International Journal of Laboratory Hematology
The Official journal of the International Society for Laboratory Hematology

ORIGINAL ARTICLE INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

ICSH recommendations for identification, diagnostic value, and quantitation of schistocytes

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doi:10.1111/j.1751-553X.2011.01380.x

Received 18 February 2011; accepted for publication 19 September 2011

Keywords
Schistocytes, cytomorphology, red blood cell fragments, thrombotic microangiopathy, peripheral blood film

SUMMARY

Schistocytes are fragments of red blood cells (RBCs) produced by extrinsic mechanical damage within the circulation. The detection of schistocytes is an important morphological clue to the diagnosis of thrombotic microangiopathic anemia (TMA). Reporting criteria between different laboratories, however, are not uniform, owing to variability of shape and nature of fragments, as well as subjectivity and heterogeneity in their morphological assessment. Lack of standardization may lead to inconsistency or misdiagnosis, thereby affecting treatment and clinical outcome. The Schistocyte Working Group of the International Council for Standardization in Haematology (ICSH) has prepared specific recommendations to standardize schistocyte identification, enumeration, and reporting. They deal with the type of smear, method of counting, morphological description based on positive criteria (helmet cells, small, irregular triangular, or crescent-shaped cells, pointed projections, and lack of central pallor). A schistocyte count has a definite clinical value for the diagnosis of TMA in the absence of additional severe red cell shape abnormalities, with a confident threshold value of 1%. Automated counting of RBC fragments is also recommended by the ICSH Working Group as a useful complement to the microscope, according to the high predictive value of negative results, but worthy of further research and with limits in quantitation.

INTRODUCTION

Schistocytes, or schizocytes (from the Greek word *schisto*, broken or cleft, or the correspondent verb *schizo*), are circulating fragments of red blood cells (RBCs) or RBCs from which cytoplasmic fragments have been lost (Bessis, 1976). They are usually absent or very rare in blood films of healthy individuals. The finding of schistocytes in the peripheral blood, especially in the absence of additional severe morphological RBC abnormalities, should lead to a prompt investigation for the presence of a thrombotic microangiopathy (Moake, 2002). Thrombotic microangiopathic anemia (TMA) includes two major syndromes: thrombotic thrombocytopenic purpura (TTP) and hemolytic-uremic syndrome. The prognosis for TTP, in particular, is poor without specific and immediate treatment. In recent years, the efficacy and life-saving potential of early plasma exchange for TTP have increased the diagnostic importance of a schistocyte count even before the development of overt symptoms (Rock, Porta & Bobbio-Pallavicini, 2000). A new developing area for schistocyte counting is monitoring of patients after transplantation of hematopoietic stem cells, in which TMA is a frequent and severe complication (Zomas *et al.*, 1998; Martinez *et al.*, 2005; Ruutu *et al.*, 2007; Lesesve *et al.*, 2011).

Schistocytes are also a typical consequence of mechanical damage from structural abnormalities of the heart and great vessels, often a malfunctioning prosthetic valve (Marsh & Lewis, 1969), HELLP syndrome, malignant hypertension, and metastatic cancer. RBC fragments similar to schistocytes can be found in non-TMA-related genetic or acquired RBC disorders (e.g. RBC membrane defects, thalassemia, megaloblastic anemia, primary myelofibrosis, and thermal injuries). In these cases, they show highly variable shapes and are associated with marked anisopoikilocytosis and a wide range of additional RBC size and morphological changes, not specific for the diagnosis of TMA (Bain, 2006a, 2010).

Schistocytes are detected in the peripheral blood smear stained using standard procedures and observed by microscopy. Precise morphological criteria for the recognition and enumeration of schistocytes still remain poorly defined, and there is variability in the morphological interpretation and reporting between laboratories and observers (Lesesve *et al.*, 2005). Lack of standardization may lead to inconsistent diagnostic information, thereby affecting treatment and clinical outcome. Although identification of RBC fragments apparently seems straightforward, there is no published consensus definition about what a schistocyte actually is (Lesesve, Salignac & Lecompte, 2001). Observer bias because of the specific request for schistocyte count has also been described (Burns, Lou & Pathak, 2004). Besides inconsistency of identification criteria, this variability may also depend on differences in the counting method (i.e. general overview or accurate enumeration), the area of the smear assessed, the number of the RBCs counted, the use of counting aids such as the Miller disk, the heterogeneity of reporting to physicians (present/absent, qualitative or quantitative assessment). To decrease the interobserver variability and improve accuracy and reproducibility, a group of French morphologists tried to set up national consensus rules for schistocyte identification and counting (Lesesve *et al.*, 2005). In addition, automated RBC fragment count has been implemented recently in the latest generation of blood cell counters, as a screening tool with good sensitivity but poor specificity for the detection of TMA-associated schistocytes (Saigo *et al.*, 2002; Lesesve *et al.*, 2004a,b; Banno *et al.*, 2005; Abe *et al.*, 2009). As prognostic scores that include a schistocyte count have been proposed in the context of TMA related to hematopoietic stem cell transplantation (Zeigler *et al.*, 1995; Ruutu *et al.*, 2007), this makes it even more important that a standardized approach to quantitation be introduced in clinical laboratories.

With the aim of improving the accuracy and reproducibility of a schistocyte count for the diagnosis of TTP and related TMA, an international Schistocyte Working Group was formed in November 2008 by the new ICSH (McFadden *et al.*, 2008).

Detailed goals of the ICSH Schistocyte Working Group were as follows:

- to define standardized morphological criteria for the recognition of schistocytes;
- to standardize the method for counting schistocytes;
- to indicate a consensus threshold value for the diagnosis of TMA;
- to evaluate the reliability and clinical utility of automated fragment count.

Recommendations, as agreed by the ICSH Schistocyte Working Group, are presented in this article. Only criteria on which there was full consensus have been included in the recommendation.

ICSH RECOMMENDATIONS FOR MICROSCOPIC IDENTIFICATION OF SCHISTOCYTES (TABLE 1)

Schistocytes should be identified on the basis of specific positive morphological criteria

Inconsistencies in identification and classification are one of the main causes of poor reproducibility of schistocyte counting. Schistocytes are described in the literature with an infinite variability in shape, which encompasses all irregularly distorted RBCs, bizarre poikilocytes, burr-shaped erythrocytes, atypical hyperchromic spherocytes, RBC ghosts, etc. (Dacie & Lewis, 1968; Glassy, 1998). Two different positions emerged from the discussion within the ICSH Working Group: (i) the inclusion under the definition of schistocytes of any type of small fragment of RBC cytoplasm; (ii) the requirement of specific morphology for RBC fragments to be included in the definition of schistocyte. After discussion and review of the published evidence, finally, the Group agreed that a few main types of schistocytes should be identified, using positive morphological criteria. A consensus was obtained on the origin of schistocytes as fragments of erythrocytes or amputated erythrocytes, from which those fragments have arisen. The univocal mechanism of schistocyte formation is mechanical damage to the membrane caused by fibrin strands on the endothelial surface and/or excess of turbulence of blood (Means & Glader, 2009).

According to ICSH, thus, schistocytes are always smaller than intact RBCs and should be defined as follows (Figure 1a,b):

(i) small fragments of varying shape, sometimes with sharp angles or spines (triangles), with straight borders or sometimes with a round outline on one side (microcrescents), often distorted, usually staining darkly, occasionally pale as a result of loss of hemoglobin at the time of fragmentation (Lesesve *et al.*, 2005; Bain, 2006a); microcrescents should be distinguished, on the basis of their size, from the irreversibly sickled cells (drepanocytes) of sickle cell disease;
(ii) helmet cells, which are damaged erythrocytes with one single, rarely a double, amputated zone highlighted by a straight border, with sharp angulated edges: the missing cell portion corresponds to the fragments that have been split off as a result of a break on a fibrin strand (Lynch, 1990);
(iii) damaged cells larger than small fragments, which have a pair of spicules separated by a semicircular

Table 1. International Council for Standardization in Haematology recommendations for schistocyte counting

1. Schistocytes should be evaluated on peripheral blood smears using an optical microscope at medium magnification and estimated as a percentage after counting at least 1000 red blood cells
2. A schistocyte count should be requested and carried out when a diagnosis of thrombotic microangiopathies caused by red cell mechanical damage is suspected, usually in patients with thrombocytopenia
3. Schistocytes should be identified by specific positive morphological criteria. 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, keratocytes, or microspherocytes*
4. A schistocyte count should be considered clinically meaningful if schistocytes represent the main morphological red blood cells abnormality in the smear (other than signs of erythropoietic regeneration)
5. A robust morphological indication for the diagnosis of thrombotic microangiopathic anemia in adults should be recognized when the percentage of schistocytes is above 1%
6. Fragmented red cell enumeration by automated blood cell counters should be considered a useful complement to microscopic evaluation, as it provides rapid results with a high predictive value of negative samples. A microscope check is needed for positive and macrocytic samples†

*Microspherocytes only in the presence of other mentioned RBC shapes.
†Macrocytic samples are at risk of underestimation or absence of flag ('false-negative' test).

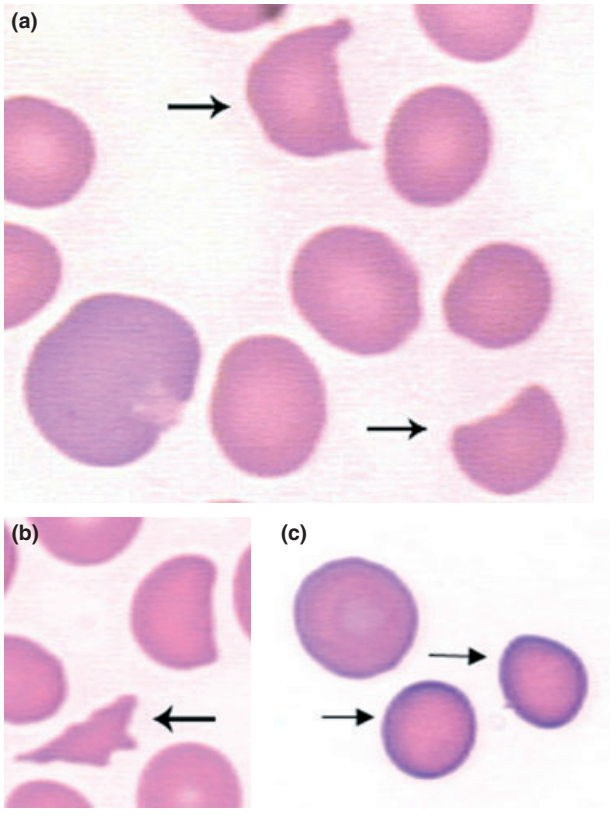

Figure 1. Typical shapes for specific identification of schistocytes. (a) keratocyte (upper arrow) and helmet cell (lower arrow), close to a polychromatophilic erythrocyte in the left lower corner; (b) a triangle schistocyte (arrow) with a helmet cell on the upper right; (c) two microspherocytes (arrows); they are derived, in a context of thrombotic microangiopathic anemia, from schistocytes.

concave segment of membrane, sometimes even two or three pairs: they are usually named keratocytes (cells with horns). They occur in the same conditions as triangles, crescents, and helmet cells and have been formed by rupture of one or more peripheral pseudovacuoles and subsequent fusion of the cell membrane (Bessis, 1972, 1976). Morphologically identical cells occur in Heinz body hemolytic anemia (e.g. glucose-6-phosphate dehydrogenase deficiency), as a result of removal of a Heinz body by a macrophage (Bain, 2006b; Barth & Hirschmann, 2007): they are distinguished from keratocytes as part of TMA by assessing the context in which they occur.

(iv) small-sized hyperdense RBCs with a round shape and increased staining (Figure 1c): they are named microspherocytes (Bain, 2006a) or spheroschizocytes (Bessis, 1972). A pale central zone is absent. Microspherocytes are a secondary manifestation of fragmentation and should be included within the schistocyte count only in the presence of the schistocyte shapes mentioned in points (i) to (iii): they are likely formed when the rupture reduces the membrane surface in relation to the remaining cytoplasmic volume (Bain, 2006b) or as a result of a shape change of other schistocytes, which takes place in the more flattened areas of the smear, close to the feathered edge of the smear (Lesesve *et al.*, 2002). They should not be confused with spherocytes of hereditary spherocytosis or immune hemolysis, which have decreased diameter but are not so small (although partial overlapping in both morphology and terminology does exist).

A schistocyte count, as a percentage of RBCs, should be carried out microscopically according to a standardized method

Schistocytes should be identified and counted on a peripheral blood smear using optical microscopy. The blood smear should be spread, air-dried, fixed, and stained according to standard procedures with panoptical stains, as reported by ICSH (1984) and confirmed by international studies (Barnes *et al.*, 2005). Automated blood film makers and stainers are equally acceptable. A quantitative assessment is important for diagnosis and monitoring of patients. The results should be expressed as a percentage, after counting at least 1000 RBCs in optimal areas of the film. However, the quantitative assessment should be reported only when schistocytes are the dominant RBC abnormality on the smear.

The 95% confidence limits (essential statistical error) of an observed percentage of cells when the total number of events varies from 100 to 10 000 were reported by Rümke (1979) (Table 2). The ICSH Schistocyte Group agreed that a count on 1000 RBCs offers a reasonable compromise between the precision required and the time needed for the count. This 1000 RBC threshold was confirmed after a practical

Table 2. Confidence limits of an observed percentage of schistocytes when the total number of events varies from 1000 to 10 000 (adapted from Rümke, 1979)

Schistocytes (%)	100 RBC	1000 RBC	10 000 RBC
0	0.0–3.6	0.0–0.4	0.0–0.1
1	0.0–5.4	0.5–1.8	0.8–1.3
2	0.2–7.0	1.2–3.1	1.7–2.3
3	0.6–8.5	2.2–4.3	2.6–3.4
4	1.1–9.9	2.9–5.4	3.6–4.5
5	1.6–11.3	3.7–6.5	4.5–5.5
6	2.2–12.6	4.6–7.7	5.5–6.5
7	2.9–13.9	5.5–8.8	6.5–7.6
8	3.5–15.2	6.4–9.9	7.4–8.6
9	4.2–16.4	7.3–10.9	8.4–9.6
10	4.9–17.6	8.2–12.0	9.4–10.7
15	8.6–23.5	12.8–17.4	14.3–15.8

RBC, red blood cells.

check performed by the Group members. To avoid the addition of imprecision from inconsistent classification to the inherent statistical error, on the other hand, identification criteria have to be standardized.

The region where the smear is examined influences the identification of schistocytes. Angular fragments, like other types of normal and abnormal RBCs, have a tendency to become spherical near the tail of blood smears (Lesesve *et al.*, 2002). Counts should be performed within a smear area of correct thickness, usually behind the tail, where RBCs are just beginning to separate from each other. This excludes the 'feather edge' at the tail of the smear.

Microscope evaluation should be carried out at medium magnification with a 40×, 50×, or 60× power objective, using immersion oil if necessary, in combination with 10× or 12.5× eyepieces (Bain, 2006a). In these conditions, all atypical shapes should be quite easily seen within a few fields.

A schistocyte count has a specific diagnostic value in the clinical context of TMA and related conditions caused by RBC mechanical damage

The origin of RBC fragments may be as heterogeneous as their shapes:

(i) mechanical damage by filaments of fibrin in the microvessels (Bull & Kuhn, 1970; Bessis, 1976), as in TMA, but also increased turbulence, shear stress, and RBC adhesion to abnormal endothelium, e.g. within the heart or great vessels modified by foreign surfaces or pathological abnormalities or in a renal dialysis circuit;
(ii) cytoskeletal abnormality and resultant membrane fragility, often in the context of dyserythropoiesis, either genetic or acquired (Bain, 2006a);
(iii) thermal injury of the RBC membrane in burns, which causes the formation of spherocytes and microspherocytes, with separation of fragments often spherical (microvesicles, blebs, and buds), but sometimes close to angular schistocytes (Lawrence & Atac, 1992; Bain & Liesner, 1996; Muller, Salignac & Lesesve, 2009).

However, for the diagnosis of TMA, the ICSH Working Group has agreed that only schistocytes formed by the first of these three mechanisms (i.e. mechanical damage) are relevant.

A schistocyte count is feasible and meaningful in practice if the schistocytes represent the main morphological RBC abnormality on the film

As there is morphological overlap of schistocytes because of RBC fragmentation syndromes and fragments originated from intrinsically defective RBCs, it is essential, before performing a schistocyte count, to establish that schistocytosis is the dominant abnormality rather than schistocytes being just one feature of another syndrome. A schistocyte count is irrelevant if the underlying pathological process is, for example, oxidant-induced RBC damage or hereditary pyropoikilocytosis. Rare case of myelodysplastic syndromes even present with fragments and thrombocytopenia and should not be misdiagnosed as TMA.

Thus, the ICSH Group agreed that the overall RBC morphology should be taken into account in the context of schistocyte enumeration for the diagnosis of TMA (Figure 2). In TTP and related disorders, schistocytes are seen as the main morphological abnormality, sometimes in association with moderate signs of stimulated erythropoiesis, such as polychromasia, basophilic stippling, and circulating nucleated RBCs (Tsai, 2009). The ICSH group recommends providing only a qualitative report for those samples where schistocytes are observed in a context of multiple heterogeneous RBC shape aberrations. This is especially true

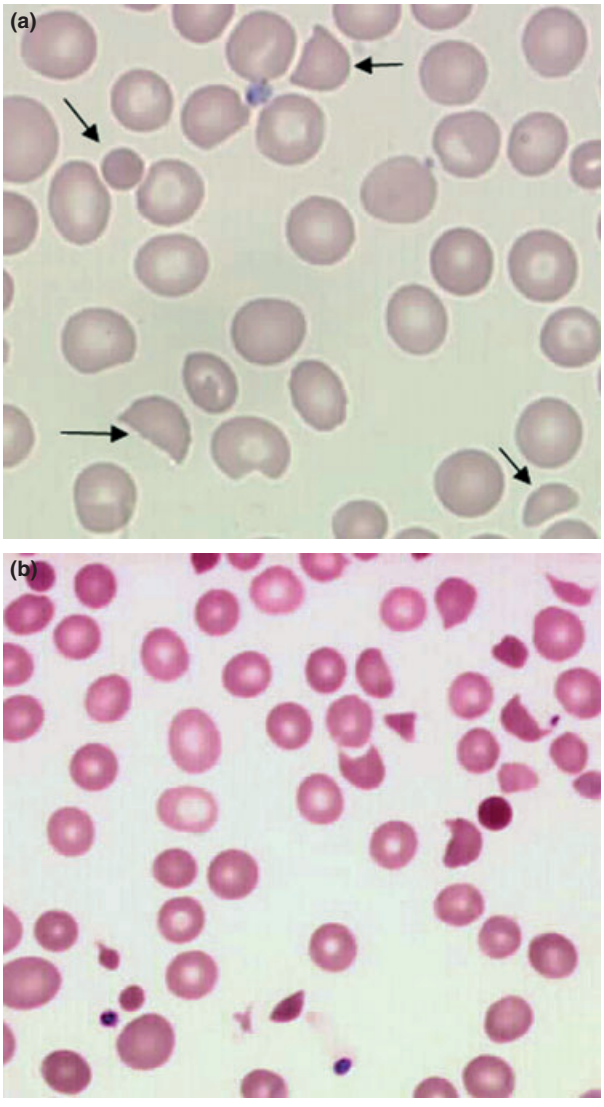

Figure 2. Peripheral blood smear from a case of thrombotic thrombocytopenic purpura. (a) arrows indicate a helmet cells (lower left), a microspherocyte (upper left), a keratocyte (center top), and a microcrescent (lower right angle); (b)morphological abnormalities include microspherocytes, keratocytes, helmet cell, and several crescent and triangular schistocytes.

when quantitative data are needed to monitor patients, such as those with hematopoietic stem cell transplant–associated TMA. In the final report, it should be stated whether schistocytes are the main morphological RBC abnormality in the smear or are part of a severe, generalized anisopoikilocytosis or occur in the context of other abnormalities, suggesting an alternative diagnosis. In the latter cases, a diagnosis of TMA is unlikely and should be confirmed with other tests.

According to the ICSH Schistocyte Working Group, spherocytes, irregularly contracted cells, dacryocytes, acanthocytes, and echinocytes, as well as bite cells that are a feature of oxidant damage, should not be included within the schistocyte count. Bite cells, in particular, can be defined as cells with a small gap in the profile, which has the shape of a bite. The missing portion is lost when precipitated hemoglobin (correspondent to Heinz bodies invisible on fixed and stained films) is removed by the spleen, after oxidative damage, and in patients with glucose-6-phosphate dehydrogenase deficiency or unstable hemoglobins (Bain, 2006b; Barth & Hirschmann, 2007): during hemolytic crisis, bite cells are often found in the company of irregularly contracted cells, dense echinocytes, and hemighosts. The ICSH Group, however, recognizes that the terms keratocyte, helmet cell, and bite cell are used as synonyms by some authors (Bain, 2006a). Very small rounded, irregular, crenated or distorted poikilocytes, and RBC microvesicles should also not be included in the count in the context of anisopoikilocytosis. They can be generated by a markedly dyserythropoietic matrix, formed in a fibrotic bone marrow at the time of release in blood or produced by severe thermal or chemical injury. They are observed, sometimes together with typical schistocytes, in burns, thalassemia major or intermedia, primary myelofibrosis, megaloblastic anemia, congenital sideroblastic and dyserythropoietic anemias, advanced and long-lasting iron deficiency, and severe myelodysplasia. Some confusion may arise in similar conditions, where poikilocytes and bizarre fragments may be morphologically close or even indistinguishable from the typical schistocytes produced by mechanical damage (Bain, 2010). Schistocytes can be seen, usually in very low number, in other conditions such as disseminated intravascular coagulation and sepsis. However, the count of schistocytes in these cases rarely has a specific clinical diagnostic value.[1]

[1]''Schistocytes are practically counted as fragmented red cells in clinical laboratories'' (Japanese Society of Laboratory Hematology, personal communication.

A percentage of schistocytes above 1% is a robust cytomorphological indicator for the diagnosis of TMA in adults

Schistocyte normal ranges in adults are poorly defined and variably reported in different laboratories. There is no consensus in the literature on the numerical upper limit for healthy adults, which is set in general practice below 1% (Klein *et al.*, 1975). Burns, Lou and Pathak (2004) have found an upper limit of 0.2% in normal individuals, 0.6% in patients with renal failure, 0.45% in pre-eclamptic women, and 0.48% in patients with normally functioning prosthetic valves; they have also found schistocytes in all six patients with TTP with a range of 1.1–9.4%. Lesesve, Salignac and Lecompte (2007) have found a maximum value of 0.19% in 119 healthy controls.

The ICSH Schistocyte Working Group agreed that a schistocyte percentage above 1% in a peripheral blood smear in adults is a robust cytomorphological indication in favor of a diagnosis of TMA, when additional features suggesting an alternative diagnosis are absent.

If schistocytes are absent and there is a high suspicion of TMA, blood smear screening for schistocytes should be repeated daily, as the appearance of schistocytes can occasionally be delayed for several days. In rare cases, schistocytes do not appear at all during the course of TMAs (Fava & Galizia, 1995; Akiyama *et al.*, 1997; Daram *et al.*, 2005). For the diagnosis of transplant-associated TMA, however, a higher threshold value is often adopted (Figure 3). An International Working Group recommended a 4% threshold (together with thrombocytopenia, increased lactate dehydrogenase, decreased hemoglobin concentration, and decreased haptoglobin) (Ruutu *et al.*, 2007). Figure 4 reports a flow chart as a

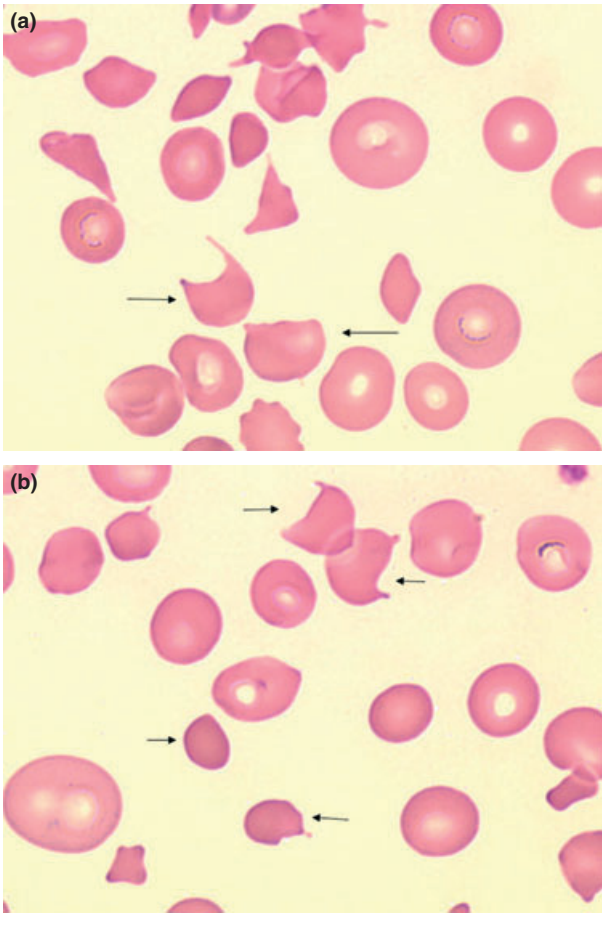

Figure 3. Peripheral blood smear from a case of post-transplant thrombotic microangiopathic anemia. (a) a keratocyte (left arrow), a helmet cell (right arrow), and several hyperchromatic triangular erythrocytes are present; (b) two keratocytes (upper arrow) and two deformed microspherocytes (lower arrow) are present, together with more bizarre red cell fragments.

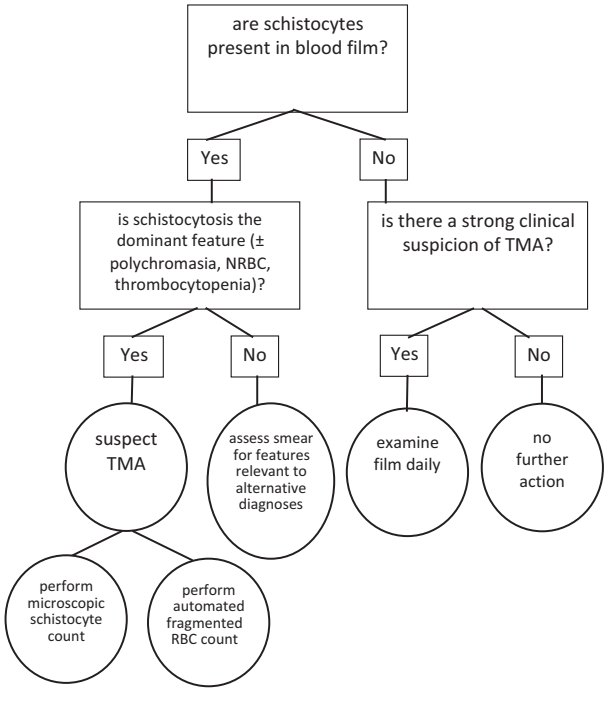

Figure 4. Flow chart for schistocyte count according to ICSH Guidelines (originally drafted by B. J. Bain).

practical recommendation for the use of schistocyte count in diagnosis of TMA.

Red blood cells fragments are frequent soon after birth, with values up to 1.4–1.9% in normal newborns and up to 4.9–5.5% in preterm newborns (Garzia *et al.*, 2005; O. Fenneteau, unpublished data). However, the present ICSH recommendations apply only to adult patients.

Fragmented red cells enumeration by automated blood cell counters is a useful complement to microscopy, as it provides rapid results with a high negative predictive value

All the latest generation, automated hematology analyzers have a flag for fragmented red cells (FRC), and some of them also provide methods for an automated FRC count on EDTA-anticoagulated blood samples, through the measurement of forward scatter and the intensity of fluorescence (Jiang *et al.*, 2001; Abe *et al.*, 2009) or 2-dimensional optical analysis (Lesesve *et al.*, 2004a,b).

The automated FRC count is a promising new parameter for a routine screening because of its low cost and rapid availability. Its reproducibility is excellent, especially at high counts; the stability over time is limited to <24 h (Banno *et al.*, 2005). Automated methods show good correlation with the percentages of schistocytes in patients with TMA or after transplantation (Saigo *et al.*, 2002; Lesesve *et al.*, 2004a,b). However, one of the two presently available methods tends to slightly underestimate the FRC count compared with microscopy (Banno *et al.*, 2005), while the other one tends to slightly overestimate it (Lesesve *et al.*, 2004a,b; Garzia *et al.*, 2005). False-negative results occur in samples with an MCV $\geq$ 105 fL, probably due to the fact that large FRC are included by the analyzers in the count of microcytic RBC. Sensitivity and specificity for the diagnosis of TMA vary according to the selected threshold. However, the predictive value of negative results is reported as high (Garzia *et al.*, 2005; Lesesve, Salignac & Lecompte, 2007).

On the basis of the published scientific studies and personal experience of the Group members and expert reviewers, the ICSH Schistocyte Working Group recognizes the utility of the automated FRC count as a screening method in the routine laboratory. All samples with a positive automated FRC count at diagnosis as well as macrocytic samples with a negative automated FRC count need to be confirmed by microscopy. One major advantage of the automated FRC count is the possibility of easily obtaining an accurate and precise follow-up of true-positive samples.

NOTE ON ICSH SCHISTOCYTE GROUP WORKING METHOD

To develop the present recommendations, the ICSH Working Group proceeded through the following steps:

- exhaustive review and analysis of the literature on TMA, RBC fragments, and schistocytes: this confirmed the inconsistency of the existing definition criteria;
- questionnaire survey of schistocyte counting in 31 laboratories worldwide, which indicated poor uniformity of schistocyte-counting reports, ranging from a semi-quantitative description (few/moderate/many or presence/absence) to a percentage quantitation;
- extensive exchange of opinions and intermember comparisons by internet e-conferencing, including circulation of photographs of stained blood smears, aimed at defining criteria for nomenclature and morphology;
- practical verification and refinement of proposed criteria on six films of peripheral blood with low, intermediate, and high schistocyte counts mailed to all Group members;
- discussion and slide projection with agreement on morphological aspects of selected cells and cell fragments on blood smears during three ICSH general meetings, in May 2009, November 2009, and May 2010.

Consensus recommendations were shared with an international panel of experts (see 'Acknowledgements') for further comments prior to being submitted for peer-reviewed publication.

ACKNOWLEDGEMENTS

The Committee gratefully acknowledges the following reviewers for their expert comments:

Barbara J. Bain, Department of Hematology, St. Mary's Hospital Campus of Imperial College, Faculty of Medicine, London, UK. Yohko Kawai, International University of Health and Welfare, Sanno hospital, Tokyo, Japan. Jin-Yeong Han, Dong-A, University College of Medicine, Busan, Korea. S. Mitchell Lewis, Department of Haematology, Hammersmith Hospital, London, UK. Samuel J. Machin, The University College London Hospitals, London, UK.

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