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

Letter to the Editor

Classification of several morphological red blood cell abnormalities by DM96 digital imaging

Sir, Manual light microscopy remains the gold standard for morphological analysis of peripheral blood smears. However, this method is labour-intensive and time-consuming and requires highly trained personnel. Digital microscope systems contribute in realizing a more rapid, standardized and efficient morphological analysis of peripheral blood smears [1, 2].

Automatic classification of white blood cells (WBCs) using digital imaging in a peripheral blood smear is already well established for the five main classes (neutrophils, lymphocytes, monocytes, eosinophils and basophils) and blast cells [2–4]. These cell classes can be recognized with a high degree of reliability; less common classes such as promyelocytes, myelocytes and metamyelocytes are also recognized by the system but with less accuracy [2].

The use of digital microscopy systems could also improve morphological analysis of aberrant red blood cells (e.g. schistocytes, teardrop cells), eventually leading to a faster diagnosis and treatment of diseases in which these abnormalities are the hallmark of these diseases. Moreover, digital microscopy will lead to a more standardized way of performing these kinds of analyses.

The recent introduction of a novel application module for the digital microscope system DM96 (Advanced RBC application, CellaVision, Lund, Sweden) allows for the automatic detection and classification of morphological abnormalities in red blood cells. This application was developed using an artificial neural network, and it considers 80 features such as size, roundness, size and shape of inner pallor and distribution of notches around the border for the morphological classification of erythrocytes. The system generates an image (Figure 1), which corresponds with an area of eight microscopic fields (100× objective and a 22 mm ocular). While producing this image, the system also performs a preclassification of the red blood cells (RBCs). The classification results of the morphological abnormality are displayed on the screen and can subsequently be altered or confirmed by the operator. The module has already been validated for the detection and subsequent classification of teardrop cells and schistocytes in peripheral blood smears [5–7].

To date, standardization of red blood cell morphology is still limited [8, 9]. In this study, we continued our ongoing validation of the RBC module by comparing postclassification results (after manual intervention by three morphological experts) from the RBC module on the DM96 to results from manual assessment, despite all still the gold standard. We also compared the RBC module postclassification results to the preclassification results by the software (without manual interference). For this, a cohort of patient samples and normal samples were used.

Result Laboratory serves as the reference laboratory for blood cell morphology proficiency testing for the Netherlands and morphology experts from this department are highly experienced, trained and skilled technicians. In addition, the department participates in internal and external quality control procedures on a routine basis. The morphological experts using the RBC module were tested for competence and examined (score of minimal 90% concordance with examinator) before working on this study. No clinical significant discrepancies were present between the morphological experts.

A total of 316 peripheral blood smears were used for analysis, including 198 patient samples and 118 normal samples (according to manual assessment). Due to limited availability of positive samples, the morphological abnormalities elliptocytes, ovalocytes, echinocytes, pappenheimer bodies, basophilic stippling and parasites were excluded from this study. The included abnormalities are listed in Table 1.

Due to clinical relevance, different guidelines were used for the percentages of abnormal cells. The cut-off values for the various red blood cell abnormalities described in this article are the values currently used in our laboratory.

The peripheral blood smears were prepared from venous blood samples collected in EDTA tubes, using the SP-10 (slide maker-stainer, Sysmex, Etten-Leur, the

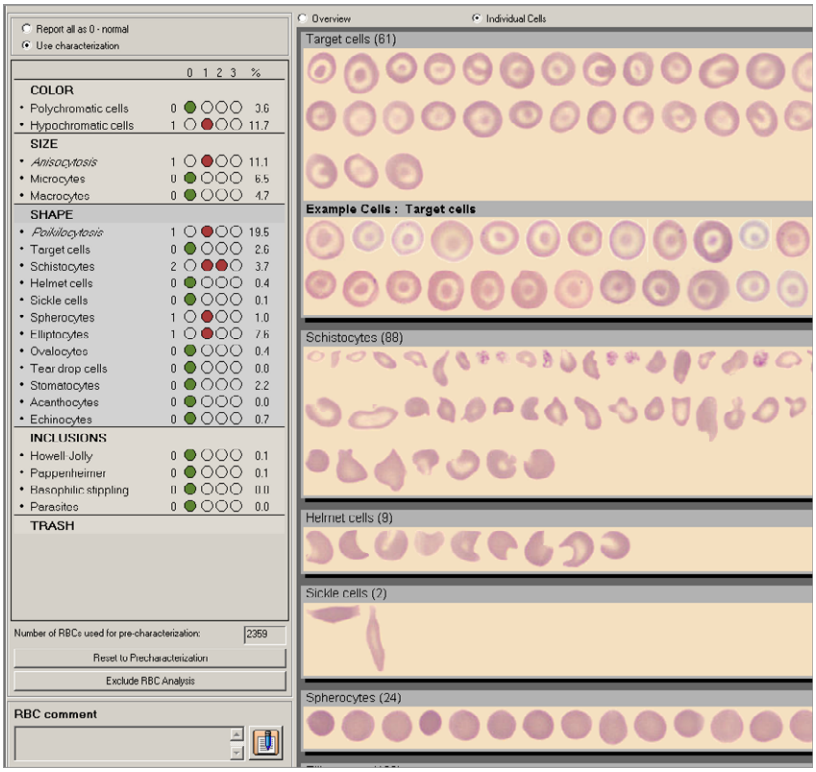

Figure 1. Partial screenshot of the Advanced RBC application software. Several individual cell classes are displayed in which the RBCs were classified by the software without manual intervention (preclassification).

Table 1. Morphological red blood cell abnormalities with the corresponding percentages and grading defined as positive according to the gold standard used in this study

Abnormality	Percentage	Grading	Number of samples
Microcytosis*	≥25%	++	14
Macrocytosis*	≥25%	++	23
Target cells*	≥20%	++	17
Schistocytes*	≥1%	+	11
Spherocytes*	≥1%	+	15
Teardrop cells*	≥1%	+	67
Howell Jolly bodies*	≥1%	+	36
Acanthocytes*,†	≥5%	+	12
Stomatocytes†	≥20%	++	8
Polychromasia†	≥5%	+	108
Hypochromasia†	≥20%	++	30
Sickle cells†	≥1%	+	13

*Percentage and grading according to the CellaVision guidelines.
†Percentage and grading according to the Dutch national guidelines [10].

Netherlands) and stained according to the May–Grünwald–Giemsa staining procedure.

Approximately 2000 to 4000 erythrocytes per blood smear were evaluated and preclassified by the advanced RBC module on the DM96; analysis time is about one minute. The percentage of abnormal blood cells was converted to a grading system [9], ranging from 1+ to 3+. The different grades differ per morphological abnormality. Postclassification was performed by three morphological experts who adjusted or verified the grading suggested by the RBC module, using the overview image and individual cell classes. The samples were divided amongst the morphological experts wherein each expert analysed about one-third of the samples.

For the manual microscopic assessment of these samples, a similar analysis was performed in which eight fields were screened per sample, using a 100× objective and a 22 mm ocular.

Classification results were divided into two categories: positive and negative, according to the CellaVision and Dutch national guidelines [10] (Table 1). Sensitivity, specificity, confidence intervals (using the Wilson score method) and agreement (accuracy of the classification) were calculated for both the DM96 postclassification versus manual classification and

preclassification (DM96) versus postclassification (DM96) results (Tables 2 and 3).

When comparing manual classification with postclassification results from the RBC module, the sensitivity varied per morphological abnormality (ranging from 25.0% to 88.9%), with high sensitivity rates such as 88.9%, 88.0% and 83.6% for Howell Jolly bodies, polychromasia and teardrop cells. Morphological abnormalities like macrocytes and stomatocytes displayed a lower sensitivity of 25.0%. In contrast, the specificity was over 90% for all morphological abnormalities used in this study.

Comparison of preclassification versus postclassification results revealed sensitivity rates ranging from 17.6% to 100.0%. Abnormalities such as target cells, microcytosis, macrocytosis and acanthocytes revealed a poor sensitivity rate; 17.6%, 41.2%, 28.6% and 33.3%, respectively. Nevertheless, sensitivity rates for spherocytes, schistocytes and stomatocytes each scored 100%. Specificity rates ranged from 46.3% to 100.0%; high specificity rates were seen for target cells, sickle cells (both 100%) and acanthocytes (99.0%). Polychromasia, spherocytes and schistocytes showed lower specificity of 46.3%, 59.9% and 64.0%, respectively.

Comparison between preclassification and postclassification using the RBC module yielded variable results in agreement and specificity, depending on the type of morphological abnormality. This is due to the fact that the software does not fully classify all abnormalities correctly

Table 2. Sensitivity, specificity, confidence intervals (CI) and agreement of the DM96 postclassification results vs. manual classification results

Abnormality	Sensitivity in %	95% CI sensitivity	Specificity in %	95% CI specificity	Agreement in %	False-positive ratio in %	False-negative ratio in %
Microcytosis	64.3	38.8–83.7	97.4	94.9–98.7	95.9	2.6	35.7
Macrocytosis	25.0	12.0–44.9	99.7	98.0–99.9	94.0	0.3	75.0
Target cells	82.4	59.0–93.8	99.0	97.1–99.7	98.1	1.0	17.6
Schistocytes	72.7	43.4–90.2	94.8	91.7–96.8	94.0	5.2	27.3
Spherocytes	80.0	54.8–93.0	91.7	88.0–94.3	91.1	8.3	20.0
Teardrop cells	83.6	72.9–90.6	94.4	90.8–96.6	92.1	5.6	16.4
Howell Jolly's	88.9	74.7–95.6	97.5	94.9–98.8	96.5	2.5	11.1
Acanthocytes	75.0	46.8–91.1	100.0	98.8–100.0	99.1	0.0	25.0
Stomatocytes	25.0	7.2–59.1	99.7	98.2–99.9	97.8	0.3	75.0
Polychromasia	88.0	80.5–92.8	97.6	94.5–99.0	94.3	2.4	12.0
Hypochromasia	83.3	66.4–92.7	96.2	93.2–97.8	94.9	3.8	16.7
Sickle cells	69.2	42.4–87.3	99.7	98.1–99.9	98.4	0.3	30.8

Table 3. Sensitivity, specificity, confidence intervals and agreement of the DM96 preclassification results vs. DM96 postclassification results

Abnormality	Sensitivity in %	95% CI sensitivity	Specificity in %	95% CI specificity	Agreement in %	False-positive ratio in %	False-negative ratio in %
Microcytosis	41.2	21.6–64.0	95.3	92.3–97.2	92.4	4.7	58.8
Macrocytosis	28.6	8.2–64.1	91.9	88.3–94.5	90.5	8.1	71.4
Target cells	17.6	6.2–41.0	100.0	98.7–100.0	95.6	0.0	82.4
Schistocytes	100.0	86.2–100.0	64.0	58.4–69.3	66.8	36.0	0.0
Spherocytes	100.0	90.6–100.0	59.9	54.0–65.4	64.6	40.1	0.0
Teardrop cells	91.4	82.5–96.0	80.1	74.6–84.6	82.6	19.9	8.6
Howell Jolly's	92.3	79.7–97.4	76.2	70.8–80.8	78.2	23.8	7.7
Acanthocytes	33.3	12.1–64.6	99.0	97.2–99.7	97.2	1.0	66.7
Stomatocytes	100.0	43.9–100.0	92.4	89.0–94.9	92.5	7.7	0.0
Polychromasia	96.0	90.2–98.4	46.3	39.8–53.0	62.0	53.7	4.0
Hypochromasia	94.4	81.9–98.5	91.4	87.6–94.2	91.8	8.6	5.6
Sickle cells	50.0	23.7–76.3	100.0	98.8–100.0	98.4	0.0	50.0

yet (e.g. polychromasia, schistocytes, spherocytes). However, sensitivity rates for these RBC abnormalities were excellent, of which the high sensitivity for schistocytes was previously described in other papers [6, 7].

Comparing schistocyte DM96 postclassification versus manual microscopy resulted in a sensitivity of 72.2% with a false-negative rate of 27.3%. Three out of 11 positive samples (defined by manual microscopy) were defined as negative by DM96 postclassification. Further analysis of the raw data revealed that the amount of schistocytes in these samples were close to the cut-off value of 1.0%. The probability of missing a TTP in a clinical situation is therefore very small, because TTP cases most commonly present with more than 1.0% schistocytes in the peripheral blood smear [11].

This observation is very significant, for example in the case of a suspected thrombotic thrombocytopenic purpura (TTP), in which rapid detection of schistocytes is essential to guarantee immediate treatment and improved survival.

The specificity for sickle cells and target cells is very high (100%). Although preclassification by the software results in a high false-negative ratio, no false positives are given for these abnormalities.

Comparison between the two methods, postclassification using the manual microscopic method and the RBC module, revealed a high specificity and agreement (both >90%).

Analysis of several morphological red blood cell abnormalities with the novel RBC module correlates well with the manual microscopic method. Routine analysis of several morphological RBC abnormalities is now also possible using this device. Classification of some morphological abnormalities by the software requires further development (polychromasia, spherocytes and schistocytes). The morphological abnormalities such as elliptocytes, ovalocytes, echinocytes, pappenheimer bodies, basophilic stippling and parasites were not discussed in this study due to a lack of positive samples. The novel RBC application module has proven to be a useful tool for the morphological analysis of a number of red blood cell abnormalities [5–7]. This module makes use of individual RBC characteristics and numbers (2000 to 4000 RBCs) and generates percentages instead of only grading estimations. Moreover, the RBC module is easy to use and leads to a more standardized way of analysis. Combining cell counter results and digital imaging of RBCs will undoubtedly result in an even higher sensitivity and specificity for the detection and recognition of haematological diseases.

A. Egelé, K. Stouten, L. van der Heul-Nieuwenhuijsen, L. de Bruin, R. Teuns, W. van Gelder, J. Riedl

Result laboratory, Albert Schweitzer hospital, Dordrecht, Netherlands
E-mail: angelique_egele@hotmail.com

doi: 10.1111/ijlh.12530

References

1. Ceelie H, Dinkelaar RB, van Gelder W. Examination of peripheral blood films using automated microscopy; evaluation of Diffmaster Octavia and Cellavision DM96. J Clin Pathol 2006;60:72–9.
2. Stouten K, Riedl JA, Levin M-D, van Gelder W. Examination of peripheral blood smears: performance evaluation of a digital microscope system using a large-scale leukocyte database. Int J Lab Hematol 2015; 37:e137–e140.
3. Lee LH, Mansoor A, Wood B, Nelson H, Higa D, Naugler C. Performance of CellaVision DM96 in leukocyte classification. J Pathol Inform 2013;4:14.
4. Kratz A, Bengtsson HI, Casey JE, Keefe JM, Beatrice GH, Grzybek DY, Lewandrowski KB, van Cott EM. Performance evaluation of the Cellavision DM96 system, WBC differentials by automated digital image analysis supported by an artificial neural network. Am J Clin Pathol 2005;124:770–81.
5. Egelé A, van Gelder W, Riedl JA. Automated detection and classification of teardrop cells by a novel RBC module using digital imaging/microscopy. Int J Lab Hematol 2015; 37:e153–e156.
6. Egelé A, van Gelder W, Riedl JA. Automated detection and classification of schistocytes by a novel RBC module using digital imaging/microscopy. J Hematol 2015;4:184–6.
7. Hervent AS, Godefroid M, Cauwelier B, Billiet J, Emmerechts J. Evaluation of schistocyte analysis by a novel automated digital cell morphology application. Int J Lab Hematol 2015; 37:588–96.
8. Constantino BT. Reporting and grading of abnormal red blood cell morphology. Int J Lab Hematol 2014;37:1–7.
9. Palmer L, Briggs C, McFadden S, Zini G, Burthem J, Rozenberg G, Proytcheva M, Machin SJ. ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features. Int J Lab Hematol 2015;37:287–303.
10. Ermens AAM, Mulder A, van Gelder W, van de Berg G. "Diff magazine", Dutch National Guidelines. Amsterdam: Nvkc, 2013.
11. Burns ER, Lou Y, Pathak A. Morphologic diagnosis of thrombotic thrombocytopenic purpura. Am J Hematol 2004;75:18–21.