Research regarding the toxic effects of heroin consumption on human homoleucogramma

RADIANA TAMBA-BEREHOIU, N. C. POPA, STELA POPESCU, C. POPA
Faculty of Biotechnology, Bd. Marasti No. 59, 71331, Bucharest, Romania

Abstract

Due to the dynamics of drug consumption, it is important to understand the main toxicological aspects of blood, noticed in drug addicts, in order to establish the most adequate, efficient, preventive and therapeutic methods so as to diminish this phenomenon.

The products resulting from the two antagonistic metabolic processes (anabolism and catabolism) are continuously introduced or eliminated in blood, because blood maintains the connection between different organs and tissues and between different cells and digestive organs.

As a consequence, we may consider that the entire composition of blood deviations from normal (deviations that surpass sanguine homeostasis), are significantly correlated with body pathology. Moreover, blood is one of the most accessible tissues in the human body, and it can be collected and analyzed without harming the patient.

Keywords: Heroin, homoleucogramma, assymetry coefficients, regression

Introduction

In this paper we proposed:

- An analysis of the homoleucogramma of people consuming opium drugs, in order to emphasize the characteristic aspects that appear, regarding the level of several blood parameters (the number of leucocytes, the number of red cells, the quantity of hemoglobin, haematocrit, the number of platelets, the percent of monocytes, the percent of lymphocytes, the percent of eosinophiles, the percent of basophiles, the percent of granulocytes);
- the specific correlations between blood parameters, for consumers of opium drugs;
- An analysis of specific relationships between sanguine parameters, for consumers of opium drugs;
- An analysis of the sanguine parameters characteristics for consumers of opium drugs, in order to emphasize the importance of toxicological control of blood, so as to highlight the pathology of narcotics and their derivatives.

Material and Method

The tested material was human blood collected from a homogeneous lot of subjects, males between 20 and 30 years old, known to have consumed heroin at least 6 months before collecting the samples.

We determined the number of leucocytes (WBC), the number of erythrocytes (RBC), the quantity of hemoglobin (HgB), haematocrit (HCT), the number of platelets (PLT), the

Bibliotheque de Humanisme et Renaissance | ISSN : 0006-1999
Volume 81, Issue 5, 2021

percent of eosinophiles (EO), the percent of lymphocytes (LY), the percent of granulocytes (NEUT), the percent of monocytes (MO) and the percent of basophiles (BASO). [2]

The number of leucocytes and red cells has been evaluated under microscope, using a hemocytometer. The haematocrit has been determined using a microcentrifuge, by evaluating volume of the sediment.

We can use for the other elements the acid, alkaline or neutral colouring kits (May Grűnwald-Giemsa colours). The May Grűnwald solution colours cytoplasm very well, but do not color well the nucleus and red cells. The Giemsa solution renders evident the nucleus and certain blood parasites. All sanguine components will colour depending on their tinctorial affinities, as follows. Red cells are biconcave disks, with rounded margins, and colors the margins in pink-orange and the center less intensively.

Leukocytes are unsegmented and segmented granulocyte elements, differentiated by specific granularity, which divides them into neutrophiles, eosinophiles, basophiles, lymphocytes and monocytes. [2]

Neutrophiles color in pale pink, eosinophiles are big, round, and color in orange red. Basophiles have a nucleus with several lobes, club shaped (polymorphonuclear), and color in violet-blue. Lymphocytes are big and small, chromatin disappears as accumulations from the nucleus, and cytoplasm colors in sky-blue. Monocytes have a reniform core, without nucleoli, and present a gray adundant cytoplasm, with fine azure granulations and many vacuoles. Platelets (platelet plaquettes) are the smallest elements in blood; they are spherical or oval shaped, having in the middle reddish azure granulations, surrounded by a blue protoplasm zone.

Final results were interpreted by employing the methods of mathematical statistic analysis applied in the experimental technique, using the professional COHORT program.

We used both the testing methods of the estimates of the mean variability, estimates of the repartition of noticed frequencies, reported to theoretical calculated frequencies (asymmetry tests, χ^2 test etc), as well as specific variance analysis methods (correlations, regressions etc). [1]

Results and Discussions

Tables 1 and 2 show the estimates of variability, calculated for the characteristic parameters of homoleucogramma, for 30 tested subjects.

Table 1. The estimates of variability of the red cells and platelets parameters for the tested subjects

PARAMETER	$\overline{X} \pm s_{\bar{x}}$	s	CV %	NORMAL LIMITS
Red cells number (RBC) (M/µl)	4.390 ± 0.634	0.403	14.456	4 – 5.2
Quantity of Hemoglobin (HgB); (g/dl)	13.886 ± 1.507	2.272	10.855	12 – 15
Haematocrit (HCT) (%)	40.616 ± 4.836	23.392	11.907	34 – 45
Number of platelets (PLT) (K/µl)	218.166 ± 75.377	5681.730	34.550	150 - 300

In table 1, we can notice that none of the tested parameters deviates from normal limits of variation. More than that, for the **red cell parameters** (number of red cells, quantity of hemoglobin and haematocrit), we could see their stability regarding the associated variation coefficients. This fact suggests a smaller influence of the narcotics upon the associated variation coefficients. As regards the parameter "**number of platelets**", it developed a big variation coefficient (34.55 %), and this coefficient shows the influence of heroin consumption.

In table 2, we present the estimates of variability for the total number of leucocytes and for the components of leukocyte formula.

Table 2. The estimates of variability of the total number of leucocytes and the components of the leukocyte formula for the tested subjects

PARAMETER	$\overline{X} \pm s_{\bar{x}}$	s	CV %	NORMAL LIMITS
Number of leucocytes (WBC) (K/µl)	6.115 ± 1.374	1.887	22.462	4 - 8
Percent of eosinophiles (EO) (%)	2.650 ± 1.803	3.251	68.028	0 – 5
Percent of lymphocytes (LY) (%)	28.063 ± 7.681	59.000	27.373	37.5 - 45
Percent of granulocytes (NEUT) (%)	58.140 ± 8.454	71.469	14.540	42 - 70
Percent of monocytes (MO) (%)	9.633 ± 2.960	8.766	30.866	0 – 8
Percent of basophiles (BASO) (%)	0.64 ± 0.600	0.363	94.162	0 – 1

In table 2, we can notice that certain parameters are situated outwards the normal variation limits: the **percent of lymphocytes** (28.063), which is under the minimum limit, and the **percent of monocytes**, which exceeds the upper limit.

Moreover, for all the **leukocyte parameters,** excepting the percent of granulocytes (neutrophiles), the variation coefficients are big or very big.

The conclusion places the modified values of the variation coefficients as a direct or indirect consequence of heroin consumption, unless the subjects have other diagnosed diseases that may cause the variations. These values suggest sensitization of the immune system in heroin consumers, but also the active implication of heroin in the **detoxification process**. [3, 4, 5]

The increasing of the number of monocytes participating both in the phagocyte processes of microbes and other cell remains, and in the anti-infectious reactions, is correlated with the decrease of the number of lymphocytes, whose main role is to produce antibodies.

These modifications suggest a direction of the immune system orientation, maybe as a result of the general condition of the body, which is sensitized towards infections.

In figure 1, we present comparatively the average values of the variation coefficients for the parameters depending on: red cells (number of red cells, haematocrit and hemoglobin), platelets and leucocytes (number of leucocytes, number of eosinophiles, percent of lymphocytes, percent of granulocytes, and percent of basophiles).

Bibliotheque de Humanisme et Renaissance | ISSN : 0006-1999
Volume 81, Issue 5, 2021

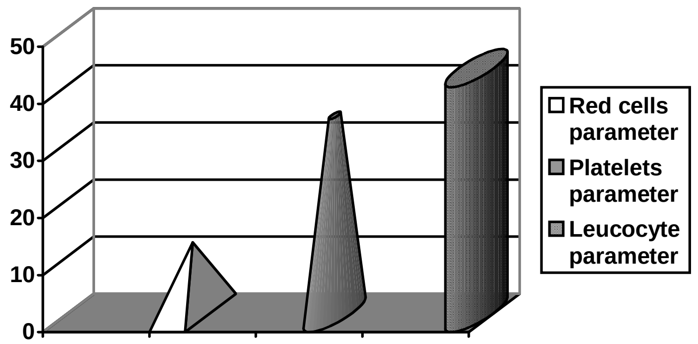

Figure 1. Average variation coefficients for red cells parameters, platelets parameters and leukocyte parameters, for the tested subjects

In figure 1, we can see that platelets parameters and leukocyte parameters were for the heroin consumers the least stable to the variation factors in body, while red cells parameters were almost inert to these factors.

If clinical status of tested subjects does not show other diseases, we think that the modified values of the variation coefficients are a consequence of direct or indirect heroin consumption.

Table 3 presents the results after comparing the effects of the distribution of parameters in series boarded by their inferior and superior limits. By applying statistical methods, we characterized the asymmetry tendencies, as well as the deviations appeared in the frequency of certain values, towards their theoretical distribution.

Table 3. Minimum, maximum values of the asymmetry coefficients, associated to the series of values for the red cells parameters and platelets parameters, for the tested subjects

Parameter	**Average**	**Minimum**	**Maximum**	**Asymmetry coefficients**		**NORMAL LIMITS (average)**
				β_1*	β_2**	
Red cells number (RBC) (M/µl)	4.390	3.18	5.73	-0.143	2.860	4.0 – 5.2 (4.6)
Quantity of Hemoglobin (HgB); (g/dl)	13.886	11.20	17.00	0.200	2.708	12 – 15 (13.5)
Haematocrit (HCT) (%)	40.616	25.50	49.30	-0.834	4.644	34 – 45 (39.5)
Number of platelets (PLT) ; (K/µl)	218.166	75.00	349.00	0.039	2.210	150 – 300 (225)

- β_1* represents an asymmetry coefficient whose standard value for a perfectly symmetrical curve is 0. Any deviation from 0, in one direction or another, is directly proportional with the degree of asymmetry of the curve.
- β_2** is another characteristic measure of the asymmetry, whose value for the perfect symmetrical curve is 3. Any deviation from this value is associated to the flatting degree of the curve.

In table 3, we can notice that the degree of asymmetry for **red cells parameters** varies from little significant for the number of red cells and for hemoglobin, to significant for **haematocrit**. The parameter "number of platelets" shows insignificant asymmetry.

For all these parameters, the variation range excels the normal variation limits; it means that the inferior limits of the studied indices are smaller than the inferior limits of the normal variation range, and the upper limits are higher than the normal superior limits.

The average percent difference between the average associated to each parameter and the median of normal variation range is shown in figure 2. We notice that for the tested subjects the parameters **number of red cells and number of platelets** registered a slight decrease towards the median of the normal variation range, while the parameters **hemoglobin** and **haematocrit** registered an increase almost equal towards the median.

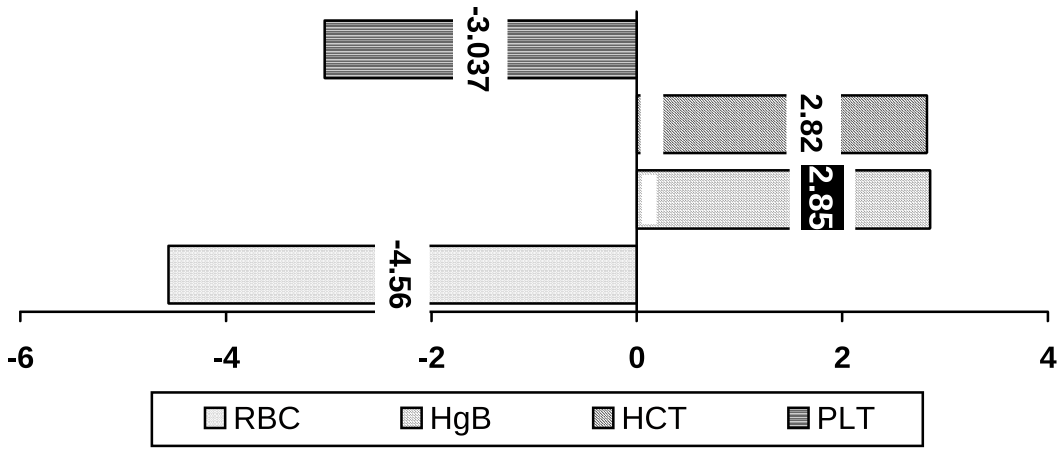

Figure 2. Percent average difference between the mean value for the tested red cells and platelets parameters and the median of the normal variation range

Figure 3 shows the distribution of the frequencies of the haematocrit parameter for the tested heroin consumers.

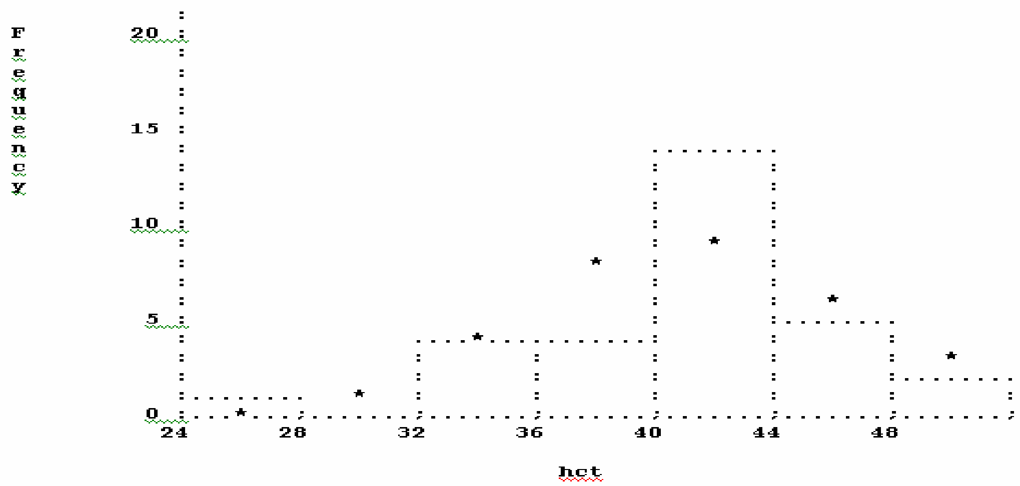

Figure 3. Distribution of the values of the haematocrit parameter, for the tested subjects

We may point out a positive asymmetry, its increasing tendency (most values are around average and bigger). The tendency for this parameter is strong enough to produce a visible asymmetry. The value of χ^2 for the repartition we noticed is 3.028*** and represents a

Bibliotheque de Humanisme et Renaissance | ISSN : 0006-1999
Volume 81, Issue 5, 2021

significant deviation from the theoretical repartition. Accordingly, we may conclude that the **haematocrit parameter is very significantly influenced by heroin.**

The correlation tests emphasized existing relations between the erythrocyte parameters we previously related. So, the parameter **number of erythrocytes** correlated, as expected, with the **quantity of hemoglobin**, the correlation coefficient (r) attached to this relation being of 0.576**. We are surprised by the relative small value of the correlation coefficient and the value of the determination coefficient (R^2 = 0.376), as the only blood hemoglobin source is represented by the erythrocytes.

The equation of regression, number of red cells – quantity of hemoglobin is shown in figure 4.

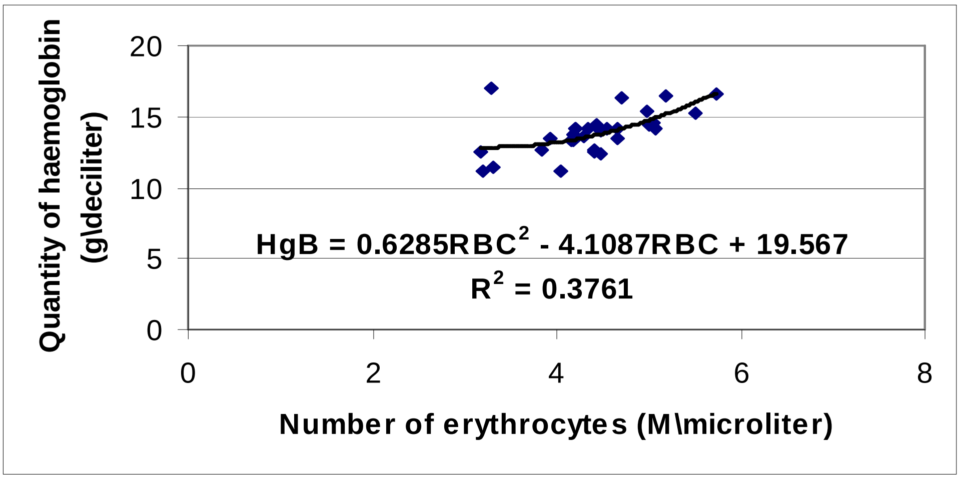

Figure 4. The equation of regression number of erythrocytes – hemoglobin

The relative small value of the correlation coefficient for the relation between the two parameters, as the test of frequencies did not show significant influences of the heroin consumption, on the number of erythrocytes and on the hemoglobin quantity, may be a new idea, for investigating the influence of heroin on the relationship between the blood parameters.

In contradiction with the previous correlation, the relation between the number of red cells (cells of a majority in blood) and haematocrit is very significant, being described by correlation and determination coefficients, with theoretically expected values: r = 0.859***, respectively R^2 = 0.74. The regression curve with the appropriate equation, number of erythrocytes - haematocrit is shown in figure 5.

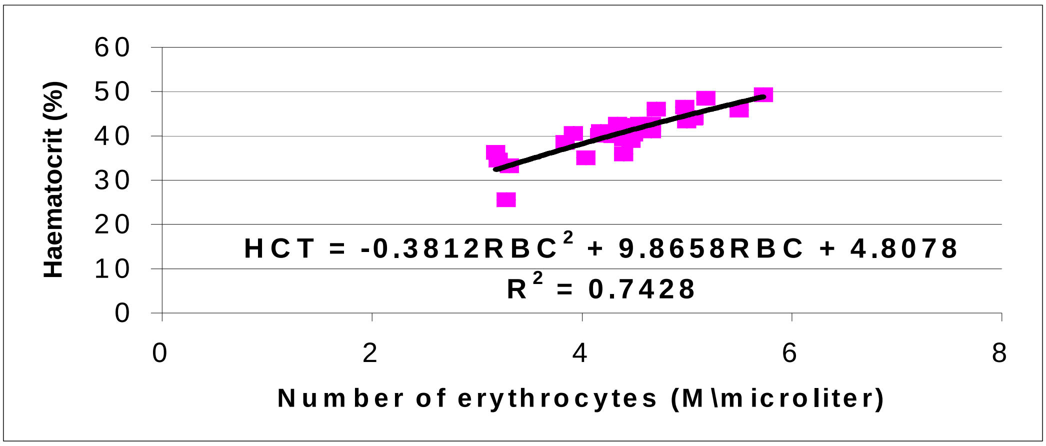

Figure 5. Regression between the parameters - number of erythrocytes and haematocrit, for the tested subjects

Bibliotheque de Humanisme et Renaissance | ISSN : 0006-1999
Volume 81, Issue 5, 2021

The correlation between the quantity of hemoglobin and haematocrit is illustrated in figure 6. This case also shows the unexpectedly low level of the associated correlation coefficient, which confirms the weak representation of the link between the number of erythrocytes and the quantity of hemoglobin, previously discussed.

In conclusion, as far as the effect of heroin consumption on the erythrocytes parameters is concerned, we may observe their apparent stability to the effects of drug. However, there are certain deviation tendencies from the standard values, which do not appear in the average value, but in the characteristics of the distribution of the frequencies attached to each parameter. The most sensitive parameter from those discussed so far is the **haematocrit**, whose asymmetric distribution, oriented to increase of average and over average values, was found significant from statistically point of view, by the χ^2 test.

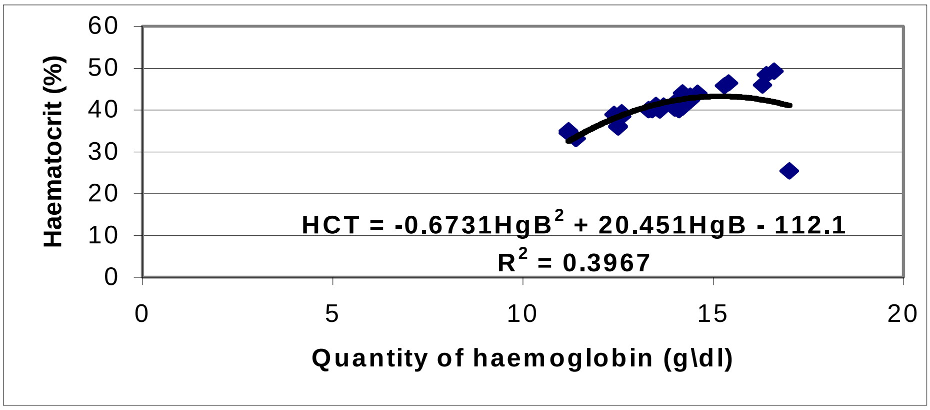

Figure 6. Regression of the parameters quantity of hemoglobin – haematocrit, for the tested subjects

According to the former presented data, the most sensitive blood parameters of the subjects consuming heroin, taking into consideration the value of the variation coefficients and the tendencies of the averages, were those related to the immune system.

Table 4 presents the minim, maxim, respectively the asymmetry coefficients, associated to the values obtained for the **leukocyte parameters**.

Table 4. The minim, maxim, respectively the asymmetry coefficients, associated to the values obtained for the leukocyte parameters.

Parameter	Average	Minim	Maxim	Asymmetry coefficients		Normal limits (median)
				β_1*	β_2**	
Number of leucocytes (WBC) ; K/µl)	6.115	3.86	9.07	-0.124	2.234	4 – 8 (6)
Percent of eosinophiles (EO) (%)	2.650	0.0	8.6	1.55	6.11	0 – 5 (2.5)
Percent of lymphocytes (LY) (%)	28.063	13.0	43.4	0.166	2.414	37.5 – 45 (41.25)
Percent of granulocytes (NEUT) (%)	58.140	43.4	74.1	-0.181	1.972	42 – 70 (56)
Percent of monocytes (MO) (%)	9.633	5.3	17.9	0.867	3.425	0 – 8 (4)
Percent of basophiles (BASO) (%)	0.64	0.1	2.9	2.642	9.741	0 – 1 (0.5)

Bibliotheque de Humanisme et Renaissance | ISSN : 0006-1999
Volume 81, Issue 5, 2021

In table 4, we observe that for the leukocytes parameters, the asymmetry coefficients have relatively high values. We registered the most **asymmetric repartitions** for the values of the parameters **percent of monocytes, percent of eosinophiles and percent of basophiles**. These observations suggest an active implication of the immune blood system in the **detoxification** process for the tested subjects.

Also, for the parameters number of **leucocytes and percent of eosinophiles**, the observed variation interval includes the normal variation interval, its two extremes being smaller or equal, respectively higher than the extremes corresponding to the normal interval.

For the **percent of lymphocytes,** the minimal observed value is much smaller than the normal variation interval (13 observed against 37.5 normally), and the maximum value is also smaller than the normal interval maximum.

For the **percent de granulocytes** the minimal observed value is higher than the minimum of the normal interval, and the maximum noticed value is higher than the normal interval maximum.

The percent of monocytes has, for the tested heroin consumers, a different distribution from the normal one. The minimum observed value (5.3) is higher than the normal one, for the heroin consumers. So, the minimum noticed value is higher than the median of the normal interval (4), and the maximum registered value is higher than twice the value of the normal interval.

The percent of basophiles is distributed in close limits to the minimum normal value, for the minimum noticed limit, and almost three times higher than the normal maximum value, for the maxim noticed limit.

Figure 7 shows the percentage deviation of the average investigated leukocyte parameters, from the median of the normal variation interval.

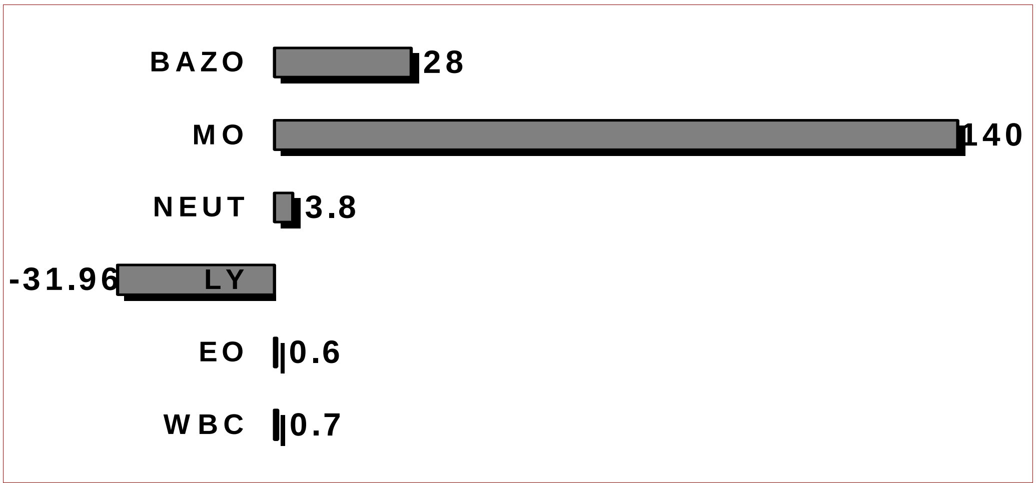

Figure 7. Percentage deviation of the average investigated leukocyte parameters, from the median of the normal variation interval

In figure 7, we can observe that the parameters percent of **eosinophiles**, percent of **leucocytes**, percent of **granulocytes**, presented positive minimal deviations from the median of the normal interval of variation. The parameters percent of **basophiles** and percent of **neutrophiles** registered however positive high and very high deviations from this median.

The percent of **lymphocytes** also had a negative significant deviation.

In figure 8, we can observe that the most asymmetric distributions of the values were noticed in the case of leucocytes parameters. In the graphical representation, we can see the

Bibliotheque de Humanisme et Renaissance | ISSN : 0006-1999
Volume 81, Issue 5, 2021

average values of the two investigated asymmetry coefficients, for the three categories of blood parameters.

The highest asymmetry, registered for the leucocytes parameters, together with the rest of the observations, proves the implication of the blood immune system in the **detoxification antinarcotics process** at the heroin consumers.

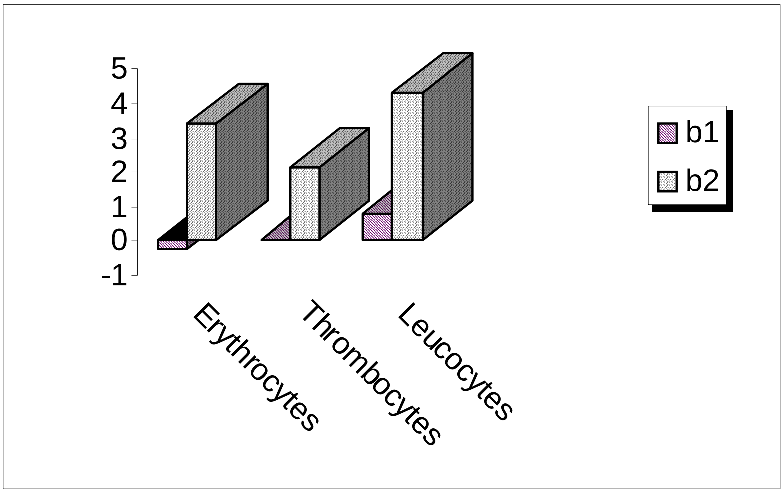

Figure 8. The average values of the two asymmetry coefficients, (b_1 and b_2) for the three categories of investigated parameter, namely: erythrocytes, thrombocytes and leukocytes

Figure 9 shows the distribution of frequencies for the parameter **"percent of eosinophiles".** This parameter has been mainly characterized by very high values of the variation and asymmetry coefficients. We can notice that in this case, the registered deviations from the theoretical curve can be considered as significant, the value of the $\chi 2$ coefficient being 4.922, significant. The distribution of frequencies for this parameter can be consequently considered anomalous, for the heroin consumers.

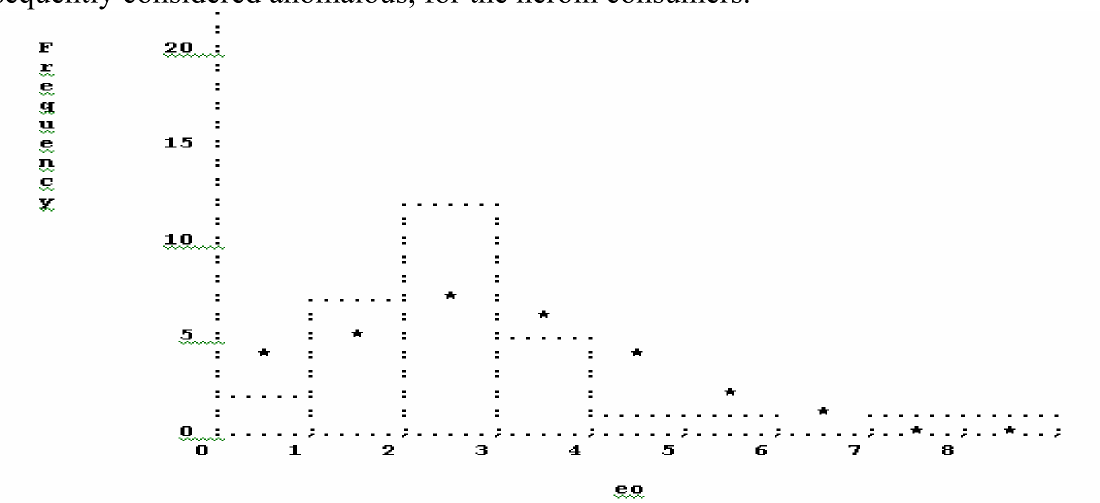

Figure 9. Distribution of frequencies for the parameter percent of eosinophiles, for the investigated heroin consumers

An interesting aspect refers to the existence, for the investigated leukocyte parameters, of a **very significant correlation** between the parameters "percent of **lymphocytes**" and "percent of **neutrophiles**". The value of the correlation coefficient (r) for this relationship is – 0.764*** for this relationship.

Figure 10 presents the regression of the parameters "percent of lymphocytes" and "percent of neutrophiles", as well as the corresponding regression equation.

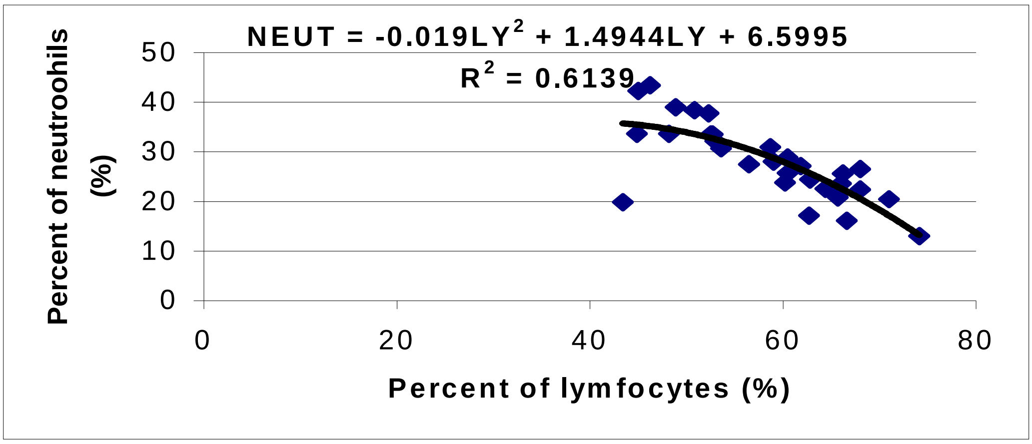

Figure 10. Regression of the parameters percent of lymphocytes - percent of neutrophiles, for the tested heroin consumers

The regression curve in figure 10 shows that the percentage of neutrophile increases in subjects in which the percentage of lymphocyte decreased. As the decreasing of the number of lymphocytes represents an indicator of heroin consumption (as noticed previously when analyzing the distribution of frequencies), this regression suggests the immune sanguine system of the organism is suffering an adaptation process to its detoxification needs.

On the other side, the neutrophiles present positive chemotactism to toxins and anti-infection properties, their role in decreasing the number of lymphocytes (producers of antibodies) being associated to the increase of the percent of monocytes (which also have anti-infection phagocyte properties).

Conclusions

1. The consumption of drugs does not cause major variations of the parameters of tested **erythrocytes:** number of red cells, quantity of hemoglobin and haematocrit, and we can notice no deviations from the normal variation limits.

2. The parameter number of **thrombocytes** presents a high variation coefficient, being susceptible to be influenced by the heroin consumption, although its average value situated in the normal physiological limits.

3. All the **leukocyte** parameters presented high and very high variation coefficients, excepting the neutrophiles percent. This fact suggests the active implication of the immune system in the detoxification antinarcotics process, as well as its possible injuring by the heroin consumption.

4. The percent of **lymphocytes** had average values smaller than the inferior limit of the normal variation interval; the frequencies distribution test shows that this deviation represents an important characteristic of the heroin consumers.

5. The percent of **monocytes** exceeded normal limits, being higher than the superior limit of the normal variation interval. Additionally, in this case, the frequencies distribution test confirmed that the obtained value is normal for the heroin consumers.

Bibliotheque de Humanisme et Renaissance | ISSN : 0006-1999
Volume 81, Issue 5, 2021

6. The parameters least stable to the variation factors in the organism, were, in average the **thrombocytes** and **leukocytes** parameters for the heroin consumers, while the **erythrocytes** parameters were almost inert to these parameters.

As the clinical status of the tested subjects did not indicate other diseases, we may think that these modified values of those variation coefficients are due to the heroin consumption, directly or indirectly.

7. The erythrocytes and **thrombocytes** parameters showed normal distributions of the frequencies, excepting haematocrit, which had a significant deviation from the theoretical distribution curve. This fact suggests that the haematocrit parameter is influenced by the heroin consumption.

8. For the **leucocytes** parameters, the asymmetry coefficients showed relatively high values. The most asymmetric repartitions of values were registered for the following parameters:
percent of monocytes, percent of eosinophiles and percent de basophiles. These observations suggest the active implication of the blood immune system in the detoxification process for the tested subjects.

9. As for the percent of lymphocytes, the minimum observed value was much smaller than the appropriate value of the normal variation interval (13 noticed against 37. 5 normally), and the maxim value is also under the maxim of the normal interval.

10. The percent of monocytes has for the studied heroin consumers a different distribution from the normal one. So, the minim noticed value (5.3) is higher than the median of the normal interval (4), and the maxim registered value is higher than the double maxim value of the normal interval.

13. The percent of basophiles is distributed in close limits to the normal observed minim limit and almost three times higher than the observed normal maxim value

14. The percent of eosinophiles and the percent of basophiles are the only leukocyte parameters of the investigated population, which presented significant deviations from the normal distribution curves. This means that the immune system of heroine consumers is directed towards the detoxification processes, associated to the mentioned parameters.

16. The reducing of the percent of lymphocytes is very significantly correlated with the increasing of the number of monocytes; this may be an argument for the hypothesis that the immune system is directed towards detoxification processes.

References

1.Balaban C., 1993, Strategia experimentării şi analiza datelor experimentale. Editura Academiei Române, Bucureşti,.
2. Fodor, O., 1974, Tratat elementar de medicină internă, Ed. Dacia, Cluj Napoca
3. Jurcoane, Şt., Cornea, P., Stoica, I., Vassu, T.,….Tamba-Berehoiu R….., 2006, Tratat de Biotehnologie II, Ed. Tehnică, Bucureşti.
4.Vasile, D., Gheorghe, M. D.,Voicu, V., 2001 Dependența de heroină, Editura Amaltea, Bucureşti
5.Voicu, V., 1997, Toxicologie Clinică, Ed. Albatros, Bucureşti.