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Letter to the Editor

Automated detection and classification of teardrop cells by a novel RBC module using digital imaging/microscopy

Sir, In a number of diseases, morphological analysis of abnormal red blood cells is of vital importance, contributing to a rapid and correct diagnosis. An example is teardrop cells, which may indicate dyserythropoiesis due to circulation through altered bone marrow sinuses and splenic cords. Teardrop cells in a peripheral blood smear are significant and correlate with several diseases, such as iron deficiency anemia, hemolytic anemia, megaloblastic anemia, and metastatic carcinoma due to bone marrow infiltration and myelofibrosis [1, 2].

Myelofibrosis is characterized by a progressive anemia, pancytopenia, and thrombocytopenia. Typically present are splenomegaly and/or hepatomegaly due to extramedullary erythropoiesis, leukoerythroblastosis caused by the lack of space in the bone marrow due to fibrosis, and the presence of myeloid precursors and teardrop cells in peripheral blood.

Nowadays, the grading of abnormal red blood cells is semi-quantitative, usually indicated by a score ranging from 1 to 3 + correlating with the percentage of abnormal cells present. A consistent and standardized international grading system for abnormal red blood cells is still lacking, resulting in scoring percentages that differ per laboratory [2]. In our laboratory, the amount of teardrop cells is indicated with 1 + (which stands for 0.5–2% teardrop cells), 2 + (2–5%), and 3 + (>5%) [3].

To date, no cutoff value is established for the percentage of teardrop cells in a peripheral blood smear, discriminating pathology from nonpathology. If a reliable cutoff value can be determined for this red blood cell abnormality, this could lead to a faster and more accurate detection of myelofibrosis and in distinguishing this disease from benignity.

How to realize a good, reliable, and automated screening tool for the detection of morphological red blood cell abnormalities (e.g., teardrop cells)?

Automated morphological analysis and classification of leukocytes with the use of digital microscopy is nowadays a routine procedure in a large number of laboratories globally [4]. Recently, a novel software tool has been developed to detect and classify morphological abnormalities of red blood cells (advanced RBC application). The advanced RBC application (CellaVision) is basically a RBC cell-locating device. The system identifies and segments red blood cells. Each red blood cell is then characterized for size, shape, color, and inclusion. The characterizations for shape, color, and inclusion are performed by an artificial neural network. This network is trained by a number of highly qualified experts, and the network uses 80 features computed for each RBC image. Examples of used features are size, roundness, distribution of notches around the border, size, and shape of inner pallor. The user can verify or change any suggested morphology. The results are represented as a percentage value and a grading which is based on a conversion table defined by the user in the settings file.

We evaluated and validated this module, using a cohort of patient samples and healthy controls, and compared pre- and postclassification results. Preclassification was performed by the RBC classification module without manual intervention (Figure 1), and the postclassification was performed by a morphological expert. Subsequently, statistical analysis was performed to determine the accuracy and correlation.

Classification analysis of teardrop cells was performed on 46 peripheral blood smears from patients in which teardrop cells were present and a cohort of normal blood smears (n = 10). The slides were prepared using the SP-10 (slide maker/stainer; Sysmex, Etten-Leur, the Netherlands) from venous blood samples collected in EDTA tubes and stained according to the May–Grünwald–Giemsa stain. Of these 46 patients, fifteen were diagnosed with myelofibrosis, confirmed by bone marrow analysis. Myelofibrosis evolved from polycythemia vera (PV) in four patients and from essential thrombocythemia (ET) in five patients. Three patients were diagnosed with primary myelofibrosis (PMF) and three with idiopathic myelofibrosis (IMF). Among the other 31 samples, six were diagnosed with a myelodysplastic syndrome (MDS), four with chronic lymphocytic leukemia (CLL), three with an iron deficiency, one with ET, two with PV,

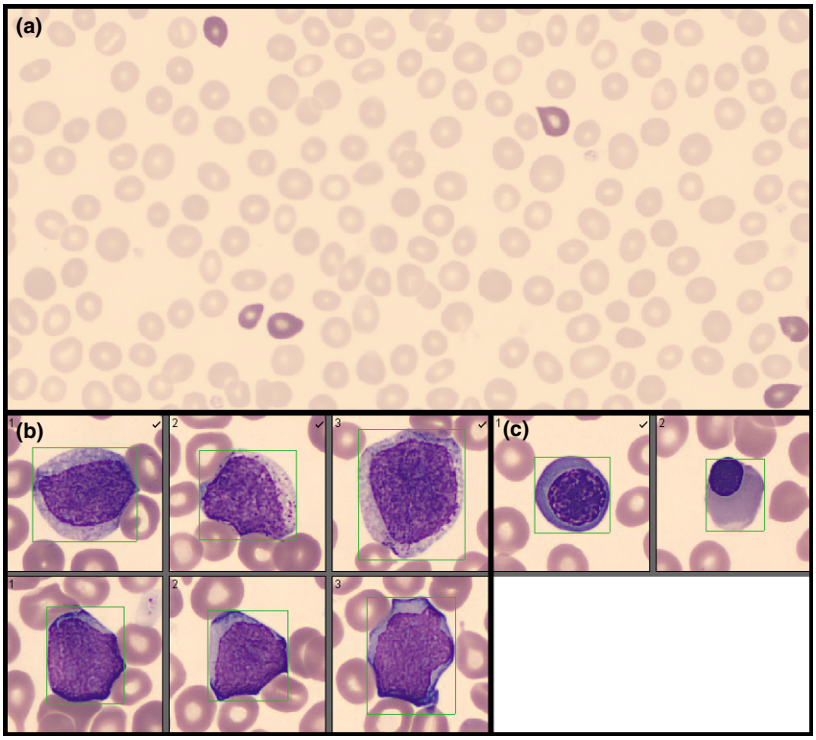

Figure 1. Peripheral blood smear of a patient with primary myelofibrosis. Panel a shows an overview of the red blood cells used for the classification in which the teardrop cells (highlighted) are preclassified by the software without manual intervention. In panel b, myelocytes and blasts are displayed, and panel c shows erythroblasts.

and six with metastatic carcinoma. The remaining nine patients had different diagnoses varying from sickle cell anemia to thalassemia (Table 1). The red blood cell classification module (CellaVision) classified approximately 2000–4000 red blood cells per blood smear in about 1 min. Statistical analysis was performed using the EP Evaluator software from Siemens Healthcare Diagnostics and the Passing–Bablok method for regression analysis. The Passing–Bablok method is a linear regression procedure with no special assumptions regarding the distribution of the samples and the measurement errors [5].

Preclassification of these 46 samples resulted in detecting teardrop cells in a range of 0.5% to 10.4% and postclassification in a range of 0.2% to 6.8% teardrop cells (Table 1). Analysis of normal blood smears ($n = 10$) resulted in a range of 0.1% to 0.5% teardrop cells in preclassification and 0% to 0.1% in postclassification.

When classified according to the VHL guidelines [3], none of the ten normal blood smears and twelve of the 46 patient blood smears were false-positive, leading to an overall specificity of 45%, regardless of classification. Moreover, no false-negative samples were generated, leading to an overall sensitivity of 100%. Data analysis revealed three preclassification categories with 100% sensitivity: <0.5% teardrop cells, 0.5% to 3% (1 + and 2 + according to the VHL guidelines), and >3%. When using the VHL guidelines, no false-positive samples are present above 5% teardrop cells in preclassification. Based on our own data, no false positives are present above 3% and no false negatives are present below 0.5%.

In between, in the intermediate category with 1 + and 2 + scores, the specificity gradually increases. When comparing the 1 + and 2 + scores in the preclassification with the corresponding 1 + and 2 + scores in the postclassification, the concordance is low. This is due to the increased sensitivity of the RBC classification module for teardrop cells as compared to manual microscopy. However, the specificity for the category 1 + is 52.6% and the specificity for the category 2 + is 76.9%. The specificity for 3 + preclassification *vs.* 3 + postclassification is 71.4%. The 3 + preclassification compared to positive samples (e.g., 2 + and 3 +) in postclassification leads to a specificity of 100%.

Results from pre- and postclassification showed a good correlation (correlation coefficient of 0.95), albeit a 20–30% overestimation of teardrop cells by the classification software ($y = 0.76x - 0.4$) (Figure 2). Regression analysis showed a statistically significant proportional bias (1–0.766) with a confidence interval of 0.673–0.913 and a constant bias of −0.36%.

Reproducibility was tested using ten samples with teardrop cells varying from 1.4% to 3.3%. These samples were analyzed in 10 consecutive runs, resulting in a variation coefficient (VC) of lower than 0.3%. In 10 samples where no teardrop cells were present, a similar analysis resulted in a VC which stayed below 0.7%.

Table 1. Pre- and postclassification results of the teardrop cells in patient blood smears (n = 46). MF-staging was obtained by pathological analysis (MF1-4)

Diagnosis	Extent of marrow fibrosis	Preclassification in %	Postclassification in %
Primary myelofibrosis	MF-3	5.0	4.1
	MF-3	6.0	4.3
	MF-3	4.6	4.1
Idiopathic myelofibrosis	MF-4	3.0	1.4
	Not available	1.2	0.7
	MF-2	1.7	1.1
Post-ET myelofibrosis	MF-2	4.4	2.8
	MF-2/3	6.9	4.6
	Not available	6.6	5.9
	Not available	9.9	6.5
	MF-2/3	4.9	2.3
Post-PV myelofibrosis	Not available	1.0	0.3
	Not available	0.9	0.4
	MF-3/4	10.4	6.8
	MF-3/4	7.9	5.8
ET	No fibrosis	3.3	1.6
PV	MF-1	2.0	1.6
	Not available	2.6	0.3
MDS		1.8	1.0
		1.7	0.8
		1.3	0.4
		0.6	0.2
		1.0	0.3
		2.2	0.8
CLL		1.1	0.5
		2.6	2.9
		1.7	0.4
		2.0	1.0
Iron deficiency anemia		0.5	0.3
		1.8	0.3
		2.0	0.9
Metastatic carcinoma			
Prostate carcinoma		1.1	0.8
		1.3	0.9
Ovarium carcinoma		1.8	1.4
		1.5	1.4
Lung carcinoma		1.3	1.0
		2.2	0.3
Remaining		1.8	1.2
		1.9	1.7
		0.5	0.6
		1.1	0.7
		0.7	0.2
		2.3	0.8
		2.9	0.7
		2.1	0.3
		2.7	1.4

Results show an excellent correlation (0.95) between the pre- and postclassification in detecting teardrop cells in peripheral blood smears (Figure 2). We also show that the determination of a cutoff value for teardrop cells is feasible. Healthy controls always score less than or equal to 0.5% teardrop cells in the preclassification, whereas in

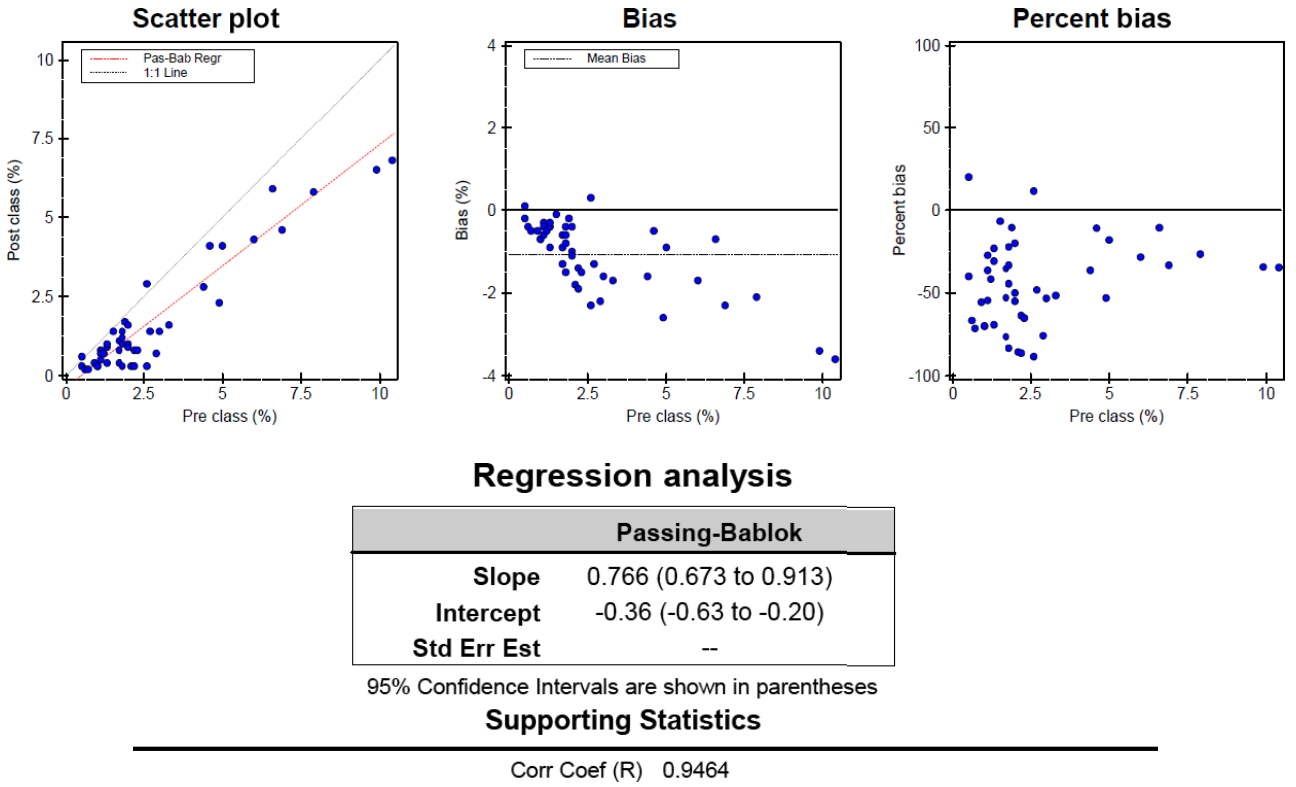

Figure 2. Graphic representation of the comparison of the preclassification of teardrop cells by the DM96 and postclassification by a morphological expert (normal samples were excluded).

patient samples the percentage of teardrop cells always exceeds 0.5%. The 20–30% overestimation of teardrop cells by the RBC classification module may result in a high sensitivity. This will lead to some false-positive samples but little to none false-negative samples, which is more clinically relevant.

Automated screening of blood smears using digital microscopy provides an objective, more standardized and reproducible morphological tool to assess red blood cell morphology. This contributes to rapid and efficient detection of teardrop cells (and possibly other RBC abnormalities) using digital imaging.

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