MORPHOLOGICAL AND CHEMICAL CHANGES IN BLOOD CELLS IN ACUTE LEUKAEMIA IN HUMANS NOTE 1. ANALYSIS OF BLOOD COUNTS IN PATIENTS WITH SUSPECTED ACUTE LEUKAEMIA

Olga-Alina RADA[1], CorinaLuminiţa MĂRAN[2], Mihaela OSTAN[1]

University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Calea Aradului Street, no. 119, 300645, Timişoara, Romania
[2]Municipal Clinical Emergency Hospital from Timisoara, Gheorghe Dima Street, no. 5, Romania
radaolga2005@gmail.com

Abstract: *acute leukaemias are a heterogeneous group of clonal neoplastic nature with undifferentiated stem cells, or partially differentiated, characterized by stopping differentiation and maturation of these cells, associated or not with their passage in the peripheral blood. The disease has a high incidence, affecting all age groups. Investigate automatic CBC gives extremely precise and clear indications of the presence of a number of cells outside the limits of variation or of the presence of a distribution or abnormal morphology. Comparing the data obtained from processing CBC from patients investigated with biological reference values from literature the authors have found that leukocytes reached a value six times higher (602 %) than the maximum reference value;these values were associated with thrombocytopenia and hypochromia.In all evaluated patients, CBC signalled the presence of blasts and undifferentiated cells that motivate further investigation.*

Key words**:** *CBC, acute leukaemia, leukocytes, thrombocytopenia, hypochromia*

INTRODUCTION

Leukaemia is an extremely severe disease with a rather low survival rate even with properly administered treatment and that address any group of age, no matter the gender or the occupation; they are related to an anomaly at cell level for which outer factors are often responsible. The incidence of leukaemia currently varies between 1-6.5 cases/100,000 inhabitants/year; acute lymphoblastic leukaemia is more frequent in children and youth (60% of the cases in people aged below 20), while acute myelogenous leukaemia occurs commonly in adults with a higher incidence in the elderly (HOFFMAN, 2006).

Diagnosing patients with one of acute leukaemia forms (myelogenous or lymphoblastic) is decisive in establishing therapy and prognosis. To do so as properly as possible, we need to corroborate the data of two large word bodies that research these diseases and that have established accurate diagnosis criteria – the mainly descriptive French-American-British (FAB) classification (BAIN *ET AL.*, 2010; KINNEY AND LUKENS, 1999) and the World Health Organization (WHO) classification delivered in 2001 and renewed in 2008; the latter includes, besides morphological criteria and cytogenetic, molecular, and immune-phenotypical data, clinical information, making up a genuine diagnosis algorithm (MUNTEANU *ET AL.*, 1999; VARDIMAN, 2009; FEY AND BUSKE, 2013).

MATERIALS AND METHODS

The study of blasts was carried out on blood sampled through vein puncture in vacutainers for blood counts, with EDTA anti-coagulant, without previous centrifugation, or through finger puncture (peripheral blood) from 25 patients suspected of leukaemia. Automatic blood counts were made with an automatic Sysmex XN-1000 haematology analyser. The device runs complete blood counts measuring a set of haematologic parameters from vein

blood entirely sampled on K_2EDTA as anticoagulant (ratio 9:1). The samples are analysed within 2 hours after sampling without being refrigerated (SR EN ISO 15189, 2013).

To characterise numeric data used in the study, we used as statistic indices arithmetic mean and standard deviation, their ratio – variation coefficient, as well as spread indices, minimum, maximum, and medium. The link between variables was established with Pearson correlation coefficient and the z test for shares established the frequency of leukaemia in the two sexes. Analyses were carried out at the Haematology Compartment of the Laboratory of Medical Analyses of the Municipal Clinical Emergency Hospital from Timisoara, accredited RENAR.

RESULTS AND DISCUSSION

Investigating automatic blood counts is indispensable in diagnosing acute leukaemia because it provides extremely accurate indices on the number of cells below or above the variation limit or on the incidence of abnormal distribution or morphology. If this is the case, the blood count is completed by an examination of a peripheral blood smear and of a bone marrow smear.

The automatic blood counts of the 25 patients investigated are shown in Table 1. Their analysis (Table 2) shows that all investigated parameters have very high variation coefficients, which suggests an extremely uneven haematological picture (Figure 1).

Table 1

Automatic blood counts of investigated patients

Patient number	**Patient age (years)**	**Patient gender (M/F)**	**Number of leukocytes (*10^3/µl)**	**Number of thrombocytes (*10^3/µl)**	**Haemoglobin (g/cll)**	**Other types of undifferentiated cells**	**Blasts in peripheral blood (%)**
1	62	F	201.28	42	3.8		95
2	66	F	19.65	34	13.3	53	7
3	73	M	20.39	11	9.9	57.5	-
4	36	F	12.54	30	6.5	82	-
5	81	F	45.91	16	7.6	-	58
6	50	F.	19.34	5	6.9	-	57
7	64	F.	8.7	72	8.3	-	61
8	32	F.	102.01	37	9.4	-	65
9	82	F	17.52	126	11.4	-	70
10	65	M	36.23	18	5.7	-	58
11	38	F	0.52	11	9	-	2/smear
12	48	M	62.38	18	8.7	-	92
13	51	F	86.03	27	7.8	-	97
14	71	F	102.01	37	9.4	-	65
15	80	M	187.7	94	9.1	-	62
16	81	F	106.87	23	5.6	-	61
17	54	M	167.84	187	11.1	-	28
18	68	F	5.39	51	6.4	-	90
19	59	F	2.42	442	10.5	-	16
20	71	M	90.38	16	6.2	-	77
21	67	M	68.73	296	13	82	-
22	51	F	86.03	27	7.8	-	97
23	22	M	19.52	136	8.9	-	90
24	41	F	25.23	12	7.5	-	4
25	19	M	10.50	156	9.5	2	-
Reference biological interval/ measure unit[2]			**4-10 /*10^3/µL**	**150-410 /*10^3/µL**	**12-15 /g/dL**		

Table 2

Statistical processing of investigated patients' blood counts

Parameter	Patient age (years)	Number of leukocytes (thousands)	Number of thrombocytes (thousands)	Amount of haemoglobin (g/cl)	Blasts or undifferentiated cells (%)
Maximum	82	201.28	442	13.3	97
Mean	62	36.23	34	8.7	62
Minimum	19	0.52	5	3.8	1
Average	57.28	60.2048	76.96	8.532	61.1
Standard deviation	18.21	58.96	103.14	2.28	29.67
C.V. (%)	**31.79%**	**97.92%**	**134.02%**	**26.78%**	**48.57%**

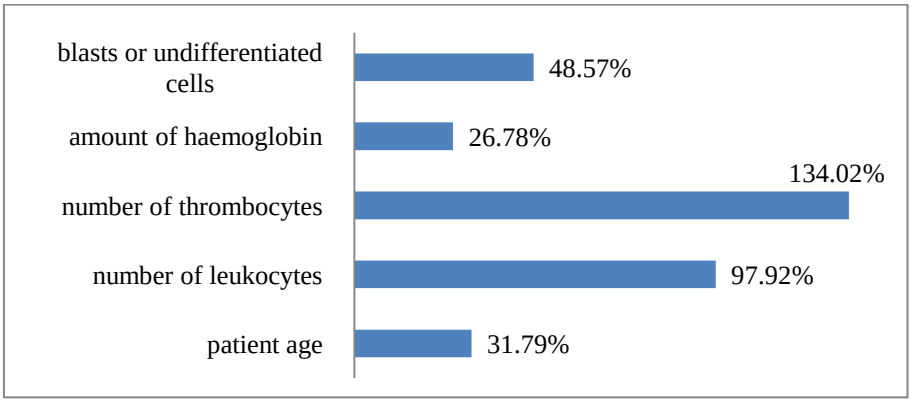

Figure 1. Variation coefficients of investigated parameters

Comparing the data with reference biological values in literature, we can see that leukocytes had a mean value of 60.20±58.96/$^{*}10^{3}$/µL, i.e. a value 6 times larger (602%) than the maximum reference value (4-10/$^{*}10^{3}$/µL) (Figure 2).

Thrombocytes had a mean value of 76.96±103.14/$^{*}10^{3}$/µL, i.e. 51.30% of the minimum reference value (150-410/$^{*}10^{3}$/µL), suggesting an association of leukaemia with thrombocytopenia (Figure 3).

Haemoglobin had a mean value of 8.53±2.28/g/dL, i.e. 71.08% of the minimum reference value (12-15/g/dL) (Figure 4).

In all investigated patients, blood counts pointed to the presence of blasts or of other undifferentiated cells; compared to the other types of blood cells, they had a mean of 61.1±29.67% (Figure 5), suggesting the need to run other investigations to establish the morphological type of atypical cell; the age of investigated patients varied between 19 and 82 years, with an average of 57.28 years (Figure 6).

When calculating the correlation coefficients between the variables, we identified a single statistically significant correlation between the amount of haemoglobin and the number of thrombocytes (Table 3 and Figure 7).

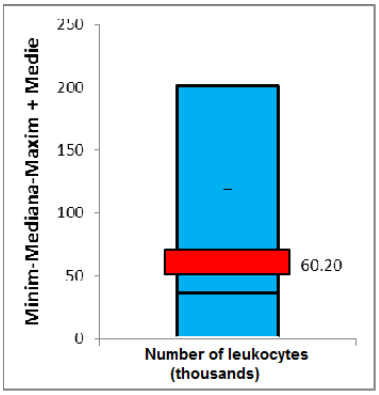

Figure 2. Mean of the number of leukocytes after the processing of blood counts

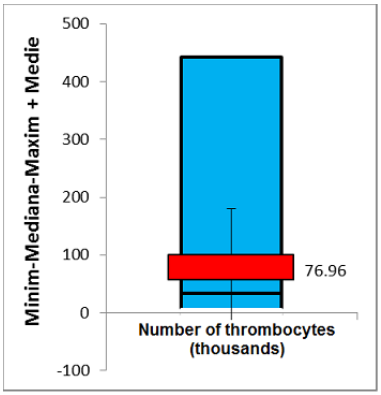

Figure 3. Mean of the number of thrombocytes after the processing of blood counts

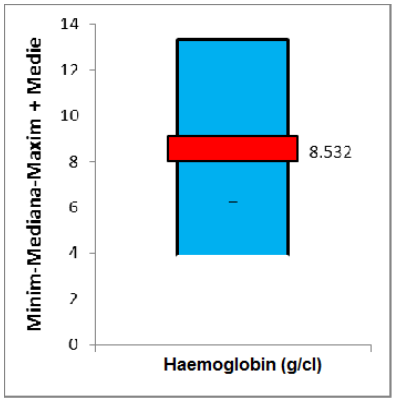

Figure 4. Mean of the amounts of haemoglobin after the processing of blood counts

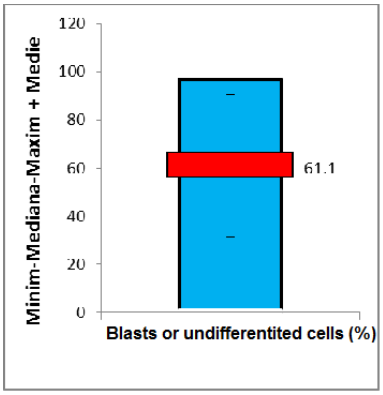

Figure 5. Percentage of blasts or undifferentiated cells after the processing of blood counts

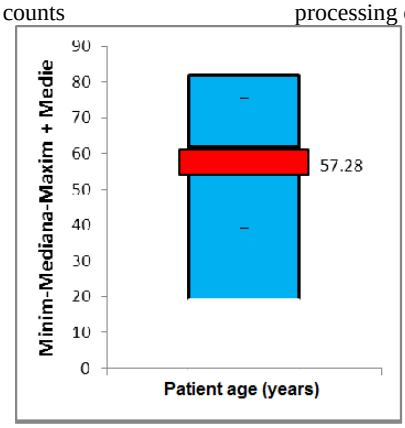

Figure 6. Mean of the age of investigated patients

Table 3.

Statistical correlation of studied parameters

Variables	Patient age (years)	Number of leukocytes (*10³/µl)	Number of thrombocytes (*10³/µl)	Amount of haemoglobin (g/cl)	Blasts or other types of undifferentiated cells in peripheral blood (%)
Patient age (years)		0.270	-0.019	0.008	0.060
Number of leukocytes (*10³/µl)	0.270		-0.003	-0.155	0.351
Number of thrombocytes (*10³/µl)	-0.019	-0.003		**0.490**	0.151
Amount of haemoglobin (g/cl)	0.008	-0.155	**0.490**		-0.262
Blasts or other types of undifferentiated cells in peripheral blood (%)	0.060	0.351	0.151	-0.262	
Values in bold are different from 0 with a significance level alpha=0.05					

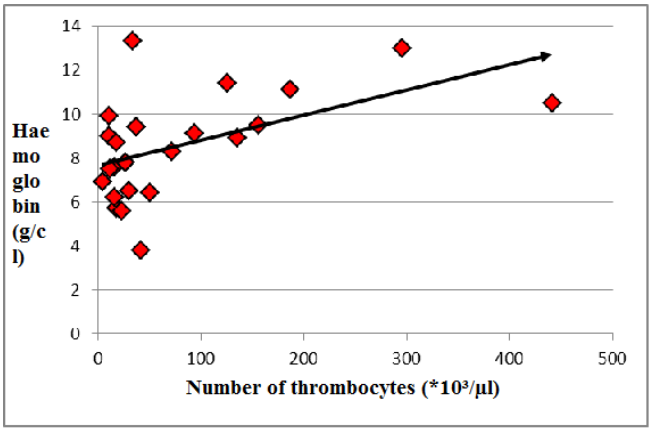

Figure 7. Statistic correlation between the amount of haemoglobin and the number of thrombocytes

Z Test for shares compared the distribution per gender of the subjects with the distribution per gender of the reference population; we could see the existence of a highly statistically significant difference ($p<0.001$), thus establishing a share of 64.00% in women, much more than the share of the general female population (in Romania, there are 51.4% women and 48.6% men with acute leukaemia, IONIȚĂ *ET AL.*, 2015) (Figure 8).

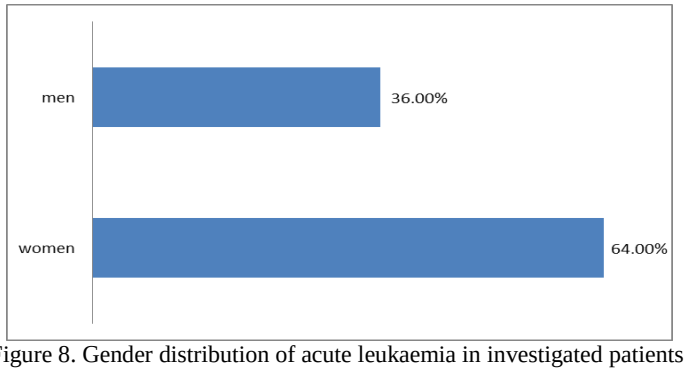

Figure 8. Gender distribution of acute leukaemia in investigated patients

CONCLUSIONS

Comparing the data from processing of blood counts with reference biological values in literature, we could see that leukocytes had a value of 60.20±58.96/*10^3/µL, i.e. six times more than the maximum reference value (4-10/*10^3/µL).

These values were associated with the number of thrombocytes – half of the minimum reference value (51.30%) and a low value of the amount of erythrocyte haemoglobin (71.08%); the two parameters correlated statistically significantly, suggesting that acute leukaemia is accompanied by thrombocytopenia and anaemia.

Comparing the distribution per gender of this disease pointed out the existence of a highly statistically significant difference ($p<0.001$), i.e. 64.00% women, much above the mean of Romania (51.4% in women and 48.6% in men).

In all investigated patients, blood counts pointed to the presence of blasts or undifferentiated cells, which asks for further investigation (microscopic examination of peripheral blood and haematogenous bone marrow smears).

BIBLIOGRAPHY

1. BAIN J. B., DAVID, M., CLARK, B. S.,2010 - Bone Marrow Pathology, Wilkins 4th Edition, Willy-Blackwell, Oxford, UK.
2. BAIN J. B., BATES,I., LAFFAN, M.A., LEWIS, S.M., 2012 - Dacie and Lewis- Practical hematology 12th Edition, ChurchillLivingstone,Edinburgh, London, Melbourne and New York.
3. FEY, M. F., BUSKE, C., 2013 - Acute myeloblastic leukaemias in adult patients: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, Annals of Oncology, 24 (6): p.138-143.
4. HOFFMAN, R., 2006 - Hematology - basic principles and practice, New York: Elsevier:59-66.
5. IONIȚĂ, H. ET. AL., 2015 – Hematologie clinică, Ed. Victor Babeș, Timișoara.
6. KINNEY, M.C., LUKENS, J.N., 1999 - Wintrobe's Clinical Hematology,10th Edition.
7. MUNTEANU N., RADU P., COLITA, D., 1999 - Tratat de medicină internă–Hematologie Clinică, partea a-II-a, Editura Medicală, București.
8. VARDIMAN, J.W., THIELE, J., ARBER, D.A., BRUNNING, R.D., BOROWITZ, M.J., PORWIT, A., HARRIS, N.L., LE BEAU, M.M., HELLSTROM-LINDBERG, E., TEFERI, A., BLOOMFIELD, C.D., 2009 - The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes, Blood, 114 (5):937-51.
9. SR EN ISO 15189, 2013 – Regulament specific de acreditare în domeniul acreditării laboratoarelor medicale, Asociația de Acreditare din România.