CHANGES OF BLOOD CELLS IN THROMBOCYTOPENIA IN HUMANS

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Abstract. Thrombocytopenia is a condition characterized by low number of platelets (thrombocytes) and leads to disruption of coagulation or haemostasis, manifested by increased bleeding time and clotting. The incidence of thrombocytopenia reported in the literature is between 13% and 41% and is associated with increased mortality and length of hospitalization. The purpose of this paper is to highlight changes in quantitative and qualitative of platelets and emphasizing the importance of haematological investigations (cell blood count, peripheral blood smear, bone marrow smear) in orientation for subsequent diagnosis thrombocytopenia. The material used in the study was the 25 blood smears, respectively 9 bone marrow smears and clusters crushed by bone marrow. Blood count (CBC) examination results revealed severe thrombocytopenia, platelets representing 27.25% of the minimum reference value in healthy individuals. Associated it was observed 74.89% reduction in red blood cells count, in haemoglobin 77.91%, haematocrit to 82.80% and reduction with 29.20% of total leukocytes from the minimum reference value. The blood count was found to drastically reduce the proportion of granulocytes, monocytes 25% reduction and the increase of lymphocytes twice. Examination of blood smears revealed changes in platelet morphology (presence of macro-thrombocytes and platelet aggregates) associated with morphologic changes in white blood cells (blasts presence on smear) and in erythrocytes (dacrocytes, anisocytosis and hypochromia). Bone marrow smear revealed that approximately 33.33% of study smears present thrombocytopenia mega-karyocyte activity, which indicates that bone marrow is functional and marrow produces platelets; 66.66% of smears from bone marrow mega-karyocyte examined were absent, probably stored in the spleen or their production was blocked by the presence of other inhibitors, may immune or hereditary cause.

Keywords: thrombocytopenia, CBC, blood smear, marrow smear

INTRODUCTION

Thrombocytopenia is a frequent complication in critical patients that, most often, is a clinical challenge. Its incidence, as reported in specialty studies, ranges between 13% and 41% and is associated with an increase of death rate and of hospitalisation (Gilău, 2013).

Thrombocytopenia occurs because of the failure of thrombocytes at marrow level or because of the increase of lysis phenomena at blood or spleen level. Marrow tumours can cause secondary thrombocytopenia. After treatment with antibiotics, antihistamines, or sulphanamides, there can be idopathic thrombocytopenia. Thrombocytopenia is accompanied by clogging and haemostasis failure (increase of bleeding and clogging times) (Demott and Tilzer, 1994; Fischbach, 2009; Rodgers, 2004, Smock and Perkins, 2013).

In case of suspicion of thrombocytopenia, it can be easily confirmed by laboratory tests (Oltean et al., 2009). A complete blood count is a compulsory primary investigation to which we should add, in certain cases (established by the laboratory doctor depending on the aspect of the blood count), by a blood and bone marrow smear.

A complete blood count covers the parameters that supply useful information warning on the causes of a thrombocytopenia. After confirming it through the determination of the number of blood platelets and of the other figurate elements, examining blood and bone
marrow smears is a valuable means in the diagnosis and evaluation of the condition (clarifying the disease aetiology).

The goal of this paper is to point out the quantitative and qualitative changes in figurate elements and the importance of haematological investigation (blood count, peripheral blood smear, bone marrow smear) in later establishment of thrombocytopenia.

Blades with blood and haematogenous bone marrow smears were obtained with the help of the Laboratory Haematology Compartment of the Municipal Clinical Emergency Hospital from Timișoara.

MATERIAL AND METHODS
The material used in this study consisted in 25 blood counts of peripheral blood smears and 9 haematogenous bone marrow and grain smears.

The study of figurate elements was done on blood sampled through vein puncture in vacutainers for blood count with EDTA anti-clogging with no previous centrifugation or through fingertip puncture. The blood counts were analysed with a SYSMEX XT 2000 automatic analyser to determine the number of figurate elements in the blood and to calculate the erythrocyte indices. If there are differences between the results of the automatic analyser and the number of thrombocytes per smear (the platelets look agglutinate), they recommend the sampling of blood in a vacutainer containing as anti-clogging natrium citrate. This unbinds the groups of thrombocytes.

Blood and marrow smears were coloured using the panoptical May-Grünwald-Giemsa (MGG) coloration and they were examined with an Olympus CX 41 microscope.

To photograph blood platelets, we used a Quickphoto Micro 2.2, caption and statistical measurement soft of the Olympus CX 41 microscope.

Statistical processing of experimental data was done using the non-parametric correlation index of Spearman ranks.

RESULTS AND DISCUSSIONS
Investigation of the blood count is completed by the examination of the peripheral blood smear: if, on the blood smear studied with the microscope, there are other anomalies than thrombocytopenia, such as nucleated erythrocytes or abnormal or immature leukocytes, then we should examine bone marrow. The investigation can be completed through haemostasis tests.

Initiated to check the number of thrombocytes, a defining criterion in diagnosing thrombocytopenia, this study supplies details on the association of thrombocytopenia with other haematological diseases such as different forms of anaemia and leukaemia.

After processing the blood counts of the studied patients, the mean values calculated for each parameter point to very high variability coefficients in the number of leukocytes (221.55%), eosinophils (162.83%), basophiles (130.98%), lymphocytes (352.61%) and monocytes (252.77%), suggesting a completely confused haematological image (Figure 1).
Figure 1. Variability coefficients of trial parameters

The comparison of the types of leukocytes (granulocytes, agranulocytes) was done in relation to the total number of leukocytes; the mean leukocyte formula pointed out a decrease of the number of neutrophils to 44.11%, of eosinophils to 9.20%, of basophiles to 15.00% and of monocytes to 75.00%. In exchange, the number of lymphocytes increased twice pointing to leukaemia (Figure 2).

Figure 2. Comparison of white figurate elements with maximum reference values (%)

Based on the values of Spearman correlation coefficients, we established the following correlations between the different blood figurate elements:
- There is positive, statistically insignificant correlation between thrombocytes and erythrocytes, leukocytes, neutrophils ($r=0.208430$, $r=0.171989$ and $r=0.118090$, respectively);
- There is positive, low correlation between thrombocytes and eosinophils and basophiles, respectively ($r=0.523309$ and $r=0.459988$, respectively);
- There is negative, statistically insignificant correlation between thrombocytes and lymphocytes and monocytes, respectively ($-0.251222$, and $-0.047046$, respectively).
Examining the blood smear to evaluate thrombocyte morphology usually associated with morphological changes of erythrocytes (anisocytosis: macro- and microcytosis, hypochromia) and of leukocytes (the presence of hyper-segmented granulocytes and of blasts) can support the changes observed in the study of blood counts.

The changes noticed upon thrombocyte examination were:

a) The presence of macrothrombocytes: they have a diameter >4 µm (→) and they occur in the presence of a high thrombocyte turnover (idiopathic thrombocytopenic purple); the macrothrombocytosis associated with morphological changes of erythrocytes: anisocytosis and hypochromia (Figure 3).

A particular case was that of a patient later diagnosed with May Hegglin anomaly where, besides macrothrombocytosis, there were also Döhle body neutrophils (the aspect is common to central thrombocytopenia because of the lack of thrombocytes - Drachman, 2004). (Figure 4).
b) Presence of thrombocyte aggregates: they can occur because of microcoagules in the sample and it can be induced by EDTA/cold agglutinin (Figure 5).

![Figure 5. Aspect of thrombocyte aggregates (col. MGG, x100, original)](image)

The changes noticed upon examination of other blood elements were:

a) The presence of blasts (in chronic lymphatic leukaemia and acute lymphoblast leukaemia, where thrombocytopenia was secondary) (Figure 6).

![Figure 6. Presence of blasts in leukaemia secondary thrombocytopenia (col. MGG, x100, original)](image)

b) The presence of hypersegmented granulocytes: we noticed neutrophils with hypersegmented nucleus, with or without satellite thrombocytes (Figure 7).
Figure 7. Aspect of numerous, hypersegmented neutrophils (col. MGG, x100, original)

c) The presence of erythrocytes with shape changes (dacryocytes, tear-shaped erythrocytes) (←), with diameter changes (anisocytosis: micro- and macrocytosis) and with haemoglobin load changes (hypochromia) (←) (Figure 3).

Examination of the bone marrow smear was done in 9 of the 25 subjects (36%). The changes noticed upon examination of bone marrow smears were:

a) in 3 of the 9 bone marrow smears (33.33%) there were megakaryocytes with thrombocytopenia activity, which shows that haematogenous bone marrow is functional and produces thrombocytes (Figure 8).

Figure 8. Presence of thrombocytopenia megakaryocytes (col. MGG, x100, original)

b) in the other 6 smears (66.66%), there were no megakaryocytes, probably because they were stored in the spleen or because their production had been blocked by the presence of other inhibiting factors possibly immune or hereditary.
CONCLUSIONS

- Blood count in 25 patients pointed out to serious thrombocytopenia, with thrombocytes representing 27.25% of the minimum reference values in healthy individuals. There was also a decrease of the number of erythrocytes to 74.89%, of haemoglobin to 77.91% and of haematocrit to 82.80%;
- Previous observations were accompanied by changes of the white line: decrease of total leukocytes with 29.20% compared to the minimum reference value, and 3.23 times less leukocytes compared to the maximum total leukocytes;
- In the leukocyte formula, there was drastic decrease of the share of granulocytes, decrease with 25% of monocytes and increase two times of lymphocytes, respectively;
- Examining blood smears pointed out changes of thrombocyte morphology (the presence of macro-thrombocytes and of thrombocyte aggregates) associated with morphological changes of leukocytes (presence of blasts on the smear that confirmed the observations regarding the abnormal increase of the number of lymphocytes and pointed to leukaemia associated with thrombocytopenia) and of erythrocytes (shapedacrocytes, size-anisocytosis and load with haemoglobin-hypochromia, suggesting as main diagnosis anaemia associated secondarily with thrombocytopenia).
- Examining the bone marrow smear pointed out that in about 33.33% of the smears there were megakaryocytes with thrombocytopenia activity, which means that haematogenous bone marrow is functional and produces thrombocytes; in 66.66% of the bone marrow smears, megakaryocytes were absent, maybe because of being stored in the spleen or because their production had been blocked by the presence of other inhibitors (immune or hereditary).

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