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# Analysis of changes in the morphology of erythrocytes – microscopic evaluation of peripheral blood smears

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## Abstract

Microscopic assessment of peripheral blood smears belongs to the basic techniques of quantitative and qualitative analysis of leukocytes, erythrocytes and platelets. Modern hematology analyzers allow accurate and precise assessment of blood cells, flagging parameters that are beyond the limit of the norm. Despite advanced technologies, the automatic peripheral blood smear is devoid of detailed data on abnormalities in the shape of erythrocytes, the presence of inclusions and erythroblasts, which can only be obtained by manual evaluation of peripheral blood smears. The following work aims to highlight the role of manual evaluation of peripheral blood smears, with particular emphasis on the assessment of abnormalities in the morphology of erythrocytes, which despite the passage of time should still be routinely performed as a supplement to modern diagnostic tools.

*Key words: peripheral blood smear; erythrocytes; anemia.*

Complete blood counts are routinely performed in every medical (diagnostic) laboratory, which is usually accompanied by the automatic evaluation of peripheral blood smears. The presence of modern hematology analyzers enables more and more accurate and precise assessment of blood cell counts, but it does cannot manual evaluation of peripheral blood smear performed by an experienced laboratory diagnostician.

Evaluation of peripheral blood smear should include the assessment of leukocytes, erythrocytes and thrombocytes. However, the most attention is paid to the quality disorders of white blood cells (the Schilling percentage formula), omitting other leukocytes. This paper aims to

emphasize the value of manual evaluation of peripheral blood smears, and in particular to attract attention to deviations that may occur in the morphology of red blood cells.

Proper interpretation of the microscopic image (irrespective of the type of material evaluated) is strictly connected with the proper collection and protection of the material, and in the case of peripheral blood also with a properly performed smear. The blood is taken to a test tube with an anticoagulant: diaminotetraacetic acid (EDTA), but one should remembered about the correct ratio of blood volume to anticoagulant (excess of EDTA results in erythrocyte relaxation, consequently the MCV, HTC and MCHC values are reduced) [1]. Performing a peripheral blood smear should be done within 3 hours after collection (Appendix 3 to the MZ Decision from 23 March 2006 [2]), ideally 2 hours after collection are recommended[3]. It has to be performed on degreased and clean slides with a field for signature. The specimen should end with a tail so that an objective assessment of the morphology of all blood cells is possible. The first stage is the evaluation of the specimen under low magnification (x 10 lens). This allows the evalutaion of the "arrangement" of erythrocytes, in which particular attention should be paid to rouleaux formation of erythrocytes and agglutination. Rouleaux formation of erythrocytes occurs among others patients with multiple myeloma, as a consequence of high monoclonal protein [4], and agglutination may caused by the the presence of autoantibodies. If there is a reasonable suspicion of cold agglutinin disease (IgM antibodies), the evaluation of the peripheral blood smears should be conducted at 37º in order to obtain a credible result [5]. The morphology of erythrocyte is evaluated under immersion (lens x 100), in a place where red blood cells lie freely, one next to the other. Blood cell size assesment is one of the criteria for the division of anemia and allows to "narrow down" the cause of its occurrence. Normal double-concave erythrocytes - normocytes (erythrocytes with the diameter of 7 μm) are easily recognized by an experienced diagnostician because their size is similar to that of small lymphocytes. The presence of microcytes (erythrocytes with a diameter of less than 6 μm) is not equivalent to the diagnosis of iron deficiency anemia. It should be remembered that their presence is also characteristic for thalassemia, therefore a further differential diagnosis is recommended [6]. The presence of macrocytes (larger than 9 μm in diameter) in the peripheral blood smear allows for the diagnosis of macrocytic anemia, the cause of which does not have to be a deficiency of vitamin B12 and / or folic acid. It may also accompany chronic alcohol abuse, liver disease and in some cases it should arouse suspicion neoplastic disease, e.g. myelodysplastic syndrome. In this case it is important to perform additional biochemical and ultrasound tests, as well as, the assessment of coexisting lesions, e.g. in leukocytes (hyper-proliferation of neutrophilic nuclei will be in favor of vitamin B12 deficiency). It should also be noted that macrocytic anemia of unknown cause is an indication of bone marrow aspiration [7].

The assessment of abnormalities in the shape of erythrocytes is an indispensable part of membranopathy diagnostics, i.e. congenital hemolytic anemia caused by defects of the erythrocyte membrane. Particularly noteworthy is congenital spherocytosis, which is the most common congenital hemolytic anemia. Usually, in order to diagnose anemia, it is sufficient to identify jaundice, splenomegaly, reticulocytosis, presence of spherocytes in the peripheral blood smear and decreased osmotic resistance of erythrocytes, with a negative result of a direct antiglobulin test and positive family history [8]. The evaluation of red blood cell morphology along with an interview, clinical symptoms and other laboratory and genetic tests is a comprehensive combination necessary in the diagnosis of hemolytic anemia. The microscopic assessment of peripheral blood smear plays an important role also in case of suspected thrombotic microangiopathy, among others thrombotic thrombocytopenic purpura (TTP). One of the criteria necessary to diagnose TTP is the presence of schistiocytes (fragmented red blood cells) in the peripheral blood smear, usually more than 10% in the field of vision [9, 10]. The assessment of erythrocyte morphology also includes erythrocyte interjections. The most frequent are the Howell - Jolly bodies, present in patients after splenectomy. The presence of Howell-Jolly bodies in the peripheral blood smear may also be a useful marker to assess the function of the spleen, especially in patients after partial spleen embolization, and indicate a searious decline in its function [11]. The presence of irregularities in the shape and color of erythrocytes and erythrocyte interjections does not have to be specific to a single disease entity.

The table below presents basic disorders of red blood cells accompanying certain disease entities.

| **Morphological changes of erythrocytes** |
|---|
| 1. Acantocytes<br>abetalipoproteinemia, severe liver disease, splenectomy,<br>disorders of absorption, hypothyroidism, vitamin E deficiency |
| 2. Echinocytes<br>uremia, pyruvate kinase deficiency, in neonates (especially premature babies),<br>microangiopathic hemolytic anemia, as artifacts |
| 3. Spherocytes<br>congenital spherocytosis, in some hemolytic anemias,<br>after transfusion, severe burns |
| 4. Codocytes<br>hemoglobinopathies, thalassemia, iron deficiency anemia,<br>splenectomy, in congestive liver diseases |
| 5. Sickle cells (drepanocytes)<br>hemozygous form of sickle cell disease |
| 6. Stomatocytes<br>congenital asthma, alcoholism, liver disease,<br>Rh null phenotype as artifacts |
| 7. Eliptocytes / ovalocytes<br>elliptocytosis / congenital ovalocytosis, iron deficiency anemia,<br>megaloblastic anemia, extra-haem haematopoiesis |
| 8. Lacrymocytes (dacriocytes)<br>myelofibrosis, thalassemia, other cases of extramedullar haematopoiesis |
| 9. Schistiocytes<br>hemanganic microangiopathic anemia (TTP, DIC, HUS),<br>severe burns, rejection of kidney transplants |
| 10. Rouleaux formation of erythrocytes<br>increased protein and / or paraprotein concentration |
| 11. Autoagglutination<br>antigen - antibody reactions |
| 12. Howell-Jolly body<br>splenectomy, splenic insufficiency,<br>megaloblastic anemia, hemolytic anemia |
| 13. Basophilic mucus<br>lead poisoning, thalassemia, heme synthesis disorders |
| 14. Pappenheimer bodies<br>splenectomy, hemolytic anemia, sideroblastic anemia, megaloblastic anemia,<br>hemoglobinopathies |
| 15. Cabot ring<br>myelodysplastic syndromes, megaloblastic anemia |

Table 1. Morphological changes of erythrocytes [12].

The analysis of peripheral blood smears is also used in parasitology, mainly in the diagnosis of malaria (ague). This study, together with the specimen of a thick drop of blood, is currently considered as a reference method (gold standard) for the final confirmation or exclusion of acute episode of ague and a superior one, compared with techniques detecting specific Plasmodium spp antigens (immunochromatographic and immunoenzymatic methods) [13]. Comprehensively, the assessment of the erythrocyte system should also include an assessment of the forms of younger erythrocytes that may appear in peripheral blood. Reticulocyte (immature non-nuclear erythrocyte) evaluation is performed by staining the cells with dyes showing RNA (e.g. new methylene blue) or using modern hematology instruments (the automatic method). The standard May-Grunwald Giemsa (MGG) staining does not allow to assess the level of reticulocytes, as opposed to

erythroblasts (nucleated red blood cells), occurring among others in hemolytic anemia, microangiopathic hemolytic anemia. The presence of erythroblasts in the peripheral blood smear is immensly important, often associated with the presence of earliest stages of granulopoiesis (it may raise the suspicion of hematological cancers), therefore the presence of even few erythroblasts should be recorded on the result [3].

Manual evaluation of peripheral blood smears is the basic technique of the quantitative and qualitative analysis of leukocytes, erythrocytes and platelets. It should be remembered that the credibility of the result depends on the quality of the specimen and the experience of the laboratory diagnostician. Compared to the automatic peripheral blood smear method, the microscopic assessment of the erythrocyte system is not limited to the diagnosis of anisocytosis (erythrocyte size abnormalities) and poikilocytosis (irregularities in the shape of erythrocytes). It allows for an accurate analysis of changes in the shape of red blood cells, the presence of inclusions and erythroblasts. Proper interpretation supplemented by the results of biochemical (laboratory) tests allows to determine the type and cause of anemia, to monitor its treatment, and, in the case of coexisting disorders of the white blood cells and / or platelet system, to plan further diagnostics. The microscopic evaluation of peripheral blood smears continues to play an important role in laboratory diagnostics and should be performed routinely as a complement to modern diagnostic methods.

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