ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features

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SUMMARY

These guidelines provide information on how to reliably and consistently report abnormal red blood cells, white blood cells and platelets using manual microscopy. Grading of abnormal cells, nomenclature and a brief description of the cells are provided. It is important that all countries in the world use consistent reporting of blood cells. An international group of morphology experts have decided on these guidelines using consensus opinion. For some red blood cell abnormalities, it was decided that parameters produced by the automated haematology analyser might be more accurate and less subjective than grading using microscopy or automated image analysis and laboratories might like to investigate this further. A link is provided to show examples of many of the cells discussed in this guideline.
INTRODUCTION
The presence of qualitative flags and/or quantitative abnormal results may indicate the need for peripheral blood (PB) film review and/or a manual differential count. The examination of a well made and stained PB film coupled with the complete blood count information and the ability and skill of the reviewer adds qualitative and/or quantitative information and is an essential part of the diagnostic work-up. Abnormal morphologic findings are reported in various ways: (i) a simple description, (ii) the use of terms such as present or absent, (iii) a semi-quantitative determination, mild (+), moderate (++), marked (+++), (iv) a quantitative percentage of the morphological abnormalities: normal (<5%), mild (5–25%), moderate (25–50%), marked (>50%) – although certain morphological abnormalities, for example schistocytes, will have a greater significance at lower percentage numbers than other abnormalities such as hypochromia and this is reflected in the proposed grading criteria.

Worldwide, there is a marked variation in blood film evaluation, reporting practices and morphology terminology with recommendations in the literature [1, 2] and in local regional publications from a number of different national societies including the College of American Pathologists (CAP), the United Kingdom National External Quality Assessment Service (UK NEQAS), the Japanese Society for Laboratory Hematology and the Royal College of Pathologists of Australasia Quality Assessment Programs (RCPA QAP).

Although there is no evidence that one reporting system is superior to the others, it has become evident that there is a need to develop a global consensus guideline for the grading of blood film abnormalities and blood film reporting as part of good laboratory and clinical practice and for use by laboratory accrediting agencies. The aim of the ICSH committee on Standardization of Peripheral Blood Cell Morphology, Nomenclature and Grading was to provide a guideline for the nomenclature and grading of red cell, white cell and platelet abnormalities.

MATERIALS AND METHODS
An international group of pathologists, haematologists and scientists with blood film morphology expertise from Europe, America, Australasia and Asia participated in a survey on blood film morphology and grading routinely used in their respective laboratories. The results of this survey were discussed at a full day meeting in New Orleans USA (May 2011).

This preliminary meeting resulted in a consensus agreement on red cell, white cell and platelet nomenclature (with a suggestion to include a short description of these cells and morphological abnormalities), and a proposal to develop a grading system for these cell types. One important recommendation was to encourage the use of grading some cell morphology using analyser parameters which can be generated with a higher level of accuracy and precision compared with observer use of the optical light microscope, for example red cell size abnormalities – mean cell volume (MCV) for microcytosis and macrocytosis, and mean cell haemoglobin (MCH) for hypochromia and hyperchromia. However, it is important that the laboratory establishes policies to review peripheral blood smears for abnormalities when the full blood count (FBC) data contain test results that indicate pathologies which must be investigated.

Laboratories that do not have advanced haematology analysers which produce morphology grading will need to establish their own guidelines for when to examine the peripheral blood smear for morphological elements.

The writing Committee collected all available data and after an exhaustive review and analysis of the literature, the preliminary draft was circulated among the members, and a final draft was fully agreed on by the committee. The Working Group communicated by internet e-conferencing. Moreover, the in-progress drafts of this guideline were discussed and finalized during the ICSH General Assembly meetings in October 2013 (Gerrards Cross, UK) and May 2014 (The Hague, the Netherlands). In addition to this document, it was agreed that an IP address for a link to the morphological image web database of Manchester Metropolitan University, UK, and ICSH.org be provided to offer reference images for some of the cell types and morphological abnormalities included in this document. The images may also be found as additional supporting information (supplementary images) with the online version of the manuscript. An ad hoc meeting in Manchester was organized to select the images.

It is not the purpose of this guideline to provide recommendations for standardized blood film prepara-
tion, staining and examination or an exhaustive description of blood cell morphology. For these aspects, the authors recommend reference to existing ISLH/ICSH guidelines and standard morphology textbooks [1–5]. Nor is it the purpose of this guideline to provide diagnostic criteria for the classification and diagnosis of haematological malignancies or other disease states.

There were a number of differences in opinion on nomenclature and practice between different world regions evident from the initial meeting in New Orleans and the committee members acknowledge this. This document reflects a consensus agreement on nomenclature and grading between the participating committee members and is intended as a guideline only. We come from laboratories of different sizes and scopes of practice, serving different populations and types of patients. One reporting system probably would not fit all and there should be some degree of flexibility in the way laboratories report. This may to some extent be dictated by the limitations of the different Laboratory Information Systems and middleware in use.

### GRADING OF MORPHOLOGICAL FEATURES

The grading of morphology elements should provide the clinician with useful information regarding the status of any abnormality in the peripheral blood. This means that it is the responsibility of the laboratory to provide information to assist in the differential diagnosis instead of providing bits of data that are not clinically significant. Therefore, the morphology grading table included contains a two-tiered grading system, for 2+ (moderate) and 3+ (many). The designation for 1+ (few/rare) is reserved only for schistocytes, as the observation even in small numbers is clinically significant. Each laboratory and laboratory system should have policies in place to ensure the consistent application of the grading criteria (Table 1).

### RED BLOOD CELLS

Automated analyser peripheral blood (PB) counts provide accurate and precise red blood cell (RBC) counts and red cell indices, information on RBC population distribution, size and haemoglobin content less than one minute after sample aspiration. Abnormalities in one or more of these parameters generate instrument flags that should be confirmed by optical microscope. In a stained PB film, normal RBC are on average 7.5 μm in diameter and round or slightly oval in shape with an area of central pallor occupying approximately the middle third of the cell. Microscopic slide review of red cells and the identification of abnormalities in size, shape and staining and the presence of inclusions, remains a fundamental way of identifying morphological abnormalities useful in the diagnostic work-up. According to the Rümkö table distribution [6], a minimum of 1000 RBC should be evaluated to provide a precise

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**Table 1. Morphology Grading Table**

<table>
<thead>
<tr>
<th>Cell Name</th>
<th>Grading System</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Few/1+</td>
</tr>
<tr>
<td><strong>RBC</strong></td>
<td></td>
</tr>
<tr>
<td>Anisocytosis</td>
<td>N/A</td>
</tr>
<tr>
<td>Macrocrits</td>
<td>N/A</td>
</tr>
<tr>
<td>Oval macrocytes</td>
<td>N/A</td>
</tr>
<tr>
<td>Microcytes</td>
<td>N/A</td>
</tr>
<tr>
<td>Hypochromic cells</td>
<td>N/A</td>
</tr>
<tr>
<td>Polychromasia</td>
<td>N/A</td>
</tr>
<tr>
<td>Acanthocytes</td>
<td>N/A</td>
</tr>
<tr>
<td>Bite cells</td>
<td>N/A</td>
</tr>
<tr>
<td>Blister cells</td>
<td>N/A</td>
</tr>
<tr>
<td>Echinocytes</td>
<td>N/A</td>
</tr>
<tr>
<td>Elliptocytes</td>
<td>N/A</td>
</tr>
<tr>
<td>Irregularly contracted cells</td>
<td>N/A</td>
</tr>
<tr>
<td>Ovalocytes</td>
<td>N/A</td>
</tr>
<tr>
<td>Schistocytes</td>
<td>N/A</td>
</tr>
<tr>
<td>Sickle cells</td>
<td>N/A</td>
</tr>
<tr>
<td>Spherocytes</td>
<td>N/A</td>
</tr>
<tr>
<td>Stomatocytes</td>
<td>N/A</td>
</tr>
<tr>
<td>Target cells</td>
<td>N/A</td>
</tr>
<tr>
<td>Teardrop cells</td>
<td>N/A</td>
</tr>
<tr>
<td>Basophilic stippling</td>
<td>N/A</td>
</tr>
<tr>
<td>Howell-Jolly bodies</td>
<td>N/A</td>
</tr>
<tr>
<td>Pappenheimer bodies</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>WBC</strong></td>
<td></td>
</tr>
<tr>
<td>Döhle bodies</td>
<td>N/A</td>
</tr>
<tr>
<td>Vacuolation (neutrophil)</td>
<td>N/A</td>
</tr>
<tr>
<td>Hypogranulation (neutrophil)</td>
<td>N/A</td>
</tr>
<tr>
<td>Hypergranulation (neutrophil)</td>
<td>N/A</td>
</tr>
<tr>
<td>Platelets</td>
<td>N/A</td>
</tr>
</tbody>
</table>

percentage of the cells having a particular morphological abnormality.

As a general recommendation, the ICSH group recommends providing only a qualitative report for those presenting with RBC abnormalities; however, a schistocyte count may be of clinical value for the diagnosis and follow-up of thrombotic thrombocytopenic purpura (TTP) and haemolytic uraemic syndrome (HUS) [7]. There is wide variation in red cell nomenclature in common use and a table showing the recommended nomenclature and common red cell synonyms has been provided (Table 2).

<table>
<thead>
<tr>
<th>Recommended Nomenclature</th>
<th>Synonym</th>
<th>Common clinical conditions associated with</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthocyte</td>
<td>acanthoid cell, astrocyte, burr cell, prick cell, pyknocyte, star cell, spur cell, thorn cell</td>
<td>Liver disease, vitamin E deficiency, post-splenectomy, abetalipoproteinaemia, McLeod RBC phenotype</td>
</tr>
<tr>
<td>Basophilic stippling</td>
<td>punctate basophilia</td>
<td>Lead poisoning, haemoglobinopathies, thalassaemia, abnormal haem synthesis</td>
</tr>
<tr>
<td>Bite cell</td>
<td>keratocytes</td>
<td>G6PD deficiency</td>
</tr>
<tr>
<td>Blister cell</td>
<td>puddle cell, eccentrocyte</td>
<td>Oxidative haemolysis, G6PD deficiency</td>
</tr>
<tr>
<td>Echinocyte</td>
<td>berry cell, burr cell, crenated cell, mulberry cell, poikilocyte, pyknocyte, spiculated cell, spur cell, sputnik cell, star cell</td>
<td>Liver and renal disease, pyruvate kinase deficiency, storage artefact</td>
</tr>
<tr>
<td>Elliptocyte</td>
<td>bacillary cell, cigar or rod shaped cell, ovalocyte, pencil cell</td>
<td>Hereditary elliptocytosis, iron deficiency</td>
</tr>
<tr>
<td>Howell-Jolly body</td>
<td></td>
<td>Hyposplenism, post-splenectomy, haemolytic anaemia, megaloblastic anaemia</td>
</tr>
<tr>
<td>Hypochromic cell</td>
<td>anulocyte, pessary form, ring form</td>
<td>Iron deficiency, thalassaemia</td>
</tr>
<tr>
<td>Irregularly contracted cell</td>
<td>macronormocyte, megalocyte</td>
<td>G6PD deficiency, haemoglobinopathies</td>
</tr>
<tr>
<td>Macrocyte</td>
<td>micronormocyte</td>
<td>B12/folate deficiency, liver disease, MDS</td>
</tr>
<tr>
<td>Microcyte</td>
<td>bacillary cell, cigar or rod shaped cell, elliptocyte</td>
<td>Iron deficiency, thalassaemia</td>
</tr>
<tr>
<td>Ovalocyte</td>
<td></td>
<td>Hereditary elliptocytosis, iron deficiency</td>
</tr>
<tr>
<td>Pappenheimer bodies</td>
<td></td>
<td>Sideroblastic anaemia, haemoglobinopathies, hyposplenism</td>
</tr>
<tr>
<td>Poikilocyte</td>
<td>burr cell, irregular shaped cell, irregularly contracted cell, pyknocyte, spur cell</td>
<td>Haemolytic anaemia, haematinic treatment</td>
</tr>
<tr>
<td>Polychromatic cell RBC</td>
<td>polychromatophilic cell erythrocyte, normocyte, discocyte</td>
<td>Microangiopathic haemolytic anaemia, TTP, HUS, DIC, renal disease</td>
</tr>
<tr>
<td>Schistocyte</td>
<td>burr cell, helmet cell, horn cell, keratoschistocyte, pincer cell, poikilocyte, prick cell, red cell fragment, schizocyte, thorn cell, triangular cell</td>
<td>Sickle cell anaemia and other sickle cell diseases</td>
</tr>
<tr>
<td>Sickle cell</td>
<td>drepanocyte, holly leaf cell</td>
<td>Hereditary spherocytosis, ABO and warm AIHA, Clostridium perfringens sepsis, burns</td>
</tr>
<tr>
<td>Spherocyte</td>
<td>spherical cell</td>
<td>Alcoholic liver disease, hereditary stomatocytosis</td>
</tr>
<tr>
<td>Stomatocyte</td>
<td>cup cell, knizocyte, slit cell</td>
<td>Liver disease, haemoglobinopathies, thalassaemia myelofibrosis</td>
</tr>
<tr>
<td>Target cell</td>
<td>codocyte, leptocyte</td>
<td></td>
</tr>
<tr>
<td>Teardrop cell</td>
<td>dacrocyte, pear-shaped cell</td>
<td></td>
</tr>
</tbody>
</table>
Irregular distribution of RBC on the blood film

**Agglutination**

Agglutination is the irregular clumping of red blood cells into grape-like clusters, usually indicating the presence of a cold reactive anti-RBC antibody. A falsely increased MCV and falsely reduced RBC count will be obtained from the analyser leading to an erroneous elevation in the MCH and mean cell haemoglobin concentration (MCHC).

The recommendation is to report the presence of agglutination when observed.

**Rouleaux formation**

Rouleaux formation (red cells stacked up like a pile of coins) usually occurs when plasma protein concentrations are high.

The recommendation is to report the presence of rouleaux when observed.

**Abnormalities of RBC size and/or colour**

**Anisocytosis**

Anisocytosis is an increased variability in RBC size. It is nonspecific and will be reflected in an increased red cell distribution width (RDW) in automated analyser counts.

The recommendation is to use and report the RDW as a measure of the degree of variation in red cell size. However, anisocytosis can be graded if an RDW is unavailable.

**Dimorphism**

Dimorphism is the presence of two distinct RBC populations which may be clearly seen on an analyser red cell histogram with a corresponding increase in RDW. The term is most often used when there is a population of microcytic, hypochromic cells and another population of normochromic cells which may be normocytic or macrocytic, but could also be used to describe coexisting macrocytic and normocytic populations of cells.

The recommendation is to report the presence of dimorphism and describe the two populations.

**Hypochromia**

Hypochromia is a reduction in RBC staining with an increase in central pallor to greater than one-third of the RBC diameter. The MCH will be decreased as will the MCHC in severe hypochromia. Clinical conditions causing hypochromia will often have an associated microcytosis. Hypochromia may also be seen in red cells that are thinner than normal but have normal haemoglobin concentration and volume.

It is recommended that the analyser generated MCH be used to gauge hypochromia rather than grading by visual microscopic examination but grading criteria have also been included in the table for hypochromic cells for those laboratories who prefer to grade hypochromia using direct visual inspection.

**Macrocytes**

Macrocytes are enlarged red cells with a diameter greater than 8.5 μm (MCV > 100 fL). The MCV will be elevated but the MCH will be normal or elevated if there is a significant increase in MCV. They may be round or oval in shape which can have diagnostic significance. It is noteworthy that red cells in premature, newborn babies and neonates are physiologically larger than in adults. Reticulocytosis may also cause an elevated MCV.

It is recommended that the more accurate analyser generated MCV be used to gauge red cell size (degree of macrocytosis) rather than grading by visual microscopic examination but grading criteria have also been included in the table for macrocytes for those laboratories that do not have these advanced analysers. An abnormal RDW or red cell histogram that suggests the presence of macrocytes even though the MCV is normal may also prompt slide review and grading of macrocytes by visual microscopic examination. However, if oval macrocytes are present, it is recommended that these be graded.

**Microcytes**

Microcytes are small red blood cells with a diameter of less than 7 μm (MCV < 80 fL). They may be associated with decreased amounts of haemoglobin (hypochromia). It should also be noted that moderate
numbers of target cells will falsely lower the MCV due to their increased surface area to volume ratio [8].

As previously mentioned, the red cells of newborns and neonates are larger than in adults but the red cells of healthy children are physiologically smaller than in adults so it important that red cell size be interpreted in the context of the age of the subject.

It is recommended that the more accurate analyser generated MCV be used to gauge red cell size (degree of microcytosis) rather than grading by visual microscopic examination but grading criteria have also been included in the table for microcytes for those laboratories that do not have these advanced analysers. An abnormal RDW or red cell histogram that suggests the presence of microcytes even though the MCV is normal may also prompt slide review and grading of microcytes by visual microscopic examination.

**Polychromasia**

Polychromasia refers to immature red cells that are pinkish blue-grey in appearance due to residual ribosomal RNA. They are larger in size than normal mature red cells.

The recommendation is to grade polychromasia and perform a reticulocyte count if necessary.

**Abnormalities of RBC shape**

**Acanthocytes (spur cell)**

Supplementary image S1.

Acanthocytes are round, hyperchromic red cells with 2-20 irregularly spaced projections or spicules of variable length, thickness and shape. Some spicules have club-shaped rather than pointed ends.

The recommendation is to grade acanthocytosis.

**Bite cells**

Supplementary image S2.

Bite cells are RBC with peripheral single or multiple arcuate defects (bites) caused by the removal of Heinz bodies by the spleen and are a feature of oxidant haemolysis. Microangiopathic haemolytic anae-

mias and mechanical damage to the red cells may produce morphologically identical cells (keratocytes), which are formed by the rupture of peripheral pseudovacuoles and subsequent fusion of the red cell membrane.

The recommendation is to grade bite cells.

**Blister cells**

Supplementary image S3.

Blister cells are red cells in which the haemoglobin appears retracted into one half of the cell to form a dense mass leaving the remainder of the cell as an empty membrane.

The recommendation is to grade blister cells.

**Echinocytes (burr cell)**

Supplementary image S4.

Echinocytes are red cells that have lost their disc shape and are covered with 10-30 short blunt projections or spicules of fairly regular form.

The recommendation is to grade echinocytes.

**Elliptocytes and ovalocytes**

Supplementary image S5.

Elliptocytes are cells with an elliptical shape (the long axis is more than twice the short axis), while ovalocytes have an oval shape (the long axis is less than twice the short axis). The recommendation is to grade elliptocytes and ovalocytes.

**Irregularly contracted cells**

Supplementary image S6.

Irregularly contracted cells are smaller and denser RBC which lack an area of central pallor but are not as regular in shape as spherocytes.

The recommendation is to grade irregularly contracted cells.

**Poikilocyte**

A poikilocyte is a red cell of abnormal shape. Poikilocytosis is a non-specific abnormality but poikilocytes of specific shapes may be associated with a particular disorder e.g. elliptocytes in hereditary elliptocytosis.
The recommendation is to report the abnormal specific cell shape rather than use poikilocytosis.

**Schistocyte**

Supplementary image S7.

Schistocytes are fragments of red blood cells produced by extrinsic mechanical damage within the circulation and are a diagnostic feature of microangiopathic haemolytic anaemia (MAHA). Schistocytes are always smaller than intact red cells and can have the shape of fragments with sharp angles and straight borders, small crescents, helmet cells or keratocytes. Microspherocytes may also be a feature of MAHA [7].

The recommendation is to grade schistocytes. A schistocyte count may be of value when schistocytes are the dominant feature (± polychromasia, NRBC, thrombocytopenia) for the diagnosis and follow-up of MAHA.

**Sickle cells**

Supplementary image S8.

Sickle cells are red cells that become crescent or sickle-shaped with pointed ends as a result of polymerization of HbS.

The recommendation is to grade sickle cells. A sickle screen or haemoglobinopathy screen may be recommended.

**Spherocytes**

Supplementary image S9.

Spherocytes are of small diameter (<6.5 μm) and are dense spheroidal RBC with a normal or decreased MCV and an absence of central pallor. They may be formed as a consequence of an abnormality of the RBC cytoskeleton and membrane, immune and microangiopathic haemolysis and direct damage to the red cell membrane.

The recommendation is to grade spherocytes.

**Stomatocytes**

Supplementary image S10.

Stomatocytes are uniconcave cup-shaped red blood cells that appear on a stained blood film with a slit-like area of central pallor. In South East Asian ovalocytosis, the stomatocytes may have two stomas per cell which may be longitudinal, transverse, V or Y shaped.

The recommendation is to grade stomatocytes.

**Target cells**

Supplementary image S11.

Target cells are thin cells with an increased surface area to volume ratio that have an area of increased staining which appears in the middle of the area of central pallor.

The recommendation is to grade target cells.

**Teardrop cells**

Supplementary image S12.

Teardrop cells are red cells that are pear or teardrop in shape.

The recommendation is to grade teardrop cells.

**Inclusions in RBC**

**Basophilic stippling**

Supplementary image S13.

Basophilic stippling describes the occurrence of fine, medium, or coarse blue granules due to abnormally aggregated ribosomes, uniformly distributed throughout the RBC.

The recommendation is to grade basophilic stippling.

**Howell-Jolly bodies**

Supplementary image S14.

Howell-Jolly bodies are usually single, small (1 μm), dense, perfectly round basophilic inclusions that are fragments of nuclear material (DNA).

The recommendation is to grade Howell-Jolly bodies.

**Intracellular haemoglobin crystals**

Crystalline aggregates of haemoglobin may be seen in HbC and HbSC disease. These crystals stain densely, vary in size and have straight edges with pointed ends.

The recommendation is to report the presence of intracellular haemoglobin crystals when observed.
Micro-organisms in RBC

Micro-organisms may be seen free between or within RBC in patients with bacterial, fungal, protozoan or parasitic infections.

The recommendation is to report their presence when observed.

Malarial species identification should be made and reported. For patients with malaria, determination of parasite density is useful in clinical management and in monitoring a patient’s response to treatment, particularly in *Plasmodium falciparum* and *Plasmodium knowlesi* [9].

Pappenheimer bodies

Supplementary image S15.

Pappenheimer bodies are ferritin aggregates in red cells, visible in Romanowsky stained PB films as multiple basophilic inclusions of variable size, shape and distribution usually in a limited cytoplasmic area. They stain positively for iron (Perls Prussian blue reaction).

The recommendation is to grade Pappenheimer bodies.

Nucleated Red Blood Cell (NRBC)

Supplementary image S16.

A nucleated red blood cell is a red cell precursor and is used to describe an erythroblast in the peripheral circulation.

The recommendation is to report NRBCs as an absolute count within the differential with the WBC corrected for the presence of NRBCs, or, count and report the number of NRBC per 100 WBC.

WHITE BLOOD CELLS

In nearly all cases, modern haematology analysers provide accurate white cell counts and white cell differentials. The differential may be suppressed or inaccurate when there are abnormal white cell populations present but this will cause abnormal flags to be triggered [10]. Automated instruments cannot enumerate and classify abnormal white cell populations or recognize many significant morphological abnormalities necessitating the microscopic examination of a well made and well-stained peripheral blood film for accurate white cell differentiation and classification.

White cell differentiation involves the classification of white cells based on size, nuclear shape, chromatin pattern and cytoplasmic appearance and content [11, 12]. Morphological qualitative abnormalities of the cell nucleus or cytoplasm and/or the size of the white cells can be congenital or acquired in the course of various diseases.

The 2008 World Health Organisation (WHO) Classification of Tumours of Haemopoietic and Lymphoid Tissues recommends a 200 white cell peripheral blood cell differential be performed as part of the diagnostic work-up [13] in acute myeloid leukaemia (AML) and myelodysplastic syndromes; however, a 100 white cell differential is more usual in the routine haematology laboratory.

Normal myeloid development and morphology

Myeloblast

Blast cells in normal myeloid maturation have a diameter of 12–20 μm and a relatively large round or oval nucleus with a fine chromatin pattern and one or more distinct nucleoli. The cytoplasm is basophilic with an absent Golgi zone and granules may or may not be present.

Promyelocyte

Normal promyelocytes are 15–25 μm in diameter, have an oval or round nucleus with fine/intermediate chromatin and a usually visible and prominent nucleolus. The cytoplasm is basophilic and contains blue-violet and red (primary) granules. A pale area equating to the Golgi zone is present adjacent to the nucleus.

Myelocyte

The myelocyte is slightly smaller than the promyelocyte (10–18 μm) with a round or oval nucleus which may be eccentrically placed. The nuclear chromatin shows a moderate degree of coarse clumping and nucleoli are not seen. There is a moderate amount of blue-pink cytoplasm which contains numerous red-violet granules. As the myelocyte matures, the secondary granules develop definite neutrophilic, eosinophilic or basophilic characteristics.

Metamyelocyte

The metamyelocyte is smaller than the myelocyte with an indented or kidney-shaped nucleus. Nucleoli are not observed. The cytoplasm is usually clearly pink and contains granules that are clearly differentiated as neutrophilic, eosinophilic or basophilic.

N.B. Immature granulocytes (promyelocytes, myelocytes and metamyelocytes) are not usually seen in normal peripheral blood.

Band neutrophil

Band neutrophils are 10–14 μm in diameter and have a nucleus that is nonsegmented or has rudimentary lobes that are connected by a thick band rather than a thread. Cytoplasm is abundant, pink and contains many small violet-pink neutrophilic or secondary granules distributed evenly throughout the cell.

Many laboratories do not report band neutrophils on adult patients or children due to interobserver variation in band neutrophil classification; this is a well recognized and acceptable practice.

It is recommended that band neutrophils be counted as segmented neutrophils in the differential. Appropriate comments may be made if increased numbers are seen in the blood film.

Segmented neutrophil

A granulocyte that is 10–14 μm in diameter with a lobulated nucleus (usually 3–4 lobes, but small numbers of 2 and 5 lobed neutrophils may also be seen) connected by a thin thread of chromatin. The chromatin is coarse, stains violet and is arranged in clumps. Small nuclear appendages may be seen. There is abundant pink cytoplasm with many small secondary granules.

Eosinophil

The diameter of the eosinophil is 12–17 μm. The nucleus usually only has 2 lobes with coarsely clumped, violet-staining chromatin. There is abundant cytoplasm containing many eosinophilic (orange) secondary granules that are larger than neutrophil granules and more uniform in size.

Basophil

A basophil is 10–16 μm in diameter with pale blue cytoplasm containing purple-black secondary granules. These granules are water soluble and may dissolve on staining leaving clear areas in the cytoplasm. The nucleus is segmented but is often obscured by basophilic granules which may vary in number, size and shape.

Monocyte

Monocytes are the largest cell in the peripheral blood, variable in size but usually 15–22 μm in diameter. The nucleus is irregular in outline (often kidney shaped), and the chromatin is arranged in fine strands with sharply defined margins. The cytoplasm is light blue-grey and contains numerous fine dust-like granules. Some cells may contain a small number of red-violet granules. Vacuolation may be present.

Normal lymphocyte development and morphology

Lymphoblast

The lymphoblast has a diameter of 8–20 μm. The nucleus is round or oval with fine granular chromatin and one or more indistinct nucleoli. The cytoplasm is scanty and basophilic, and cytoplasmic granules are absent. It cannot be reliably distinguished from some types of undifferentiated or minimally differentiated myeloblasts and therefore should be counted as a blast cell.

Prolymphocyte

The nucleus is round and contains a single prominent nucleolus. It has more cytoplasm than a lymphoblast and the chromatin is more condensed.

N.B. Lymphoblasts and prolymphocytes are not usually seen in the normal peripheral blood.

Lymphocyte

Lymphocytes seen in the peripheral blood are predominantly small (10–12 μm), or, less frequently large (12–16 μm).
Small lymphocytes are usually round in outline, and the nucleus is round with coarse, densely staining chromatin. Cytoplasm is scanty.

Large lymphocytes are usually irregular in outline, and the nuclear chromatin is not as coarse as in small lymphocytes. Cytoplasm is abundant and tends to be light sky blue in colour.

Large granular lymphocytes (LGLs) are of the same appearance as large lymphocytes but the cytoplasm contains prominent small red-violet granules. These cells can comprise up to 10–20% of the peripheral blood lymphocytes in normal subjects. LGLs are not routinely counted as a separate lymphocyte population.

Supplementary image S17.

It is recommended that LGLs be counted as lymphocytes but may be commented on if they are present in increased numbers. This may prompt further investigations such as flow cytometry.

N.B. Lymphocytes predominate in the blood films of infants and children until 4 years of age. These lymphocytes are more pleomorphic than those seen in normal adult blood films.

Quantitative abnormalities

Neutrophilia, neutropenia, lymphocytosis, lymphopenia, monocytosis, monocytopenia, eosinophilia, eosinopenia, basophilia, basopenia.

WBC differential counts can be performed by automated analysers or manual microscopic visual examination of a blood film. Automated analysers use multiple parameters and methods such as impedance technology and fluorescence flow cytometry to differentiate and count the 5 major white cell types found in the peripheral blood – neutrophils, lymphocytes, monocytes, eosinophils and basophils. Many modern analysers also now provide a 6 part differential with the enumeration of immature granulocytes (promyelocytes, myelocytes and metamyelocytes).

The automated WBC differential count is less time-consuming and expensive than the manual method and as an analyser counts thousands of cells compared to the usual 100–200 WBC by the microscopic method, it will also be more precise in the absence of abnormal cell populations. Very low or high white cell counts may also cause the manual differential to be less accurate and reproducible.

Each laboratory should establish their own reference ranges as these will vary depending on population, laboratory, instrumentation and methods used.

It is recommended that the automated analyser WBC differential count be reported in patients with normal cell populations in the absence of analyser flags or abnormal cell populations that cannot be reliably differentiated and classified by automated instruments. The automated differential may also be reported after viewing a blood film due to flags or other indicators where the automated values are found to be accurate.

Qualitative abnormalities in myeloid cells

Cytoplasmic abnormalities

Auer rod. Supplementary image S18.

A sharply defined red rod or needle-like cytoplasmic inclusion formed by abnormal primary granule development. Found mainly in leukaemic myeloblasts or abnormal promyelocytes, they stain positively for myeloperoxidase and are a specific marker for myeloid lineage neoplasms. There may be several in a cell and may be arranged in bundles (faggots).

The recommendation is to report the presence of Auer rods when seen.

Döhle body. Pale light blue or grey, single or multiple, cytoplasmic inclusions found near the periphery of the neutrophil. Döhle bodies are a non-specific reactive change but may also indicate May-Hegglin anomaly if associated with thrombocytopenia and giant platelets. Döhle bodies may also be seen in patients on growth factor therapy such as granulocyte colony-stimulating factor (G-CSF).

The recommendation is to grade Döhle bodies when seen.


Coarse, purple staining primary (azurophilic) neutrophil cytoplasmic granules which occur as a response to infection and inflammation. A non-specific reactive change, it is a result of abnormal primary granule maturation with retention of their azurophilic staining properties.

The recommendation is to grade hypergranulation when seen.

Hypogranulation – neutrophil. Supplementary image S20.

Reduced or absent neutrophil granulation causing the cytoplasm of mature neutrophils to appear blue-grey.

The recommendation is to grade hypogranulation when seen.

Vacuolation – neutrophil. Neutrophil cytoplasmic vacuolation in infection is due to granule fusion with a phagocytic vacuole and release of lysosomal contents to kill bacteria. This vacuolation may appear as ‘pin-hole’ vacuolation – small, discrete vacuoles, but the vacuoles may be larger. Other causes of neutrophil vacuolation include alcohol toxicity and prolonged exposure to EDTA anticoagulant (storage artefact).

The recommendation is to grade neutrophil vacuolation when seen.

Nuclear abnormalities

Hypersegmented neutrophils. Normal neutrophils usually have 3–4 lobes (occasionally 2 and 5 lobes). Hypersegmented neutrophils have an increased number of distinct nuclear lobes with increased numbers of neutrophils having 5 or more nuclear segments.

Neutrophil hypersegmentation is defined as any neutrophil having 6 or more lobes or more than 3% of neutrophils having 5 lobes, when 100 neutrophils are examined.

The recommendation is to comment on the presence of hypersegmented neutrophils when seen.


Hyposegmented neutrophils are marked by the failure of normal nuclear lobe development during terminal differentiation and have coarse clumped nuclear chromatin.

It is important that these hyposegmented neutrophils not be confused with myelocytes, metamyelocytes or band neutrophils. They are mature neutrophils and can be differentiated by their smaller nucleus and lower nuclear: cytoplasmic ratio (N:C ratio) and condensed nuclear chromatin [14].

It is recommended that hyposegmented neutrophils be counted and reported as mature segmented neutrophils but with a suitable interpretive comment.

Myeloid cells in haematological neoplasms

Leukaemic myeloblasts. Supplementary image S22.

Leukaemic myeloblasts vary in appearance. They can be large or small in size. Some may have a high N:C ratio, uncondensed chromatin and usually one or more prominent nucleoli. Others may have a lower N:C ratio and a few red-purple granules or Auer rods. Nuclear and cytoplasmic irregularities may be present, for example nuclear folding, cytoplasmic basophilia and cytoplasmic blebbing or pseudopods.

The recommendation is to count these as blasts and describe them in the film report with a suitable interpretive comment.

Abnormal promyelocytes in acute promyelocytic leukaemia (APL). Supplementary images S23 and S24.

The promyelocytes in the hypergranular variant of APL have nuclei that vary in size and shape and are often kidney shaped or bilobed. The cytoplasm is packed with large coalescent pink-purple granules and may contain Auer rods. These may be grouped in bundles or “faggots” within the cytoplasm.

In the hypogranular or microgranular variant, the nuclear shape is usually bilobed but the cytoplasm contains few or no granules.

The recommendation is to count these abnormal promyelocytes as blast equivalents in the differential but it is important that a suitable description of the abnormal promyelocytes and an interpretive comment is added to the film report and a likely diagnosis of APL communicated directly to the clinician.

Monoblasts. Supplementary image S25.

Monoblasts are larger than myeloblasts (20-30 μm), with a round/oval nucleus, fine chromatin and one or two prominent nucleoli. The cytoplasm is basophilic and usually lacks granules.

The recommendation is to count these as blasts and describe them in the film report with a suitable interpretive comment.
Promonocytes. Supplementary image S26.

Promonocytes may be rarely seen in the peripheral blood in reactive conditions as well as in some leukaemias. They are large cells with a nucleus that is convoluted/indented with a delicate, lace-like chromatin pattern and prominent nucleolus. The cytoplasm is blue-grey and may contain a small number of fine red-violet granules.

The recommendation is to count promonocytes in the differential and comment on their presence with a suitable interpretive comment. Leukaemic promonocytes should be summated with blast cells when making a diagnosis of AML.

Abnormal monocytes. Monocytes produced under conditions of bone marrow stress or stimulation, for example infections, growth factor (GM-CSF) administration, show an increased N:C ratio, a more delicate chromatin pattern, nucleoli and increased numbers of vacuoles. Granulation and cytoplasmic basophilia may also be increased.

Abnormal monocytes can be seen in a number of haematological neoplasms. In contrast to monoblasts and promonocytes, the abnormal monocytes are larger, have irregular nuclei and increased cytoplasm.

The recommendation is to count these as monocytes with a comment on their morphology and a suitable interpretive comment.

Dysplastic changes. Dysplasia refers to morphologically abnormal cells or tissues that are due to abnormal cell development and maturation. Examples of dysplasia include abnormally large or small cells, nuclear hyposegmentation (hypolobation), nuclear hypersegmentation, hypergranulation, hypergranulation and abnormal granulation (large fused granules, Auer rods).

The recommendation is to comment on the presence of dysplasia with a suitable interpretive comment.

It should be noted that the diagnosis and classification of myelodysplastic syndrome as per the WHO classification 2008 requires a percentage count of dysplastic changes per lineage. This, however, is not routinely performed by the scientist reviewing the peripheral blood film. Further investigations and clinical correlation by the clinician is required.

Qualitative abnormalities in lymphoid cells

Lymphocyte morphology is subject to wide variability due to various immunological stimuli both in inflammatory and infectious diseases (particularly viral) as well as in neoplastic disorders (leukaemias and lymphomas), resulting in circulating lymphocytes with morphological abnormalities in various quantities. Terminology for these lymphocytes has been varied and confused with many different terms being used to describe the same thing including variant, reactive, abnormal, activated and atypical lymphocytes, Downey cells Type I–3, Turk cells, immunoblasts and even combinations of cells, for example monocytoid lymphocytes. This highlights a need to simplify this terminology.

It is recommended that reactive lymphocyte is used to describe lymphocytes with a benign aetiology and abnormal lymphocyte with an accompanying description of the cells is used to describe lymphocytes with a suspected malignant or clonal aetiology.

Reactive lymphocytes (atypical lymphocyte, suspect reactive – European LeukemiaNet classification) [15].

Supplementary image S27.

Abnormalities include increased cell size, immaturity of the nucleus including a visible nucleolus and lack of chromatin condensation, irregular nuclear outline or lobulation, cytoplasmic basophilia and vacuolation and irregular cell outline. The cytoplasm may be abundant with staining varying from pale blue to markedly basophilic especially at points of contact with adjacent cells.

The recommendation is to comment on the presence of reactive lymphocytes. They may be counted as a separate population in the differential if they are present in significant numbers.

Abnormal lymphocytes (atypical lymphocyte, suspect neoplastic – European LeukemiaNet classification) [15].

A comprehensive classification and description of lymphocytes in malignant lymphoid neoplasms is beyond the scope of this article. For this, the reader is advised to refer to the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, Fourth Edition [13].

It is recommended that abnormal lymphoid cells that can be identified as a particular neoplastic cell type (as described below), for example hairy cells, lymphoma cells and prolymphocytes (based on dis-
distinctive morphology and confirmed by immunophenotyping), and plasma cells in plasma cell myeloma or other plasma cell dyscrasias be included in the differential as that cell class. Other abnormal lymphoid cells can be described in the film comment and counted as a separate population of ‘abnormal lymphocytes’ in the differential if present in significant numbers.

The use of this nomenclature underlines the limited diagnostic value of morphology in the lymphoproliferative neoplasms where the final diagnosis is determined by immunophenotyping by flow cytometry.

**Hairy cells**

Supplementary image S28.

Hairy cell leukaemia is a chronic B cell lineage leukaemia with morphologically distinctive neoplastic cells. Hairy cells are larger than normal lymphocytes and have abundant pale blue-grey cytoplasm with fine hair-like projections. The nucleus varies in shape and may be round, oval, bean-shaped or bilobed.

It is recommended that hairy cells are counted as abnormal lymphocytes on first presentation with a detailed description of the cells included in the film comment. After immunophenotyping, the cells may be counted as hairy cells in the WBC differential.

**Lymphoma cells**

Lymphoma is a neoplasm of B, T or Natural Killer (NK) lymphocytes and is more often found in tissues other than bone marrow and peripheral blood. Lymphoma may have a leukaemic phase, however, in which morphologically abnormal cells appear in the peripheral blood. A comprehensive classification of lymphoma is beyond the scope of this document, but some specific examples include the following:

**Follicular lymphoma** – These lymphoma cells are often small with scanty, weakly basophilic cytoplasm and have nuclei with notches or deep narrow clefts. Sometimes the cells are larger and more pleomorphic with small but distinct nucleoli and nuclear clefts or notches.

Supplementary image S29

**Mantle cell lymphoma** – These lymphoma cells are pleomorphic varying in size and N:C ratio. Chromatin condensation is less than in CLL lymphocytes and some cells may appear blastic with cleft or irregular nuclei and a prominent nucleolus.

**Burkitt lymphoma** – These lymphoma cells are large with dispersed nuclear chromatin, one or more prominent nucleoli and moderately abundant, deeply basophilic and vacuolated cytoplasm.

**Sézary syndrome** – Sézary syndrome is a mature T-cell lymphoma with neoplastic T lymphocytes in the peripheral blood. The cells are present in variable numbers ranging from a few cells to a frankly leukaemic picture with a marked leucocytosis. The cells may be large or small but the nuclear morphology, classically described as cerebriform, is the characteristic cytological feature of both cell types. The nucleus has deep narrow clefts with superimposed and folded lobes giving it a very convoluted appearance.

**Adult T-cell leukaemia/lymphoma (ATLL)** – ATLL is characterized by a broad spectrum of cytological features but the characteristic ATLL cells have been described as ‘flower cells’ with many nuclear convolutions and lobules.

It is recommended that lymphoma cells are counted as abnormal lymphocytes on first presentation with a detailed description of the cells included in the film comment. After immunophenotyping, the cells may be counted as lymphoma cells in the WBC differential.

**Plasma cells**

Supplementary image S30.

A plasma cell is larger than a normal small lymphocyte, has deeply basophilic cytoplasm, an eccentric round or oval nucleus, coarsely clumped chromatin and a pale Golgi zone or perinuclear halo adjacent to the nucleus.

It is recommended that plasma cells be counted as a separate population in the WBC differential.

**Prolymphocytes**

B-prolymphocytes are twice the size of a lymphocyte and have a round nucleus, moderately condensed nuclear chromatin, a prominent central nucleolus and a relatively small amount of faintly basophilic cytoplasm.
Supplementary image S31.

Prolymphocytes are smaller and more pleomorphic than B prolymphocytes. Nuclei are irregular or lobulated. The cytoplasm is scanty and moderately basophilic and cytoplasmic blebs may be present. Nucleoli are usually not as large or prominent as B-lineage prolymphocytes.

It is recommended that prolymphocytes be counted as a separate population in the WBC differential.

Smudge cells (smear cells)

Smudge cells result from shearing forces on the cells during the spreading of blood films. They are the disrupted nuclei of fragile cells. A repeat film made with one part albumin added to four parts blood may prevent cell disruption and allow identification of the fragile cells and their inclusion in the WBC differential.

When the nature of the smear cell is apparent, it is recommended that they should be counted as the cell from which they are derived. Large numbers of smudge cells may be seen in CLL PB films (supplementary image S32). It is recommended that the automated differential be reported if available in this instance but the presence of smudge cells may be commented on in the film report.

Leukaemic lymphoblasts

Leukaemic lymphoblasts range from those with a high N:C ratio, clumped chromatin, inconspicuous nucleoli and scanty basophilic cytoplasm to those that are heterogeneous in appearance and have a nuclear chromatin pattern varying from finely dispersed to coarsely condensed. The nuclear outline may be irregular and nuclear clefting, indentation and folding are common. Nucleoli vary in size and number but are often indistinct. A small number of lymphoblasts may have more abundant cytoplasm containing coarse azurophilic granules.

Lymphoblasts cannot be reliably distinguished from myeloid blasts, lymphoma cells and sometimes, reactive lymphocytes. Additional information from cytochemical stains or immunophenotyping may be required to make an accurate diagnosis.

The recommendation is to count and report these as blasts and describe them in the film report.

PLATELETS

Platelets are small, blue-grey granular fragments derived from megakaryocytic cytoplasm, containing many small, reddish-purple granules.

Qualitative abnormalities

Platelet size

Platelet size is of diagnostic significance particularly when considered in relation to the platelet count.

A normal platelet measures 1.5–3 μm in diameter. Large platelets measure 3–7 μm (roughly the diameter of a normal sized red cell), whilst giant platelets, (supplementary image S33), are larger than normal sized red cells at 10–20 μm in diameter and are flagged by automated analysers. In a normal person, usually less than 5% of the platelets appear large. Platelet size increases gradually during storage in EDTA anticoagulated venipuncture tubes.

It is recommended that giant platelets be graded.

A comment about the platelet count and the presence of small, large and/or giant platelets can be made with an additional interpretive film comment if appropriate.

Hypogranular platelets, (supplementary image S34), exhibit little, if any, of the purple-red granules found in normal platelets.

It is recommended that a comment about the presence of hypogranular platelets be made if seen in the PB film.

Megakaryocytes and megakaryoblasts are rarely seen in normal peripheral blood. Small megakaryoblasts may be indistinguishable from lymphoblasts whereas larger ones will have a nucleus with a diffuse chromatin pattern and a variable amount of basophilic cytoplasm which may form blebs.

Abnormal megakaryocytes, megakaryoblasts and bare megakaryocyte nuclei may be seen in the PB in pathological conditions. Micromegakaryocytes, (supplementary image S35), are seen in some patients with haematological neoplasms. The micromegakaryocyte is defined as a megakaryocyte approximately the size of a promyelocyte or smaller with a nonlobated or a bilobed nucleus and a variable amount of weakly basophilic cytoplasm. The nucleus may appear ‘bare’ but a small rim of cytoplasm can be demonstrated by
electron microscopy. The cytoplasm is weakly basophilic. Cytoplasmic vacuolation and variable numbers of granules may be present, and there may also be small cytoplasmic protrusions or ‘blebs’. Platelets may appear to be ‘budding’ from the surface.

It is recommended that a comment about the presence of megakaryoblasts, megakaryocytes and micro-megakaryocytes be made if seen in the PB film.

**CONCLUSION**

These recommendations deal with the need for a global standard in naming, grading and reporting abnormal cells or morphological abnormalities which are observed at the time of the PB film review and manual differential count.

Their primary goal is to produce clear guidelines for scientists who perform analysis of haematology samples. This issue is becoming more important as hospitals and laboratories join with others, forming large hospital and laboratory systems, with physicians practicing at multiple sites and with patients able to gain access to the healthcare system from multiple sites. There are also laboratories which are crossing international borders. Laboratory services can now span multiple countries. The need for consistent nomenclature and appropriate grading standards are therefore more important than ever.

This document is the result of an exhaustive and comprehensive review, analysis and consensus agreement by the ICSH ad hoc Committee on Standardization of Peripheral Blood Cell Morphology Nomenclature and Reporting in accordance with the basic aim of the ICSH to achieve reliable and reproducible results in laboratory analysis and, in particular, diagnostic haematology. The existence of traditional and geographical differences in nomenclature is recognized and accepted, thus favouring some flexibility in the suggested methods of reporting for a number of parameters.

Links for examples of cells discussed. (i) http://www.morphology.mmu.ac.uk, (ii) www.icsh.org.

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**CONFLICT OF INTEREST**

All authors declare that they have no conflict of interests.

**REFERENCES**


APPENDIX

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Supplementary Images: ICSH Recommendations for Peripheral Blood Cell Morphology Standardization and Grading

Image S1. acanthocytes
Image S2. Three bite cells
Image S3. blister cells
Image S4. echinocytes
Image S5. ovalocytes and elliptocytes
Image S6. irregularly contracted cells
Image S7. schistocytes

Image S8. sickle cells
Image S9. spherocytes
Image S10. stomatocytes
Image S11. target cells
Image S12. tear drop cells
Image S13. basophilic stippling
Image S14. Howell-Jolly bodies
Image S15. Pappenheimer bodies (Copyright: Microscopic haematology: a practical guide for the laboratory 3e (c) 2011, Sydney, Elsevier Australia)
Image S16. nucleated red blood cell
Image S17. large granular lymphocyte
Image S18. Auer rods
Image S19. hypergranulation (neutrophils)
Image S20. hypogranulation (neutrophils) (Copyright: Microscopic haematology: a practical guide for the laboratory 3e (c) 2011, Sydney, Elsevier Australia)
Image S21. Pelger Huet neutrophils

Image S22. leukaemic myeloblasts
Image S23. abnormal promyelocytes in APL (1)
Image S24. abnormal promyelocytes in APL (2) (Copyright: Microscopic haematology: a practical guide for the laboratory 3e (c) 2011, Sydney, Elsevier Australia)
Image S25. monoblasts
Image S26. abnormal promonocytes
Image S27. reactive lymphocytes
Image S28. hairy cells
Image S29. follicular lymphoma cells
Image S30. plasma cells
Image S31. prolymphocytic leukaemia cells
Image S32. chronic lymphocytic leukaemia cells
Image S33. giant platelets
Image S34. hypogranular platelets
Image S35. micromegakaryocytes