

## Leukocyte Cytochemistry and Hematometric Indices in Chronic Heroin Addicts

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The influence of diacetylmorphine (heroin) on white blood cell morphological/functional characteristics and some hematometric indices — leukocyte count (WBC), differential leukocyte count, granulocyte/lymphocyte ratio, whole blood viscosity (WBV) as well as some cytological/cytochemical changes of polymorphonuclears (granulocytes) from the peripheral blood of chronic heroin addicts has been investigated, comparing data to those of healthy individuals. The role of WBC and some morphological/cytochemical changes in granulocytes of chronic heroin abusers has been discussed as factors for the elevated WBV, the acute allergic states and the high sensitivity of heroin addicts to bacterial and viral infections.

*Key words:* chronic opiate (heroin) addicts, hematometric indices, whole blood viscosity, leukocyte count, leukocyte cytology/cytochemistry.

### Introduction

Narcotics such as morphine, diacetylmorphine (heroin) etc., influence white blood cells changing morphological and functional characteristics of leukocytes, as well as blood viscosity and hematometric indices of drug abusers [4, 5, 7, 9, 14, 15, 19]. Recent studies [14] show an increase in monocyte, neutrophil and eosinophil counts in drug abusers compared to healthy individuals. Chruvasta and Ehrmann [3] find positive correlation between elevated number of eosinophils and intravenous drug use. The aim of the study is to investigate leukocyte hematometric indices — white blood cell count (WBC — g/l) and differentiation count (%), granulocyte/lymphocyte ratio (Gr/Ly), mean whole blood viscosity (WBV) as well as some cytological/cytochemical characteristics of polymorphonuclears (granulocytes) in chronic opiate users, comparing data to those of healthy individuals.

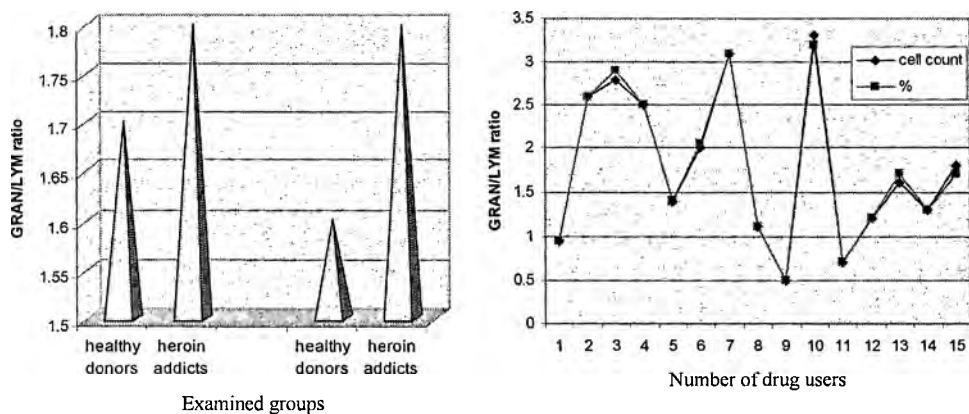
### Material and Methods

Whole blood heparinized samples from 15 chronic heroin addicts (3 female and 12 male - mean age  $26.53 \pm 7.34$  years, HIV-seronegative, under methadone mainte-

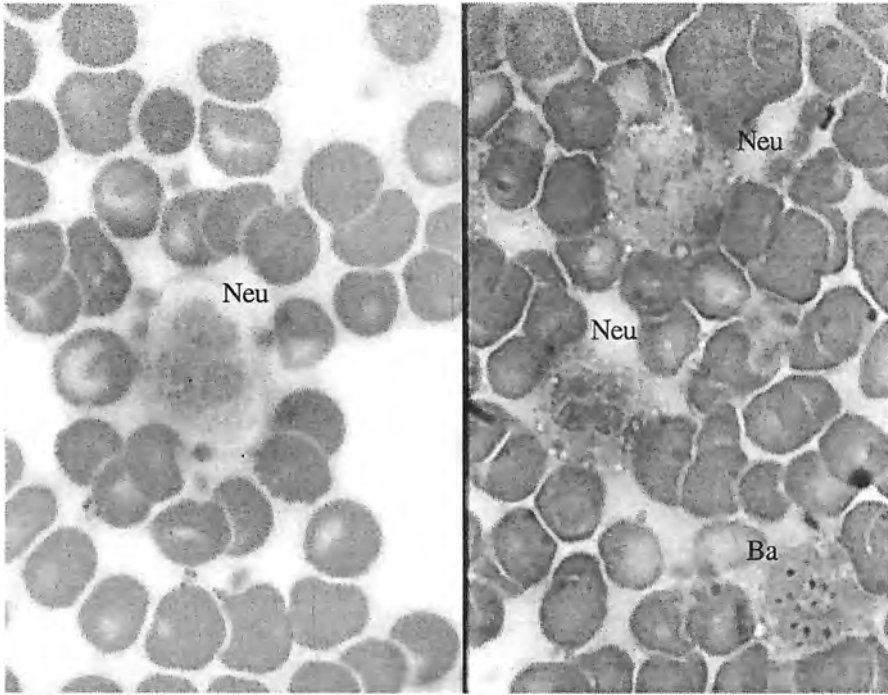
nance therapy), obtained from the Bulgarian National Center for Addictions, were studied. Blood samples were collected in heparinized tubes and rheological measurements were completed within 3 hours after blood preparation. Whole blood viscosity (WBV) was measured at 37°C using a rotational viscometer Contraves Low Shear 30 (Switzerland) with the standard measuring system MS1/1, at a steady flow over a shear rate range of 0.0237 s<sup>-1</sup> to 128.5 s<sup>-1</sup>. The results were compared with a control group of 19 healthy individuals (9 female and 10 male; mean age — 34.84 ± 4.06 years). White blood cell hematometric indices — white blood cell count (WBC) and granulocyte/lymphocyte ratio were analyzed by automated cell counter. Cytological characteristics of granulocytes were examined by May-Grünwald-Giemsa technique and by the cytochemical method for cytoplasmic cationic proteins [23]. Student's t-test for determining differences in the mean values of the parameters examined at level of significance  $p < 0.05$  was used. Relationships between leukocyte parameters and WBV were evaluated using simple correlation coefficient  $r$ . Statistical analysis was done on MATLAB 6.5.

## Results

The results from total white blood cell count (g/l) showed differences in leukocyte number of chronic heroin addicts (6.89 ± 1.86) and healthy individuals (7 ± 1.2). Based on the total WBC, the granulocyte/lymphocyte ratio (Gr/Ly) is 1.8 for the drug users, versus 1.7 — for healthy donors (Fig. 1). Differential leukocyte count (%) also showed changes in the granulocyte/lymphocyte ratio of drug users compared to that of healthy donors: increased ratios (1.8) were determined in cases of drug abuse, versus 1.6 — in healthy individuals (Fig. 2). Mean whole blood viscosity (WBV) values of the investigated group of heroin abusers were elevated compared to these of healthy persons and the elevation was statistically significant at higher shear rates ( $\gamma = 20.4 \text{ s}^{-1}$ ,  $p = 0.1$ ;  $\gamma = 94.5 \text{ s}^{-1}$ ,  $p = 0.05$ ). Statistical correlation between granulocyte differential count (%) and WBV at low shear rates was also determined. Our cytological/cytochemical data showed changes in the condensation and distribution of the nuclear chromatin in granulocytes: unevenly dispersed and distributed nuclear DNP - containing irregularly condensed spots of chromatin, were obtained in neutro-



Figs. 1, 2. Granulocyte/lymphocyte ratios in heroin abusers and healthy donors

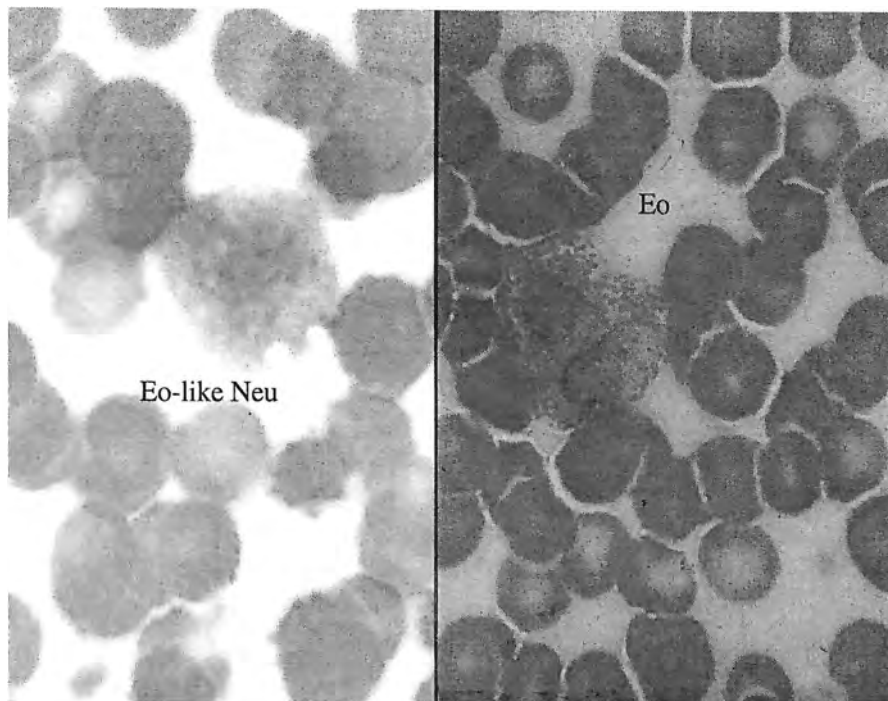


Figs. 3, 4. Neutrophils (Neu) and one basophil (Ba) from the peripheral blood smears of chronic heroin addicts. Changes in the condensation and distribution of DNP in the nuclear chromatin and reduced quantity of cytoplasmic granules, containing cationic proteins could be seen in neutrophils; the cytoplasmic granules of basophil were partially degranulated.  $\times 450$

phils and eosinophils of chronic heroin addicts (Figs. 3—6). Low quantity of cytoplasmic granules and diffusely stained cationic proteins are visible in the neutrophils from peripheral blood of heroin abusers (Figs. 3, 4). Abundant fast-green positive granules, containing large amount of cationic proteins, could be seen only in the cytoplasm of eosinophils as well as in a population of eosinophil-like neutrophils (Figs. 5, 6). The percentage of partially degranulated basophils (Fig. 4) is often higher in the peripheral blood of chronic heroin abusers, compared to data of the control group.

## Discussion

The reduced total number of leukocytes, increased granulocyte/lymphocyte ratio as well as decreased quantity of cationic protein-containing granules in neutrophils correlate with data [6, 14] for the high sensitivity of heroin addicts to bacterial and viral infections. It is well known fact [9, 16, 22] that narcotics lead to a significant decrease in the phagocytic index and chemotaxis of granulocytes, as well as to the reduced production of superoxide anions ( $O_3$ ,  $H_2O_2$ ,  $NO$ ) which is independent of the co-existence of HIV-infection in drug addicts. On the other hand, the abundant amount of cationic protein-containing cytoplasmic granules in the eosinophilic granulocytes may contribute to the acute allergic states as eosinophilic aseptic arachnoiditis, acute eosinophilic pneumonia, pulmonary edema, urticaria, asthma, etc., often



Figs. 5, 6. Eosinophil (Eo) and eosinophil-like neutrophil (Eo-like Neu) containing abundant cytoplasmic granules with large amount of basic cationic proteins in peripheral blood smears of chronic heroin abusers.  $\times 450$

observed in heroin addicts [2, 8, 12, 13, 17, 19]. Our data that partially degranulated basophils are frequently seen in blood smears of drug abusers are in agreement with those of other researchers [1, 10, 18] about the increased number of tissue mast cells leading to anaphylactoid reactions, typical for drug addiction. There are speculations about the relationship between white blood cell count (WBC) and blood viscosity: prevails the opinion that not only the hematocrit, hemoglobin and RBC, but also WBC and platelet count could affect WBV [11]. In our previous experimental studies [21] we hypothesized that changes in RBC (macrocytic anemia) are main factors for elevated WBV, but the role of WBC and morphological/cytochemical changes in granulocytes of chronic heroin addicts should be also considered and evaluated. In this regard the statistical correlation obtained between granulocyte differential count (%) and WBV at low shear rates is important. The influence of chronic heroin use on the proliferation and differentiation of early myeloid precursor cells in bone marrow has been studied [20], but the effect should be further examined.

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