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RESEARCH ARTICLE

INCLUSIONS IN ERYTHROCYTES: INTERPRETATIONS

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ABSTRACT

Erythrocyte inclusions are seen in many hematological disorders, both benign and malignant. Some of the common hematological disorders are anemias, which often have vague and frequently overlapping symptoms. Erythrocyte inclusions may be pathological evidence of specific diseases or disorders when interpreted within the context of presenting clinical symptoms and signs. Characterizing and identifying RBC inclusions can provide or support the diagnosis of various clinical conditions. The main aim of this review is to study the morphological characteristics, staining patterns, and clinical correlation of the most common erythrocyte inclusions.

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INTRODUCTION

Red blood cell inclusions can arise from a variety of sources. Correct identification of these abnormalities is important since it can provide insights into metabolic, physiologic, and pathologic conditions affecting the red blood cell. Most RBC inclusions can be identified and characterized on a routine peripheral blood smear using standard hematological Romanowsky stains, such as Wright or Giemsa. On the basis of their appearance, structural composition, and associated pathophysiology, the most common erythrocyte inclusions are categorized as Howell-Jolly, Heinz, or Pappenheimer bodies, basophilic stippling, Cabot rings, or precipitated hemoglobin inclusions, such as HbH[1].

DISCUSSION

HOWELL- JOLLY BODIES

History: William Henry Howell was born in Baltimore in 1860 and received a PhD from Johns Hopkins in 1884. His thesis was on the origin of fibrin in blood coagulation. During his work on coagulation, Howell began to observe how granules stained like nuclei. He went on to describe larger granules that would later be described as "Howell's nuclear particles".

Justin Marie Jolly was born in 1870 in Melun, now a Paris suburb, obtaining his doctorate in 1898 at the University of Paris. During his work, Jolly refined and detailed many of Howell's descriptions. He described fragmentation of nuclei during expulsion which left behind "pieces" of nuclei, and he additionally noted smaller basophilic granules. The first report connecting the presence of a Howell-Jolly body to splenectomy was by Schur in 1908 [2].

Morphologic characteristics: Howell-Jolly bodies are small (0.5-1 micron) purple inclusions that contain DNA. They are thought to represent chromosomes that have separated from the mitotic spindle that are left behind when the red cell nucleus is extruded. They are nuclear remnants that are usually removed when blood cells are in the spleen. These are small, dark, basophilic, smooth, round to oval, punctate cytoplasmic bodies[3]. (Fig:1)

Staining pattern: Howell-Jolly bodies stain purple with Romanowsky dyes, such as May-Grünwald-Giemsa, Wright-Giemsa, Leishman, and Diff-Quik® stains[3].

Clinical significance: They are associated with splenectomy, hyposplenism, megaloblastic anemia, hemolytic anemia, myelothisic anemia.

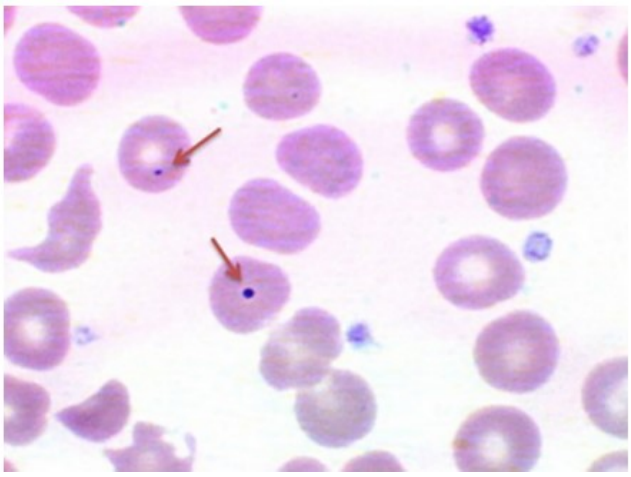

Figure 1. Howell Jolly bodies

HEINZ BODIES

History: Heinz bodies, initially known as Heinz-Erlich bodies are named after Robert Heinz (1865–1924), a German physician who in 1890 described these inclusions in connection with cases of hemolytic anemia.

Morphologic characteristics: Heinz bodies are precipitated, irreversibly denatured hemoglobin deposits within RBCs. These inclusions are seen attached or close to the cellular membrane, may be single or multiple, and are small, round, refractile inclusions measuring 1 to 3µm in size. Heinz bodies may protrude through the cellular membrane; in a wet blood film, they may be seen moving around in the cell[4]. (Fig:2)

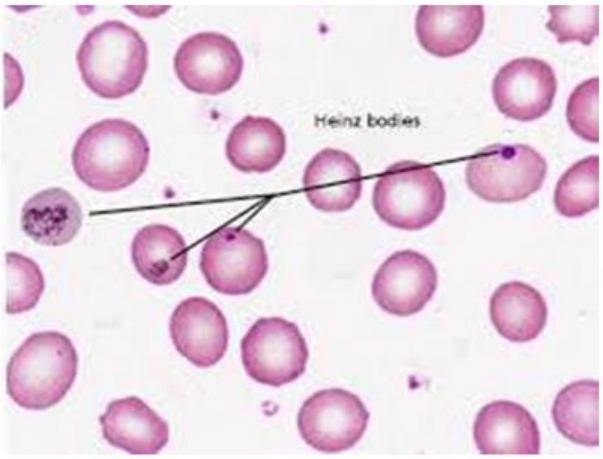

Figure 2. Heinz bodies

Staining pattern: Heinz bodies are poorly visualized with Romanowsky dyes. They may appear as pink-brown nodular structures inclusions. Heinz bodies will stain dark purple-blue and are better visualized with supravital stains such as methylene blue, brilliant cresyl blue, and methyl violet[5].

Clinical significance: Heinz bodies presence can result in hemolytic anemia. It may be present in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency and those with methemoglobinemia. Heinz bodies may also indicate unstable hemoglobins such as Hb Koeln or Hb Wien. They are evident in beta-thalassemias. Heinz body production is amongst the dysmorphisms brought on by the oxidative stress of COVID infection[6,7].

Erythrocyte oxidative damage with resulting Heinz body formation can occur in various clinical conditions, especially in patients experiencing diabetic ketoacidosis. Drugs acting as oxidants also cause Heinz body formation, such as acetaminophen, vitamin K1, propafol, and phenothiazines. Mineral deficiency, for example, selenium, also increases susceptibility to oxidative damage and Heinz body formation[8].

BASOPHILIC STIPPLING

Morphologic characteristics: Incomplete or failure of ribosomal degradation leads to precipitation of ribosomes or ribosomal remnants in circulating erythrocytes, which is visible as basophilic stippling on microscopy. Ribosomes and fragments of ribosomal RNA/ribonuclear proteins can form aggregates in circulating reticulocytes and erythrocytes as a consequence of disease. It is an indicative of disturbed erythropoiesis[9]. (Fig:3).

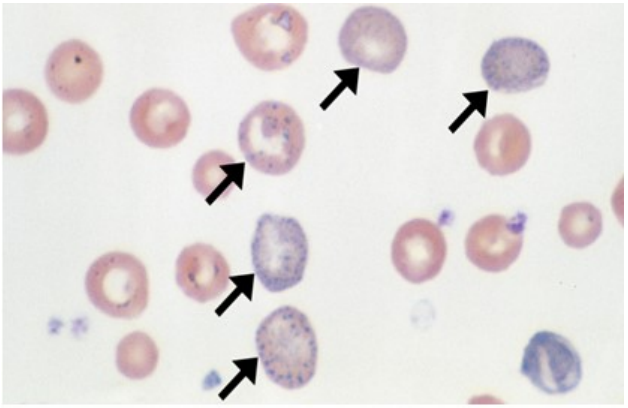

Figure 3. Basophilic stippling

Staining pattern: They appear dark blue to purple, fine to coarse punctate granules, numerous with fairly even distribution with Wright Giemsa and Methylene blue stain. Fine stippling is often noted in polychromatophilic red cells[10].

Clinical Significance: Coarse basophilic stippling may be seen in sideroblastic anemias, lead poisoning, myelodysplasias, abnormal heme synthesis and thalassemias[10].

PAPPENHEIMER BODIES

History: In 1945, Alwin Max Pappenheimer J described three patients whose red blood cells, after splenectomy, showed inclusions when stained with Giemsa stain or Wright's stain. They described these bodies as red–purple, usually coccoid, and adjacent to the cell membrane, and they demonstrated that the cells stained for iron with Prussian blue stain. Several other investigators had described siderotic granules in erythrocytes prior to Pappenheimer's report. Gruneberg found Prussian blue-positive inclusions in red cells of human fetuses and later splenectomized adults. He coined the term ''siderocyte'' for these red cells with Prussian blue-staining inclusions[11].

Morphologic characteristics: Pappenheimer bodies, or siderotic granules, are hemosiderin-containing, small, round, dark, basophilic or black-colored inclusions situated at the periphery of RBCs. It is the result of sideroblastic erythropoiesis producing siderotic, non-heme iron intracytoplasmic granules. The granules contain ferric iron, lipids, proteins, and carbohydrates. If many Pappenheimer

bodies are present, it can easily be confused with punctate basophilia. However, because Pappenheimer bodies contain hemosiderin, they will stain blue with Perls Prussian blue. In contrast, punctate basophilia will appear pink[12]. (Fig:4)

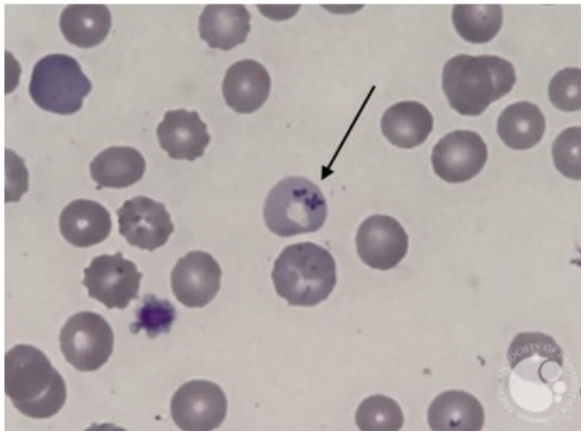

Figure 4. Pappenheimer bodies

Staining pattern: They are visible under both Wright/Romanowsky stains and Perls Prussian Blue stain. Pappenheimer inclusions appear as clusters of fine and irregular granules located at the periphery of the red blood cell and are much smaller than Howell- Jolly bodies[12].

Clinical significance: Splenectomy, Sideroblastic Anemia, Thalassemia, Sickle Cell Disease, Hemachromatosis, Myelodysplastic syndrome[13].

CABOT'S RING

History: Also known as Cabot Schleip ring is named after Richard Clarke Cabot and Karl Friedrich Wilhelm Schleip. Richard Cabot in 1903 first described Cabot ring in the blood smears of anemic patients as ring-shaped structures[14].

Morphologic characteristics:They are thin,thread like red-violet-staining strands in the shape of rings, figure eights, or shapes of the letter B may on rare occasions be seen in erythrocytes. Their presence indicates defect in erythrocyte production. They are probably microtubules remaining from a mitotic spindle. It is reported to involve arginine rich histone and non hemoglobin iron[15].

Staining pattern: They stain dark blue to purple, 1-2 per cell with Wright Giemsa and New Methylene blue stain. They are a rare finding and may be confused with malaria[3]. (Fig:5)

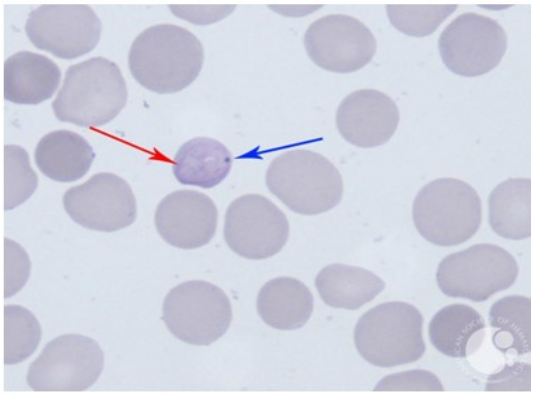

Figure 5. Cabot' Ring

Clinical characteristics: Cabot's ring may seen in Hemolytic anemia, lead poisoing, Pernicious anemia, Thalassemaia, Megaloblastic anemia.

RETICULOCYTES

History: The first description of reticulocytes was made in 1865 by Wilhelm Heinrich Erb, a German neurologist when he discovered the population of granulated erythrocytes while observing the effect of acetic and picric acid on the development of erythrocytes. In 1922, Edward Bell Krumbhaar, first coined the term "Reticulocyte"[16].

Morphologic characteristics: They are still developing immature anuclear erythrocytes produced in the bone marrow and released into the peripheral blood, where they mature into RBCs within 1 to 2 days.

Breakdown and expulsion of organelles begin while in the bone marrow and continue when the reticulocyte is in the bloodstream; it includes the endoplasm reticulum, Golgi apparatus, lysosomes, mitochondria, and ribosomes via both autophagic and non-autophagic pathways. Once in the bloodstream, RNA breakdown occurs, facilitated by ribonucleases. Some rRNA will remain for RBC formation. Changes in cell volume and membrane remodeling are thought to occur via exosomes. All these changes occur selectively, so the necessary proteins are available during the life of a reticulocyte but can be expelled when necessary to create a mature biconcave RBC. Reticulocytes have greater volume, higher hemoglobin content, and lower hemoglobin concentration[17] (Fig:6)

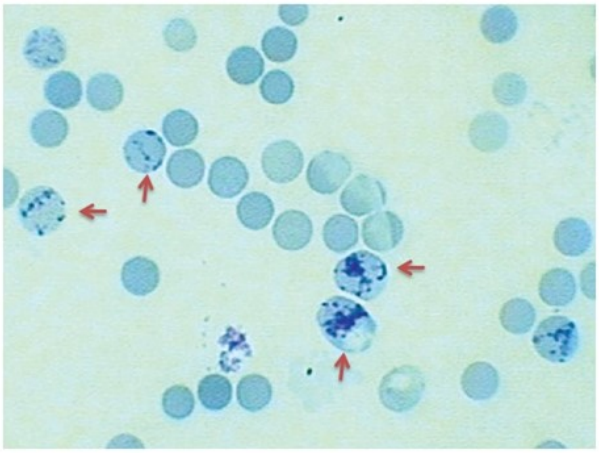

Figure 6. Reticulocytes

Staining pattern: They stain dark blue granules, precipitated RNA, two or more per cell with New Methylene Blue or Brillant Cresyl Blue. On staining with Giemsa or Wright stain, causes the RNA to disappear from alcohol fixation and reticulocyte acquire slightly larger size than mature RBC, with polychromatic dark blue color[18].

Clinical significance: Increased reticulocyte index is associated with hemolytic anemias, recent haemorrhage, chemotherapy induced anemias. Decreased reticulocyte index is associated with aplastic anemia, ineffective erythropoiesis, megaloblastic anemia[19].

HEMOGLOBIN H

Hemoglobin H (HbH) inclusions are precipitated beta-chain tetramers seen in alpha-thalassemia. Alpha-chain inclusions represent alpha-chain precipitation in beta-thalassemia major.

Morphologic characteristics: Hemoglobin H is made of four beta chains, and HbBarts is made of four gamma chains. HbH may denature and form greenish, round inclusion bodies when staining for reticulocytes with new methylene blue[20] (Fig:7).

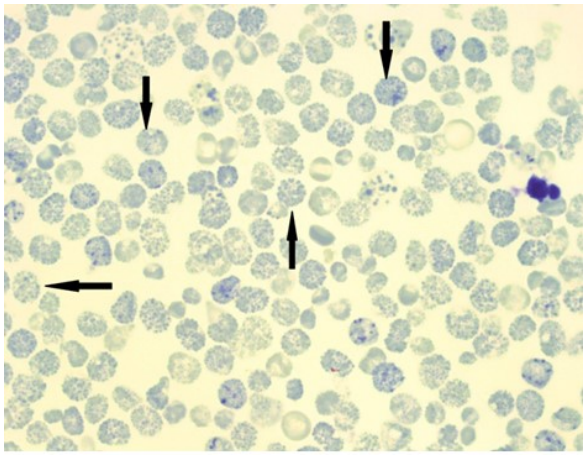

Figure 7. Hemoglobin H

Staining pattern: They appear as dark blue irregular nodules close to the nucleus of the marrow erythroblasts when stained with supravital methyl violet or brilliant cresyl blue stains often described as 'Golf balls or Raspberries'.

Clinical significance: Inclusions of hemoglobin are abundant in the normoblasts of patients with beta-thalassemia and other thalassemias. Hemoglobin H can cause chronic hypochromic microcytic anemia and hemolytic anemia, which can worsen in periods of oxidant stress[21].

CONCLUSION

Erythrocyte inclusions are elements that may be present in red blood cells (RBCs). The appearance, composition, and associated physiology of the inclusions are specific for each type of inclusion. Identification and reporting of these inclusions are important because their presence may indicate diseases or disorders. Many erythrocyte inclusions can be visualized on a Wright-stained smear. However, some erythrocyte inclusions can only be observed by using a special stain.

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