

## REVIEW ARTICLE

# Morphologic changes in red blood cells: An illustrated review of clinically important light microscopic findings

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### Abstract

In this article, we provide an illustrated review that may serve as a microscope companion, as well as a reference for the diagnosis of red blood cells alterations and the interpretation of their significance. Beginners in the fields of clinical haematology and haematopathology may benefit from this manuscript's brevity and practical points, while the more advanced will find it useful as a teaching tool.

**Keywords:** Erythrocytes, anaemia, leukaemia, morphology

### INTRODUCTION

Laboratory testing is an important component of patient evaluation. The complete blood count (CBC) is the most common diagnostic lab test performed worldwide.<sup>1</sup> The information gained from a CBC can be greatly enhanced by an examination of a peripheral blood smear (PBS). At most academic centres, a pathologist or trained technologist evaluates and reports PBS findings. Clinicians (particularly haematologists) can benefit greatly from reviewing smears, either independently or jointly with lab personnel.

With more clinical sub-specialisation and increased reliance on advanced testing, the practice of a clinician evaluating peripheral blood smears has become less common. Nevertheless, it is still necessary for clinicians to have familiarity with PBS findings and ascertain important diagnostic information when a pathologist is not available. Whether in low-resource settings without trained pathologists readily available or in major medical centres after regular work hours, PBS review remains a relevant skill for clinicians.

A careful review of a well-prepared PBS is a relatively simple, cost-effective way to quickly obtain important and clinically consequential information. PBS review of red blood cell (RBC, also known as erythrocyte) morphology can narrow the differential diagnosis for unexplained

anaemia, point to certain infections or toxicities, and identify signs of systemic diseases. It can even be used to supplement or replace other lab tests in low-resource settings.

### Peripheral blood smears preparation and examination

Proper technique in the preparation and evaluation of a peripheral blood smear is essential in properly interpreting red blood cell morphology. Ethylenediaminetetraacetic acid (EDTA) is the preferred anticoagulant for CBC testing and making peripheral blood smears. Fresh blood from a capillary puncture may also be used if the smear is immediately made on-site, however, even minor delays will result in the specimen clotting. Even in anticoagulated specimens, morphological changes start to develop one hour after collection, so prompt processing is preferable. Smears should be made within 8 hours if the specimen is kept at room temperature or within 24 hours if it is refrigerated at 2-8°C.

Smearing may be performed manually or by using automated devices. If available, a properly validated smearing device is generally preferable due to the ability to create reproducible smears with ample reading areas and low cellular destruction. Typically, peripheral blood smears are stained with variations of the Romanowsky

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stain, which include Giemsa, May-Grünwald-Giemsa, Wright, and Wright-Giemsa (the latter is used at our institution).

Each blood smear contains a thick end and gradually transitions to progressively thinner regions, terminating in the somewhat parabolically-shaped “feathered-edge”. Morphology is best assessed in the “reading area,” between which there is no significant erythrocyte overlapping (as seen in thicker regions), nor is there a linear arrangement of the erythrocytes with large acellular gaps (as seen in thinner regions). Figure 1 shows the reading area on a properly prepared and stained smear.

Selecting an optimal reading area is essential for proper interpretation of RBC morphology and for avoiding pitfalls. For example, erythrocytes in the thinner areas of a PBS may artifactually appear to lose central pallor, resembling spherocytes.<sup>2</sup> Additionally, artifactual rouleaux may be encountered in thick areas but should only be reported when seen in the reading area.

In contrast with reviewing peripheral smears for the presence of blasts, where even a single cell may require clinical justification, many

findings in erythrocytes have little significance when limited in scope. For instance, identification of a single schistocyte or ovalocyte on a smear is typically clinically insignificant. However, certain other red blood cell findings are always significant and require investigation or clinical correlation, no matter how rare. Examples of the latter include nucleated red blood cells outside of certain neonatal populations or finding Howell-Jolly bodies.

Finally, good practice merits evaluating all cellular components of peripheral blood (RBCs, WBCs, and platelets) upon PBS review, regardless of the indications for smear review. A thorough review should be performed on every slide.

### Normal red blood cells

Normal red blood cells are biconcave disks, containing abundant cytoplasmic haemoglobin. This shape allows for a surface area to haemoglobin volume ratio that is optimal for function and circulation,<sup>3</sup> resulting in a doughnut-like appearance on PBS. Normally, red blood cells vary only slightly in shape and

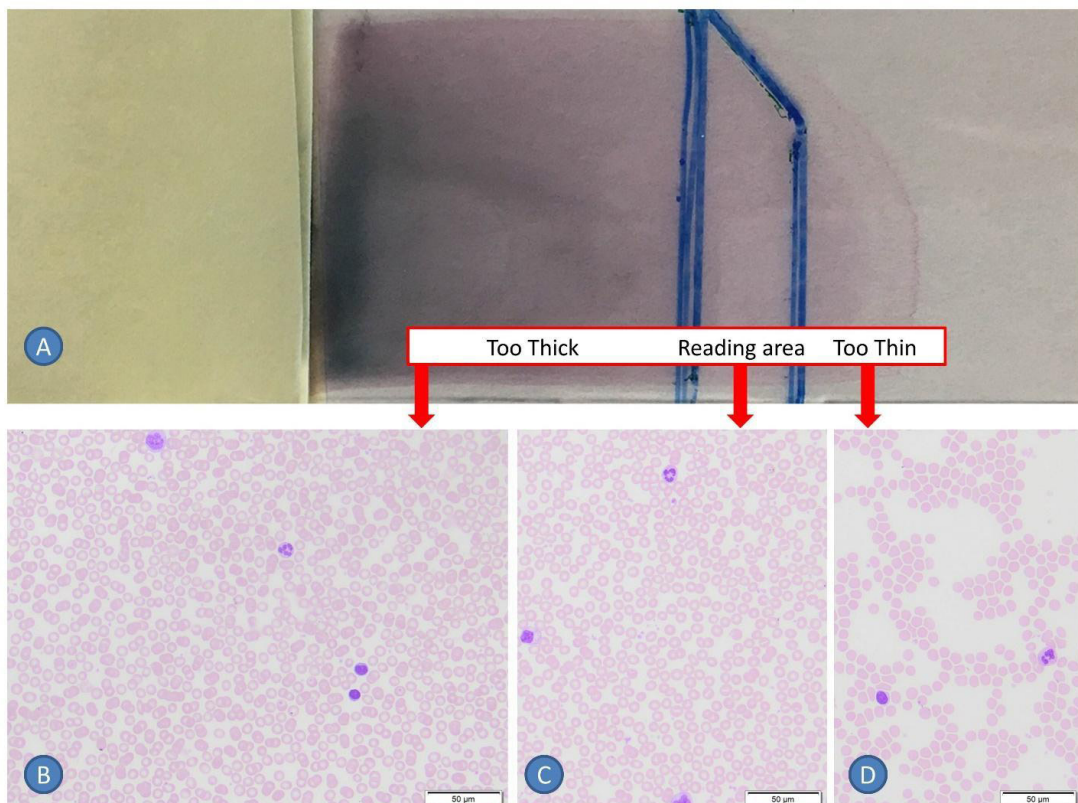


FIG. 1: The reading area (C) of a well-prepared PBS slide is an area where RBCs do not overlap (B – too thick) nor leave large empty spaces (D – too thin).

size. The central area (with the least amount of haemoglobin) appears pale (central pallor) that occupies one-third of the RBC diameter (Fig. 2). The outer two-thirds normally exhibit a homogeneous distribution of haemoglobin. The RBC outer membrane is smooth and indistinct from the haemoglobin-rich body of the cell.

**Factors that influence morphologic changes of red blood cells**

Alterations in the ratio of RBC haemoglobin content to RBC volume:

- A decrease in RBC haemoglobin content to volume ratio usually results in flaccid red blood cells that take the shape of a target on PBS. Keeping in mind that haemoglobin is composed of two major components, a *heme* molecule (consisting of a porphyrin ring with iron) and *globin* (a tetramer of amino acid chains) can help explain the list of conditions associated with increased target cells. Iron deficiency (leading to lower production of *heme*), reduced production of protein (as in

liver disease), or genetic limitations on globin chain synthesis (as in thalassaemia) constitute the major categories.

- An increase in the haemoglobin-to-RBC volume ratio is common in RBC membrane abnormalities. An example is hereditary spherocytosis (HS), an RBC membrane abnormality that results in round cells tensely engorged with haemoglobin.

Alterations in the structure of haemoglobin leading to RBC deformity:

- Abnormal haemoglobin variants such as sickle cell haemoglobin, haemoglobin C and unstable haemoglobin variants.
- Polymerisation and precipitation of normal haemoglobin due to deficiency of a protective enzyme (G6PD, pyruvate kinase, etc.)

Increased peripheral RBCs destruction or demand:

- Results in the release of young, incompletely haemoglobinised RBCs (i.e. polychromatophilic RBCs)

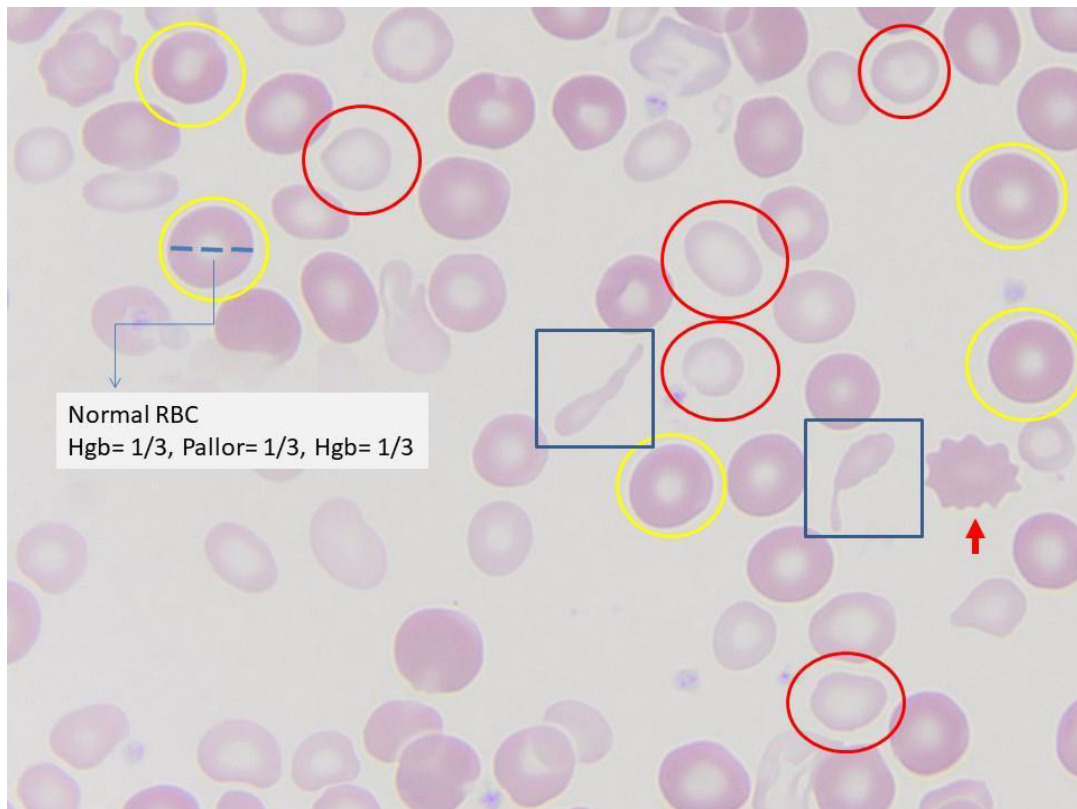


FIG. 2: A microscopic view from a PBS of a child who presented with iron deficiency anaemia and required transfusion. Compare the patient’s hypochromic microcytic RBCs (in red circles) with the transfused normochromic normocytic RBCs (yellow circles). Also seen are “pencil” shaped RBCs typical of iron deficiency anaemia (squares). An echinocyte (burr RBC) is seen in this field (arrow).

**Regulatory system dysfunction:**

- An example is the hypofunctional reticuloendothelial system (as in absent, hypo-functional spleen or even a normal spleen overwhelmed by severe haemolytic anaemias) resulting in the circulation of RBCs with Howell-Jolly bodies.

**Red blood cells in hypochromic microcytic anaemias**

Microcytosis is the term used to describe red blood cells that are inappropriately small. A good morphologic rule of thumb is that normal red blood cells should be roughly the size of a non-activated lymphocyte nucleus. If most RBCs are smaller than a lymphocyte nucleus, this suggests microcytosis. It is important to note that mean corpuscular volume (MCV, a measure of red blood cell size) that would be considered microcytic in adults may fall within the normal range in paediatric populations, especially from 2 months to 2 years of age.<sup>4</sup> Thus; age-appropriate reference ranges should be consulted. Microcytosis commonly coexists with hypochromasia, which morphologically refers to red blood cells with central pallor occupying more than one-third of their diameter.

In paediatric clinical practice, most hypochromic microcytic anaemias are caused by iron deficiency and thalassaemias. Iron deficiency anaemia is the most common form of anaemia in children beyond the first months of life. During the neonatal period, microcytic anaemia is so rare that if encountered, unusual causes should be suspected, such as  $\alpha$ -thalassaemia with a three-gene deletion.<sup>5</sup> While there are some morphological differences between iron deficiency anaemia and the thalassaemias which will be expanded on below, reviewing the CBC haematologic indices is also extremely useful for distinguishing these two entities. The red blood cell distribution width (RDW) is generally higher in iron deficiency anaemia. The red blood cell count tends to be increased in thalassaemias, in contrast to being decreased in iron deficiency anaemia. The MCV is typically lower in thalassaemia relative to the degree of anaemia. The Mentzer index, calculated by dividing the MCV (in fL) by the RBC count (in millions/ $\mu$ L) can be used to help differentiate iron deficiency (Mentzer index > 13-15) from thalassaemia (Mentzer index < 13).

The morphology of iron deficiency anaemia can range from being nearly normal to

drastically altered depending on the severity. The aforementioned increase in RDW is demonstrated by marked anisocytosis, or size variation between erythrocytes. Additionally, relative to the severity, hypochromasia tends to be more pronounced in iron deficiency anaemia. Iron deficiency anaemia should be suspected when the combination of hypochromasia and marked RBC size variability is seen.

Morphologically, RBC forms known as “pencil cells”, or “cigar cells” are typically seen in iron deficiency anaemia. The term refers to hypochromic elliptocytes that may demonstrate a marked increase of the long-to-short axis ratio. While the mere presence of these cells is non-specific, they are relatively more frequent in iron deficiency anaemia than the other microcytic anaemias. It is our experience that “pencil cells” with tapered blunt ends are most characteristic of iron deficiency anaemia (Fig. 2). It is recommended to limit using the phrase “pencil cell” only to the context of iron deficiency anaemia due to the strong implication of the terminology. Target cells, while more classically associated with thalassaemia, may also be seen in iron deficiency anaemias.<sup>2,6</sup>

As previously mentioned,  $\alpha$ - and  $\beta$ -thalassaemia tend to have smaller red blood cells and less anisocytosis relative to iron deficiency when at similar degrees of anaemia. Due to the varying severity based on the number of mutations inherited, morphology may range from normal to markedly abnormal. Target cells are usually seen but are non-specific, being also common in severe hepatic disease, haemoglobin C disease, post-splenectomy, iron deficiency anaemia, lead intoxication, and sickle cell anaemia.<sup>2</sup>

Another feature seen in thalassaemia is basophilic stippling.<sup>6</sup> While not a specific finding, in the context of “thalassaemic” indices it may serve to increase pre-test clinical suspicion<sup>7</sup> and support triage to additional workup such as haemoglobin electrophoresis. Basophilic stippling may also be seen in sideroblastic anaemias. Sideroblastic anaemias are a much less common cause of microcytic hypochromic anaemia. They are seen mainly in the elderly, but also rarely in children as an inherited disorder (most commonly with *ALAS2* mutations) or acquired (particularly through lead intoxication).

Anaemia of chronic disease is typically normocytic; however severe cases may occasionally be slightly microcytic. Severe microcytosis or marked anisocytosis generally

argues against anaemia of chronic disease as RBCs usually appear unremarkable in the latter.

Finally, a dimorphic population of red blood cells is typically seen after transfusion in a patient with microcytic hypochromic anaemia. While the RDW may be increased in these cases, this represents artifactual anisocytosis. A review of PBS in such patients provides a unique opportunity to compare microcytic hypochromic and normocytic normochromic RBCs (Fig. 2).

### Red blood cells in haemoglobinopathies

In countries with advanced health care systems, most haemoglobinopathies are usually detected through newborn screening programs, so initial diagnosis based on examination of a PBS is rare, but the latter remains important for the assessment of patients in crises or explaining clinical findings.

Sickle cell anaemia refers to a sickling/haemolytic disorder that can be caused by biallelic inheritance of HbS (i.e. HbS/S) or double heterozygous inheritance of one HbS allele and another Beta globin chain abnormality on the other allele (such as HbS/C or HbS/beta-thalassaemia). The hallmark morphologic feature of sickle cell disorders is the presence of elongated or banana-shaped erythrocytes with sharp pointed ends and dark condensed haemoglobin (due to polymerisation of HbS in response to low oxygenation states) (Fig. 3A).

Upon examination of PBS, the presence of haemoglobin C crystals helps separate HbS/C (Fig. 3B). Furthermore, microcytosis (low MCV) can help separate HbS/Beta thalassaemia (Fig. 3C) from HbS/S and HbS/C. Howell-Jolly bodies may be seen with increased frequency correlating with disease chronicity and splenic hypofunction resulting from recurrent auto-infarction.

During sickle/haemolytic crises, a robust erythropoietic response may be seen, demonstrated by polychromasia and occasional nucleated red blood cell precursors. Patients receiving appropriate therapy may show limited-to-no evidence of sickle cell anaemia on peripheral smear. Due to the predominance of HbF during the first weeks of life, morphological features of sickle cell anaemia are not typically seen during the early post-natal period. Those with sickle cell trait typically have a normal peripheral blood smear, although rare, sickled RBCs may be encountered.

The main morphologic findings of haemoglobin

C disease (HbC/C) include the predominance of target or clam-shell-shaped RBCs and occasional haemoglobin C crystals. The latter are elongated rod-shaped precipitated haemoglobin that deform and distort RBC morphology (Fig. 3B). PBS in the HbC trait commonly shows target cells (sometimes up to 20-30% of RBCs) but no HbC crystals and it is usually clinically innocuous.<sup>8</sup>

Haemoglobin E disease is common in people of Southeast Asian descent and typically results in mild anaemia. The peripheral smear shows microcytosis and abundant target cells. Those heterozygous for haemoglobin E typically have no anaemia or clinical symptoms: the peripheral smear is generally unremarkable besides microcytosis with few target cells.<sup>9</sup> Unstable haemoglobins are rare but may be associated with haemoglobin precipitation and “bite” cells (Fig. 3D) similar to changes seen in G6PD haemolytic crisis (see below).

### Red blood cell membrane abnormalities

#### Hereditary spherocytosis

Spherocytes are red blood cells that lack central pallor (Fig. 4A and B). This morphologic change can be inherited or exist as a transient phenomenon (secondary spherocytosis). Hereditary spherocytosis (HS) forms a group of disorders that vary in clinical severity, mode of inheritance, and mutation. They are generally characterised by some degree of haemolytic anaemia and the presence of numerous spherocytes on PBS. HS can be inherited in either autosomal dominant or recessive patterns or may occur as a result of *de-novo* mutations. It is the most common RBC membrane disorder in people of Northern European descent but can be seen in patients of any ethnicity.<sup>10</sup> HS is caused by a functional defect or deficiency of one of the RBC cytoskeleton proteins (typically ankyrin, band 3, or spectrin) which causes reduced deformability and subsequent splenic sequestration and destruction.

The clinical severity of HS is variable, even among people that share the same mutation. Classically, HS will manifest with haemolytic anaemia and splenomegaly in a child, though some present with severe or prolonged neonatal jaundice while others are not diagnosed until adulthood. Common lab findings include signs of extravascular haemolysis (anaemia, elevated lactate dehydrogenase, and unconjugated hyperbilirubinaemia), reticulocytosis, and elevated mean corpuscular haemoglobin

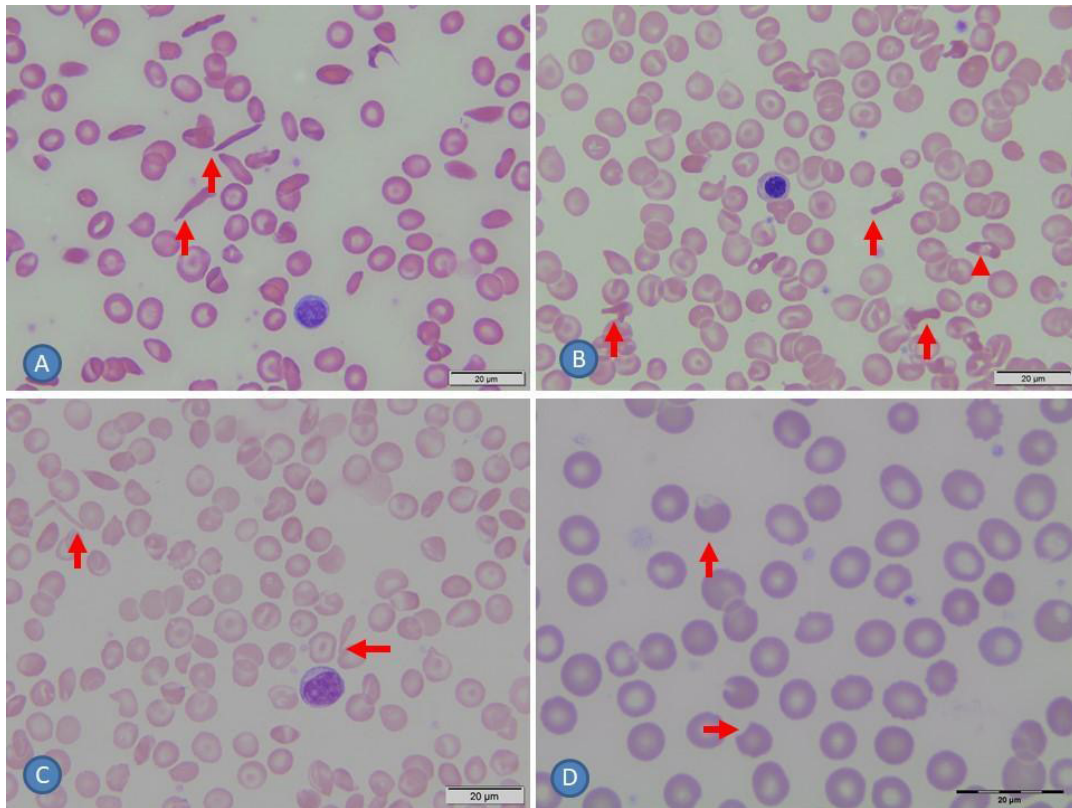


FIG. 3: (A) PBS from a patient with sickle cell disease (Hb S/S) showing typical sickle RBCs with sharp pointed ends; and (B) from a Hb S/C patient showing additional RBCs with haemoglobin C crystals (arrows) and a “safety pin” shaped RBC (arrowhead); (C) from a patient with Hb S/Beta thalassaemia (note the target cells and microcytosis, with most RBCs smaller than the lymphocyte nucleus); (D) from a patient with unstable haemoglobin showing “blister” and “bite” RBCs (arrows).

concentration (MCHC) and RDW. MCV may be normal or low. Mildly affected individuals may have normal haemoglobin (Hgb) and bilirubin values, but will usually have reticulocytosis indicative of mild, chronic haemolysis.<sup>11</sup>

On the PBS from a patient with HS, most RBCs will be spherocytes, with some microspherocytes and immature (polychromatophilic) red blood cells. A subpopulation of normal red blood cells is typically present. Additional abnormal RBC forms, such as ovalocytes or acanthocytes, may be seen in small numbers as well and may be indicative of the specific affected cytoskeleton protein. The percentage of the micro-spherocytes present is an indicator of HS severity.<sup>11</sup> Additional testing, such as eosin-5-maleimide (EMA) binding or specific genetic testing can be performed if the diagnosis is not clear, but within the appropriate clinical context, a CBC with peripheral blood smear review is often sufficient to make a presumptive diagnosis.<sup>12</sup> Osmotic fragility testing will often be positive, but it is not specific for HS and has

limited diagnostic utility. Of note, nutritional anaemias and certain haemoglobinopathies may mask the appearance of spherocytes on PBS due to their effects on the RBC surface-to-volume ratio.<sup>13</sup>

Secondary spherocytosis can be seen in several haematological disorders, but most commonly occurs in autoimmune haemolytic anaemia. Severe hypophosphatemia and clostridia sepsis can also be associated with secondary spherocytosis.<sup>13</sup>

#### **Ovalocytosis / Elliptocytosis**

An elliptocyte, also known as an ovalocyte, is an RBC that is twice as long as it is wide (Fig. 4C). The two terms are almost always used interchangeably in the literature. The presence of some ovalocytes is common in nutritional anaemias,  $\beta$ -thalassaemia major, sickle cell disease, and myelophthisis.<sup>10</sup> However, if >25% of circulating RBCs are ovalocytes, hereditary elliptocytosis (HE) should be strongly considered.<sup>10</sup> Echinocytes may be seen on PBS

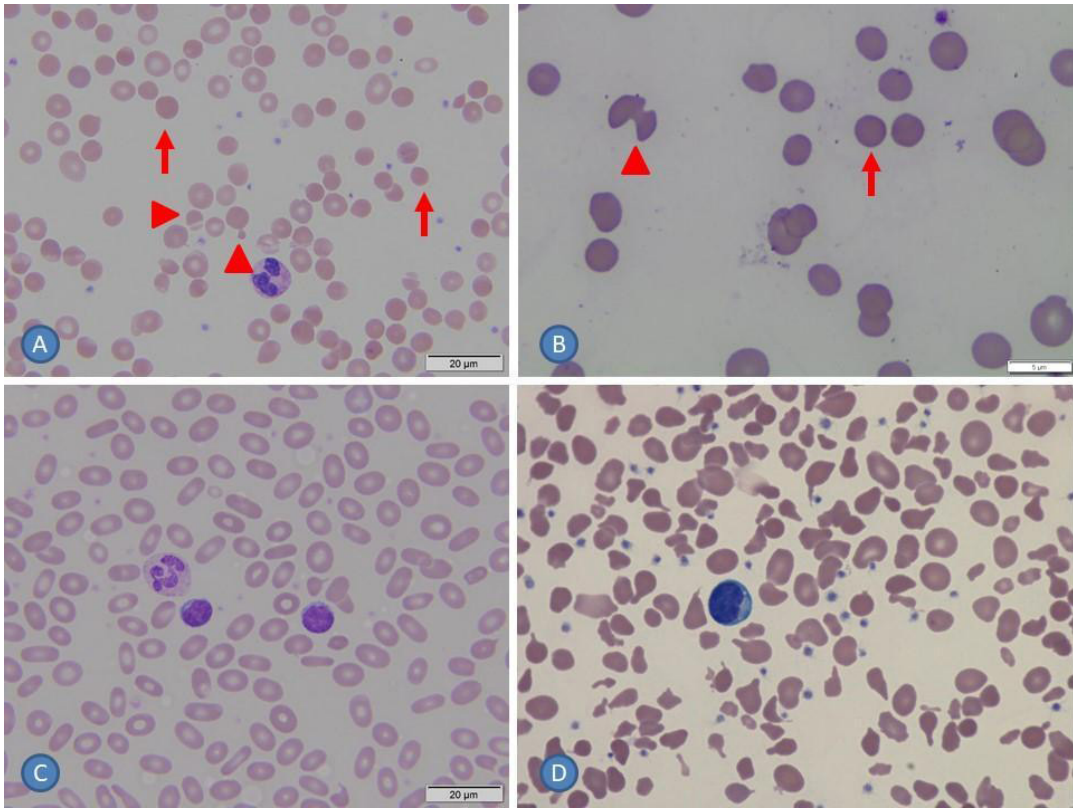


FIG. 4: (A, B) PBS from a patient with hereditary spherocytosis showing spherocytes (arrows) and “mushroom” or “bowling pin” shaped RBCs (arrowheads); (C) Hereditary elliptocytosis; (D) Pyropoikilocytosis.

of HE patients as well. HE is a clinically and genetically heterogeneous group of disorders with presentations ranging from asymptomatic to fatal hydrops foetalis.<sup>14</sup> HE can result from mutations affecting  $\alpha$  or  $\beta$  spectrin, ankyrin, band 3, or protein 4.1R.<sup>14</sup> HE variants are inherited in an autosomal dominant pattern and are more common in people with African, Mediterranean, or Southeast Asian ancestry. The HE variant found frequently in Southeast Asia (intuitively called Southeast Asian ovalocytosis) exists only in the heterozygote form, presumably because homozygosity is incompatible with life.<sup>15</sup> For most other HE variants, homozygotes or compound heterozygotes will display some degree of splenomegaly and haemolytic microcytic anaemia that improves after splenectomy.<sup>13</sup> Heterozygotes are typically asymptomatic outside of infancy but may experience neonatal haemolytic anaemia with transient poikilocytosis.<sup>14</sup> PBS review of samples from the affected infant’s parents may be helpful, but may not be able to distinguish common HE from hereditary pyropoikilocytosis (see next section).<sup>14</sup>

#### Pyropoikilocytosis

A poikilocyte is an abnormally shaped RBC. The term “pyropoikilocytosis” was coined to describe the array of bizarre RBC morphology resulting in changes similar to those seen in patients with severe burns (Fig. 4D). Hereditary pyropoikilocytosis (HPP) is a subset of hereditary elliptocytosis in which the RBCs are unusually sensitive to heat and vary greatly in their morphology.<sup>13,15</sup> It typically presents with moderate to severe haemolytic anaemia in infancy that usually becomes less severe by 2 years of age. CBC will typically show markedly microcytic anaemia. Spherocytes, RBC fragments, elliptocytes, and other abnormally shaped RBCs will be evident on PBS.<sup>13</sup> These RBCs consistently display thermal sensitivity (unlike HE with transient pyropoikilocytosis in infancy, in which the thermal sensitivity resolves).<sup>16</sup> The inheritance pattern is not always clear.

#### Hereditary Stomatocytosis

A stomatocyte is an RBC with a rectangular or slit-like area of central pallor. Hereditary

stomatocytosis is inherited in an autosomal dominant manner and is caused by various mutations affecting different ion channels in the RBC membrane.<sup>16</sup> It is relatively rare, occurring more frequently in patients with African ancestry. There are two subtypes of hereditary stomatocytosis: hydrocytotic (overhydrated) type and xerocytotic (dehydrated) type. The hydrocytotic type is characterised by mild to severe haemolytic anaemia with a high MCV, low MCHC, and abundant stomatocytes on PBS review. The xerocytotic type is characterised by high-normal MCHC, mild to moderate haemolysis (with or without anaemia), and splenomegaly, with target cells and only occasional stomatocytes noted on PBS review.<sup>16</sup> Unlike other RBC membrane disorders, splenectomy is contraindicated in hereditary stomatocytosis due to an increased risk of thromboembolic complications post-splenectomy.<sup>13,16</sup>

#### Red blood cell enzyme abnormalities

RBC enzymopathies encompass a wide range of disorders that generally result in some degree of haemolytic anaemia. The two most common, clinically relevant RBC enzyme disorders are glucose-6-phosphate dehydrogenase (G6PD) deficiency and pyruvate kinase deficiency. The rare pyrimidine 5' nucleotidase (P5'N) deficiency is discussed due to its unique identifying characteristics.

#### G6PD Deficiency

G6PD deficiency is the most common red blood cell enzyme disorder and affects approximately 400 million people worldwide.<sup>17</sup> It is an X-linked recessive disorder with global distribution, though is more common in populations with the Mediterranean, African, Middle Eastern, or South Asian ancestry. The Kurdish population has the highest prevalence globally (70% of males).<sup>17</sup> In the United States, approximately 10% of African-American males are affected.<sup>17,18</sup>

Common clinical manifestations include neonatal jaundice, followed by episodes of haemolysis triggered by oxidative stress. Though fava beans are thought of as the classic precipitant, viral infections are actually the most common triggers of acute haemolysis in G6PD deficiency.<sup>17</sup> Exposure to certain medications (particularly sulfa drugs, rasburicase, methylene blue, certain antimalarial drugs, and nitrofurantoin) can also trigger haemolysis.<sup>17</sup> The sensitivity to oxidative stress and the severity

and frequency of haemolytic episodes varies.

Most patients with G6PD deficiency have no signs of haemolysis or altered RBC morphology between triggered haemolytic episodes. During haemolytic episodes, however, typical signs of haemolysis manifest (acute-onset anaemia, reticulocytosis, elevated LDH, hyperbilirubinemia, etc.), and PBS will show characteristic blister cells and bite cells (Fig. 5). Heinz bodies, which are inclusions composed of denatured haemoglobin, are visible only with supravital stains.<sup>10</sup> The blister/bite cells are the result of the removal of precipitated haemoglobin as the blood is filtered through the spleen. Diagnosis is made by measuring enzyme activity. G6PD can be falsely normal/elevated during the haemolytic crisis, so it is prudent to repeat testing of suspected cases at least 3 months after an episode.<sup>13,17</sup> Severity of disease generally correlates with the percentage of G6PD enzyme activity present in RBCs.<sup>13</sup>

#### Pyruvate Kinase (PK) Deficiency

PK deficiency is a rare autosomal recessive disorder most commonly seen in people of northern European descent and the Amish population of the U.S.A.<sup>10</sup> Deficiency of this enzyme causes ATP depletion within the erythrocyte, resulting in chronic haemolysis and all of its typical sequelae (splenomegaly, gallstones, hyperbilirubinaemia, iron overload, etc.). Most patients will have significant neonatal hyperbilirubinaemia. PBS may show abnormally shaped RBCs, including echinocytes and acanthocytes. Unlike G6PD deficiency, acute episodic worsening of haemolysis rarely occurs, though it has been observed following large doses of aspirin.<sup>13</sup> Like all disorders with a decreased lifespan of the RBC, aplastic crisis precipitated by parvovirus or other viruses can occur. The severity of chronic haemolysis varies, with some patients experiencing mild haemolytic anaemia and others requiring chronic transfusion therapy to survive.<sup>13</sup> A paradoxical increase in reticulocyte count occurs after splenectomy, due to increased reticulocyte survival in the absence of the spleen.<sup>17</sup> Morphologic abnormalities of the RBC may also become more apparent following splenectomy.<sup>13</sup>

#### Pyrimidine 5' Nucleotidase (P5'N) Deficiency

P5'N deficiency is an autosomal recessive condition that results in mild to moderate chronic haemolytic anaemia. Though rare, it is notable for two reasons. First, it is the



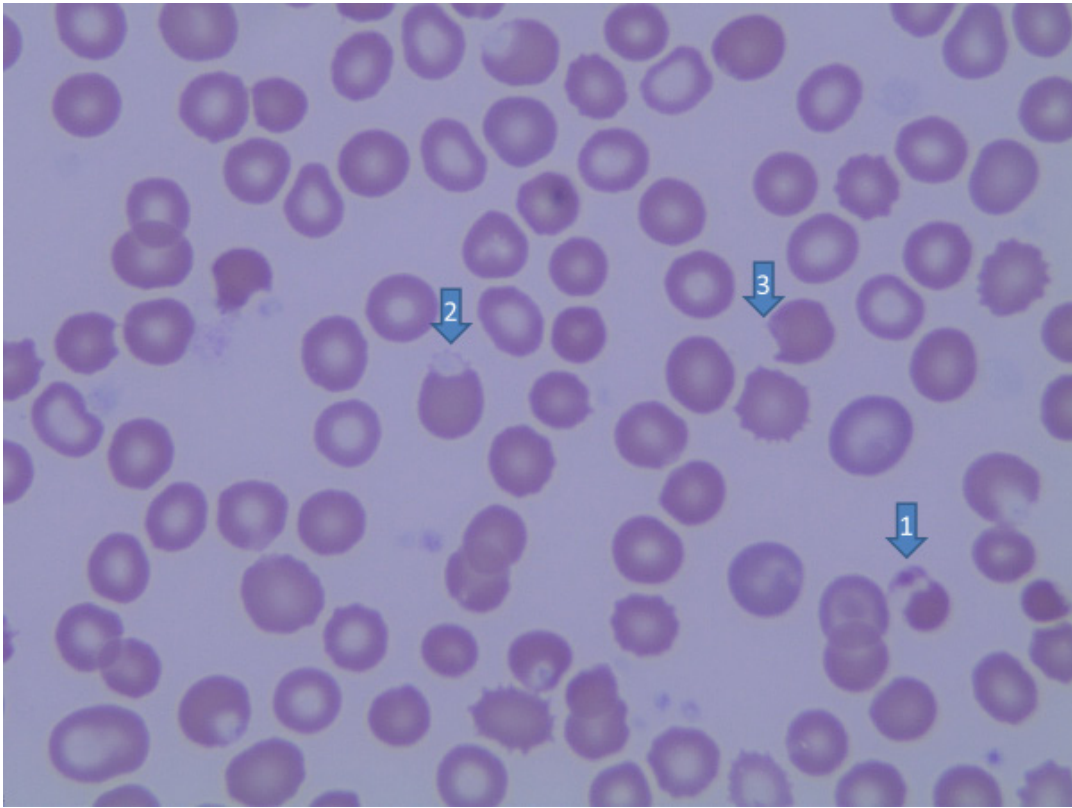


FIG. 5: A microscopic view of a stained PBS of a G6PD deficient patient during a haemolytic crisis, showing an RBC with precipitated haemoglobin (1), another RBC whose precipitated haemoglobin was removed leaving a blister (2), and a third whose blister burst leaving a “bite” RBC (3).

most common enzyme abnormality affecting nucleotide metabolism.<sup>17</sup> Second, it is notable for causing haemolytic anaemia with marked (>5% of RBCs) basophilic stippling.<sup>19</sup> The exact pathogenesis and prevalence of this disorder are not currently known.

#### **Red blood cells in microangiopathic haemolytic anaemia**

Microangiopathic haemolytic anaemia (MAHA) is the process of damage and destruction of RBCs (Fig. 6A) as they circulate through a small blood vessel (small vessel intravascular haemolysis). MAHA is most commonly caused by thrombi in the microvasculature, also referred to as thrombotic microangiopathy (TMA), which presents as haemolytic anaemia and thrombocytopenia. Microvascular thrombosis causes platelet consumption (thrombocytopenia) and mechanical haemolysis of RBCs as they flow through the affected vessel.

TMA is mainly seen in three life-threatening conditions: thrombotic thrombocytopenic purpura (TTP), haemolytic uremic syndrome (HUS), and

disseminated intravascular coagulopathy (DIC). Less commonly, TMA can result from certain rheumatic diseases (lupus, antiphospholipid antibody syndrome, and scleroderma) or medications, such as calcineurin inhibitors, quinine, and chemotherapy drugs. Other causes of MAHA include vasculitis, prosthetic cardiac valves, cardiac septal defects, burn injuries, Kasabach-Merritt phenomenon in neonates, severe vitamin B<sub>12</sub> deficiency, and HELLP syndrome in pregnant women.<sup>20</sup> The presence of *schistocytes* (RBC fragments resulting from intravascular haemolysis) in a patient with anaemia and thrombocytopenia should raise suspicion of TMA; secondary spherocytes may also be seen. Intravascular haemolysis is suggested by elevated LDH, elevated free Hgb, and low haptoglobin.<sup>10</sup> RBC morphology cannot distinguish between the types or causes of MAHA, nor can it accurately predict the severity of MAHA. However, recognizing the morphologic abnormalities associated with MAHA/TMA is key to early diagnosis.<sup>20</sup> Of note, schistocytes may be rare or absent on the

initial blood work, so it is important to review subsequent peripheral blood smears if clinical suspicion of MAHA remains.

### Red blood cell morphology in immune-mediated haemolytic anaemia

Immune-mediated haemolytic anaemias can be divided into autoimmune, alloimmune, or drug-induced. Common to all these at the morphologic level is a variable RBC line left shift, with an increase in polychromatophilic RBCs noted on PBS. In addition, secondary spherocytes are commonly seen with warm autoantibodies (usually IgG in nature with resultant extravascular haemolysis). Agglutination of RBCs is the hallmark of cold autoantibodies (usually IgM in nature with resultant intravascular haemolysis).

*Red blood cell agglutination* describes the phenomenon of abnormal clumping of RBCs on PBS (Fig. 6B and C). Cold agglutinins typically occur as a post-infectious phenomenon (Mycoplasma, Epstein-Barr), in plasma cell dyscrasias, or idiopathically.<sup>1</sup> RBC agglutination is also seen in paroxysmal cold haemoglobinuria,

which is caused in most cases by antibodies to RBCs P antigen. Red blood cell indices in a CBC performed on a sample with agglutination may be artifactually affected due to RBCs clumping (e.g. artificially increased MCV).

### Red blood cell morphology in systemic diseases

*Rouleaux* formation describes RBCs that adhere to one another lying side by side in a roughly linear arrangement, similar to a stack of coins (Fig. 6D). This contrasts with the patternless clumping seen with agglutination. *Rouleaux* is caused by abnormal proteins in the plasma, such as immunoglobulins or excess fibrinogen. Unlike immune-mediated agglutination, *Rouleaux* does not affect automated CBC results and is reversible with dilution in the lab.<sup>10</sup> *Rouleaux* formation is a common artifact if the PBS is too thick or if the reading area was not properly selected. True *rouleaux* will be associated with a high sedimentation rate (ESR).<sup>21</sup> The classical clinical associations are myeloma and macroglobulinaemia, but *rouleaux* can also be

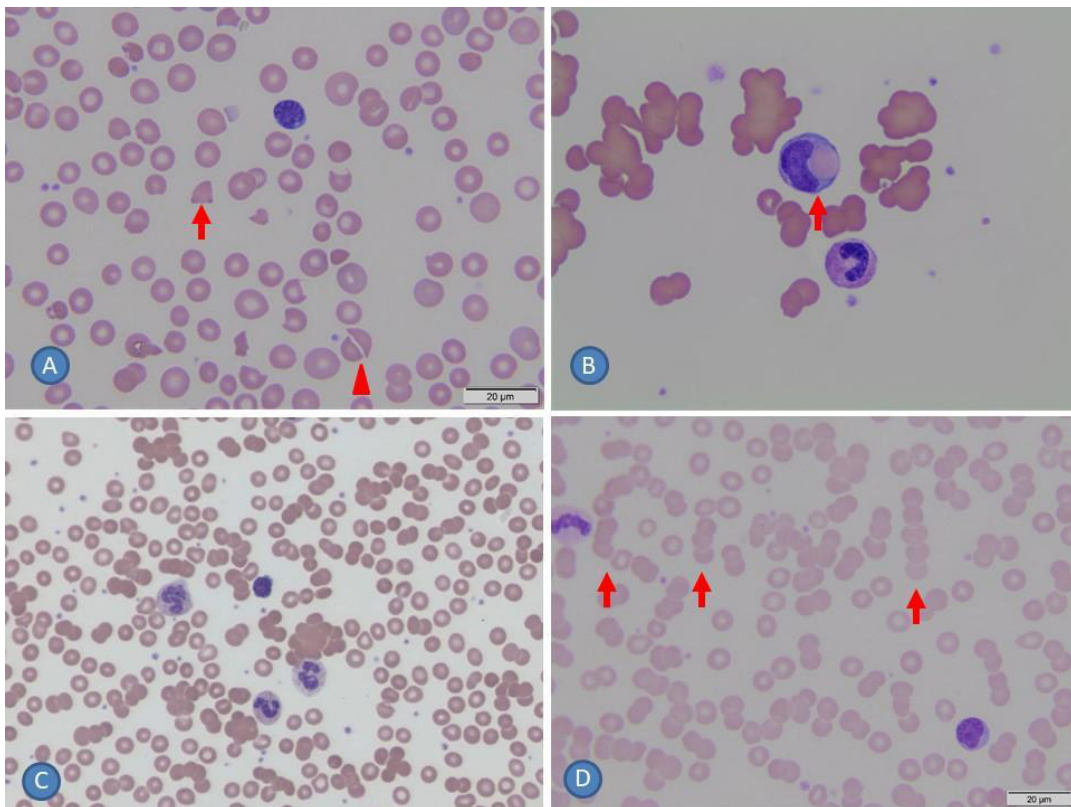


FIG. 6: (A) PBS of a child with haemolytic uremic syndrome showing triangular (arrow) and helmet-shaped (arrowhead) schistocytes; (B) RBCs agglutination in DAT-positive haemolytic anaemia – a macrophage with phagocytised RBC is shown (arrowhead); (C) Large clumps of RBCs in paroxysmal cold haemolytic anaemia; (D) *Rouleaux*.

seen in settings of infection, chronic liver disease, amyloidosis, or connective tissue diseases.<sup>21</sup>

Abetalipoproteinaemia is an extremely rare (1 in 1,000,000) autosomal recessive disease characterised by low/absent levels of plasma cholesterol. Fat malabsorption, chronic diarrhoea, growth restriction, and hepatic steatosis are commonly seen. Deficiency of fat-soluble vitamins subsequently leads to anaemia, spinocerebellar degeneration, vision loss, retinitis pigmentosa, and occasional bleeding symptoms.<sup>13,22</sup> Acanthocytosis is evident on PBS, with at least 50% of RBCs displaying acanthocytic (spur cell) morphology.<sup>13</sup>

Acute and chronic liver disease can cause a multitude of haematologic abnormalities. There is often normocytic or macrocytic anaemia. Erythropoietin is typically elevated unless there is concurrent kidney disease.<sup>13</sup> Acanthocytes, target cells, and/or stomatocytes can be present in variable numbers on PBS review.<sup>13</sup> As noted above, Rouleaux formation is commonly seen in chronic liver disease.

Chronic kidney disease often causes

normocytic, normochromic anaemia due to anaemia of chronic disease and low erythropoietin production. Burr cells can also be seen if moderate-to-severe uraemia occurs.<sup>13</sup>

Hypothyroidism can cause microcytic, normocytic, or macrocytic anaemia, which may co-occur with iron deficiency or pernicious anaemia, confounding RBC indices.<sup>13</sup> Hyperthyroidism can increase red blood cell mass in general, but there are generally no morphologic red blood cell abnormalities.

### Red blood cell morphology in myeloid dyscrasias

Abnormalities in red blood cell morphology are frequently seen in myeloid dyscrasias (Fig. 7). Circulating nucleated red blood cells may be seen secondary to marrow underproduction of mature erythroid elements, resulting in the compensatory release of immature erythroid elements; however, this is not specific to myeloid neoplasia. In myelodysplasia, the atypical morphology of circulating RBCs can be initial clues towards accurately diagnosing the process.

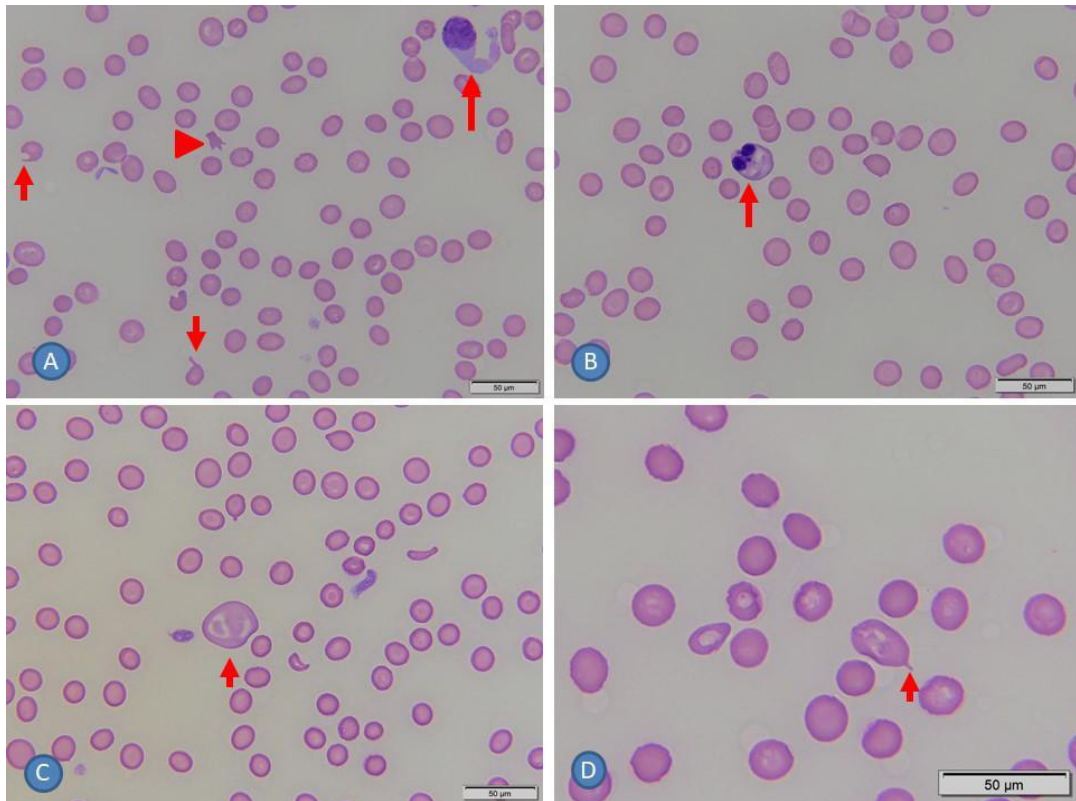


FIG. 7: PBS from a patient with myelodysplastic syndrome. (A) Abnormal bizarre leukocyte (arrow), tailed RBCs (short arrows), bizarre pushpin-shaped RBC (arrowhead); (B) abnormal bi-nucleated NRBC; (C) abnormally large (macrocytic) target cell; (D) abnormally large (macrocytic) and tailed target RBC.

Anisocytosis, poikilocytosis, and basophilic stippling are features that are commonly seen in peripheral blood smears from patients with myelodysplastic syndrome (MDS)<sup>23</sup> and should raise suspicion in the appropriate clinical picture – especially with concurrent irregularities of peripheral myeloid cells and platelets. However, this diagnosis requires correlation with the bone marrow aspirate smear. In the context of PBS review for a new leukaemia presentation, significant morphologic RBC abnormalities and variability in size and shape favour myeloid leukaemia over lymphoid lineage. This is likely due to the closer erythroid and myeloid kinship to a common progenitor along the lineages of haematopoietic differentiation (in comparison with the more distant lymphoid line).

#### **Polychromatophilic and nucleated red blood cells (“left-shift”)**

In response to increased peripheral demand, healthy bone marrow responds by releasing immature red blood cells lacking full haemoglobin content, which manifests on PBS as polychromatophilic RBCs (PRBCs). On routine stains, these appear

paler (with a somewhat “glassy” appearance) and larger in size than mature RBCs. The number of PRBCs correlates with the reticulocyte count, hence estimating PRBCs is sometimes called the “poor man’s” reticulocyte count. More significant peripheral demand (due to bleeding, haemolysis, severe sepsis, etc.) prompts the marrow to release more PRBCs in addition to nucleated RBCs (which represent an even earlier stage of RBC maturation) and in some cases, even polychromatophilic and basophilic normoblasts. Nucleated RBCs may superficially resemble lymphocytes but can be distinguished by an almost perfectly circular nucleus and distinct chromatin clumping. In the severe fulminant loss of RBCs (such as severe immune-mediated haemolytic anaemia) a massive release of erythroid cells is accompanied by the release of early myeloid cells to peripheral blood (left-shift) mimicking leukaemia (hence the term erythro-leukaemoid reaction, (Fig. 8). The latter phenomenon is a manifestation of the bone marrow reacting as an organ, and all three cell lines acting in unison.

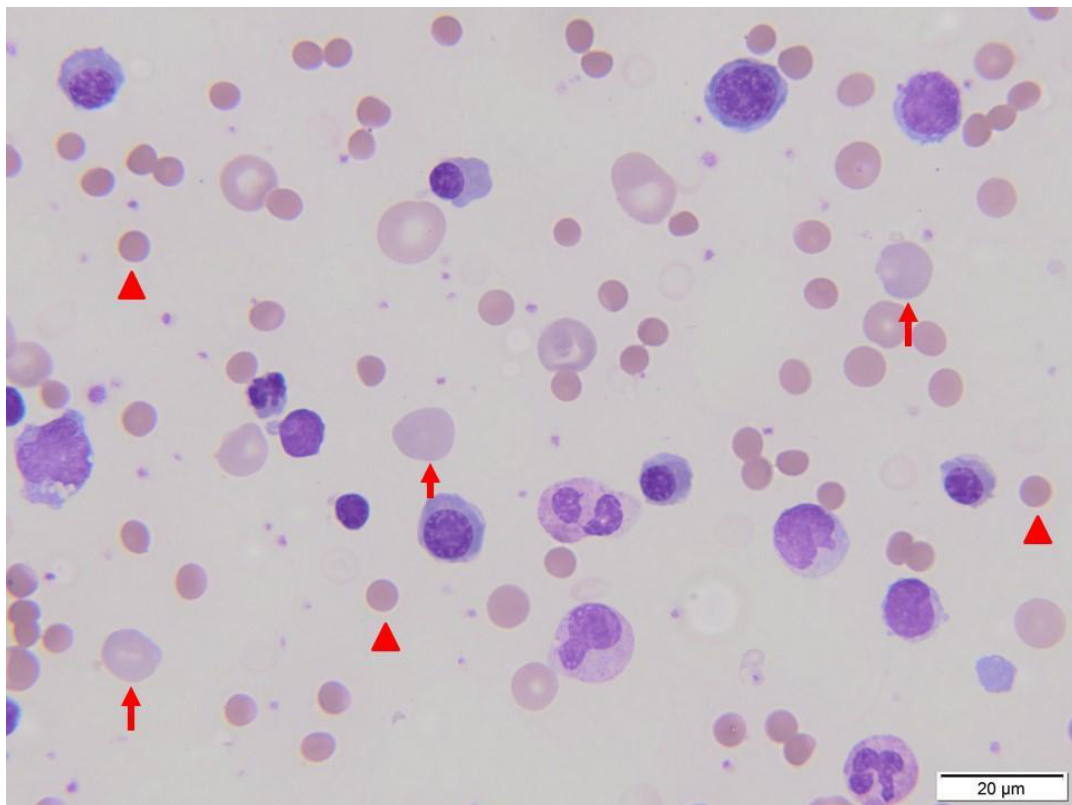


FIG. 8: PBS from a child with erythro-leukaemoid reaction resulting from a severe bout of immune haemolytic anaemia. Note the polychromatophilic RBCs (arrows), secondary spherocytes (arrowheads) along with nucleated RBCs, and left-shifted erythroid and myeloid precursors.

### Red blood cell inclusions

Red blood cell inclusions occur in a variety of clinical scenarios. The correct identification of specific RBC inclusions can provide valuable diagnostic information. Howell-Jolly bodies are black, rounded remnants of DNA encountered in the RBC (Fig. 9A). They indicate splenic dysfunction (hyposplenism) or absence (asplenia). Congenital asplenia (and associated conditions), surgical asplenia, and conditions associated with splenic dysfunction (such as auto-infarction secondary to sickle cell disease) should be considered if Howell-Jolly bodies are present. Recognizing splenic failure/absence is important to allow prophylaxis against encapsulated bacteria, such as pneumococcus. A delay in the diagnosis of asplenia secondary to missing Howell-Jolly bodies can put patients, particularly paediatric patients, at risk of serious infections. Therefore, the identification of Howell-Jolly bodies in a patient with no known clinical explanation should prompt additional evaluation. The homogeneity and lack of surrounding “halo” are helpful in distinguishing Howell-Jolly bodies from their most common mimics – platelets superimposed on top of RBCs (see “common artifacts” section).

Pappenheimer bodies (Fig. 9B) are dark blue or black, irregularly sized and shaped structures composed of iron-containing mitochondria. One notable feature is their tendency to cluster and their classic location at the periphery of the red blood cell. Several conditions are associated with these structures, most prominently asplenia. They can be encountered in other conditions, such as sideroblastic anaemia, myelodysplasia syndrome, alcoholism, and lead poisoning. Pappenheimer bodies stain positive for iron, which can help distinguish them from basophilic stippling.<sup>24</sup>

Basophilic stippling (Fig. 9C) refers to the small punctate granule-like structure seen in the cytoplasm of affected red blood cells. These are made up of ribosomes, and thus are largely rRNA. Stippling granules are generally smaller than Pappenheimer bodies and are much more evenly distributed throughout the erythrocyte. In addition, basophilic stippling will not stain with iron. Basophilic stippling may be seen in many diseases, including megaloblastic anaemia, thalassaemia, haemolysis, MDS, and alcoholism. Primary pyrimidine 5' nucleotide deficiency and certain heavy metal poisoning (such as lead and arsenic toxicity) are also causes of basophilic stippling; however, in these cases, the stippling

tends to be much coarser and heterogeneous in size (Fig. 9D). In lead poisoning, stippling is a result of precipitation of RNA due to an acquired (lead-induced) pyrimidine-5'-nucleotidase deficiency.<sup>25</sup>

### Parasites involving red blood cells

Babesiosis (Fig. 10, caused by *Babesia* species) and malaria (Fig. 11, caused by *Plasmodium* species) are the two main parasitic infections that involve red blood cells on peripheral smear. These two diseases are endemic in distinct regions of the world, a fact that helps simplify the differential diagnosis. While morphologic distinctions can be made between certain *Plasmodium* species (refer to Fig. 12 for a suggested algorithm), an accurate travel history remains valuable for the clinical suspicion of RBC parasitic infections. Although molecular-based tests have recently been approved for clinical diagnosis of malaria infection, final identification and speciation continue to rely heavily on morphology.

In babesiosis, ring forms are mostly seen within normal-sized red blood cells, frequently with multiple organisms infecting the same cell. Occasional extracellular organisms may be seen. These ring forms are frequently pleomorphic, vacuolated, or pear-shaped. Tetrads of organisms forming a “Maltese Cross” are characteristic but are rarely seen in practice. Individual *Babesia* species cannot be distinguished morphologically. While *Babesia* species appear similar to the ring forms of malaria, there are several distinguishing features. Ring forms are smaller in *Babesia* and no mature parasitic forms are encountered, unlike malaria. Additionally, extracellular ring forms are common (Fig. 10D), in contrast with malaria. Finally, *Babesia* organisms lack the pigment typically seen in malaria.<sup>26</sup>

Unlike babesiosis, the five most clinically significant species of malaria (*Plasmodium falciparum*, *P. knowlesi*, *P. malariae*, *P. ovale*, and *P. vivax*) show markedly different morphology depending on the life stage of the organisms. Early speciation can be very important, given the differences in management.

The thin and delicate *P. falciparum* typically infects normal-sized red blood cells. The ring form trophozoites measure ~ 20% of the red blood cell diameter (Fig. 11A). They have one or two chromatin dots, and some are described as “headphones.” They commonly are seen in the red blood cell periphery (“appliqué” forms), and multiple organisms can infect the same red blood cell. Occasionally, infected RBC may show

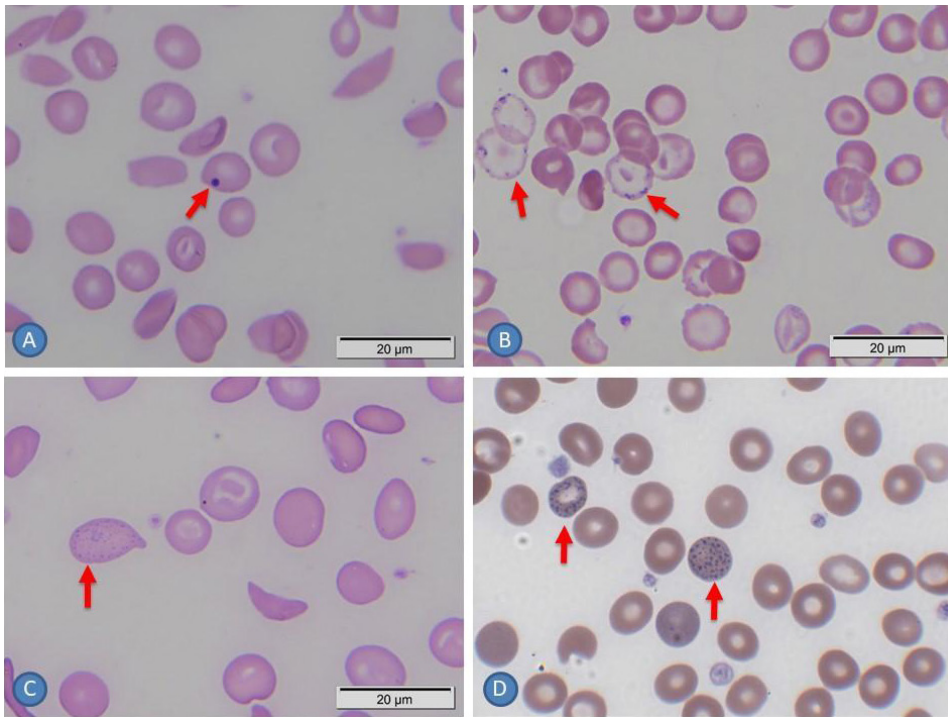


FIG. 9: (A) Howell-Jolly body: usually single round and deeply basophilic-to-black; (B) Pappenheimer bodies: small, deeply basophilic-to-black, usually in clusters located at RBC periphery; (C) Basophilic stippling: small, dot-like, blue and evenly distributed through a teardrop-shaped RBC; (D) Lead poisoning coarse stippling: blue, variable in size and mostly larger than the fine dot-like basophilic stippling.

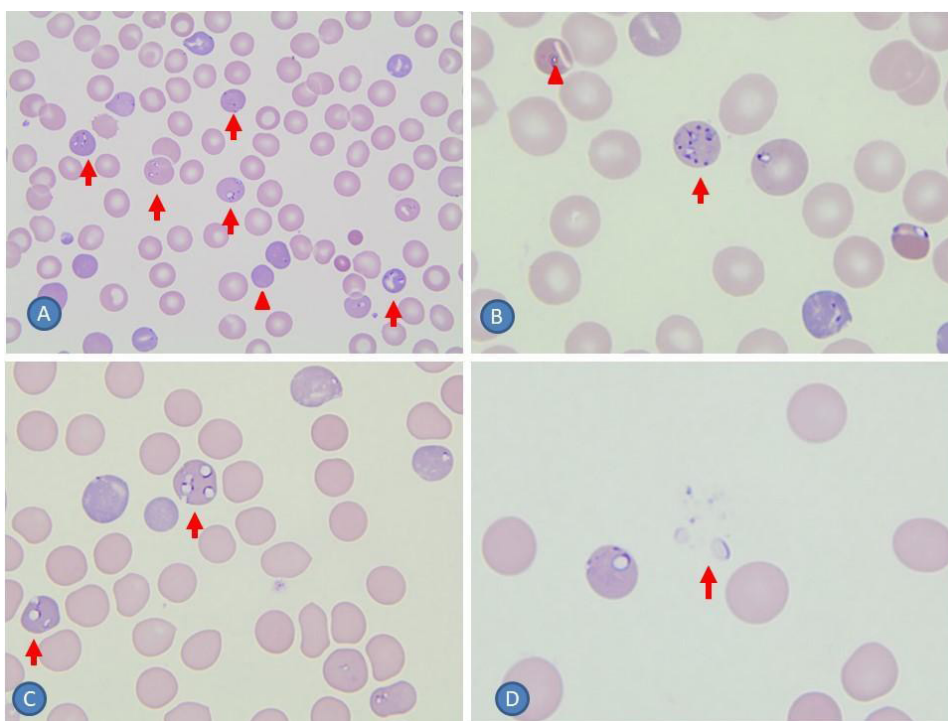


FIG. 10: (A, B, and C) *Babesia*, note that only small ring forms are seen; (D) Extracellular babesial ring forms can be seen. A refractile foreign particle, likely dust, is seen in (B) (arrowhead).

“Maurer’s clefts,” which are larger and coarser than the Schüffner’s dots of *P. ovale* and *P. vivax*. The classic, banana-shaped gametocytes may be seen, and measure approximately 1.5x the diameter of an RBC.<sup>27</sup> This species is unique, as late-stage trophozoites and schizonts are rarely seen circulating.

*P. ovale* and *P. vivax* show somewhat overlapping morphologic features and cannot always be confidently distinguished on a peripheral smear; however, these two species have different geographic ranges. *P. vivax* infected RBCs are noticeably enlarged, and those infected by *P. ovale* tend to be oval with some showing fimbriated borders. The ring forms of these two tend to be larger than those seen in *P. falciparum* and *P. malariae*. They usually have one chromatin dot, though occasional forms with two dots can be seen. RBCs infected with multiple ring forms can be seen, though less frequently than in *P. falciparum*. Fine Schüffner’s dots may be seen in the RBC cytoplasm as the trophozoites mature. The schizonts of *P. vivax* on average contain more merozoites (12-24) (Fig. 11B) than those of *P. ovale* (4-16) (Fig. 11C), and the gametocytes are extremely

similar (with those of *P. vivax* being slightly larger).<sup>27</sup>

*P. malariae* infects small-to-normal-sized red blood cells, and the ring forms tend to be thicker than in *P. falciparum*. There is usually only one chromatin dot (with only rare ring forms with two), and some rings may have a “bird’s eye” morphology, with the dot located in the center of the ring. The mature trophozoites can show oval-to-rectangular “bands” which completely traverse the infected RBC (Fig. 11D). The schizonts have 6-12 merozoites, and the gametocytes are round and usually fill the RBC.<sup>27</sup>

*P. knowlesi* tends to involve roughly normal-sized red blood cells. The early ring forms of *P. knowlesi* are very similar to those seen in *P. falciparum*, with one-to-two chromatin dots, and multiple infected RBCs may be seen. However, these ring forms can get quite large, and can eventually occupy >50% of the host RBC. Maturing trophozoites occasionally may show band forms similar to *P. malariae*. Schizonts have up to 16 merozoites per cell, with an average of 10. The gametocytes are round and occupy much of the host RBC.<sup>27</sup>

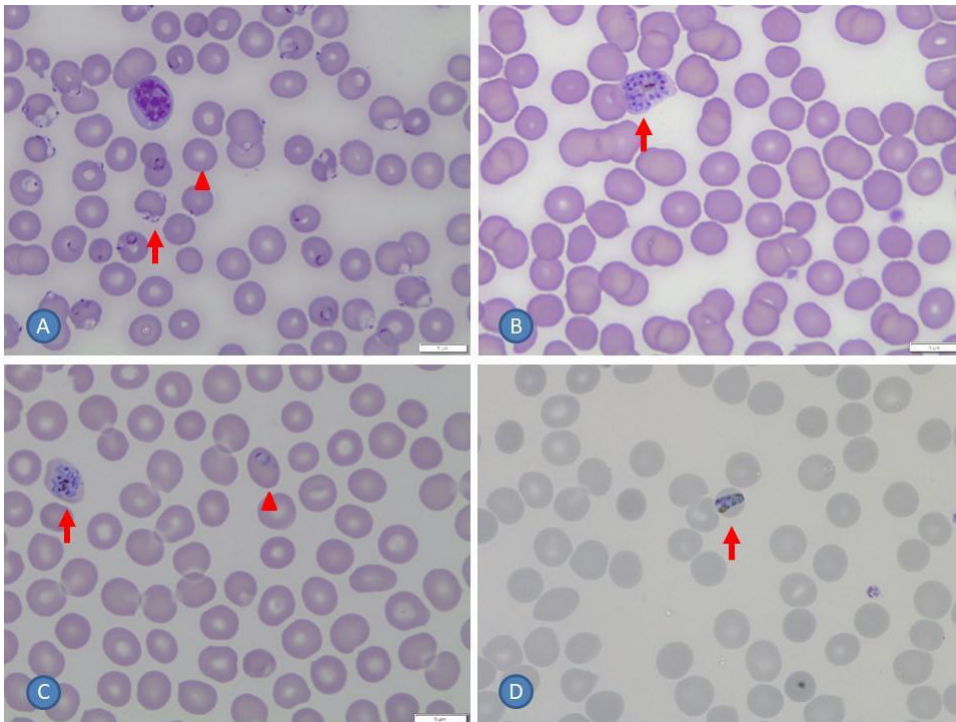


FIG. 11: (A) *Plasmodium falciparum*, note the high parasitaemia and that infected RBCs (arrow) are similar in size to non-infected (arrowhead); (B) *Plasmodium vivax*, a schizont with more than 12 merozoites is noted (arrow); (C) *Plasmodium ovale*, infected RBC is larger and oval (arrowhead), a schizont with only 6 merozoites is seen (arrow); (D) *Plasmodium malariae* “band” form (arrow)

A suggested approach to the morphologic identification of Plasmodium(Malaria subtypes)

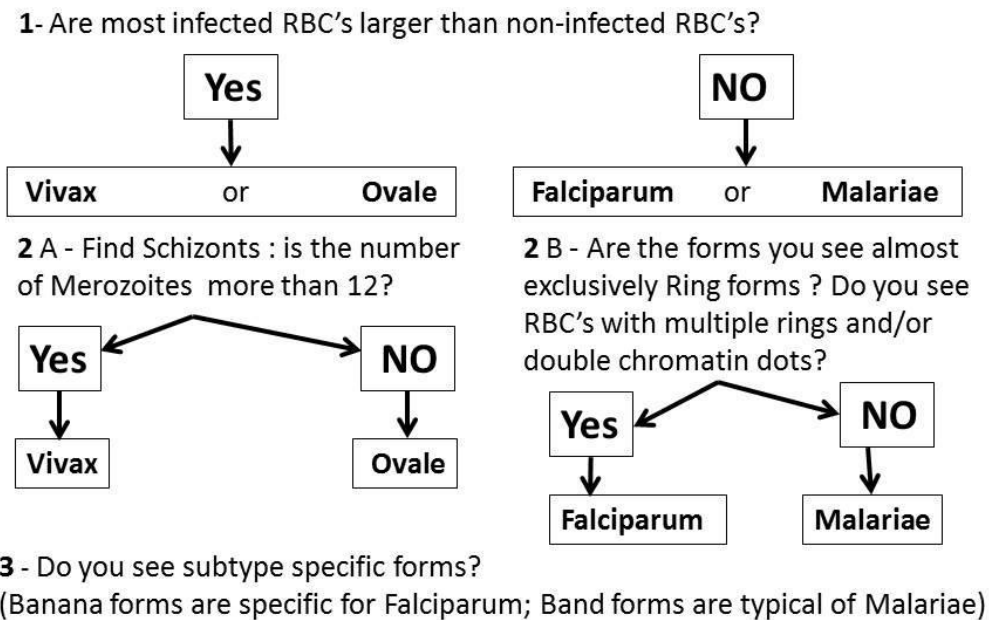


FIG. 12: An algorithmic approach to the morphologic diagnosis of Plasmodium spp.

**Common artifacts**

Artifacts of EDTA anticoagulation are among the most commonly seen on peripheral smears and tend to involve leukocytes (agglutination) and especially platelets (agglutination, degranulation, or satellitism) more so than erythrocytes. EDTA may directly induce platelet agglutination, or through the presence of EDTA antibodies.<sup>28</sup> Identifying this process allows for the correction of falsely altered values reported by automated analyzers and can usually be confirmed by demonstrating the absence of agglutination with citrate anti-coagulated blood. Cold reacting antibodies may result in severe erythrocyte agglutination – this effect can often be eliminated by maintaining the collected blood at body temperature (37°C) until the specimen is tested, allowing for accurate CBC results and improved RBC morphologic assessment.

Echinocyte-like changes (“burr cells”) are a common artifactual phenomenon on peripheral smears. Factors associated with this change include a prolonged interval between specimen collection and smearing,<sup>28</sup> high potassium,<sup>28</sup> EDTA exposure,<sup>28</sup> low cellular density,<sup>29</sup> insufficiently hydrophobic glass surfaces (slide or coverslip),<sup>29</sup> warm temperatures,<sup>29</sup> high pH,<sup>2,30</sup> or hypertonicity of the staining

solution.<sup>2</sup> Identifying other changes suggestive of prolonged specimen storage (such as neutrophil vacuolation or karyorrhexis) further supports an interpretation of pseudoechinocytosis.<sup>28</sup> Artifactual stomatocytosis is frequently seen, commonly as a drying artifact on Wright-stained smears<sup>31</sup> or due to acidity of the staining solution.<sup>2,30</sup> One should review multiple fields because in true cases of stomatocytosis, these cells should be omnipresent.<sup>31</sup> Likewise, in some instances, target cells may be seen, but focal distribution suggests these are artifactual, as true target cells should display even distribution throughout the reading area.<sup>2</sup>

Platelets are frequently superimposed on top of red blood cells in both normal and abnormal smears. With experience, this feature is typically simple to identify, and is readily dismissed. However, it can sometimes be mistaken for a red cell inclusion and is especially troublesome when assessing for intra-erythrocytic parasitaemia (Fig. 13). The morphologic similarity to the surrounding platelets can help with the distinction. Other common artifacts include bubbles, stain precipitation, and dust particles. The latter appears refractile upon adjusting the focus (Fig. 11B – arrowhead).



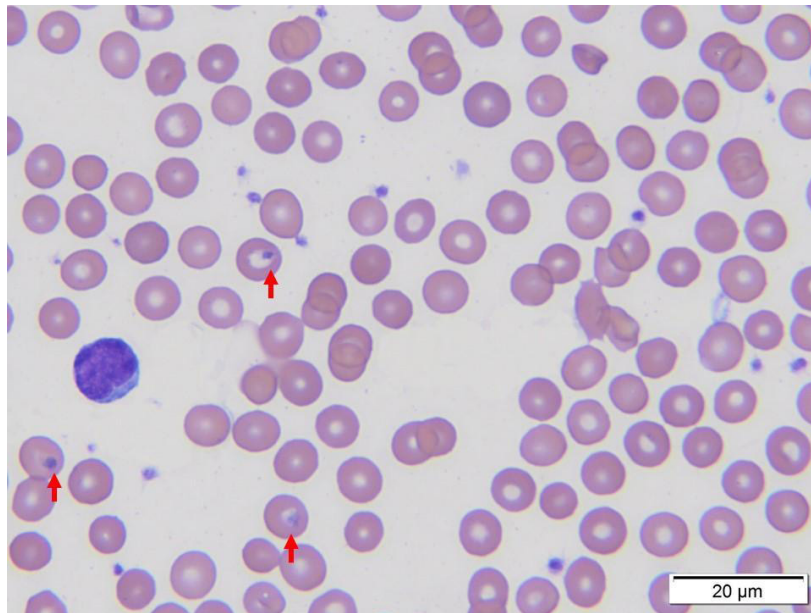


FIG. 13: Platelet on top of RBCs mimicking ring form of *Plasmodium spp.* (arrows)

**Summary/Index Table**

<b>PBS Finding</b>	<b>Definition/Figure #</b>	<b>Clinical Association</b>
Acanthocytes (spur cell)	RBCs that have few irregular spicules; no central pallor	<ul style="list-style-type: none"> <li>- Abetalipoproteinaemia</li> <li>- Liver disease (neonatal hepatitis, cirrhosis)</li> <li>- PK deficiency</li> <li>- McLeod neuroacanthocytosis</li> <li>- Severe malnutrition/anorexia nervosa</li> <li>- Artifact</li> </ul>
Basophilic stippling (coarse)	Blue, granule-like cytoplasmic inclusions  Figure 9D	<ul style="list-style-type: none"> <li>- Pyrimidine 5' nucleotide deficiency</li> <li>- Lead poisoning</li> <li>- Arsenic toxicity</li> <li>- Other heavy metal poisonings (mercury, zinc, silver)</li> </ul>
Basophilic stippling (fine)	Small, blue, dot-like cytoplasmic inclusions  Figure 9C	<ul style="list-style-type: none"> <li>- Haemolysis</li> <li>- Megaloblastic anaemia</li> <li>- Thalassemia</li> <li>- Alcoholism</li> </ul>
Blister/bite RBCs (pyknocyte)	RBC with a peripherally located vacuole on one side and concentrated haemoglobin on the other  Figure 5 and Figure 3D	<ul style="list-style-type: none"> <li>- G6PD deficiency</li> <li>- Unstable haemoglobin</li> <li>- NADPH deficiency</li> <li>- Glutathione synthetase deficiency</li> <li>- Drug-induced oxidative damage</li> <li>- Infantile pyknocytosis</li> <li>- Viremia</li> </ul>
Echinocytes (burr cell)	RBC with multiple short spicules; central pallor is present (unlike acanthocytes)  Figure 2	<ul style="list-style-type: none"> <li>- PK deficiency</li> <li>- Artifact</li> <li>- Uraemia</li> <li>- Surgical asplenia</li> </ul>

<b>PBS Finding</b>	<b>Definition/Figure #</b>	<b>Clinical Association</b>
Elliptocytes/ovalocytes	Oval-shaped RBC with normal central pallor  Figure 4C	- Hereditary elliptocytosis - Megaloblastic anaemia - Sideroblastic anaemia - Iron deficiency - Thalassemia - Myelophthistic anaemia - Congenital dyserythropoietic anaemia
Haemoglobin C crystals	Rectangular/rod-shaped RBC inclusions  Figure 3B	- Haemoglobin C disease - Haemoglobin SC disease
Howell-Jolly Bodies	Single round black inclusion in RBC (composed of DNA)  Figure 9A	- Asplenia/hyposplenism - Myelodysplastic syndrome
Hypochromic RBCs	RBCs with increased central pallor (pallor >50% of cell diameter)  Figure 2	- Iron deficiency - Thalassemia - Haemoglobinopathies
Microcytic RBCs	Abnormally small RBC (low MCV for age)  Figure 2	- Iron deficiency - Thalassemias - Lead poisoning - Anaemia of chronic disease (some)
Macrocytic RBCs	Abnormally large RBC (high MCV for age)  Figure 7	- B12 or folate deficiency - Hypothyroidism - Reticulocytosis - Drug-induced - Asplenia - Myelodysplastic syndromes - Liver disease
Nucleated RBCs	RBC with retained nucleus  Figure 8	- Term newborn up to one week of age (longer if premature) - Haemolytic anaemias - Space occupying processes of bone marrow (myelofibrosis, primary or metastatic tumours) - Increased peripheral demand/increased production
Pappenheimer bodies	Small, dark gray to black, irregularly shaped RBC inclusions composed of iron-containing mitochondria located at RBC periphery  Figure 9B	- Asplenia/hyposplenism - Iron-overload - Thalassemia - Haemolytic anaemias - Sideroblastic anaemia
“Pencil cells”	Elliptocytes with increased central pallor and one tapered blunt end  Figure 2	- Iron deficiency

<b>PBS Finding</b>	<b>Definition/Figure #</b>	<b>Clinical Association</b>
Poikilocytes	Abnormal RBCs bizarre in shape and variable in size Figure 4D	- Hereditary pyropoikilocytosis - Myelodysplastic syndromes - Severe burns - Hereditary elliptocytosis (neonatal period)
RBC agglutination	Patternless clusters of RBCs Figures 6B and 6C	- Cold agglutinins - Paroxysmal cold haemoglobinuria - Artifact
Rouleaux	Linear formation of RBCs partially “stacked” like coins on top of one another Figure 6D	- Hyperfibrinogenaemia - Multiple myeloma - Macroglobulinaemias - Artifact - Chronic liver disease - Connective tissue diseases - Infection
Schistocytes	Fragmented RBCs (helmet-shaped, triangle-shaped, etc.); typically lack any central pallor Figure 6A	<u>Intravascular haemolysis:</u> - DIC - TTP - HUS - HELLP syndrome - Traumatic/mechanical haemolysis (heart valve, etc.) - Kasabach-Merritt phenomenon - Severe burns - Vasculitis
Sickled cells	Crescent-shaped RBCs Figure 3A	- Sickle cell disease - Sickle cell trait with environmental trigger (dehydration, high altitude)
Spherocytes	Round RBC lacking central pallor Figures 4A and 4B Figure 8	I. Primary: - Hereditary spherocytosis II. Secondary: - Immune-mediated haemolytic anaemias - Extravascular haemolysis - Haemolytic transfusion reactions - Hypophosphatemia - Bartonella infection - Rh-null phenotype - MAHA (secondary microspherocytes)
Stomatocytes	RBC with rectangular-shaped/slit-shaped area of central pallor	- Hereditary stomatocytosis - Artifact - Absence of Rh antigens - Dilantin toxicity - Alcoholism
Tailed RBCs <sup>32</sup>	RBC with elongated projection from one side with consistent diameter and rounded end (compare to “teardrop cell”)	- Myelodysplasia - Myelofibrosis - Artifact (all “tails” will point in same direction)

PBS Finding	Definition/Figure #	Clinical Association
Target cells	RBC with central area of haemoglobin surrounded by area of pallor within an outer rim of haemoglobin  Figure 3	- Haemoglobinopathies (most prominent in HbC) - Thalassemia - Iron deficiency anaemia - Liver disease
Teardrop cells (dacrocyte) <sup>32</sup>	RBC with a shorter projection than tailed RBCs that steadily narrows to a point at the terminus (compare to “tailed RBC”)  Figure 9	- Splenomegaly - Extramedullary haematopoiesis - Megaloblastic anaemia - Thalassemia - Artifact (all “tails” will point in same direction)

**Authors' contribution:** Laura Tyrrell wrote sections of the manuscript, selected references and edited manuscript drafts. Gary Rose wrote sections of the manuscript selected references and edited manuscript drafts. Amal Shukri wrote one section of the manuscript and reviewed manuscript. S Kahwash drafted the outlines, wrote sections of the manuscripts, took and selected microscopic photos, and edited the manuscript.

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