
MCH (pg)	31.49 ± 1.73	30.83 ± 1.81	30.11 ± 2.96	2.164	0.120
MCHC (g/L)	346.05 ± 11.88	342.58 ± 9.74	345.87 ± 13.98	1.140	0.324
Platelets (x10 ⁹ /L)	238.52 ± 38.25	213.53 ± 46.61	236.26 ± 61.75	2.990	0.055

[†]mean ± SD; *ANOVA

met two criteria: indicative results on the Montgomery-Asberg Depression Rating Scale (MADRAS)²³ and sufficient number of symptoms shown in the structural clinical interview based on DSM-IV criteria for MDD²¹. Diagnostic balance between the MADRAS and clinical interview based on DSM-IV criteria for MDD was $\kappa=0.98$. MADRAS questionnaire was used because of its simplicity and reliability; in our study alpha coefficient was 0.93. Two psychiatrists performed this part of patient evaluation, each of them examining independently all study subjects. The agreement between the two psychiatrists was high ($\kappa=0.97$).

Leukocyte count measurements

Blood samples were collected from forearm vein, in glass red-topped vacuum tubes without anticoagulant, in the morning at 8.00-9.00 a.m., after a 12-h overnight fast and 30-min rest immediately before blood collection. WBC and other hematology parameters were measured on an automatic blood counter (Abbott Cell-Din Y 3200, CA, USA). Our laboratory reference intervals for hematology parameters are as follows: WBC $3.4-9.7 \times 10^9/L$, neutrophils $2.1-6.5 \times 10^9/L$, lymphocytes $1.2-3.4 \times 10^9/L$, monocytes $0.100-0.800 \times 10^9/L$, eosinophils $0.00-0.400 \times 10^9/L$, basophils $0.00-0.100 \times 10^9/L$, RBC $4.34-5.72 \times 10^{12}/L$, hemoglobin 138-175 g/L, hematocrit 0.415-0.530 l/L, MCV 83.0-97.2 fL, MCH 27.4-33.9 pg, MCHC 320-345 g/L, and platelets $158-424 \times 10^9/L$.

Statistical analysis

Differences in sociodemographic variables among three study groups were analyzed by use of χ^2 -test. Normal distribution of all study parameters and in each group was estimated by Kolmogorov-Smirnov test.

Student's t-test was used to compare patients with PTSD and those with PTSD + MDD according to age, total WBC and leukocyte subpopulation counts, RBC count, hemoglobin, hematocrit, MCV, MCH, MCHC, and platelet count. Statistical significance was set at $p < 0.01$. Statistical analysis was done by use of SPSS software (SPSS for Windows 8.0, SPSS, Chicago, IL, USA).

Results

There was no statistically significant difference among veterans with combat-related chronic PTSD, combat-related chronic PTSD + MDD, and patients

with MDD only according to age, marital status, level of education, and place of residence (Table 1). There was no statistically significant difference in WBC, neutrophil, lymphocyte, monocyte, eosinophil, basophil, RBC counts, hemoglobin, hematocrit, MCV, MCH, MCHC, and platelet counts among veterans with combat-related chronic PTSD, combat-related chronic PTSD + MDD, and patients with MDD only either (Table 2).

Discussion

Our results showed no statistically significant difference in WBC, neutrophil, lymphocyte, monocyte, eosinophil, basophil and RBC counts, hemoglobin, hematocrit, MCV, MCH, MCHC and platelet count among veterans with combat-related chronic PTSD, veterans with combat-related chronic PTSD + MDD, and patients with MDD only. The objective of the study was to show how exposure to chronic stress in veterans with combat-related PTSD led to a number of changes in leukocyte count and other hematology variables such as RBC, hemoglobin, hematocrit, MCV, MCHC, platelets or WBC subpopulations (neutrophils, lymphocytes, basophils, monocytes and eosinophils). The above mentioned changes in the hematologic/immune system would be mediated by numerous biochemical processes, leading to changes in the immune system *via* nervous and endocrine systems. However, our results were contrary than expected, differing from the results of other studies reporting elevated leukocyte counts in Vietnam veterans with chronic combat-related PTSD²⁴. Yet, the above mentioned studies in Vietnam veterans were conducted after a longer period of time of primary stress experience than our study, so the exposure to chronic stress was much longer. Besides, unlike our study, these studies did not include analysis of RBC, hemoglobin, hematocrit, MCV, MCH, MCHC and platelets.

The inconsistency in the results of our study and of those carried out in Vietnam veterans may be due to a number of other possible comorbid conditions present in the latter. Also, our study, in contrast to those conducted and reported elsewhere, was so designed as to categorize patients according to comorbidity into those suffering from PTSD and those with PTSD + MDD. It is important to note because previous studies have shown that MDD is a very common comorbidity with PTSD, recorded in as many as 70% of cases.

Our study also had several limitations. For example, we had no control group of healthy individuals, so it

would be interesting to see the results obtained in such a study design. It would also be interesting to observe PTSD veterans over a prolonged period of time to estimate the length of time after the onset of the disorder needed for the effect of stress on the immune system to manifest.

The present study was the first one to show hematology/immunology parameters in Croatian war veterans with PTSD or PTSD + MDD comorbidity. Moreover, the hematology/immunology parameters were for the first time analyzed in PTSD patients at a shorter period of time elapsed from stress exposure.

In conclusion, we found to statistically significant differences in total WBC, neutrophil, lymphocyte, monocyte, eosinophil, basophil and RBC counts, hemoglobin, hematocrit, MCV, MCH, MCHC and platelet count among veterans with combat-related chronic PTSD, those with combat-related chronic PTSD + MDD, and patients with MDD only. Additional studies are needed to investigate the open question of the role of leukocytes and other hematology parameters in patients with PTSD.

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Sažetak

LEUKOCITI I DRUGI HEMATOLOŠKI POKAZATELJI U HRVATSKIH RATNIH VETERANA
S POSTTRAUMATSKIM STRESNIM POREMEĆAJEM*M. Miklič-Bublić, D. Karlović, M. Martinac i D. Marčinko*

Cilj ove studije bio je ispitati razlike u broju leukocita, neutrofila, monocita, bazofila, eozinofila i limfocita u hrvatskih ratnih veterana s post-traumatskim stresnim poremećajem (PTSP) uzrokovanog ratnim traumama u usporedbi s istim poremećajem uz istodobno prisutan velik depresivni poremećaj (VDP). Dijagnoza PTSP i/ili VDP postavljena je pomoću strukturiranog kliničkog upitnika prema kriterijima DSM-IV. Kao dopunski kriteriji primijenjena je Clinician Administered PTSD ljestvica (CAPS) za PTSP te Montgomery-Asberg Depression Rating Scale (MADRAS) za VDP. Broj leukocita izmjeren je na automatskom hemaološkom brojaču. Rezultati nisu pokazali nikakvu statistički značajnu razliku u broju leukocita, neutrofila, limfocita, monocita, eozinofila, bazofila, eritrocita, trombocita, kao ni u razini hemoglobina, hematokrita, MCV, MCH i MCHC između veterana s PTSP uzrokovanog ratnim traumama, veterana s PTSP uzrokovanog ratnim traumama i istodobnim VDP i bolesnika koji boluju samo od VDP. Zaključuje se kako ne postoje razlike u broju leukocita, neutrofila, limfocita, monocita, eozinofila, bazofila, eritrocita, trombocita, kao ni u razini hemoglobina, hematokrita, MCV, MCH i MCHC između veterana s PTSP uzrokovanog ratnim traumama, veterana s PTSP uzrokovanog ratnim traumama i istodobnim VDP i bolesnika koji boluju samo od VDP.

Ključne riječi: Stresni poremećaji, post-traumatski – imunologija; Anksiozni poremećaji – imunologija; Preosjetljivost – imunologija; Veterani – psihologija; Broj leukocita; Hrvatska; Rat