Anne Winther-Larsen*, Else Marie Vestergaard and Anders Abildgaard

Clinical value of smear review of flagged samples analyzed with the Sysmex XN hematology analyzer

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Abstract

Objectives: A smear review is typically made in flagged differential counts performed with hematology analyzers although the clinical value of such reviews is uncertain. Therefore, we evaluated the differences in differential counts between Sysmex XN-9000 and a smear review in flagged samples. Furthermore, the clinical value of blasts identified was investigated.

Methods: Data on all differential counts performed in a two-year period were identified at two laboratories. In patients with blasts, the electronic health record was reviewed. Agreement between automated and manual differential counts was evaluated by Bland-Altman plots. Concordance between the two methods categorized according to reference intervals was evaluated and adjusted for irrelevant non-concordance caused by random analytical error.

Results: In total, 5,500 flagged differential counts were identified from 4,092 patients. A good agreement between the automated and manual differential count was found for all cell types (-0.480×10^9/L to 0.297×10^9/L). The concordance between the two methods was excellent for all cell types, except for monocytes (82 %) where the automated estimates were higher than the manual in 19 % of samples. Blasts were identified in 241 (1 %) of smear reviews. Acute leukemia was diagnosed in 13 (5 %) patients, and only in one patient contributed the detection of blasts to the suspicion of acute leukemia.

Conclusions: Our findings indicate that routine smear review of all flagged samples do not contribute with additional, significant information. After local validation and dialogue with clinical departments, such reviews may potentially be omitted to increase cost-effectiveness and reduce turn-around-time.

Keywords: white blood cell differential count; blasts; smear review; Sysmex hematology analyzer

Introduction

Differential count of white blood cells (WBCs) in the peripheral blood is a very common analysis used for investigating quantitative and morphologic abnormalities of the WBCs [1]. Prompt results are often needed, and to achieve this, high-throughput automated hematology analyzers that can perform differential counts of WBCs based on thousands of cells within a very short time and with a high degree of precision are widely used [2–4]. However, if no differential count of cells can be performed due to abnormal cells in the sample, a manual count of cells is needed. Furthermore, if a differential count of cells can be performed, but pathological WBCs are suspected in the sample, a smear review is still indicated before the result can be released [5]. In such cases, the instrument reports a “flag” highlighting the suspicion. Even though these flags have a relatively high sensitivity, particularly the blast flag, the specificity is only moderate [3]. This leads to a large number of false-positive samples flagged, which requires manual labor to confirm, or more likely disconfirm, the presence of blast cells etc. [6, 7]. This is a laborious procedure that significantly increases the turn-around-time (TAT) of the analysis [6] and requires a trained laboratory technician on duty 24-7. Consequently, there is a constant desire to optimize the number of WBC differential counts that needs a manual review.

A great concern in optimizing the smear review process is the risk of misclassification of cells as well as overlooking blasts, which may be an early warning of hematological malignancies. Nonetheless, as the manual differential count

***Corresponding author: Anne Winther-Larsen**, Department of Clinical Biochemistry, Aarhus University Hospital, Palle Juul-Jensens Boulevard 99, 8200 Aarhus, Denmark; and Department of Clinical Medicine, Aarhus University, 8200 Aarhus, Denmark, E-mail: anlarsen@rm.dk. https://orcid.org/0000-0002-2763-595X

Else Marie Vestergaard, Department of Clinical Biochemistry, Regional Hospital Horsens, Horsens, Denmark; and Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

Anders Abildgaard, Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark; Department of Clinical Medicine, Aarhus University, Aarhus, Denmark; and Department of Clinical Biochemistry, Regional Hospital Horsens, Horsens, Denmark. https://orcid.org/0000-0001-7272-0953

suffers from imprecision due to the counting of typically only 100 cells and the subjective interpretation of the examiner [8, 9], it is of outmost importance to evaluate if smear reviews actually add additional clinical value for clinicians and patients. Previous studies have shown that the neutrophil count in flagged samples from the automated hematology analyzer is reliable and can be released without a manual review [10, 11]. However, no real-world data exist on the differences in WBC counts between the automated differential count on the Sysmex XN-9000 analyzer and a subsequent manual differential count in flagged samples. Additionally, information on how often the finding of blasts in flagged samples actually is a key finding in the diagnosis of a new hematological disease is missing. Hence, the clinical value of smear reviews in flagged samples in a high throughput laboratory is difficult to determine. Therefore, the aim of this study was to evaluate the differences in WBC differential count between the automated hematology analyzer Sysmex XN-9000 and a subsequent smear review in flagged samples during a two-year period at two different laboratories in Denmark analyzing samples from general practitioners as well as from hospital in- and outpatients. Furthermore, the clinical value of blasts identified in the smear review was evaluated. Lastly, the frequency of blasts found due to flagging of the WBC differential count at time of diagnosis in acute leukemia patients during a 2.5-year period was evaluated.

Materials and methods

Subjects and data collection

In this registry-based multicenter study, all WBC differential counts performed in a two-year period were identified in the laboratory information system at two laboratories in Denmark; Department of Clinical Biochemistry at Regional Hospital Horsens (RHH) (1st of October 2019–30th of September 2021) and Department of Clinical Biochemistry at Aarhus University Hospital (AUH) (1st of January 2021–31st of December 2022). Both laboratories are accredited according to ISO 15189 and perform analyses for both general practitioners and the hospital. RHH has a catchment area of 217.000 individuals and consists of an internal medicine and several surgery departments. AUH has a catchment area of 370.000 individuals and comprise a broad number of specialized departments including pediatric, hematology and oncology departments besides internal medicine and surgery departments. Both hospitals receive emergency admissions.

Results from the WBC differential count made on the Sysmex XN-9000 automated hematology analyzer were extracted on all patients together with the WBC differential count results from a subsequent smear review, if performed, as well as the corresponding hematological quantities (thrombocytes, hemoglobin and leukocytes). In patients where the smear review had identified blasts (independent of which flag had released the smear review), data on medical diagnoses was extracted from the electronic health record in a period from two years before until two years after the date of the smear review. These data together with the complete electronic health record were reviewed to evaluate the value of the blast result on the diagnosis of a new hematological disease. If a patient had additional samples showing blasts, only the first sample identifying blasts was considered. A hematological disease diagnosed within 90 days after the WBC differential count was classified as a "new hematological disease".

To characterize hematological findings in blood samples from patients with acute leukemia in general, including the presence of "alarm signs" such as bicytopenia, pancytopenia, blasts etc., consecutive patients diagnosed with acute lymphoblastic leukemia or acute myeloid leukemia and without a previous known hematological disease (such as myelodysplastic syndrome) in The Central Region of Denmark (1.365.262 habitants [12]) from January 2021 to June 2023 were extracted from the electronic health record. Hematology laboratory results from samples obtained immediately before the time of diagnosis were extracted.

The study was approved as a quality assurance project by the hospital managements. According to the Danish Health Care Act, requirement for informed consent was waived.

Hematology analyses

All hematology quantities including WBC differential counts were performed within 24 h after blood sample collection in K-EDTA tubes (Becton Dickinson A/S, Herlev, Denmark) with a Sysmex XN-9000 automated hematology analyzer (Sysmex Corporation, Kobe, Japan). At AUH, the white blood cell differential (WDF) channel was used. For the smear review, a Sysmex SP-10 automated stainer (Sysmex Corporation, Kobe, Japan) was used to stain samples with May-Grünwald Giemsa, and a preclassification of 100 WBCs was performed by the cell image analyzer CellaVision DM96™ (CellaVision AB, Lund, Sweden). Subsequently, reclassification of smears was performed by highly trained laboratory technicians. The "flags" releasing a smear review were the following: atypical lymphocytes?, basophils

>0.3 × 10^9, lymphocytes >5.0 × 10^9, monocytes > 3.0 × 10^9, abnormal eosinophils, abnormal lymphocytes, blasts flag and immature granulocytes >10 %.

Daily internal quality controls at two levels were analyzed. The analytical coefficient of variation (CV) of the two WBC controls was 2.3 % and 1.9 % at RHH, and 2.3 % and 1.8 % at AUH during the time period. Both laboratories participated in external quality assurance schemes (10 rounds per year).

Statistical analyses

All values are presented as medians with interquartile ranges (IQR), unless otherwise stated. Bland-Altman plots were used to assess the agreement between the automated and manual WBC differential count [13]. To further evaluate the concordance between automated and manual WBC differential counts for each cell type, the differential counts were categorized according to the reference intervals (i.e. below/within/above). Reference intervals from [14] were used for adults, whereas intervals from [15] were used for children. To adjust for clinically irrelevant non-concordance that may arise from random analytical error as well as the lack of biological variation in a single sample, we also evaluated the concordance between automated and manual differential counts when only samples with differences exceeding the reference change value (RCV) were considered non-concordant. The RCV was calculated based on the inter-laboratory variation (CV_a) observed in an external quality assurance scheme for manual differential counts during the study period (Equalis program #027, Sweden). As expected, the CV_a mainly depended on the absolute number of cells counted of each cell type (sampling uncertainty) (Supplementary Figure 1). The estimated median within-subject biological variation (CV_i) for each cell type was extracted from the EFLM Biological Variation Database [16], and RCV was calculated as $2.77 \times \sqrt{(CV_a^2 + CV_i^2)}$. Statistical analyses were performed using Excel 2016 (Microsoft Corporation, Washington, US) and the add-in software Analyze-It for statistical analyses in Microsoft excel (Analyze-It Software, Leeds, UK).

Results

WBC differential counts

A total of 636,040 WBC differential counts were identified at the two departments during the time period. A manual differential count had been performed in 4.8 % of all samples (Figure 1). In the majority of samples (73 %), the manual differential count was triggered by the Sysmex XN-9000 analyzer due to flagging. In 75 % of the flagged samples, the WBC differential count made by the Sysmex analyzer was confirmed due to concordance with a manual smear review

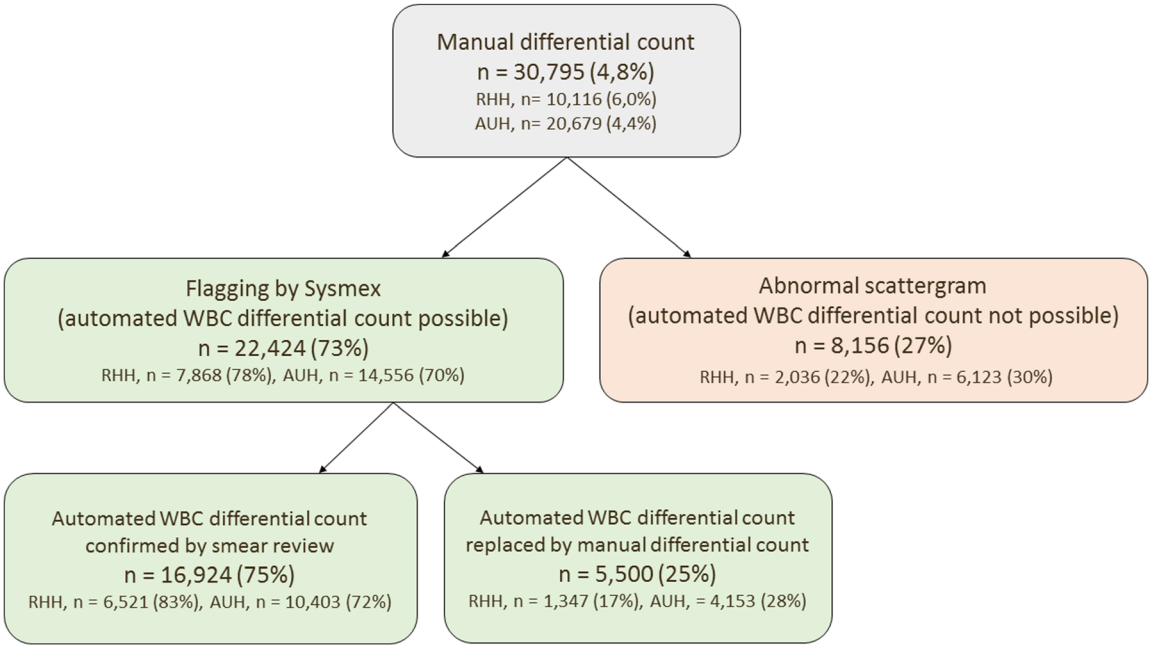

Figure 1: Flow chart showing the number of manual white blood cell differential counts performed at two Departments of Clinical Biochemistry in Denmark in a 2-years period. RHH, Regional Hospital Horsens; AUH Aarhus University Hospital.

Table 1: Characteristics of study samples with results available from automated and manual white blood cell (WBC) differential count (n=5,500). The samples comprised 4.8 % of all requested WBC differential counts in the 2-year period (n=636,040).

Characteristics	
Sex, m/f	51 %/49 %
Age, years, median (IQR)	49 (13–71)
0–18	1,623 (30)
18–40	860 (16)
41–60	865 (16)
61–80	1,600 (29)
80–98	552 (10)
Hematological parameters, median (IQR)	
Hemoglobin, mmol/L	7.3 (5.8–8.4)
Leukocytes, $\times 10^9$/L	9.24 (6.0–12.8)
Platelets, $\times 10^9$/L	217 (42–321)
Requesting department	
General practitioners	1,515 (28 %)
Emergency department	1,391 (25 %)
Depts. of internal medicine	1,174 (21 %)
Pediatric department	556 (10 %)
Intensive care units	234 (4 %)
Surgical department	201 (4 %)
Hematological department	56 (1 %)
Other	373 (7 %)

All values are presented as n (%), unless otherwise stated. m, male; f, female.

performed by a trained technician, and in 5,500 samples (25 %), the automated WBC differential count was supplemented with a manual differential count based on the smear review (Figure 1). The characteristics of these 5,500 sample are shown in Table 1. The median WBC count was 9.24×10^9/L (IQR, 6.0–12.8).

Concordance between automated WBC differential count by Sysmex and the smear review

Results from the automated and the manual differential counts performed in the 5,500 samples are shown in Figure 2 and Supplementary Table 1 for each cell type. Bland-Altman plots were conducted to evaluate the agreement between the automated and manual counts, and overall, a good agreement between the two methods was found for all cell types with mean bias deviating from -0.480×10^9/L (neutrophils, 95 % limits of agreement (LoA): -2.83–1.87×10^9/L) to 0.297×10^9/L (monocytes, 95 % LoA: -0.870–1.46×10^9/L) (Supplementary Figures S2–S7). To further evaluate the concordance between the two methods, samples were categorized according to the reference intervals (below/within/above) for each of the two methods (Table 2). When the crude results were categorized and compared, a relative low concordance was found for monocytes (64 %), basophils (61 %) and immature granulocytes (51 %). Typically, an overestimation of cells was seen in the automated WBC differential counts for these cell types. However, when the potential contribution of sampling uncertainty was considered and only differences exceeding the RCV were assessed, the concordance was excellent for all cell types (97–100 %), except for monocytes (82 %) for which the automated estimates still remained higher than the manual in 19 % of samples (Table 2).

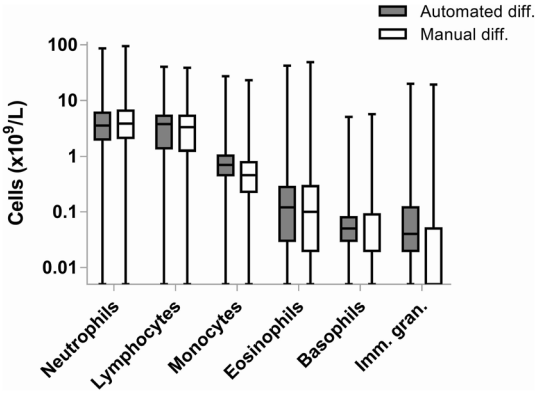

Figure 2: Results of flagged samples after automated and manual WBC differential count according to cell types. Horizontal lines represent median value and boxes represent interquartile range; whiskers represent range (min-max). n=5,500.

Regarding neutrophils, clinical decision limits for neutropenia of 0.5 or 1.0 $\times 10^9$/L are commonly used in e.g. oncology and hematology. When these cell concentrations were used as cut-off, a concordance of 99% and 98 %, respectively, was observed. After correction for sampling uncertainty, a concordance of 100 % was seen for both decision limits.

Clinical value of blasts identified in smear reviews

Among all the smear reviews performed due to flagging by the Sysmex analyzer, blasts were identified in 1 % (328/22,424) of samples. By review of the corresponding electronic health records, the clinical importance of the blast result was thoroughly evaluated in each case, and patients were classified according to a present or a subsequent diagnosis (Table 3). As shown, the majority of patients either had follow-up from previously diagnosed malignant hematological disease (35 %), or the blast result was an incidental finding with no previous or subsequent finding

Table 2: Concordance between flagged automated and manual white blood cell differential counts for each cell type categorized according to the reference interval (below/normal/above). In the left part, concordance was evaluated based on the actual results. In the right part, non-concordance was only considered when the difference between manual and automated estimates exceeded the reference change value (RCV) to adjust for random analytical and biological variation.

		Concordance, over-all			Concordance, RCV exceeded		
	Automated / Manual	Below	Normal	Above	Below	Normal	Above
Neutrophils	Below	825 (15 %)	95 (2 %)	0 (0 %)	981 (18 %)	24 (<1 %)	0 (0 %)
	Normal	158 (3 %)	3,055 (56 %)	32 (1 %)	1 (<1 %)	3,338 (61 %)	5 (<1 %)
	Above	1 (<1 %)	215 (4 %)	1,119 (20 %)	0 (0 %)	3 (<1 %)	1,145 (21 %)
Lymphocytes	Below	1,068 (19 %)	243 (4 %)	2 (<1 %)	1,155 (21 %)	85 (2 %)	2 (<1 %)
	Normal	95 (2 %)	1,915 (35 %)	294 (5 %)	5 (<1 %)	2,211 (40 %)	29 (1 %)
	Above	2 (<1 %)	142 (3 %)	1,739 (32 %)	2 (<1 %)	4 (<1 %)	2,004 (36 %)
Monocytes	Below	705 (13 %)	1,059 (19 %)	91 (2 %)	746 (14 %)	674 (12 %)	86 (2 %)
	Normal	40 (1 %)	2,097 (38 %)	642 (12 %)	0 (0 %)	2,646 (48 %)	253 (5 %)
	Above	2 (<1 %)	166 (3 %)	698 (13 %)	1 (<1 %)	2 (<1 %)	1,092 (20 %)
Eosinophils	Below	892 (16 %)	783 (14 %)	18 (<1 %)	1,025 (19 %)	46 (1 %)	15 (<1 %)
	Normal	134 (2 %)	2,934 (53 %)	101 (2 %)	3 (<1 %)	3,879 (71 %)	21 (<1 %)
	Above	3 (<1 %)	210 (4 %)	425 (8 %)	1 (<1 %)	2 (<1 %)	508 (9 %)
Basophils	Below	1 (<1 %)	597 (11 %)	164 (3 %)	1 (<1 %)	0 (0 %)	0 (0 %)
	Normal	0 (0 %)	2,799 (51 %)	540 (10 %)	0 (0 %)	4,231 (77 %)	19 (<1 %)
	Above	0 (0 %)	838 (15 %)	561 (10 %)	0 (0 %)	3 (0 %)	1,246 (23 %)
Immature granulocytes	Below	6 (<1 %)	1,024 (19 %)	239 (4 %)	7 (<1 %)	0 (0 %)	0 (0 %)
	Normal	1 (<1 %)	1,685 (31 %)	1,126 (20 %)	0 (0 %)	3,008 (55 %)	25 (<1 %)
	Above	0 (0 %)	310 (6 %)	1,109 (20 %)	0 (0 %)	11 (<1 %)	2,449 (45 %)

Table 3: Diagnoses in patients with blasts (n=241) identified in the manual differential count.

Diagnosis	n (%)
New hematological disease[a]	19 (8 %)
Acute leukemia (n=13)	
Lymphoma (n=2)	
Lymphoproliferative diseases of UNC (n=1)	
Myelodysplastic syndrome (n=1)	
Myelofibrosis (n=1)	
Monoclonal B-cell lymphocytosis (n=1)	
Hematological disease[b]	85 (35 %)
Cancer disease (non-hematological)	32 (13 %)
Infection	28 (12 %)
Unknown cause[c]	77 (32 %)

[a]Patients were classified with a new hematological disease if the disease was diagnosed within 90 days after the white blood cell (WBC) differential count. [b]Follow-up for previously diagnosed hematological disease. [c]No blasts were identified in repeated WBC differentials and no subsequent hematological disease was found.

of blasts or malignant diseases (32 %). In 19 patients, the blast result was followed by diagnosis of a new hematological disease, including 13 cases of acute leukemia. According to the patient record, the blast finding contributed to the suspicion of acute leukemia in only one of the latter 13 cases. On the contrary, overt leukocytosis as well as cytopenias were in all cases the most important findings that raised clinical suspicion of a malignant diagnose.

Peripheral blasts in patients with leukemia

A total of 193 patients diagnosed with acute leukemia were identified in the time period (Table 4). Results on hematological blood parameters, including a WBC differential count, were available at the time of diagnosis for all patients. In 60 patients (31 %), a flagged automated WBC differential count performed by the Sysmex analyzer was observed and a smear review had been performed. Again, a higher monocyte count was identified in the automated differential compared with the manual in these patients, but only minor differences between the two methods were seen for the other cell types (Table 4).

Blasts were found in 29 (48 %) of the flagged samples, and in 72 (78 %) of the samples where no automated WBC differential was possible. Not surprisingly, the median number of blasts found in flagged samples was considerably lower than in the samples where no automated WBC differential count was possible (median 0.03 (IQR: 0–0.53) vs. median 5.11 (IQR: 0.54–25)). Interestingly, for all 29 patients with blasts identified in the flagged samples, at least one

Table 4: Hematological parameters in 193 consecutive patients presenting with acute leukemia without previous known hematological disorder. Results from the blood sample obtained at the time of diagnosis were extracted from each patient. Data were stratified according to whether an automated differential count was possible and/or flagged.

	All patients	Automated diff. without flags	Flagged automated diff.	Automated diff. not possible
n (%)	193 (100 %)	41 (21 %)	60 (31 %)	92 (48 %)
Diagnosis (AML/ALL)	69 %/31 %	59 %/41 %	75 %/25 %	71 %/29 %
Age, years	64 (24–75)	47 (12–69)	67 (43–76)	66 (22–75)
Sex, m/f	56 %/44 %	56 %/44 %	48 %/52 %	62 %/38 %
Hemoglobin, mmol/L	5.8 (4.8–7)	6.3 (5.7–7.4)	6.0 (5.1–6.9)	5.3 (4.3–6.8)
Leukocytes, $\times 10^9$/L	6.5 (2.5–33)	2.7 (1.8–4.3)	2.8 (1.7–5.5)	33 (11–80)
Platelets, $\times 10^9$/L	70 (32–130)	90 (56–175)	88 (43–157)	50 (20–89)
WBC differentiation count, $\times 10^9$/L				
Neutrophils	1.3 (0.34–3.1)	0.79 (0.36–1.6)	M: 0.80 (0.35–1.6) A: 0.69 (0.32–1.3)	2.6 (0.89–6.7)
Lymphocytes	2.6 (1.10–6.2)	1.1 (0.77–2.2)	M: 1.8 (0.75–2.9) A: 1.9 (0.79–2.9)	5.8 (2.8–12)
Monocytes	0.33 (0.07–1.6)	0.19 (0.08–0.46)	M: 0.07 (0.03–0.46) A: 0.36 (0.10–1.1)	0.91 (0.07–3.1)
Eosinophils	0.03 (0.02–0.07)	0.03 (0.02–0.06)	M: 0.05 (0.02–0.07) A: 0.05 (0.03–0.07)	0.02 (0.01–0.17)
Basophils	0.02 (0.02–0.05)	0.02 (0.02–0.03)	M: 0.02 (0–0.05) A: 0.03 (0.02–0.05)	0.02 (0–0.05)
Immature granulocytes	0.03 (0.02–0.47)	0.02 (0.02–0.03)	M: 0 (0–0.03) A: 0.04 (0.02–0.18)	0.25 (0–1.8)
Blasts	0.03 (0–4.8)	NA	M: 0.03 (0–0.53) A: NA	5.11 (0.54–25)
Alarm signs, n (%)				
Bicytopenia	97 (50 %)	9 (22 %)	23 (38 %)	65 (71 %)
Blasts[a]	101 (52 %)	0 (0 %)	29 (48 %)	72 (78 %)
Leukocytosis	81 (42 %)	2 (4.9 %)	10 (17 %)	69 (75 %)
Neutropenia	117 (61 %)	32 (78 %)	48 (80 %)	37 (40 %)
Pancytopenia	59 (31 %)	22 (54 %)	28 (47 %)	9 (9.8 %)

All values are presented as medians (IQR), unless otherwise stated. AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; m, male; f, female; WBC, white blood cell; M, manual differential count; A, automated differential count. [a]No patients presented with blasts as the only of the listed "alarm signs".

further "alarm sign" was present among the hematological laboratory results, including pancytopenia (n=10), bicytopenia (n=15), leukocytosis (n=6), or neutropenia (n=22).

Discussion

Even though the workflow around WBC differential counts have improved considerable in hematology laboratories during the last decades, there still is a constant desire to decrease the number of samples that require smear reviews in order to decrease the TAT and decrease the labor-intensive procedures in the laboratory. Therefore, we conducted a large multicenter study comprising two hospitals with different patient populations to estimate the differences in WBC differential counts between the Sysmex XN-9000 and the subsequent smear review in flagged samples as well as to assess the clinical importance of the finding of blasts during the smear review. Overall, we found excellent agreement between the two methods except for monocytes, which were generally underestimated in the manual differential counts compared with the automated. Blasts were detected in only 1 % of all flagged samples, and the clinical value of the blast results appeared to be rather limited for the diagnosis of acute leukemia.

We found very low biases between the Sysmex result and the smear review for all cell types. In comparison with Schapkaitz et al. [3], who evaluated the performance of the Sysmex XN-9000 analyzer, our bias was lower for all cell types. However, their material was a mixture of samples

with normal and abnormal hematology profiles and not flagged samples as in our study. Such differences in sample selection may explain the differences in bias found. Despite the high agreement between the automated and manual differential counts in our study, we expanded the comparison and evaluated the number of samples deviating according to the reference intervals. We found relatively large differences when results were categorized according to above, within or below the reference interval based on raw data. However, the WBC differential count is affected by a high analytical variation, which has to be accounted for in a comparison [8, 9]. Indeed, a manual differential count of 100–200 cells is subject to an inherent huge sampling error, particularly for the rare cell types [17]. Therefore, we calculated a level-dependent analytical variation which allowed us to correct the non-concordant results for minor and clinically insignificant differences caused by such random error. The estimates of analytical variation were obtained from an external quality scheme which also encompasses any inter-operator variation introduced by the counting technician. In turn, we observed a highly improved concordance when the differences between automated and manual estimates were adjusted accordingly. Concordant results were seen for at least 97 % of all samples for each cell type, except for monocytes where automated estimates were found to be somewhat higher. Similarly, a higher monocyte count on Sysmex XN-9000 compared with a manual differential count has been shown previously [18]. Remarkably, these authors compared manual monocyte concentrations obtained by microscope as well as by DI-60 (Cellavision) with automated results from Sysmex XN and flow cytometry. Interestingly, the number of CD45+CD14+ cells identified by flow cytometry, which is considered the gold standard, were comparable with results from Sysmex XN, whereas both manual methods caused underestimation of the monocyte concentrations. It is well-known that monocytes are often unreliably detected in manual differential counts, partly due to heterogeneous distribution and partly due to misclassification [19]. Nonetheless, falsely reported monocytosis by the Sysmex analyzer cannot be definitively excluded in some cases. Since the criteria for the diagnosis of the clinically important condition chronic myelomonocytic leukemia partly depends on an absolute monocyte concentration as low as $>0.5 \times 10^9$/L [20], the role of manual differential counts still remain limited due to the high sampling error for rare cells. Further studies should be conducted to evaluate the optimal method for the reliable detection of peripheral monocytes. Taken together our findings suggest that the WBC differential count of flagged samples by the Sysmex XN instrument is reliable and do not warrant smear review to achieve more accurate estimates of cell concentrations.

Several previous studies have assessed the sensitivity and specificity of the blast flag on automated hematology analyzers [6, 7, 21]. Blasts cannot be automatically measured by the Sysmex instrument, but no previous study has evaluated the actually clinical value of a blast finding in smear reviews performed due to flagging by Sysmex XN. In this study, blasts were present in only 1 % of the more than the 22,000 flagged samples. As few as 13 patients were consequently diagnosed with acute leukemia, and, in solely one of these patients, the blast result was mentioned in the electronic health record. In contrast, all patients with an undiagnosed severe hematological disorder had other significant abnormalities among the hematology lab results that were likely to raise clinical suspicion of malignancy. Hence, based on our data, the significance of a blast finding in flagged samples seems negligible in raising clinical suspicion of malignant hematological disease. To further substantiate our findings, we extracted data from all acute leukemia patients over a 2.5-years period and showed that in only 29/193 of these patients, blasts were identified following a flagged sample on the Sysmex XN instrument. Reassuringly, all these patients showed at least one further significant abnormal hematology parameter potentially serving as a clinical “alarm sign”. Intriguingly, approximately one third of all flagged samples with blasts actually appeared to be clinically irrelevant since the blasts were an incidental finding not associated with new or ongoing disease. Such findings should, therefore, be considered false positives. According to the Choosing Wisely Campaign, false positive results could result in significant economic and emotional burden and may lead to additional and unnecessary testing [22].

Our findings challenge the state of art where blast detection is considered a key feature of the WBC differential count [5–7]. Historically, WBC differential count by manual microscopy has played an important role in the diagnosis of malignant hematological diseases. However, with the development of more complex cytochemical, molecular and immunophenotypic tests, the impact of a WBC differential count has changed. As an example, our local hematological department at AUH does not even require a full differential count performed on their patients since they only need the neutrophil count to treat and monitor the patients sufficiently. Nowadays, the value of the analysis lies, thus, primarily in the screening for blasts as a non-specific alarm sign of malignancy. As the resources of the health care systems are not unlimited, the costs associated with a laboratory test should be proportional to the clinical value added by the test. When it comes to smear reviews of flagged samples, we believe that this is not the case, and our local clinical departments agreed with us. Indeed, such changes should

always be made in close dialogue with the relevant clinical departments. However, based on our findings, it appears reasonable to consider whether routine smear review of all flagged samples may be omitted.

As stated previously, manual differential counts are laborious and time-consuming. Another issue relates to the competences needed to perform an accurate differential count without misclassification of cells. It may be difficult to maintain sufficient competences to correctly identify rare cells 24/7 and immature cells may be infrequently observed at hospitals without a dedicated hematological department. Consequently, manual differential counts are only performed in daytime working hours on weekdays at many Danish hospitals. In cases of e.g. acute leukemia, manual differential count results reported with a time delay of 12–72 h are of limited clinical usefulness. However, our present findings support the assumption that results from automated differential counts of flagged samples are reliable and can be immediately released without a time delay due to manual smear review.

In this study, we included a large number of samples from the routine workflow at two diverse hospitals with different patient populations which increases the impact and generalizability of our data. A limitation in the study was the retrospective nature of the patient data as the clinical value of the blast results was evaluated based on a retrospective examination of the electronic health records. Potentially, the blast results might have had an impact on the suspicion of malignancy even though it was not explicitly stated in the health records.

In conclusion, we found excellent agreement between the automated and manual differential count in flagged samples, even though a higher monocyte count was observed in the automated differential count. Furthermore, blasts were rarely observed in patients without a prior diagnosis, and the significance of a blast finding seemed negligible in the screening for malignant hematological disease. Thus, our data suggest that omission of routine smear review of all flagged samples may be considered without significant clinical risk or loss of relevant information, but further validation of our conclusions is warranted. Indeed, omission of smear reviews would improve TAT and decrease the labor-intensive procedures in the laboratory.

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