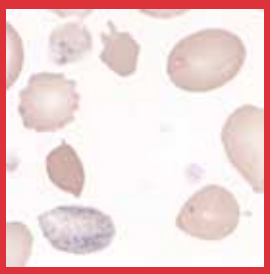
Spotlight on hematology

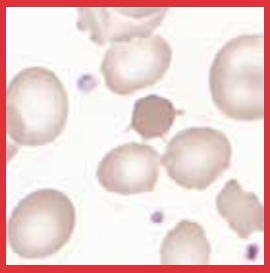
Irregularly contracted cells

Some laboratories distinguish between round spherocytes and „irregularly contracted cells." These are similar to spherocytes: small, dark red and lacking central pallor. However, their contours are not round but irregular. In some, small protuberances are seen. These protuberances can be identified by special staining as „Heinz bodies" (oxidative denatured hemoglobin which leads to aggregate formation).
Accordingly, common causes for the formation of „irregularly contracted cells" are:
- Hemolysis due to G6P-DH deficiency
- Exposure to oxidizing drugs or chemicals
- Hemoglobinopathies (e.g., HbC, unstable hemoglobin)

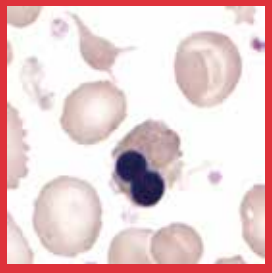
With congenital dyserythropoietic anemia, the pronounced colorful picture of this anisotype poikilocytosis and the presence of „irregularly contracted cells" can be an important indicator for excluding hereditary spherocytosis; CDA patients are frequently first misdiagnosed as having hereditary spherocytosis.



a) Irregularly contracted cell



b) Acantho-spherocytes



c) Binucleated erythroblasts

Congenital dyserythropoietic anemia

MQZH 2015-02

Introduction

Congenital dyserythropoietic anemias (CDA type I, II and III) are rare, congenital disorders affecting erythrocyte structure (in Switzerland, Austria, and Germany there are currently around 120 patients from 98 families). This leads to the formation of atypical, multinucleated erythroblasts in the bone marrow and a notable anisotropy and poikilocytosis in the peripheral blood picture. Erythropoiesis is ineffective, i.e., a large part of the erythropoietic cells die in the bone marrow. This results in anemia, jaundice (hyperbilirubinemia) with an increased risk for developing gallstones, and secondary hemochromatosis. Splenomegaly and hepatomegaly are common.
The disease is usually already manifested in newborns; with a mild course, a diagnosis may be delayed until adulthood. Our current survey sample specimen MQZH 2015-2 is derived from a patient with known CDA type I after splenectomy.

Pathophysiology

Depending on the type of CDA, different genetic defects are found. With CDA I the defect is on chromosome 15 (15q1-q3, *CDAN1*), however the exact function of this gene is not yet understood. The defect results in an increased and ineffective erythropoiesis in the bone marrow with maturation delay at the level of polychromatic erythroblasts and formation of binuclear (more rarely polynuclear) erythroblasts. The early degradation of erythrocytes in the bone marrow and spleen causes the accumulation of increased hemoglobin that must be degraded (bilirubinemia, accumulation of iron). Infections with, for example, parvovirus B19 can cause an aplastic crisis (decompensated hemolysis) in CDA patients.
In most patients, splenectomy reduces anemia (typically with CDA II). Transfusion of packed red blood cells is more rarely required. Chronic hemolysis and possible blood transfusions can lead to accumulation of iron in the organs, leading to risk of organ damage (secondary hemochromatosis).

Laboratory findings

Bone marrow

- Hypercellular with erythropoietic hyperplasia Increased binuclear (rarely polynuclear)
- polychromatic erythroblasts and karyorrhexis forms

Peripheral blood picture

Anemia (hemoglobin between 80-110 g/L)
normochromic, initially normocytic, later usually macrocytic (MCV 100-120 fl)

Erythrocyte morphology

- Anisocytosis (increased RDW)
- Severe poikilocytosis
- (differently shaped red blood cells, frequently > 50%)
- Spherocytes and „irregularly contracted cells"
- Macrocytes with irregular contours
- Erythrocytes with basophilic stippling
- Cabot rings
- Potentially few binucleated erythroblasts
- With post-splenectomy status:
- Howell-Jolly bodies, target cells, acanthocytes, karyorrhexis forms, erythroblasts

Reticulocytes

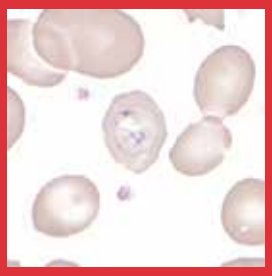
- Normal absolute and potentially increased relative reticulocyte counts
- but hyporegenerative reticulocyte production index (RPI <2.0)

Other parameters

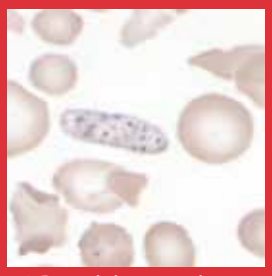
- Hemolysis parameters:
  Severe to lacking haptoglobin, indirect bilirubin elevation
- Serum acid test (in CDA type I and III negative, in CDA type II positive)
- Elevated ferritin, transferrin saturation increased

Spotlight on hematology

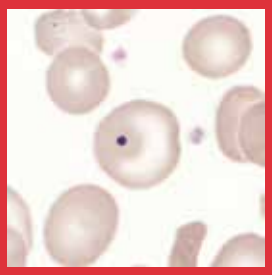
Photos of survey sample specimen



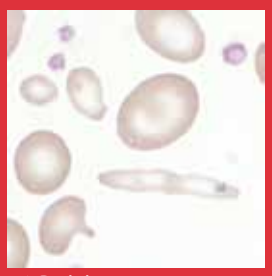
d) Cabot-ring



e) Basophilic strippling



f) Howell-Jolly bodies



g) Poikilocytosis

About
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Automated blood count

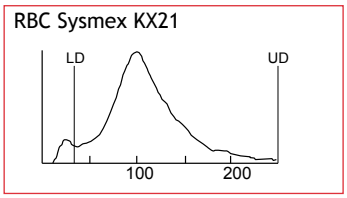
The EDTA sample of the patient was measured on a larger instrument (ADVIA) and on two small hematology instruments (Sysmex KX21 and ABX Micros CRP 200). These small instruments are typically used in laboratory practice. Are the instruments capable of distinguishing small red cells from platelets?

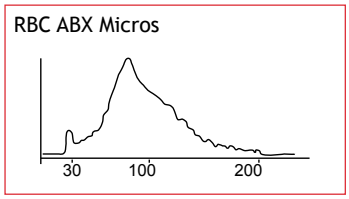
The small instruments are able to distinguish cells only on the basis of their size. This method generally works very well. If unusual curves appear during the measurement, the instruments give additional warnings (flags). Together with the histograms (curves) these flags help estimate whether the measured values are plausible.
If this is not the case, it may be necessary to send the sample to an external laboratory. Larger instruments, such as the ADVIA, measure platelet counts, for example, by a different measurement method.

Erythrocyte histogram (RBC)

The histograms produced by the two small instruments depict the number of particles with a specific volume that were counted.

- With Sysmex KX21, the boundaries (discriminators) are variably set based on the shape of the curve. Here, the lower discriminator (LD) is at 30 fl, and the upper fl at 250 (UD).
- With the ABX Micros, the measuring range that is assigned to the erythrocytes is always set between 30 and 300 fl.

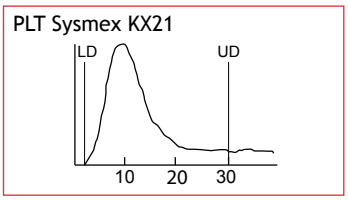


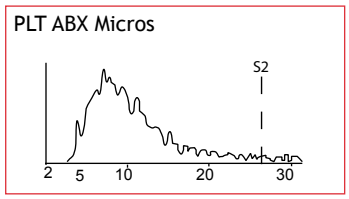


Platelet histogram (PLT)

Platelets are measured in the same measuring channel that is used for erythrocytes. The platelet histogram represents an enlarged detail of the area between 0-30 fl of the erythrocyte histogram.

- The Sysmex KX21 sets the discriminators (LD and UD) variably between 1 fl and 40 fl
- The ABX Micros counts particles as platelets from 2 fl up to a variable upper limit. In the histogram from the sample H3B the variable upper limit (discriminator S2) is seen at about 28 fl.





Discussion

The two small instruments were not able to distinguish the very small erythrocytes forms from normal platelets. This led to a falsely high platelet measurement with both instruments; however, they recognized a measurement problem:

- The Sysmex KX21 recognizes a suspiciously high number of particles at the lower Ec discriminator (LD). It therefore displays the warning „RL*“ (suspected giant platelets, micro-erythrocytes, platelet aggregates) for all parameters of red blood cell counts and for platelets.
- The ABX Micros recognizes that more particles than normal are present between 18-25 fl. It displays the warning message „PLT Flags: SCH“ (schistocytes (Ec fragments) or platelet aggregates, platelet result not trustworthy)

It is therefore important for the laboratory personnel to validate the readings before sending them to the doctor.

	ADVIA	Sysmex KX21	ABX Micros	Reference range
Leukocytes	6.05	6.3	6.1	4.0-10.0 G/l
Erythrocytes	2.53 ↓	2.4 ↓	2.39 ↓	f 4.2-5.4 T/l
Hemoglobin	95 ↓	93 ↓	90 ↓	f 120-160 g/l
Hematocrit	0.27 ↓	0.27 ↓	0.26 ↓	f 0.37-0.47 l/l
MCV	108.7 ↑	111.7 ↑	107.1 ↑	81-100 fl
MCH	37.5 ↑	38.8 ↑	37.5 ↑	28.0-34.0 pg
MCHC	345	347	350	300-360 g/l
RDW CV	27.4 ↑	29.7 ↑	10.9	< 16 %
Platelets	527 ↑	668 ↑	645 ↑	150-450 G/l