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ORIGINAL ARTICLE

Modification of the Algorithm Used by Automated Hematology Analyzer XN-3000 Improves Specificity in the Detection of Schistocytes

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SUMMARY

Background: **This study investigated the feasibility and accuracy of an automated hematology analyzer in the detection of schistocytes.**
Methods: **In total, 1,026 peripheral blood samples were collected. Schistocytes were morphologically diagnosed by manual examination of digital microscopic red blood cell images captured by a Sysmex DI-60. Automated diagnoses were performed using a Sysmex XN-3000.**
Result: **The accuracy of automated diagnosis using the XN-3000 with the default algorithm "fragments?" was determined through comparison with the findings of morphological examination. The comparison showed a sensitivity of 100% and a specificity of 41.6% for automated diagnosis. To improve the low specificity, a two-step analysis was performed. Use of the algorithm "fragments?" in XN-3000 followed by an off-line analysis using the cell parameter %FRC (percent fragmented red blood cells) yielded a sensitivity of 86.5% and a specificity of 70.3%.**
Conclusions: **Our study indicated that combined use of the %FRC parameter with the default algorithm of the Sysmex XN-3000 automated hematology analyzer can improve the low specificity of the default algorithm in rapid screening for schistocytes.**
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INTRODUCTION

Schistocytes, defined as fragmented red blood cells (FRCs), are a major sign of micro- and macroangiopathic hemolytic anemia caused by endothelial damage and deposition of fibrin strands or fibrin-platelet aggregates in small vessels [1]. Detection of schistocytes in hemolytic uremic syndrome or thrombotic thrombocytopenic purpura is clinically important for rapid diagnosis [2-5]. Currently, manual microscopic examination is the gold standard for detection of schistocytes. According to the

guidelines of the International Council for Standardization in Hematology, manual microscopic examination of at least 1,000 red blood cells (RBCs) is required to confirm the presence of schistocytes. This is a tedious and time-consuming procedure in hematology laboratories, which hinders rapid processing of critical samples. Thus, adjunctive use of automated hematology analyzers is permitted within the guidelines [6]. Automated hematology analyzers, such as the Sysmex XE-5000, Sysmex XE-2100, and Siemens ADVIA, have previously been tested for the detection of schistocytes [7,8]. However, these studies reported that automated analyses are sensitive but not specific because the percentages of microcytic and/or hypo-hemoglobinized red cells impact on FRCs count and cause FRC overestimation. [8-10].

In this study, we evaluated the performance of the automated hematology analyzer Sysmex XN-3000 (Sysmex, Kobe, Japan). We tested modified algorithms created by combining the default algorithm with off-line analyses using various cellular parameters, with the aim of improving the detection of schistocytes.

MATERIALS AND METHODS

Patient samples

The study protocol was approved by the institutional review board of Juntendo University Hospital (Tokyo, Japan). The requirement for written informed consent was waived because samples were de-identified. A total of 1,026 peripheral blood samples drawn for routine examinations sent mainly from the Departments of Hematology and Department of General Medicine: 513 samples flagged as "fragmented?" by Sysmex XN-3000 (i.e., Schistocyte positive) and 513 samples without flags. Table 1 listed the patients' underlying diseases diagnosed as schistocyte positive by human eyes (n = 148). Venous blood (2 mL) was collected in K_2-EDTA tubes. For each sample, 88 µL were used for automated analyses by the Sysmex XN-3000 and 70 µL were used to prepare slides for microscopic examinations. All procedures were completed within 2 hours from collection.

Cell analyses using Sysmex XN hematology analyzer

Automated analyses of schistocytes were performed using the Sysmex XN-3000 hematology analyzer, in accordance with the manufacturer's protocol. Cell analyses were performed by impedance channel and reticulocyte (RET) channel. The RET channel analysis was performed automatically to determine RET count using scattergrams consisting of fluorescence intensity/nucleic acid level and forward scatter/cell size. The XN-3000 also analyzed percentage of microcytic RBCs (MicroR) and hypo-hemoglobinized RBCs (Hypo-He) that are defined, as follows: RBCs with a volume < 60 fL (measured by impedance channel) and RBCs with < 17 pg of hemoglobin content (measured by RET channel). Schistocytes were flagged as "fragmented?" [11-13].

Reference methods to determine schistocytes

Peripheral blood smears were stained with May-Grünwald Giemsa stain by the automated slide maker SP-50 (Sysmex, Kobe, Japan). For morphological examinations, RBC images with more than 1,000 RBCs per slide were captured by the automated digital image analyzer DI-60 (Sysmex, Kobe, Japan) using the CellaVision Advanced RBC Software Application (CellaVision, Lund, Sweden) [14,15]. The morphological schistocyte count was determined by three board-certified hematology laboratory technologists using the digital images blindly. In accordance with International Council for Standardization in Hematology (ICSH) guidelines [6], peripheral blood smears with > 1% schistocytes were classified as positive. Schistocytes were 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 based upon the ICSH recommendation.

Statistical analysis

Statistical analysis was carried out using the Statistical Package for the Social Sciences (IBM SPSS Statistics for Mac OS version 25.0; IBM Corp., Armonk, NY, USA). The Mann-Whitney U test was used for comparisons between two groups. Unless otherwise stated, analysis of variance was used for comparisons among three or more groups. Statistical significance was set at $p < 0.05$. Descriptive statistics for continuous variables were expressed as means ± standard deviations. The sensitivities and specificities of results provided by the analyzers were derived from true/false positive/negative determination. Receiver-operating characteristic (ROC) curves were constructed to determine cutoff values for each parameter and their combinations. Area under the curve (AUC) was calculated from the ROC curves.

RESULTS

Performance of the automated hematology analyzer Sysmex XN-3000 in the detection of schistocytes

We collected 513 samples that were flagged as "fragments?" by the default algorithm of the Sysmex XN-3000, and 513 samples that were not flagged as "fragments?". Morphological microscopic examination, in contrast, revealed 148 positive samples and 365 negative samples. Compared to the morphological examination, the Sysmex XN-3000 with the default algorithm of "fragments?" yielded a high sensitivity (100%), but a low specificity (41.6%).

Contribution of various morphological parameters to the automated diagnosis by Sysmex XN-3000

Next, we sought to evaluate the contribution of morphological parameters assessed by the Sysmex XN-3000 to the detection of schistocytes. We used 148

Table 1. Schistocyte positive samples.

Diagnosis	Number
Myelofibrosis	33
Essential thrombocytosis	10
Polycythemia vera	1
Chronic myelogenous leukemia	1
Myelodysplastic anemia	21
Acute myeloid leukemia	11
Acute lymphoid leukemia	2
Diffuse large B cell lymphoma	3
Hodgkin lymphoma	1
Multiple myeloma	5
Aplastic anemia	9
Paroxysmal Nocturnal Hemoglobinuria	4
Thalassemia	3
Idiopathic thrombocytopenia	3
Liver cancer	8
Lung cancer	3
Pancreatic cancer	3
Rhabdomyosarcoma	4
Acute kidney injury	3
Systemic lupus erythematosus	2
Cogan's syndrome	1
Disseminated intravascular coagulation	1
Deep vein thrombosis	1
Pneumonia	1
Others	14

n = 148

schistocyte-positive samples and 878 schistocyte-negative samples to draw ROC curves for three basic parameters: %FRC, %MicroR, and %Hypo-He. The AUC for %FRC was 0.85 (cutoff value: 2.4%; 95% confidence interval [CI]: 0.82 - 0.88). The AUC for %MicroR was 0.75 (cutoff: 3.3%; 95% CI: 0.72 - 0.88), and for %Hypo-He was 0.77 (cutoff: 3.0%, 95% CI: 0.74 - 0.80) (Figure 1A). These data demonstrated that %FRC was the most important parameter in the automated detection of schistocytes.

Performance of combined algorithms in the automated detection of schistocytes

Currently, the default algorithms including "fragments?" of the Sysmex XN-3000 are not adjustable by users and parameter change requests are not an option. However, parameters can be extracted for %FRC, %MicroR, and %Hypo-He. To explore whether off-line analyses using combined parameters could improve accuracy, we used 148 schistocyte-positive samples and performed multiple regression analyses with various combinations of parameters to determine the best-fitting factors. Combinations of independent variables were as follows: %FRC + %MicroR + %Hypo-He; %FRC + %MicroR; and %FRC + %Hypo-He. Table 2 summarizes the calculated factors and shows that the contribution of %Hypo-He was nearly negligible. We used the optimized factors to test the accuracy of diagnosis using %FRC or %FRC + %MicroR on 1,026 samples. We observed that adding %MicroR to %FRC did not change the accuracy of %FRC (%FRC vs. %FRC + %MicroR: sensitivity, 86.5% vs. 87.2%; specificity, 68.7% vs. 68.8%) (Figure 1B).

To examine whether a two-step analysis consisting of a combination of the default algorithm "fragments?" and %FRC could improve accuracy, we tested each parameter using the 513 samples that were flagged as positive by the "fragments?" of the Sysmex XN-3000. The combination of "fragments?" and %FRC yielded a sensitivity of 86.5% and a specificity of 70.3% (Table 3). Of the samples flagged as positive by "fragments?" (n = 513), 498 (97%) were above the reference range (= cutoff value 2.4%) of %FRC and 15 (3.0%) were below the reference range; among the samples flagged as negative (n = 513), 90 (17.5%) were above the reference range of %FRC and 423 (82.5%) were below the reference range.

DISCUSSION

Manual microscopic examination of schistocytes is essential for the diagnosis of various diseases associated with micro- and macroangiopathic hemolytic anemia. However, manual examination is extremely tedious and time-consuming. Although automated hematological analyzers can be used as adjunctive methods to assist diagnosis, accuracy of currently available analyzers have not yet been extensively evaluated. In the present study, we evaluated the performance of the Sysmex XN-3000 hematology analyzer in the detection of schistocytes using peripheral blood samples that were concurrently evaluated through microscopic examinations. We performed off-line analyses using various cellular parameters extracted from the XN-3000. Our data build on previous findings that the default algorithm had high specificity but low sensitivity, leading to overestimation of schistocytes [7,9].

We extracted three cellular parameters (%FRC, %MicroR, and %Hypo-He) from the XN-3000. We endeavored to optimize the detection algorithm through multiple regression analyses using schistocyte-positive samples diagnosed by microscopic examination. Our data demonstrated that a combination of the default algorithm (i.e., "fragments?") and %FRC exhibited the best accuracy and had > 70% specificity, which is practical for clinical use.

Although the XN-series can examine approximately 30,000 RBCs within minutes, the automated hematolo-

Table 2. Multiple regression analyses of combined cellular parameters using schistocyte-positive samples.

Combinations	Factors			β_0	r^2	p-value
	%FRC	%MicroR	%Hypo-He			
%FRC + %MicroR + %Hypo-He	0.156	0.025	-0.02	0.153	0.319	< 0.0001
%FRC + %MicroR	0.147	0.005	-	0.173	0.31	< 0.0001
%FRC + %Hypo-He	0.153	-	0	0.189	0.306	0.005

Table 3. Sensitivity and specificity of combinations of %FRC and the default algorithm "fragments?".

	Sensitivity	Specificity	PPV	NPV
Fragments?	1.000	0.416	0.288	1.000
Fragments? + %FRC	0.865	0.703	0.337	0.969

PPV - Positive Predictive Value. NPV - Negative Predictive Value.

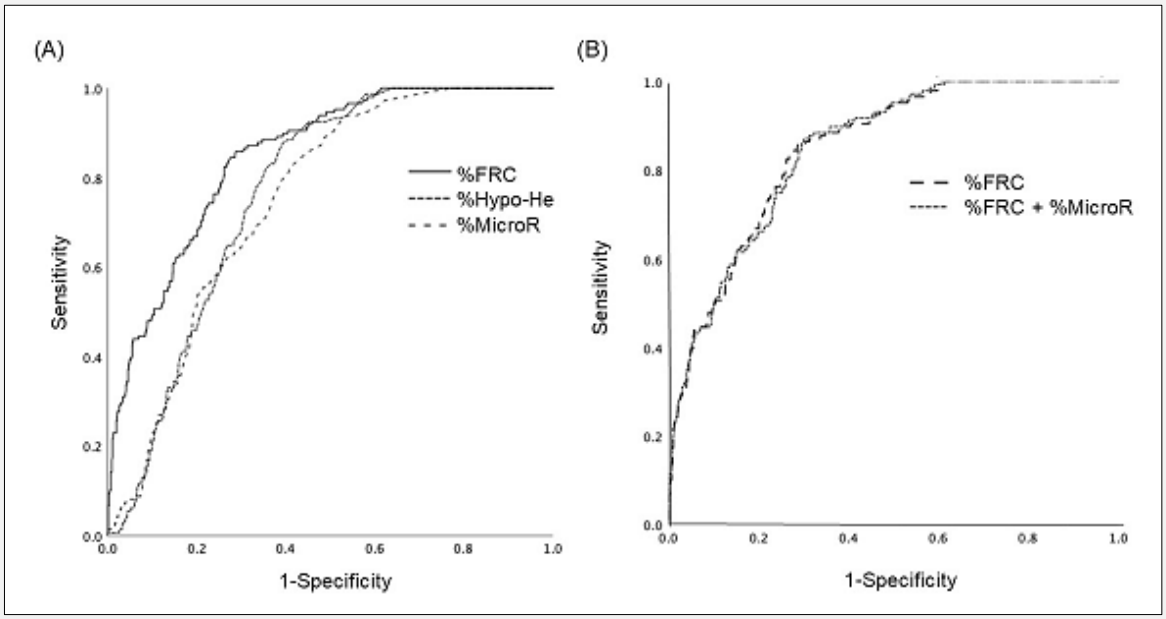

Figure 1. (A) ROC curves for prediction of schistocytes using %FRC, %Hypo-He, and %MicroR.
(B) ROC curves for prediction of schistocytes using %FRC and %FRC + %MicroR.

gy analyzers can only measure cell sizes. The reflected fluorescence signals represent structural complexity and cytoplasmic fluorescence density. The presence of microcytosis, and hypochromia in particular, could lead to misdiagnosis of FRCs because current automated analyzers cannot directly evaluate cellular morphology. Overestimation of FRCs has been reported as a result of microcytic iron deficiency anemia and microcytic hypochromic anemia [8,16]. In the future, these weaknesses may be overcome through image diagnosis using deep

convolutional neural networks [17]. We are currently upgrading the learning system for various hematological disorders.

This study had several limitations. First, it was a single-center study with a relatively small number of samples. Second, we only tested the Sysmex XN-3000, and results could differ among analyzers. Further studies are warranted to compare results generated by different algorithms. Third, when specificities become higher, sensitivities become lower (Figure 1B), leading to an underestimation of schistocytes. This may be improved using the automated image analysis system that we are developing [17].

In conclusion, these results demonstrate that a combination of the default algorithm "fragments?" with the FRC% parameter from the Sysmex XN-3000 is most effective for rapid screening of schistocyte-positive blood samples.

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Declaration of Interest:
The authors declare no competing financial interests.

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