CAT

Critically Appraised Topic

Erythrocyte Morphology: From Laboratory to Clinic

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Content

Clinical Bottom Line

The reporting of red blood cell morphology (RBC-M) is a ubiquitous feature of laboratory reports amongst hospital laboratories. It is an essential part of the hematological work-up of disorders such as anemia and can provide diagnostic clues to the etiology of anemia and other pathologies. However amongst guidelines as well as laboratories there exists significant heterogeneity in how RBC-M is evaluated and reported. The manual review of the peripheral blood smear (PBS) is a labor-intensive procedure. Scrutiny should be exercised when deciding how to conduct and report these analyses.

For a laboratory report to be meaningful it should contribute to the diagnostic process. To assess the perceived clinical utility of RBC-M we have conducted a survey amongst the clinicians of the general hospitals of Roeselare (AZ Delta) and Tielt (Sint-Andries). This has demonstrated a significant lack of knowledge of RBC-M terminology and disuse of RBC-M. It highlights a possibility for a more communicative interaction with the clinicians. Examples include: educational initiatives, the tailoring of RBC-M reporting to clinicians and adapting the grading system to provide more clinically relevant data.

Clinical/Diagnostic Scenario

The examination of the peripheral blood smear (PBS) is an inexpensive tool that can provide useful information for the diagnostic process of hematological disorders. Guidelines do not suggest to perform a PBS on all patients with a suspected hematological disorder. Common conditions (i.e. iron deficiency) can be readily diagnosed from the clinical information (presentation, course) and basic laboratory results (MCV, ferritin). (1-6) Nevertheless, there are numerous valid reasons for clinicians to request a blood smear. A PBS reflects the hematopoietic capacity of the bone marrow. Information can therefore be gleaned on erythrocytes, leucocytes and thrombocytes. In this text, we will focus on the indications for manual review of erythrocytes, their morphologic qualities and the reporting thereof. (1-3; 7-13)

In both literature and laboratory practice there is a significant heterogeneity in how the morphology of erythrocytes (RBC-M) is reported. This heterogeneity encompasses both the grading systems and the use of additional comments. The grading system determines whether the presence of an abnormality is reported qualitatively, quantitatively or graded (semi-quantitative, descriptive). This heterogeneity can supposedly exist because there is insufficient data on the prevalence of shape abnormalities in specific disease states. Additionally, no single grading system has been proven to perform better than the others. (1; 14-16)

When reporting RBC-M care should be taken to provide requesting clinicians with clinically meaningful reports that they can use in their diagnostic process. Various morphological findings can be found in small amounts in a healthy population. As an example, the reporting of rare ovalocytes in an otherwise normal PBS holds no diagnostic value and will only serve to confuse or distract the clinician. Moreover, it provides additional work-load to the laboratory. Several abnormalities are only significant when present in sufficiently large amounts. A recent guideline (ICSH 2015) suggests therefore to only report their presence when present in these large amounts. Additionally, it is essential that the report is structured and worded in a way that is comprehensible to the requesting clinician. (1; 17-18)

In AZ Delta we receive on average around 18.000 peripheral blood samples for hematological analysis (CBC + Diff) per month. Of these samples approximately 2.200 (12%) require the microscopic evaluation of a PBS for further analysis. The majority of these smears (97.4%) are requested by the laboratory due to abnormal results of automated hematology analyzers. We evaluate and report the RBC-M of every PBS examined. To assess how we can improve our laboratory work-flow we have performed a literature analysis on the significance of RBC-M and a survey on the clinician experience of the perceived clinical utility of RBC-M.

In this text, we will first summarize the various abnormalities described in RBC-M and their relevant clinical contexts. Secondly, we will discuss some defining characteristics of RBC-M reporting and compare several grading systems found in literature or available from renowned institutions. Thirdly, we will present the results of a survey conducted amongst the clinicians of AZ Delta and Sint-Andries to explore their perceived utility of these morphology reports. Lastly, we will propose several changes we can undertake to improve our method of reporting RBC-M.

Questions

1) What are the relevant clinical contexts and significance of red blood cell morphology abnormalities?
2) How should RBC-M be reported?
3) What is the perceived clinical utility of RBC-M reporting?
4) What steps could be undertaken to improve RBC-M reporting in AZ Delta?

Search Terms

Criteria for study inclusion/exclusion

Inclusion: Either of the following

- Related to the description of clinical context of morphologic abnormalities of erythrocytes.
- Related to the reporting of laboratory reports in a hematological or broader setting.

Exclusion:

- Not pertaining to the inclusion criteria as described above
- Non-English full text
- Non-human
- Sample other than peripheral blood

More recent articles and reviews were preferred over case reports and older ones.

Search strategy

1) MeSH Database (Pubmed): MeSh term:
   a. "Acanthocytes"
   b. "Spherocytes"

2) Pubmed (Medline, from 1966)

a. "Microcyte" OR "Microcytes" OR "Microcytosis"
b. "Macrocyte" OR "Macrocytes" OR "Macrocytosis"
c. "Acanthocytes" OR "Acanthocyte" OR "Spur Cell"
d. "Target cell" AND ("Erythrocytes" OR "Morphology")
e. "Stomatocyte" OR "Stomatocytes"
f. "Echinocyte" Or "Echinocytes"
g. "Sickle cell" OR "Drepanocyte" OR "Drepanocytes" AND ("Erythrocyte" OR "Morphology")
h. "Fragmentocytes" OR "Fragmentocyte" OR "Schistocytes" OR "Schistocyte"
i. "Teardrop cell" OR "Teardrop cells" OR "Dacrocyte" OR "Dacrocytes"
j. "Elliptocyte" OR "Elliptocytes" OR "Ovalocyte" OR "Ovalocytes"
k. "Spherocytes" OR "Spherocyte"
l. "Basophilic Stippling"
m. "Pappenheimer bodies" OR "Pappenheimer body"
n. "Normoblast" OR "Nucleated red blood cell"
o. "Rouleaux" AND ("Red blood cell" Or "Erythrocyte")
p. "Agglutination" AND ("Red blood cell" Or "Erythrocyte") NOT ("Column" OR "Transfusion" OR "Donor")
q. "Morphology" AND "reporting" AND "hematology"
r. Similar articles section and referred articles

These searches were summarized and evaluated separately and relevant references were extracted for use in this text. Additionally several renowned textbooks on hematology were consulted.

Appraisal

Question 1: What are the relevant clinical contexts and significance of red blood cell morphology abnormalities?

Evaluating a PBS is inherently a subjective matter, and the criteria used to classify many abnormalities have changed over time and still vary geographically. In this text we have attempted to describe the erythrocyte abnormalities in accordance with current guidelines advocating for standardization of terminology in hematology and up to date textbook descriptions. [1; 8-13]

The pre-analytic phase is crucial in evaluating RBC-M. However, a description of all important pre-analytic factors is beyond the scope of this text. Therefore we will assume samples are obtained with proper regard to the pre-analytic phase and that PBS are produced according to current best laboratory practices. For a detailed description on slide preparation we refer to Bain B.J. Blood Cells: A Practical Guide, 5th edition and Bain B.J Dacie and Lewis Practical Hematology, 11th edition as well as various guidelines on this matter. [3; 10-11; 13]

We will also assume that the erythrocyte count is performed in the smear area with the correct thickness, where erythrocytes are not overlapping nor too distant, and that at least 1.000 erythrocytes were evaluated to estimate the prevalence of abnormalities as per the current guidelines. [1;10-11]

The normal shape of the erythrocyte is a biconcave disc with an average volume (mean cellular volume or MCV) of 90 fL, diameter of 8 μm and surface area of 136 μm^2. The central third of the erythrocyte is notably paler than the outer edge as result of the biconcave shape. Erythrocytes are stained pink when stained with a classic Romanowsky stain because hemoglobin binds the acidophilic components

of the dye. This normal resting shape poorly illustrates the various shape changes erythrocytes undergo as they pass through the microvascular circulation. Both membrane and cytoskeleton should display sufficient elasticity to regain the discoid resting shape after deformation in the smaller vessels. Abnormalities in size, shape, color, inclusions or arrangement of the erythrocytes can be described. [1; 8-13]

Size abnormalities

Abnormalities of cell size can pertain to smaller erythrocytes (microcytes) or larger erythrocytes (macrocytes). Normally the erythrocytes in a given sample are of similar size. Variation of erythrocyte cell size is expressed by the red cell distribution width (RDW). A large variation in cell size is called anisocytosis. [8-13]

Microcytes ("μικρός": Small)

Microcytes are small erythrocytes with a diameter smaller than 7-7.2 μm. In clinical practice, the MCV is used to assess erythrocyte size. Microcytosis is therefore defined as a decreased MCV below the reference range. Reference range values for erythrocyte size vary by age. Microcytosis is associated with several common as well as more esoteric clinical contexts. The prevalence of microcytosis has been estimated to range from 3 to 35% depending on the tested population. Microcytosis generally reflects a decreased hemoglobin content and is therefore often associated with a reduction in mean hemoglobin content (MCH), which could even precede the MCV decrease in iron deficiency anemia according to some studies. [5-13; 19-20]

Common causes

1. Iron deficiency

Iron deficiency is the most frequent cause of microcytic anemia. It can be found in individuals of any age and due to a variety of causes. The relative frequency of these causes varies wildly on the clinical setting and age of the patient. Children are more likely to suffer from iron deficiency due to reduced iron intake, whereas in adults iron deficiency is most often caused by blood loss. This blood loss can be overt (E.g. menstruation) or occult (E.g. colon cancer, gastrointestinal parasites). Exploratory testing should be performed as indicated by the clinical context of the patient. See Table 1 for a non-exhaustive list of causes of iron deficiency. [3-7; 21]

Table 1 : Causes of Iron deficiency

Examples of causes of Iron deficiency	
Blood loss	
	Menstruation/Pregnancy
	Traumatic
	Frequent blood donation
	Hematuria
	Hematemesis/Melena
	Hemoptysis
Reduced absorption	
	Celiac disease
	Atrophic gastritis
	Bariatric surgery
	Helicobacter Pylori
Increased consumption	
	Stimulation with EPO
Congenital disorders	

2. Hemoglobinopathy

Thalassemia ("θάλασσα-αιμία"; Sea-blood) is a genetic disorder caused by a diminished production of the globin chains that constitute hemoglobin. It was given this name due to its high prevalence in the Mediterranean population. Thalassemia is the second most common cause of microcytosis. Thalassemia α (reduced production of the α globin chain) and thalassemia ß (reduced production of the ß globin chain) are of potential clinical significance because the main hemoglobin (HbA; 2α2ß) consists of 2α- and 2ß-chains. Thalassemia γ (reduced production of the γ globin chain) could potentially be significant in intrauterine or neonatal context, when hemoglobin F (HbF; 2α2γ) is the

most prevalent hemoglobin. The severity and clinical presentation of thalassemia varies depending on the amount of normally functioning genes present. [9-12; 22-23]

Aside from diminished production of hemoglobin chains, there may also be a variant hemoglobin. Most of the known hemoglobin variants are clinically silent and at most interfere with the diagnostic work-up. Many of these variants are also associated with microcytosis. [23-24]

3. Anemia of chronic disease

Anemia of chronic disease (ACD) is usually multifactorial in origin. The diagnosis is made after exclusion of other plausible causes in a patient suffering from a chronic inflammatory condition. Several pathophysiological mechanisms have been proposed. Examples are a reduced erythrocyte survival due to inflammatory cytokines, diminished erythropoiesis due to diminished erythropoietin production and altered iron metabolism due to increased production of hepcidin (iron sequestration). While ACD usually presents as a normocytic anemia, microcytosis has also been reported to some extent. Due to the sheer prevalence of chronic (inflammatory) disease, it should often be considered as a possible cause of microcytosis. [4-7; 11-12]

4. Hyperthyroidism

Several studies suggest that thyroid hormones have a significant effect on erythropoiesis by stimulating erythropoietin secretion and proliferation of erythroid progenitors. While the underlying mechanisms are unclear, an association has been described between microcytic anemia and hyperthyroidism. Thyroid induced anemia is, however, a rare disease. [25]

Several more esoteric causes of microcytosis are summarized in Table 2. A detailed description of these etiologies is beyond the scope of this text. [26-29]

Table 2 : Rare causes of microcytosis

Rare causes of microcytosis	
Hereditary causes	
	Sideroblastic anemia
	Hypotransferrinemia
	Aceruloplasminemia
	Erythropoietic protoporphyria
	Hereditary spherocytosis
	Congenital dyserythropoietic anemia
Acquired causes	
	Lead poisioning
	Cadmium poisioning
	Zinc deficieny
	Copper deficiency
	Sideroblastic anemia (Drug induced etc)

Macrocytes ("μακρός": Large)

Macrocytes are large erythrocytes with a diameter that exceeds 8.5 mm. In clinical practice, the MCV is used to assess erythrocyte size. Macrocytosis is therefore commonly defined as an elevated MCV above the reference range. Reference values for erythrocyte size vary by age. Macrocytosis is associated with several common as well as rare clinical conditions. A study has estimated it's prevalence at 1.7 to 3.6% in patients undergoing routine PBS. Macrocytosis can feature with or without anemia. [8-13; 30-31]

1. Vitamin B12 deficiency / folate deficiency

Vitamin B12 (cobalamin) deficiency is the most common cause of megaloblastic anemia. Vitamin B12 deficiency can result from a multitude of possible causes E.g. impaired absorption, impaired intake and congenital disorders. Neutrophil hypersegmentation is often found on the PBS alongside macrocytosis. Megaloblastic macrocytes are generally oval shaped. [9-13; 30-31]

Less often megaloblastic anemia is caused by folate deficiency. Causes for folate deficiency feature insufficient intake, malabsorption syndromes as well as increased metabolic requirements (as seen in pregnancy and chronic mild hemolysis). See Table 3 for a summary of the most common causes. [9-13; 30-31]

Drugs affecting the cellular availability and utilization of folate acid or vitamin B12 are also associated with megaloblastic anemia. Common drugs that can elicit macrocytosis are azathioprine, cyclophosphamide, imatinib, methotrexate, hydroxyurea, antiretroviral agents, valproate, phenytoin. A retrospective study evaluating 146 macrocytic children determined medication as the most frequent cause of macrocytosis in the pediatric population. [8-13; 30-35]

Table 3 : Causes of vitamin B12 deficiency and folate deficiency

Examples of causes of Vitamin B12 deficiency		Examples of causes of folate deficiency	
Defects in absorption		Defects in absorption	
Inadequate intrinsic factor e.g:		Disease of the small intestine e.g:	
	Pernicious anemia		Celiac disease
	Gastritis		Infiltrative disease (Lymphoma, other)
	Gastrectomy	Inadequate Intake	
Achlorhydria			Poor nutrition
Disease of the small intestine e.g:		Increased requirement	
	Resection or bypass of terminal ileum		Alcoholism
	Regional enteritis (Crohn's disease)		Pregnancy
	Celiac disease		Hemolytic anemia
	Infiltrative disease (Lymphoma, other)		Hyperthyroidism
Pancreatic insufficiency			Anti-convulsant therapy
Inadequate nutrition		Folate inhibitors	
	Strict vegetarians / vegans		Methothrexate
	Malnourishment		Trimethoprim
Congenital defects in transport /metabolism		Congenital defects	

2. Alcoholism

Alcoholism is a frequent cause of macrocytosis with or without anemia. The prevalence of alcoholism as a cause of macrocytosis varies strongly on the examined population (15-65%). Gamma-glutamyltransferase could be a valuable laboratory assay when suspecting alcoholism as a cause of macrocytosis. MCV generally normalizes within 2-4 months of abstinence. [9-12; 30; 36]

3. Myelodysplastic syndromes

Myelodysplastic syndromes (MDS) are a group of clonal hematopoietic stem cell disorders characterized by cytopenia, ineffective hematopoiesis , dysplasia in one or more of the major myeloid lineages, recurrent genetic abnormalities and increased risk of progression to acute myeloid leukemia (AML). Especially in an elderly population, macrocytic anemia is associated with MDS. Studies investigating the major causes of macrocytosis estimate 4-6% of macrocytosis can be attributed to a diagnosis of MDS. A bone marrow investigation should be considered when faced with unexplained macrocytic anemia in a patient of age. [10; 31; 37-38]

4. Hypothyroidism

Several studies suggest that thyroid hormones have a significant effect on erythropoiesis by stimulating erythropoietin secretion and proliferation of erythroid progenitor cells. While the underlying mechanisms remain unclear, it has been shown that hypothyroidism is associated with an elevated MCV. It could therefore be useful to investigate thyroid function when faced with unexplained macrocytosis. The MCV has been shown to decrease in deficient patients when pharmacological treatment is adequately maintained. [10-12; 26; 39]

5. Reticulocytosis

Reticulocytes are immature erythrocytes that have recently left the bone marrow. They are characterized by a polychromatic/pinkish-blue appearance as well as their larger size and lack of central pallor. This polychromasia is a result of the small amounts of mRNA these erythrocytes still retain. Reticulocytes typically have a MCV of 103-126 fL. Reticulocytosis is a normal physiologic response to anemia of any cause. The absence of reticulocytosis in anemia should prompt a consideration of factors that could disable the ability of the bone marrow to respond such as chronic disease, iron or vitamin deficiencies. [8-13; 30]

In practice, reticulocytes are commonly seen in various hemolytic anemias, as well as in the recovery phase post repletion of deficient iron or vitamins and after a bleeding episode. Therefore a reticulocyte count could prove useful when assessing the significance of macrocytosis. [8-13; 30]

6. Pregnancy / Newborn

In both pregnant women and newborn infants macrocytosis is a physiologic phenomenon. It is important to adjust reference ranges to account for this shift. One should also consider the possibility for pseudo normalization of MCV for example in a pregnant patient with iron deficiency. [8-10; 30; 40]

7. Cold agglutinins

The presence of cold agglutinins can cause erythrocytes to agglutinate during storage or transport. When these samples are not warmed up before measuring the cell counter will analyze clumps of erythrocytes instead of individual cells. This will result in spurious measurements of various RBC indices, amongst which a grossly elevated MCV. The formation of cold agglutinins has been associated with infections such as *M. pneumonia* and Epstein-Barr virus as well as with several lymphoid malignancies. [12; 41]

Several more esoteric causes of macrocytosis are listed in table 4. [42-48]

Table 4 : Rare or uncertain causes of macrocytosis

Rare or uncertain causes of macrocytosis	
Megaloblastic erythropoiesis	
	Multiple myeloma
	Copper deficiency
	Arsenic intoxication
Non-megaloblastic erythropoiesis	
	Congenital dyserythropoietic anemia
	Pure red cell aplasia (Diamond-blackfan)
	Aplastic anemia
	Smoking
	Chronic obstructive airway disease
	Trisomy 21
	Acquired sideroblastic anemia

Anisocytosis ("ἄνισος": Unequal)

Anisocytosis is a non-specific feature that can be present in a great many disease states. It reflects more variation in size than is normally present. The presence or lack thereof of anisocytosis could be helpful in differentiating between iron deficiency anemia and thalassemia. An elevated RDW can be indicative of the presence of anisocytosis. Considering major advances in automated cell counters the past decades it is generally deemed more accurate and reliable to report the RDW than perform a microscopic grading of anisocytosis. [1; 9-12]

Shape abnormalities

Various shape abnormalities can provide diagnostic clues to etiologies of anemia and to other pathologies.

Acanthocytes ("ἄκανθα": Thorn/Spine) / Spur cells

An acanthocyte is a densely stained erythrocyte with 2-10 irregularly positioned thorny projections of varying length and thickness. Acanthocytes should be differentiated from echinocytes, whose spicules are more uniform. Acanthocytes can be found in a diverse group of inherited or acquired diseases. Their formation is usually associated with changes in the composition of the lipid bilayer that result in an expansion of the outer leaflet. Due to their abnormal morphology, acanthocytes are more vulnerable to trapping, modulation and destruction by the spleen. This vulnerability can result in mild to severe hemolytic anemia. [1; 9-13]

Acanthocytes can be found in large amounts in liver disease, abetalipoproteinemia and neuro-acanthosis syndromes. They can also be found in smaller amounts in a wide variety of pathologies.

Common causes

1. Liver disease

In both obstructive and hepatocellular liver disease there are abnormalities in serum lipoproteins that induce changes in erythrocyte morphology by disturbing the passive exchange of lipids between plasma and erythrocytes. Cholesterol accumulation in the outer leaflet of the membrane bilayer results in an increase of membrane surface area. This superfluous membrane can manifest itself as target cells or acanthocytes. [9]

In severe liver disease the combination of acanthocytosis and hemolysis can be indicative of a spur cell anemia. This is an acquired erythrocyte abnormality that occurs in a small number of patients with end-stage liver disease. Spur cell anemia is characterized by a rapidly progressive hemolysis as well as excessive amounts of acanthocytes on the peripheral blood smear. It is most commonly seen in alcoholic liver disease, but has been described in patients with other hepatic diseases as well. [9-13; 59-50]

2. Various other causes

Acanthocytes can also be found, usually in smaller amounts, in the peripheral blood in several other disease states such as malnutrition (E.g. anorexia nervosa, cystic fibrosis), hypothyroidism, diffuse intravascular coagulation, myelodysplasia and splenectomy. Additionally, commonly prescribed drugs (statins, misoprostol) have been associated with the formation of acanthocytes. [51-58]

Rare causes

1. Abetalipoproteinemia (Bassen-Kornzweig syndrome)

Abetalipoproteinemia is a rare autosomal recessive disorder (prevalence < 1/1.000.000) caused by a mutation in the gene encoding the microsomal triglyceride transfer protein (MTTP). This defect results in the inability to produce or transport chylomicrons and VLDL. This deficiency impairs lipid uptake in the intestinal mucosa and results in low plasma triglyceride levels and markedly decreased plasma cholesterol

and phospholipid levels. An excess of sphingomyelin, preferentially inserting in the outer membrane leaflet, results in an increase of the surface area and subsequently acanthocytosis. [9; 59-60]

2. Neurological syndromes

The neuro-acanthocytosis syndromes are a rare group of syndromes (prevalence < 1-5/1.000.000) characterized by progressive neurodegeneration in adolescent or adult life and the presence of acanthocytes on the blood smear. The mechanisms underlying acanthocyte formation in the neuro-acanthocytosis syndromes are not yet fully understood. [9; 12; 61-62]

Target cells / Codocytes ("κώδων": bell)

Target cells are erythrocytes that demonstrate a dens central area surrounded by a halo of pallor. In the circulation these erythrocytes appear as bell-like shapes, upon drying the blood smear the "bulls-eye" shape is acquired. This appearance is caused by a relative excess of red cell membrane. Target cells are therefore characterized by an increased surface area to volume ratio. This ratio can be increased by superfluous membrane, as seen in liver disease, or by reduced cell volume, as seen in thalassemia. Target cells can occasionally be found in the PBS of healthy individuals. [8-13]

1. Thalassemia/ Hemoglobinopathy/ Iron deficiency

In the various thalassemia disease states there is diminished production of hemoglobin. This results in erythrocytes containing less hemoglobin than their normal counterparts. While these erythrocytes display a normal surface area, their volume is reduced, resulting in an increased surface area to volume ratio. To a lesser extent, this mechanism also applies to target cells seen in several hemoglobinopathies and in iron deficiency. [3; 9; 13; 63]

2. Liver disease

As in acanthocytes, the insertion of cholesterol in the membrane bilayer causes an expansion of the outer layer of the membrane bilayer. This expansion results in increased surface area while the volume remains constant. Lecithin-cholesterol acyl transferase (LCAT) aids in the transportation of cholesterol by converting free cholesterol to cholesteryl esters. A diminished activity of LCAT, either by destruction of hepatocytes in liver disease, inhibition by bile salts in obstructive jaundice or congenital deficiency may result in excess accumulation of cholesterol and target cell appearance. [9-13]

3. Post splenectomy state

It is observed that in the weeks following splenectomy target cells gradually increase. This is due the absence of splenic conditioning, wherein excess membrane is removed from erythrocytes as they pass through the spleen. The underlying mechanism is not yet fully understood, although lipases are assumed to play a role. [9; 13]

Stomatocytes ("στόμα": Mouth)

Stomatocytes are erythrocytes characterized by a uniconcave bowl-like shape and a central slit-like hemoglobin free area. Stomatocytosis can occur due to inherited disorders or due to several acquired abnormalities. In inherited disorders, the mechanism of origin often involves changes in the erythrocyte volume due to altered intracellular ion content. Whereas in the acquired disorders, the mechanism of origin frequently involves a decrease in erythrocyte surface area or changes in the components of the lipid bilayer. Stomatocytes can occasionally be found in the PBS of healthy individuals as well as due to drying artefacts. [8-13; 64]

Acquired conditions

A study investigating causes of acquired stomatocytosis (cut-off > 5%) in a Scottish population found a prevalence of 2,3% in patients for which hematological examination was requested (n = 4291). [64]

1. Acute alcoholism

Stomatocytes have been associated with both chronic liver disease and acute alcohol intoxication. A study investigating a series of 100 patients hospitalized for alcohol intoxication found stomatocytes (>5%) in 44%. In alcohol intoxication stomatocytosis appears to be a transient finding. In liver disease, stomatocyte formation has been attributed to a lysolecithin increase in the inner membrane layer. [9; 65]

2. Medication

In vitro research has shown that stomatocytosis can be induced in certain conditions and by various agents. These compounds are thought to preferentially insert themselves in the inner half of the membrane bilayer, and in doing so tend to cause an inner bulging of the membrane. In vitro research has also revealed the stomatocytogenic capabilities of many drugs commonly used in clinical practice. While there is significantly less literature on the in vivo effects of these drugs on erythrocyte morphology, it seems prudent to consider that, at least to a certain extent, stomatocytosis can be induced due to therapy with stomatocytogenic agents. (Table 5) [66-71]

Table 5 : Examples of stomatocyte-inducing agents

Examples of Stomatocyte-inducing conditions and agents	
Condition	
	Excess of albumin
	Low pH
Medication	
	Chlorpromazine
	Diazepam
	Citalopram
	Hydroxyurea
	Soy-based parental nutrition
	Vinca Alkaloid
	Antracycline
Intoxication	
	Arsenic

3. Myelodysplastic syndrome

An association between myelodysplastic syndrome and stomatocytes has been described in various textbooks and in at least one case report. The underlying mechanisms are unclear. [9; 13]

Congenital conditions

Hereditary stomatocytosis

Hereditary stomatocytosis (HSt), as with the other hereditary shape abnormalities, encompasses a group of inherited abnormalities (Prevalence 1/10.000) that are characterized by stomatocytosis. A common but not invariable element of these disorders is the dysregulation of erythrocyte ion concentrations, due to abnormal membrane permeability secondary to defects in channels, transporters or membrane proteins. Other genetic abnormalities that result in either loss of membrane (E.g. hereditary spherocytosis, AIHA) or defunct lipid metabolism (E.g. sitosterolemia, familial hypercholesterolemia) can also present with stomatocytosis. [9; 13; 72-74]

Echinocytes ("ἐχῖνος": Hedgehog) / Burr cells ("Burdock plant")

Echinocytes are a type of spiculated erythrocytes that present many (10-30) similar, evenly distributed projections. As with stomatocytosis in vitro research has shown that echinocytosis can be induced by various environmental factors and agents (Table 6). Contrary to stomatocytosis, echinocytes are thought to originate due to a relative expansion of the surface area of the outer membrane. Echinocytosis is generally reversible unless excessive or long lasting stimuli are present, this can be achieved by incubating echinocytes in fresh plasma or by allowing ATP synthesis. Echinocytes can be found in the PBS of healthy individuals, associated with various conditions and due to drying or prolonged storage. [8-13]

Artefacts

In clinical practice echinocytes are most commonly seen as an artefact due to extended storage in Ethylenediaminetetraacetic acid (EDTA). It is thought that this storage artefact is caused by metabolic depletion of erythrocytes (decrease of ATP) or lysolecithin formation. This is also assumed to be the mechanism causing a transient echinocytosis in patients receiving an erythrocyte transfusion. Echinocytosis can also be induced by exposing erythrocytes to glass surfaces. To minimize echinocytosis due to prolonged storage in EDTA, it is important not to delay PBS preparation unduly. [10;13]

Clinical conditions

True echinocytes are rare and have been described in a wide range of pathologies and conditions.

1. Uremia

Uremia is associated with echinocytosis. The underlying mechanism has not yet been fully elucidated. This is assumed to cause echinocytosis in patients with end stage severe renal disease as well as those with overt or obscure gastric bleeding (E.g. peptic ulcer, gastric carcinoma). [9; 12]

2. Extracorporeal circulation / hemodialysis

Several studies have shown the presence of a transient echinocytosis in patients undergoing hemodialysis or extracorporeal circulation. This echinocytosis, if found, is short lived and will normalize within 24 hours. This finding should not cause alarm or further investigations. [75]

3. Severe liver disease

It has been shown that the plasma of jaundiced patients can induce echinocytosis when incubated with normal discoid cells. It is assumed that the echinocytosis of liver disease is caused by binding of abnormal HDL and not by changes in the membrane contents like in acanthocytosis. [9; 76]

4. Neonates

Echinocytes can be found in higher amounts in the PBS of neonates as a physiological feature. [13; 77]

Several rare causes of echinocytosis are summarized in table 7. [78-80]

Table 6 : Examples of echinocyte-inducing agents

Other examples of Echinocyte-inducing conditions and agents	
Condition	
	High pH
	Excess of calcium
	Exposure to glass
	Prolonged storage
	Shortage of Albumin
	Hypophosphatemia
Medication	
	Cyclosporine

Table 7: Rare causes of echinocytosis

Other rare causes of echinocytosis	
	Dengue infection
	Pyruvate kinase deficiency (PKD)
	Burns

Sickle cells / Drepanocytes ("δρεπάνι": Sickle)

First described in 1910, sickle cells are erythrocytes that are sickle or crescent-shaped with pointed ends. They are almost always present in films of freshly drawn blood from adults with homozygosity for hemoglobin S. The typical shape change is due to the presence of polymerized hemoglobin S, which

can be induced by exposing the blood to anoxia. They are notably absent in the blood of neonates due to the high hemoglobin F percentage. [23; 81-82]

Sickle cell disorders (SS, S trait, SC, SD, S thalassemia)

Sickle cell disease is caused by a specific mutation in the gene encoding the beta-globin chain of hemoglobin. Hemoglobin S consists of 2 normal alpha and 2 abnormal beta chains. Long polymers of hemoglobin S distort the erythrocyte and hinder blood flow through capillaries. The presence of hemoglobin A, A2 and F can hinder or slow polymerization, whereas the presence of hemoglobin C, D-punjab or O-Arab can facilitate sickling. [23; 81-82]

Homozygosity for hemoglobin S is called sickle cell anemia. Heterozygosity for hemoglobin S is called sickle cell trait. Sickle cell trait is generally asymptomatic, unless there is a disadvantageous interaction with the other gene encoding the beta chain. Sickle cell trait can also lead to vascular occlusion when exposed to hypoxic conditions such as intense exercise, high fever or high altitudes. [23; 81-82]

Fragmentocytes / Schistocytes ("σχίζω": broken/cleft)

Fragmentocytes are produced by fragmentation of erythrocytes. They are a trademark feature of mechanical hemolytic anemias, of which the thrombotic microangiopathic (TMA) syndromes require rapid diagnosis and treatment. The fragmentation typically occurs due to mechanical damage within the circulation. By definition fragmentocytes are smaller than normal erythrocytes. They typically demonstrate sharp angles and borders as well as crescent or helmet shapes. [8-13]

Fragmentocytes are absent or very rare in blood smears of healthy individuals. When they are present in the blood smear as the dominant morphological abnormality a TMA should be considered. In this context it is advised to also check for hemolysis indices and thrombocytopenia and assess the kidney function. [83]

Mechanical causes

The typical TMA syndromes are thrombocytopenic purpura (TTP; ADAMST13 deficiency), hemolytic uremic syndrome (HUS; Shiga toxin), complement-mediated TMA, drug-induced TMA (DITMA), cobalamin C deficiency-mediated TMA and post-hematopoietic stem cell transplantation TMA. [83-87]

Due to a plethora of initial stimuli (E.g. endothelial damage, ADAMST13-deficiency, drugs) fibrin deposits and platelet aggregates can be formed in the small blood vessels. As erythrocytes force their way through these "damaged" vessels they undergo mechanical stress and fragmentation. [83-87]

Other systemic conditions that can present with fragmentocytes due to mechanical damage include (pre-)eclampsia, malignant hypertension, sepsis, diffuse intravascular coagulation (DIC), malignancy, etc… Foreign objects in the blood stream can also elicit fragmentocytes (macroangiopathy). This has most frequently been described with malfunctioning prosthetic heart valves. However fragmentation can occur whenever erythrocytes are exposed to high pressure gradients or high shear stress. Mechanical damage to erythrocytes can also occur due to external forces and has been reported in long distance runners and judo athletes. [88-91]

Non-mechanical causes

Erythrocyte fragmentation can also occur due to erythrocyte disorders of non-mechanical nature such as thalassemia, severe burns and erythrocyte membrane disorders. When this occurs, fragmentocytes show significant variation in shape and size and are accompanied by many additional morphological abnormalities. [23; 72; 92]

It should be noted that newborn infants and especially pre-term newborns can present with 2-5% fragmentocytes as a physiologic feature. [8-9; 13]

Fragmentocyte reference ranges are variably reported. Guidelines from the International Council of Standardization in Hematology (ICSH) suggest that a fragmentocyte count above 1% is a good argument favoring a TMA diagnosis, if features suggesting an alternative diagnosis are absent. There is little evidence that an explicit fragmentocyte count has diagnostic value in other clinical contexts. [1]

Teardrop cells / Dacrocytes ("δάκρυον": Teardrop)

Dacrocytes are erythrocytes with a single elongated or pointed extremity. Their shape is often similar to a teardrop. Dacrocytes are typically found in patients with extramedullary hematopoiesis and bone marrow fibrosis. The underlying mechanisms causing this shape change have not yet been fully elucidated. Possible causes are mechanical damage from a fibrotic bone marrow or excessive stretching of erythrocytes when the spleen removes inclusions (Pitting function). Dacrocytes can sometimes be found as an artefact due to the force applied when preparing a PBS. This should be suspected when all dacrocytes display the same orientation. [9-13; 93]

1. Myelofibrosis / Infiltration of bone marrow

Primary myelofibrosis is a myeloproliferative neoplasm associated with reactive deposition of fibrous connective tissue and extramedullary hematopoiesis. Secondary myelofibrosis can result due to a variety of conditions such as irradiation, epithelial or lymphoid infiltration, other myeloproliferative neoplasms, MDS etc… The fibrotic stage of myelofibrosis is characterized by dacrocytes and a leukoerythroblastosis. The presence of dacrocytes combined with anemia or pancytopenia should therefore prompt bone marrow investigations to evaluate for primary or secondary myelofibrosis as well as invasion by other malignancies. [93-95]

2. Extramedullary hematopoiesis

Dacrocytes have also been described in patients with extramedullary hematopoiesis that did not suffer from myelofibrosis. The fact that splenectomy leads to a significant decrease of dacrocytes in auto-immune hemolytic anemia and thalassemia suggests the spleen and the narrow spleen sinusoids play a role in dacrocyte formation. [8-13; 96]

Ovalocytes ("ᾠόν": Egg) / Elliptocytes ("ἔλλειψις": Ellipse)

Ovalocytes and elliptocytes are elongated erythrocytes with an oval and elliptical shape respectively. Erythrocytes with a long axis more than twice their short axis should be designated elliptocytes, whereas erythrocytes with a long axis less than twice their short axis should be designated an ovalocyte. In circulation, erythrocytes often undergo stress-induced elliptical deformation. Normal erythrocytes are able to return to their resting discoid shape when this stress is relieved due to elastic properties of the erythrocytes. This elasticity is a result of the interaction between the cytoskeleton and the lipid bilayer of the membrane. Up to 1% of ovalocytes and ovalocytes can be found in a PBS of a normal subject. An increase in ovalocytes and elliptocytes is associated with several inherited and acquired disease states with or without anemia. [8-13]

Prominent elliptocytosis/ovalocytosis (>25%)

Elliptocytes are most strikingly associated with several hereditary syndromes. The deformability of erythrocytes is due to how the cytoskeleton is connected to the membrane by specialized proteins. In the hereditary elliptocytosis (HE) syndromes there are mutations in the genes encoding these specialized proteins (spectrin, band 3, protein 4.1), leading to weakened cytoskeletal-protein interactions and a predilection to form new protein connections to stabilize the erythrocyte shape. Of

note is the normal formation of erythrocyte precursors, ovalocytosis/elliptocytosis is acquired as erythrocytes age in circulation and are repeatedly deformed. [8-13; 97-98]

Most of the mutations causing HE syndromes are transmitted via an autosomal dominant pattern. There is significant variation in the clinical presentation and amount of ovalocytes/elliptocytes on the PBS (15-100%). The HE syndromes have an estimated prevalence of 1-2.000 to 1-4.000. Their true incidence is difficult to assess as they can be present in fully asymptomatic patients. Hereditary pyropoikilocytosis (HPP) and Southeast Asian ovalocytosis (SAO) are generally considered subtypes of the common hereditary elliptocytosis. [8-13; 97-98]

Aside from elliptocytes common erythrocyte shape abnormalities in the HE syndromes are fragmented erythrocytes and spherocytes. This is indicative of mild hemolysis when present. In HPP there are numerous other shape abnormalities, whereas in SAO the ovalocytes are stomatocytic in nature. [8-13; 97-98]

Non-specific elliptocytosis/ovalocytosis (1-15%)

Smaller amount of elliptocytes and ovalocytes can be found in a wide variety of pathologies encompassing iron deficiency, thalassemia, megaloblastic anemia, MDS and myelofibrosis. In these pathologies elliptocytes/ovalocytes will seldom be the dominant shape abnormality. The underlying mechanisms are not always fully elucidated. The various accompanying shape abnormalities will aid in the differential diagnosis as depicted in table 8. [99-101]

Table 8 : Accompanying abnormalities of various elliptocyte/ovalocyte causes

Elliptocyte/Ovalocyte cause	Accompanying abnormalities
Iron deficiency	Microcytosis, target cells
Thalassemia	Microcytosis, target cells, basophillic stippling
Megaloblastic anemia	Macrocytosis
Myelodysplastic syndrome	Macrocytosis, dimorphism, Howell-Jolly, NRBC
Myelofibrosis	Teardrop cells, Howell-Jolly, NRBC

Spherocyte ("σφαίρα": Sphere)

Spherocytes are erythrocytes that are small and sphere-shaped rather than biconcave. When observed through a microscope these erythrocytes display a lack of central pallor due to denser hemoglobin content. Spherocytes can originate from a wide variety of pathologies and due to a plethora of mechanisms. A common theme is that in one way or another membrane is lost. Spherocytes are more susceptible to hemolysis due to reduced deformability. The degree of spherocytosis, spherocyte size and accompanying shape abnormalities can aid in differentiating possible causes. [9-13; 102-104]

1. Immune-mediated hemolysis

Erythrocytes that are sensitized with antibodies, complement or immune complexes tend to lose cholesterol and surface area due to interactions with phagocytic cells in the spleen. This mechanism is seen in the immune mediated hemolytic anemia's such as ABO incompatibility, auto-immune hemolytic anemia and hemolytic disease of the fetus and newborn (HDFN). These pathologies typically display a variety of shape abnormalities aside from spherocytes. [9; 13; 105]

2. Fragmentation

Spherocytes can also be formed due to fragmentation. These will generally be smaller than spherocytes caused by other mechanisms. We refer to the subsection on fragmentocytes for a summary of various causes of erythrocyte fragmentation.

3. Hereditary spherocytosis

A somewhat rare cause of spherocytes is hereditary spherocytosis (HS; prevalence: 1-2.000/5.000 in North European context). HS arises due to a defect in genes encoding the specialized proteins (band 3, spectrin, ankyrin) that link the cytoskeleton to the lipid bilayer. The deficiency of these proteins leads to microvesiculation and loss of membrane, what subsequently leads to a reduced surface area to volume ratio. HS is a heterogeneous disease group and can present at any age and with variable severity. The diagnosis should be considered in any coombs-negative hemolytic anemia with spherocytes on the PBS. [9; 13; 102-104]

4. Various other causes

Spherocytes have also been described to be associated with various other pathologies. Examples of these causes are summarized in table 9. [106-107]

Table 9 : Various causes of spherocytosis

Various causes of spherocytosis	
	Acute oxidant injury
	Severe burns
	Bee/Snake/Spider venom
	Dilution hemolysis
	Hypersplenism

Color abnormalities

Hypochromia ("ὐπο": under; "χρῶμα": Color)

Hypochromia is a term used to describe reduced staining of erythrocytes. This often results in the presence of an increased central pallor on microscopic evaluation. Hypochromia is most frequently a result of reduced hemoglobin content, as is microcytosis. Therefore all causes of microcytic anemia as described above can also result in hypochromia. It has been suggested that hypochromia may even precede microcytosis in iron deficiency anemia. It should be noted that in some patients with thalassemia syndromes the PBS will demonstrate microcytosis without noticeable hypochromia. [8-13; 15]

Hyperchromia ("ὐπέρ": over; "χρῶμα": Color)

The term hyperchromia is rarely used when describing blood films. It can be used when cells are more intensely stained than normal. True hyperchromic cells are typically small erythrocytes such as spherocytes. Hyperchromia can be found as an artefact when evaluating a non-optimal area of the PBS. [8-13]

Current guidelines recommend to replace a microscopic evaluation of hypochromia and hyperchromia by the mean cellular hemoglobin (MCH) and mean cellular hemoglobin concentration (MCHC) as measured by an automated hematology analyzer. [1]

Polychromasia("πολύς": many; "χρῶμα": Color)

Polychromasia is a term used to describe the presence of reticulocytes. Reticulocytes are immature erythrocytes that have recently left the bone marrow. They are characterized by a polychromatic/pinkish-blue appearance as well as their larger size and lack of central pallor. The polychromasia is a result of the small amounts of mRNA these erythrocytes still retain. Reticulocytes typically have a MCV of 103-126 fL. Polychromasia is a normal physiologic response to anemia of any cause and reflects intense erythropoietic stimulation. The absence of reticulocytosis in anemia should prompt a consideration of factors that could disable the ability of the bone marrow to respond such as chronic disease, iron or vitamin deficiencies. In practice reticulocytes are commonly seen in various

hemolytic anemias, as well as in the recovery phase post repletion of deficient iron or vitamins and after a bleeding episode. Therefore a reticulocyte count could prove useful when assessing the significance of macrocytosis. [8-13]

Inclusions

Howell-Jolly bodies (Eponym, named after William Howell and Justin Jolly)

Howell-Jolly bodies are erythrocyte inclusions that consist of nuclear remnants. They appear as a small (1 μm), spherically shaped, dense sphere on Romanowsky stain. When present there are usually no more than one per erythrocyte. Howell-Jolly bodies originate from nuclear fragmentation or incomplete expulsion of the erythrocyte nucleus. They are normally removed from reticulocytes by the spleen in a process termed "Splenic pitting". As such, they are found in disease states involving a disruption of normal splenic function such as splenectomy or hypo-/asplenism. Hypo- or asplenism can occur due to a wide variety of pathologies (Table 10). Functional hyposplenism is also commonly seen in neonates. A Howell-Jolly body and pitted cell count is sometimes used in clinical practice to assess the function of the spleen in patients with a predisposition for hyposplenism. [8-13; 108-112]

Table 10 : Examples of causes of hyposplenism

Examples of causes of hyposplenism	
Physiological	
	Neonates
Congenital	
	Absence or hypoplasia
Acquired	
Iatrogenic	
	Splenectomy
Splenic infact	
	Sickle cell anemia
	Essential thrombocytosis
Splenic atrophy	
	Coeliac disease
	Graft versus host disease
Splenic infiltration	
	Sarcoidosis
	Carcinoma
	Lymphoma
Functional hyposplenia	
	Severe hemolytic anemia

Basophilic stippling

Basophilic stippling is a term used to describe small basophilic inclusions in erythrocytes. These inclusions are typically evenly dispersed throughout the erythrocyte and are composed of aggregated ribosomes. The inclusions can be fine or coarse in appearance. They can be differentiated from Pappenheimer bodies (see below) because basophilic stippling does not tend to cluster and do not react positively with stains for iron. Occasionally fine basophilic stippling can be detected in healthy individuals. The presence of marked basophilic stippling is generally associated with acquired and congenital disorders affecting erythropoiesis and erythrocyte maturation. It is indicative of disturbed rather than increased erythropoiesis. [8-13; 15]

1. Intoxication (Lead / Heavy metal)

Coarse basophilic stippling is associated with toxicity due to heavy metals such as lead. Lead toxicity inhibits pyrimidine-5'-nucleotidase, which is an enzyme responsible for the degradation of ribosomal RNA in erythrocytes. Other heavy metals such as zinc or arsenic can present with similar findings, but are considerably less frequent. Exposure to lead can occur in varying circumstances such as contaminated soil/water, cosmetics, herbal and folk remedies or specific occupations (mining, auto-repair, battery manufacturing, painting). [9; 113-115]

2. Hemoglobinopathies

A fine basophilic stippling can be observed in patients with hemoglobinopathies such as thalassemia or other unstable hemoglobins. In this context it is likely that basophilic stippling originates due to increased erythrocyte turnover and the release of more immature erythrocytes in the bloodstream. [116-117]

To a lesser extent, basophilic stippling can be found in a variety of causes of which some examples are summarized in table 11. [118]

Table 11 : Examples of causes of Basophillic stippling

Examples of other causes of Basophilic stippling	
Congenital	
	Congenital pyrimidine 5'-nucleotidase deficiency
	(Megaloblastic anemia)
Acquired	
	Myelodysplastic syndrome
	Iron deficiency
	Severe infections
	Megaloblastic anemia

Pappenheimer Bodies (Eponym, named after A.M Pappenheimer)

Pappenheimer bodies are multiple, small basophilic inclusions of varying size and shape in erythrocytes. They are generally located in clusters in the cell periphery and consist of ferritin aggregates and hemosiderin. Their basophilic appearance on Romanowsky staining is explained due to clumps of ribosomes that are co-precipitated with the aggregates. The presence of non-heme iron in the aggregates can be confirmed by Perl's Prussian blue stain. The presence of Pappenheimer bodies is indicative of a pathology in which iron is not correctly incorporated into hemoglobin or in which aberrant erythrocytes cannot be adequately cleared by the reticuloendothelial system. An erythrocyte that contains Pappenheimer bodies can be called a siderocyte. [8-13; 119]

1. Post-splenectomy / Hyposplenism

There have been several reported cases of patients with splenectomy or hyposplenism that demonstrated Pappenheimer bodies on the PBS. Pappenheimer bodies are assumed to be produced in normal conditions as well, but the pitting function of the spleen removes these from erythrocytes. The presence of Pappenheimer bodies in a patient could therefore prompt the clinician to investigate possible causes of hyposplenism. [120-122]

2. Sideroblastic anemia

Sideroblastic anemia occurs when a pathology disrupts the normal processing of iron in erythrocytes. A typical feature are ring sideroblasts found on a bone marrow examination. Several disease-states are

associated with the production of siderocytes by the bone marrow. Possible causes entail the congenital sideroblastic anemia's, myelodysplasia, alcohol, copper deficiency and zinc excess. [123]

Micro-organisms

Micro-organisms can sometimes be found in or between erythrocytes in patients suffering from bacterial, fungal, protozoan or parasitic infections. The only micro-organisms that are observed fairly frequently are malaria parasites, but the accidental observation of other micro-organisms in a blood film can also be diagnostically useful. For a more extensive description of various micro-organisms that can be found in the PBS we refer to Karen C. Manual of Clinical Microbiology, 12th edition and various other guidelines on this matter.

Nucleated red blood cells (NRBC's) (synonym: Normoblasts)

Nucleated erythrocytes are immature erythrocytes that have not yet shed their nucleus. They can frequently be found in the PBS of neonates in the first week of life. The presence of factors such as hypoxia and prematurity can increase the NRBC count. In adults the presence of NRBC's in the PBS is associated with pathologies such as sever blood loss, malignant neoplasms, infiltrative bone marrow disease or extramedullary hematopoiesis. The presence of NRBC in the PBS is often associated with severe disease and a poor prognosis. [8-13; 124]

The presence of NRBC's in the peripheral blood will not be diagnostic for any given disease, but it should prompt consideration of the bone marrow function if no plausible explanation can be found for the circulating NRBC's. [9;13]

1. Hyposplenism

NRBC's are normally cleared from the bloodstream by the spleen. The presence of NRBC could therefore prompt investigation of splenic function. Hyposplenism can originate from a wide variety of conditions. (Table 10)

2. Compensation for anemia / hypoxia

NRBC's can also originate due to a hypoxic erythropoietin-induced compensation mechanism. The reduced capacity for transporting oxygen of the anemic patient will trigger erythropoietin production in the kidneys which will result in markedly increased erythropoiesis. The effectiveness of this compensation mechanisms depends on the underlying cause of anemia. Other common findings on the PBS include prominent reticulocytosis and polychromasia. It should be noted that this response can be elicited in any condition that reduces the oxygen supply to tissues (E.g. pulmonary or cardiac disease), and is not reserved solely for anemia. [8-13]

3. Bone marrow invasion / replacement / fibrosis

The bone marrow can be subject to overgrowth by primary hematological malignancies, invasion by circulating tumor cells or infectious agents and fibrosis due to various stimuli. This disruption of bone marrow microarchitecture can result in the untimely release of NRBC in the circulation. Common accompanying features are myeloid precursors and dacrocytes (leukoerythroblastosis). [9-13]

4. Extramedullary hematopoiesis

Extramedullary hematopoiesis can be caused by anemia, bone marrow failure or any other pathology in which the bone marrow is unable to meet the demand for accelerated erythropoiesis. It is assumed that the spleen does not retain immature precursors as well as the bone marrow. This is reflected by a release of NRBC's as well as myeloid precursors, larger immature thrombocytes and the occasional blast. [9-13; 15]

Arrangement abnormalities

Rouleaux formation

Rouleaux formation is a term used to describe the tendency of erythrocytes to align in columns alike to "stacked coins". Rouleaux are found as an artefact in all PBS when an incorrect area is used to examine erythrocyte morphology. However increased rouleaux formation can be clinically significant. It is typically seen in patients with increased plasma protein concentrations. [9-13; 125-126]

1. Inflammatory conditions

A plethora of acute and chronic inflammatory conditions result in the production of polyclonal immunoglobulins and acute phase proteins. Excess immunoglobulins and fibrinogen are associated with increased rouleaux formation of erythrocytes. [9; 126-127]

2. Pregnancy

In pregnancy there is a physiological increase of fibrinogen. Excess fibrinogen has been associated with increased rouleaux formation. [128]

3. Plasma cell dyscrasia

Various clonal plasma cell disorders can result in the excess production of monoclonal paraprotein. Examples are Waldenström's macroglobulinemia (MW), multiple myeloma (MM) and monoclonal gammopathy of unknown significance (MGUS). [126-127]

A study investigating the initial presentation of 1.027 MM patients found that 56% had rouleaux formation as an early sign of disease. Therefore it is prudent to consider a plasma cell dyscrasia when rouleaux formation is reported in a patient in whom no infectious or inflammatory explanation can be readily supplied. [129]

4. Diabetes mellitus

Erythrocytes form patients with diabetes mellitus are known to aggregate more readily. The increased blood viscosity that results is thought to be the principal cause for various vascular complications. [130]

Agglutination / Clumping

Agglutination is a term used to describe the tendency of erythrocytes to clump together in grape-like clusters. This will result in spurious measurements of MCV and erythrocyte count and derived indices. The most common cause of agglutination is the presence of antibody-coated-erythrocytes. [9-13; 131]

Cold agglutinin disease (15% of auto-immune hemolytic anemia)

The presence of cold agglutinin can cause erythrocytes to agglutinate during storage or transport. When these samples are not warmed up before measuring the cell counter will analyze clumps of erythrocytes instead of individual cells. This will result in spurious measurements of various RBC indices, amongst which a grossly elevated MCV. The formation of cold agglutinins has been associated with infections such as *M. pneumonia* and Epstein-Barr virus as well as with several lymphoid malignancies. [9; 13; 131]

Question 2: How should RBC-M be reported?

A laboratory report is vitally important because it constitutes the main communication tool between the laboratory and the requesting clinician. If this communication does not contain the necessary information or presents this information in an obscure manner the requesting clinician will be unable to make the best use of the report. It is therefore of the utmost importance to ensure that reporting practices are of the best possible quality. Laboratory reports should be standardized and be subject to

evaluation by external quality assessment instances to ensure information is communicated clearly. [17-18]

Reports on RBC-M can take several shapes. A grading system is often used to determine the prevalence of each abnormality. These descriptions can be qualitative, quantitative or graded (semi-quantitative, descriptive). These grades can be communicated directly to the clinician or they can be accompanied by interpretative comments and a summary of notable findings of the PBS. It should be noted that despite increasing harmonization and standardization in laboratory practice there still exists significant heterogeneity in RBC-M. This heterogeneity encompasses the criteria and methods used to describe and assess morphologic abnormalities, the grading systems used to describe prevalence as well as the interpretive comments used to aid clinicians. In this text we will focus on the grading systems and interpretive comments.

Grading systems

A grading system is the combination of criteria used to estimate the prevalence and significance of morphologic abnormalities. As examples of grading systems we will use the grading systems used by the following instances: AZ Delta, University hospital Leuven (UZL), Constantino et al., VHL taskforce "Difboekje" and the international council of standardization in hematology (ICSH 2015). All values are adjusted to reflect an average count of 1.000 RBC's. (See Appendix I) [14-16]

As illustrated in Appendix I, there is significant heterogeneity in the grading systems used by each instance. It is assumed this heterogeneity exists because there is insufficient data on the prevalence of specific morphological abnormalities in specific disease-states compared to a healthy population.

In AZ Delta, we report RBC-M when a review of the PBS is requested by a clinician (2.6%) or the laboratory (97.4%). The laboratory will most frequently request a review due to abnormal results of the automated cell counters or on demand of a clinical pathologist when reviewing other laboratory results. We perform grading of a select number of morphologic abnormalities: dacrocytes, spherocytes and fragmentocytes. Other abnormalities are not graded, but are reported qualitatively as "present" should their count exceed the predetermined cut-off. This method differs from some other reporting systems, where grading is used more freely.

Amongst those systems that grade more often, there are large differences in the cut-offs used. For example: Imagine a patient with an average count of 55 ovalocytes per 1.000 erythrocytes. This finding would result in a 3+ grade in UZL, a 2+ grade according to the ICSH guidelines, a 1+ grade according to the VHL taskforce and no mention of their presence according to Constantino et al. It should come as no surprise that this can be confusing to clinicians, especially when patients might consult in multiple institutions.

In an attempt to harmonize hematology reporting the ICSH has published a consensus guideline on the terminology and grading of peripheral blood features. The ICSH grading system forgoes the use of a 1+ in order to provide the clinician with less insignificant bits of data and more useful information regarding the status of abnormalities in the peripheral blood. They have elected to reserve the 1+ grade only for fragmentocytes, which are significant even in low numbers. Additionally they suggest to avoid grading hypochromasia, microcytosis, macrocytosis, anisocytosis in favor of the erythrocyte indices calculated by the automated cell counters (MCH, MCV, RDW). [1]

To our knowledge, all current guidelines concerning the grading of RBC-M are based on consensus agreement. As such significant improvements could be made by incorporating data from prospective multi-center trials that evaluate the prevalence of erythrocyte shape abnormalities in pathological and healthy populations.

Interpretative comments

The purpose of laboratory analysis is to provide the clinician with meaningful information that can be used in the diagnostic process. Historically, a great amount of attention has been devoted to the standardization of analytic processes in the laboratory. Recent publications, as well as our findings, demonstrate the vulnerability of the so called "post-post-analytical phase". This post-post-analytical phase encompasses the interpretation of the result by the clinician. [132-133]

The implementation of interpretative comments can aid clinicians in correctly interpreting results. Their use has been recommended in several guidelines as well as in the International Standard for laboratory accreditation International Standards Organization (ISO15189: 2012). The use of interpretative comments has been shown to benefit both patient safety and clinician satisfaction. Initiatives such as the Working Group on Diagnostic Hematology of the Italian Society of Clinical Chemistry and Clinical Molecular Biology (WGDH-SIBioC) have attempted to standardize the comments frequently used in RBC-M reporting. [133]

An additional benefit of the use of interpretative comments is that clinicians would be more acutely aware of the expertise of the clinical pathologists. It has been our experience that clinicians more often consult hematologists instead of the clinical pathologists to aid in the interpretation of results. The use of interpretative comments could therefore stimulate a more communicative relationship between requesting clinician and clinical pathologist.

The potential use of interpretative comments as an educational tool should not be underestimated. As medical education spends less time on pathological disciplines the benefit of recurring reminders of pathologies associated with morphological abnormalities increases.

Question 3: What is the perceived clinical utility of RBC-M reporting?

Survey design

A survey was designed to evaluate the perceived utility and experiences with RBC-M reporting of the clinicians of AZ Delta and Sint-Andries hospitals. The specific terminology (n = 24) used in the survey was based on the terminology that is used in everyday practice by the clinical laboratory of AZ Delta and Sint-Andries. Clinicians where invited to complete the survey anonymously and without external aids by an email addressing all clinicians.

An initial query explored the clinicians specialization to allow for classification of results. Respondents were asked to express qualitatively how often they are interested in erythrocyte indices and erythrocyte morphology. The available options were : "Always", "Often (>50%)", "Sometimes (15-50%)", "Rarely (<15%)" and "Never". Afterwards, they were asked to rank each term based on their opinion of its clinical value ("Very useful", "Useful", "Sometimes useful", "Clinically insignificant") and to what extent a grading would affect their opinion ("Significant even in low numbers", "Only significant if strongly present", "Only significant with other abnormalities", "Never significant"). If a respond did not know any given term, they could select the option "Unknown term".

Analysis

Descriptive statistics were calculated for all survey questions. Summary values were compared between subgroups of interest. To facilitate analyses several responses were grouped together. The options "Always" and "Often >50%" were considered to show agreement with the statements, whereas the options "Sometimes 15-50%", "Rarely <15%" and "Never" were considered to convey disagreement. In ranking morphology "Very useful" and "Useful" were combined as were "Sometimes useful" and "Clinically insignificant".

Results

45 clinicians participated in the survey. Included specializations were the following: Gynecology (5), Pediatrics (5), Gastroenterology (5), Orthopedic surgery (4), Anesthesiology (3), Neurology (3), Oncology (3), Geriatrics (2), Psychiatry (2), Emergency medicine (2), Pneumology (2), Hematology (2), Neurosurgery (1), Nephrology (1), General surgery (1), Rheumatology (1), Vascular surgery (1), Internal Resident (1). One participant elected not to disclose their specialization. Not all respondents elected to reply to all questions, resulting in varying amounts of replies for each question.

Responses to the general questions are displayed in table 12. A majority of clinicians (62%) generally consult RBC indices other than hemoglobin. Only a minority of clinicians (39%) claimed to consistently consult the morphology report. 20% claimed to never do so.

Table 12 : Responses to general survey questions

Responses to general questions on indices and morphology reporting				
Question	"Often >50%"	"Sometimes 15-50%"	"Rarely <15%"	Never
I generally review RBC indices other than hemoglobin and hematocrit.	24%	13%	13%	11%
When I review a complete blood count, I read the morphology report if present.	14%	20%	20%	20%

Surprisingly, we found that a large portion of clinicians is not familiar with standard terminology used in RBC morphology reporting. Only 2.9% (1/35) claimed to always understand the terms used in a RBC morphology report. 63% (22/35) reported that they were either "Always" or "Often >50%" confronted with terms they did not know.

Only 3 terms were designated as "Useful" by more than 50% of responders. These terms were Microcytosis (62%), Macrocytosis (61%) and Sickle cells (52%). 15 terms were reported as "Unknown term" by more than 50% of responders. See table 13 for a summary of results.

We compared results of the internal medicine clinicians (n = 14) and the remaining clinicians (n = 23) and found overall knowledge (terms known by >50%) was better in the internal medicine group. Only 3 terms (Pappenheimer bodies, anulocytes and dimorphism) were unknown by more than 50% of internal medicine clinicians compared to 16 in the non-internal medicine group. The internal medicine group also rated all individual abnormalities as more useful than the non-internal medicine group.

Table 13 : Summary results questioning clinical utility of morphology terms

All Clinicians (n=37)	Useful	Less useful	Unknown term
Microcytosis	62%	32%	6%
Macrocytosis	61%	33%	6%
Sickle cell / Drepanocyte	52%	33%	15%
Schistocyte / Fragmentocyte	45%	36%	18%
Spherocyte	30%	39%	30%
Anisocytosis	21%	38%	41%
Acanthocyte / Spur cell	21%	29%	50%
Hypochromasia	19%	47%	34%
Target cell	18%	21%	61%
Howell-Jolly body	18%	30%	52%
Rouleaux	16%	44%	41%
Tear drop cell / Dacrocyte	15%	18%	67%
Agglutination	15%	48%	36%
Stomatocyte	12%	27%	61%
Echinocyte	9%	18%	73%
Elliptocyte	9%	24%	67%
Poikilocytosis	9%	45%	45%
Pappenheimer bodies	6%	18%	76%
Anulocyte	6%	24%	70%
Basophilic stippling	6%	24%	70%
Nucleated red blood cell	6%	36%	58%
Ovalocyte	6%	39%	55%
Polychromasia	6%	39%	55%
Dimorphism	3%	30%	67%

Discussion and Conclusion

This survey has shown that the perceived clinical utility of RBC-M is low. Only a third of clinicians actually reads the morphologic report when one is provided. Furthermore, when they read these reports they are often faced with terminology they do not understand. Findings such as fragmentocytes, nucleated red blood cells, teardrop cells could indicate significant pathologies for which early recognition and treatment is crucial, however the majority of responders either does not know the term or does not find it clinically useful.

Due to the low numbers of participants of each respective specialization, we were unable to make comparisons on a specialization to specialization basis. We were able to compare the internal medicine specializations with the other clinicians and deduce they possessed a better overall knowledge of RBC-M. It is obvious that specialists dealing with specific pathologies may be more familiar with more obscure terms. However, it is alarming that less than 50% of clinicians consider fragmentocytes, agglutination or acanthocytes as useful findings.

This survey demonstrates similar findings as 2 other recent surveys that aimed to explore the clinical utility of RBC-M. It is of the utmost importance that laboratory staff are aware of this disconnect between laboratories and clinicians. Only a small part of the clinicians receiving hematology reports are hematologists. Therefore, the laboratory is spending a great amount of effort and time in evaluating and reporting RBC-morphology, whilst it is functionally useless to a majority of clinicians. No matter how import any given finding is in theory, if the result is not understood or used by the requesting clinician it holds no value. [134-135]

Question 4: What steps could be undertaken to improve RBC-M reporting in AZ Delta?

An easy take-away from the survey as described above would be that the fault lies entirely with the clinicians and that the sole means to improve the perceived clinical utility is to provide education in various shapes and forms so as to improve knowledge and understanding of RBC-M.

However, the way the laboratory formulates and shapes a report influences the ability of clinicians to understand and interpret that report. It has been shown that burying significant findings in lists of less useful terms can lead to interference of the short-term memory and reduced retention of important information. Therefore, the responsibility of the laboratory to convey important information in a clear, concise and standardized manner cannot be understated.

To this end, we suggest 3 possible changes to be implemented in our laboratory.

Firstly, we suggest adapting our reporting system as per the recommendations by the ICSH. By using a general higher cut-off as well as grading abnormalities, we hope to provide clinicians with more clinically relevant information and less distracting clutter. For a select number of terms a lower cut-off will be maintained. These include fragmentocytes, sickle cells, teardrop cells, spherocytes and parasitic inclusions as we feel these findings are sufficiently relevant even in low numbers. Other morphologic abnormalities will only be reported when present in large amounts or on specific demand of the requesting clinician. Additionally, we suggest to replace the morphologic terms of microcytosis, macrocytosis, hypochromia and anisocytosis by the erythrocyte indices MCV, MCH and RDW. It has been shown that automated hematology analyzers produce these results more accurately and more reliably than a subjective microscopic evaluation. We hope that these omissions and the increased cut-offs will result in reports that are less cluttered and of more use to the clinicians.

Secondly, we suggest to provide comments aiding in the interpretation of findings. Many clinicians commented on the survey that simply reporting the morphology was insufficient, and that they would appreciate guidance in what further evaluations should be considered. Incidentally, these comments could prove a useful way to provide ongoing education via constant reminders of associated pathologies. Examples of possible comments could be: "Basophilic stippling 3+, DD lead intoxication? Consider measuring blood lead level and questioning for exposure" or "Teardrop cells 3+ and NRBC 2+, Findings commonly associated with bone marrow disease or extramedullary hematopoiesis. Consider referring to a hematologist for further investigation of bone marrow function". These comments would be most valuable if they could be tailored to the specialization of the requesting clinician.

Lastly, we would propose to implement laboratory-driven education of the clinicians receiving laboratory reports. This education could take the form of lessons, organized on a regularly basis by laboratory workers. Another option is to send a regular newsletter to all clinicians. This newsletter could be a short description of a given abnormality and its associated clinical contexts as described under chapter 1 of this text. The comments as described in above can also serve a secondary educational purpose.

Conclusion

In this text we have first demonstrated there is a wealth of knowledge in literature and textbooks on the various morphological abnormalities of erythrocytes. We have shown that RBC-M can be used to determine possible causes of anemia, and to suspect a wide variety of acquired and inherited pathologies. Afterwards, we have shown that our clinicians do not find RBC-M very useful in their clinical practice and that a majority is unfamiliar with many terms commonly used in RBC-M. We have evaluated several reporting systems available in literature to assess how we could improve our way of reporting RBC-M. Lastly, we make several suggestions on how the reporting of RBC-M could be changed within AZ Delta to provide a service that is more tailored to the clinicians and should result in a lower overall work-load.

To do's

1) Organize meeting with clinicians to discuss the findings of the survey and what their expectations are regarding possible changes in the reporting of RBC-M.
2) Provide a framework in which the changes as described can be implemented and their efficiency can be evaluated.
3) Large, multi-center studies should be organized to determine significant cut-offs of morphology findings for specific pathologies.

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Appendix – Comparison of grading systems

RBC Morphology	AZ Delta					Uzleuven					ICSH 2015			
	Present	Rare	1+	2+	3+	Present	Rare	1+	2+	3+	Present	Few/1+	2+	3+
Acanthocyte	>3	/	/	/	/	/	1-3	3-10	11-20	>20	/	/	50-200	>200
Anisocytosis	>3	/	/	/	/	/	/	/	/	/	/	/	110-200	>200
Anulocyte	>3	/	/	/	/	/	1-3	3-10	11-20	>20	/	/	/	/
Echinocyte	>3	/	/	/	/	/	/	6-20	>20	>40	/	/	50-200	>200
Elliptocyte	>3	/	/	/	/	/	/	3-10	11-20	>20	/	/	50-200	>200
Hypochromasia	>3	/	/	/	/	/	/	10-40	40-125	>125	/	/	110-200	>200
Macrocytosis	>3	/	/	/	/	/	/	10-40	40-125	>125	/	/	110-200	>200
Microcytosis	>3	/	/	/	/	/	/	10-40	40-125	>125	/	/	110-200	>200
Ovalocyte	>3	/	/	/	/	/	/	6-20	>20	>40	/	/	50-200	>200
Poikilocytosis	>3	/	/	/	/	/	/	/	/	/	/	/	/	/
Polychromasie	>3	/	/	/	/	/	/	3-10	11-20	>20	/	/	50-200	>200
Sickle cell / drepanocyte	>3	/	/	/	/	/	/	1-3	3-7	>7	/	/	10-20	>20
Stomatocyte	>3	/	/	/	/	/	/	3-10	11-20	>20	/	/	50-200	>200
Target cell	>3	/	/	/	/	/	/	/	/	/	/	/	50-200	>200
Dimorphism	>3	/	/	/	/	/	/	/	/	/	If present	/	/	/
Pappenheimer bodies	>1	/	/	/	/	/	/	1-3	3-7	>7	/	/	20-30	>30
Howell-Jolly body	>1	/	/	/	/	/	/	1-3	3-7	>7	/	/	20-30	>30
Basophilic stippling	>1	/	/	/	/	/	/	1-3	3-7	>7	/	/	50-200	>200
Teardrop cell/ dacrocyte	/	1-3	4-10	11-20	>20	/	/	3-10	11-20	>20	/	/	50-200	>200
Spherocyte	/	1-3	4-10	11-20	>20	/	1-3	3-10	11-20	>20	/	/	50-200	>200
Schistocyte/fragmentocyte	/	1-3	4-10	11-20	>20	/	/	3-10	11-20	>20	/	<10	10-20	>20
Rouleaux	If present	/	/	/	/	If present	/	/	/	/	If present	/	/	/
Agglutination	If present	/	/	/	/	If present	/	/	/	/	If present	/	/	/
Parasites	If present	/	/	/	/	If present	/	/	/	/	If present	/	/	/

*All counts per 1000 RBC

RBC Morphology	Constantino et al 2014				VHL werkgroep - "Difboekje" 2013			
	Present	1+/Few	2+/Moderate	3+/Marked	Present	1+	2+	3+
Acanthocyte	/	10-100	110-300	>300	/	50-200	200-500	>500
Anisocytosis	/	/	/	/	/	/	/	/
Anulocyte	/	/	/	/	/	/	/	/
Echinocyte	If >300	/	/	/	/	50-200	200-500	>500
Elliptocyte	/	60-200	210-500	>500	/	50-200	200-500	>500
Hypochromasia	/	50-150	160-400	>400	/	50-200	200-500	>500
Macrocytosis	/	/	/	/	If present	/	/	/
Microcytosis	/	/	/	/	If present	/	/	/
Ovalocyte	/	60-200	210-500	>500	/	50-200	200-500	>500
Poikilocytosis	/	/	/	/	/	/	/	/
Polychromasie	/	30-50	60-200	>200	/	50-200	200-500	>500
Sickle cell / drepanocyte	If present	/	/	/	If >10	/	/	/
Stomatocyte	If >300	/	/	/	/	50-200	200-500	>500
Target cell	/	50-100	110-250	>250	/	5-20	20-50	>50
Dimorphism	If present	/	/	/	/	/	/	/
Pappenheimer bodies	If present	/	/	/	if present	/	/	/
Howell-Jolly body	If present	/	/	/	/	50-200	200-500	>500
Basophilic stippling	/	/	/	/	/	50-200	200-500	>500
Teardrop cell/ dacrocyte	if >40	/	/	/	/	5-20	20-50	>50
Spherocyte	/	10-50	60-200	>200	/	50-200	200-500	>500
Schistocyte/fragmentocyte	/	10-50	60-150	>150	/	5-20	20-50	>50
Rouleaux	/	/	110-500	>500	/	50-200	200-500	>500
Agglutination	If present	/	/	/	/	50-200	200-500	>500
Parasites	If present	/	/	/	If present	/	/	/

*All counts per 1000 RBC