Evaluation of the Automated Immature Granulocyte Count (IG) on Sysmex XE-2100 Automated Haematology Analyser vs. Visual Microscopy (NCCLS H20-A)

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The presence of immature granulocytes (IG) in peripheral blood is potentially important information indicating enhanced bone marrow activation. The quantification of immature granulocytes is routinely done by visual microscopy, which is a labour intensive and time consuming task. New software releases "XE Pro" and "IG Master" for the Sysmex XE-2100 automated haematology analyser allow simple, fast and inexpensive quantification of promyelocytes, myelocytes and metamyelocytes summarized in the "IG count", as claimed by the manufacturer.

The present study evaluated the agreement of the XE-2100 IG count with visual microscopy (NCCLS H20-A) in 246 routine blood samples containing more than 0.1 × 10^3 /μL IG as reported by the automated analyser. Passing-Bablock regression and correlation analysis showed very good agreement (r=0.900) between the two methods. A subset of pre-term and neonatal samples showed similarly good results (r=0.884). The IG count performed noticeably better in this study than with the previous XE-2100 software packages as reported in the literature. Band forms are not included in the IG count, though the absolute values of the automated counts tend to be slightly higher than those obtained by microscopy.

There is need for clinical studies on the performance of the automated IG count in routine patient care and generally accepted reference ranges in normal and diseased subjects have yet to be established.

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INTRODUCTION

The presence of immature granulocytes (IG) in peripheral blood is potentially important information indicating enhanced bone marrow activation. Besides the obvious significance of blasts for the diagnosis of leukaemia, the promyelocyte, myelocyte and metamyelocyte stages of myeloid maturation may indicate systemic inflammatory stress or leukaemic reactions. The quantification of immature granulocytes is routinely done by visual microscopy, which is a labour intensive and time consuming task. The traditional visual 100-cell differential has further disadvantages: due to the small number of cells counted it shows high imprecision, low reproducibility and a relatively wide inter-examiner variability. Thus, there is need for a reliable automated quantitative IG count.

New software releases "XE Pro" and "IG Master" for the Sysmex XE-2100 automated haematology analyser allows simple, fast and inexpensive quantification of promyelocytes, myelocytes and metamyelocytes summarized in the "IG count", as claimed by the manufacturer. The number of IG is reported both as absolute cell count and as a percentage of total white blood cells (WBC).

The present study examined the agreement, accuracy and reproducibility of the XE-2100 IG count compared to visual microscopy according to the guidelines of the NCCLS protocol (H20-A) in the routine haematology laboratory of a 700-bed teaching hospital (Barmbek General Hospital, Hamburg). Sensitivity of XE-2100 IG detection compared to visual microscopy has been described elsewhere [1].

MATERIALS AND METHODS

Blood specimen selection and processing

During a six-week period all blood specimens submitted for routine WBC differential on weekdays were screened for inclusion in the present study. Specimens were included if they showed an IG count of > 0.1 × 10^3 /μL on initial testing on the Sysmex XE-2100. All samples were submitted in 2.8 mL K_3EDTA Monovettes (Sarstedt, Germany). Repeat specimens (same patient on different days) were also included in the study.

The guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) H20-A [2] procedure for

the microscopic reference leucocyte differential count were used in our study. A set of three smears (A, B, C) for visual microscopy was prepared manually within 90 minutes of arrival of the blood specimen in the haematology laboratory.

Manual microscopy

Smears were stained by May-Grünwald-Giemsa (Pappenheim) stain. Two technicians independently performed 200-cell differentials on smears A and B (total of 400 cells). Smear C was reserved for the resolution of discordant results between smear A and B. Specially trained and experienced haematology technicians performed all microscopy after passing the examiner qualification procedure suggested by NCCLS H20-A (five samples).
The 200-cell differentials A and B were evaluated for reproducibility using confidence limits for specific %-values provided by NCCLS H20-A. Re-evaluation of smear C was performed upon violation of these 95% ranges. These calculations were done for neutrophils (NEUT), lymphocytes (LYMPH), monocytes (MONO), eosinophils (EO), basophils (BASO), and IG as a parameter for the reliability and quality of visual microscopy values. WBC counts were corrected for any nucleated red blood cells present and lymphoblasts and prolymphocytes were counted as lymphocytes.

XE-2100 IG Master software

The XE-2100 haematology analyser (Sysmex Corporation, Kobe, Japan) software version 00-20 was upgraded with the XE Pro software version 00-03 and XE IG Master software version 00-03. Both are plug–in software packs, which can be installed on the existing XE-2100 software, and both are commercially available. XE Pro is a platform software on which additional modules can be installed. XE IG Master is one of these modules. The standard XE-2100 offers the IG count as a research parameter, whereas with the IG Master module, this parameter is reportable.
The XE IG Master uses a newly developed algorithm, which employs flexible gating offering automatic separation of immature granulocytes from the mature granulocytes in the DIFF channel. WBC are stained using STROMATOLYSER-4DS, a polymethine dye which enters the cells and stains the RNA and DNA. The immature granulocytes contain more DNA than compared to mature granulocytes and therefore generate higher fluorescence signals, which are detected by a photomultiplier tube. Internal complexity of the WBC is detected by using the 90 degree scattered light signal. Both signals are plotted in the DIFF scattergram. The Adaptive Cluster Analysis System (ACAS, Sysmex Corporation, Kobe, Japan) separates the WBC sub-populations. Flexible gating of the immature granulocytes is resulting in a reportable IG count which is shown on the main display of the XE-2100 and is transferred via the HOST interface. The Immature Granulocyte population in the DIFF channel is coloured dark blue and can therefore easily be distinguished from the light blue mature neutrophil population (***Fig. 1***). "*e*-check" quality control blood provides quality control for the IG count.

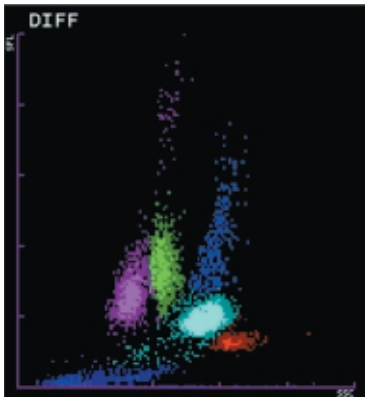

Fig. 1 *Scattergram of DIFF channel*
Note : IG in deep blue, neutrophils and bands in light blue

Reproducibility was assessed by processing clinical samples with various IG count levels. Specimens were analysed repeatedly (3-8 times depending on remaining volumes) within a time period of two hours. Analyses were not done consecutively for a given specimen, but in between other clinical samples. Thus real life reproducibility was obtained rather than a within-batch precision. Standard deviation and coefficient of variation were calculated and plotted against the mean of the IG count.

Statistical data analysis

After processing raw data on Excel worksheets, all statistical analysis was done according to Passing-Bablock linear regression and correlation analysis. All manual counts were used as combined 400-cell differentials reported as percentages ((A+B)/4) and absolute counts were calculated by multiplication with XE-2100 WBC counts.

RESULTS

258 samples initially met the criteria for study inclusion (*i.e.* reportable IG count > 0.1×10^3 /μL). This represented between 5% and 25% of all differentials requested in that period (mean 10% per day). 247 samples had complete data sheets and met the quality criteria of NCCLS H20-A and hence were included in the visual microscopy population. Seven samples were microscopically re-evaluated because of violation of confidence limits when comparing results of the two examiners in one or more specific cell populations. Only one sample had to be excluded for inconsistent results even after repeated examinations due to bad cell morphology. Thus a total of 246 samples were included in the following analysis.

Inter-examiner variation of visual microscopy

Each sample received two separate 200-cell differentials by different technicians and from different smears. Correlation between examiners is shown in ***Figs. 2a - 2b*** for separate cell types. Correlation coefficients ranged from r=0.974 for NEUT, r=0.970 for LYMPH, r=0.855 for MONO, and r=0.952 for EO. Due to low relative values no reliable Passing-Bablock statistics are given for BASO. Band forms (BAND) (r=0.874) and microscopic IG (r=0.803) showed a slightly higher, but acceptable inter-examiner variation due to variable morphology.

Correlation of XE-2100 five-part WBC differential and visual microscopy

Results for conventional cell categories compared excellently between XE-2100 and visual microscopy. Microscopic results were processed as combined 400-cell differentials. Microscopy values were converted into absolute counts by multiplication with the WBC count of the XE-2100.

Fig. 3 shows good correlation for NEUT (r=0.996), LYMPH (r=0.969; omitting one extreme value in a CLL-patient with a LYMPH count of 195.4 × 10^3 /μL by XE-2100 vs. 191.2 × 10^3 /μL by microscopy for better presentation in the graph) and EO (r=0.986). For BASO there is no reliable Passing-Bablock regression and the graph is shown without statistical data. Correlation for MONO was good (r=0.910). Passing-Bablock regression showed a slope of b=1.300 reflecting the well-known phenomenon of enhanced monocyte detection by analysers compared to microscopy.

Correlation of XE-2100 IG count and visual microscopy

The microscopic definition of immature granulocytes

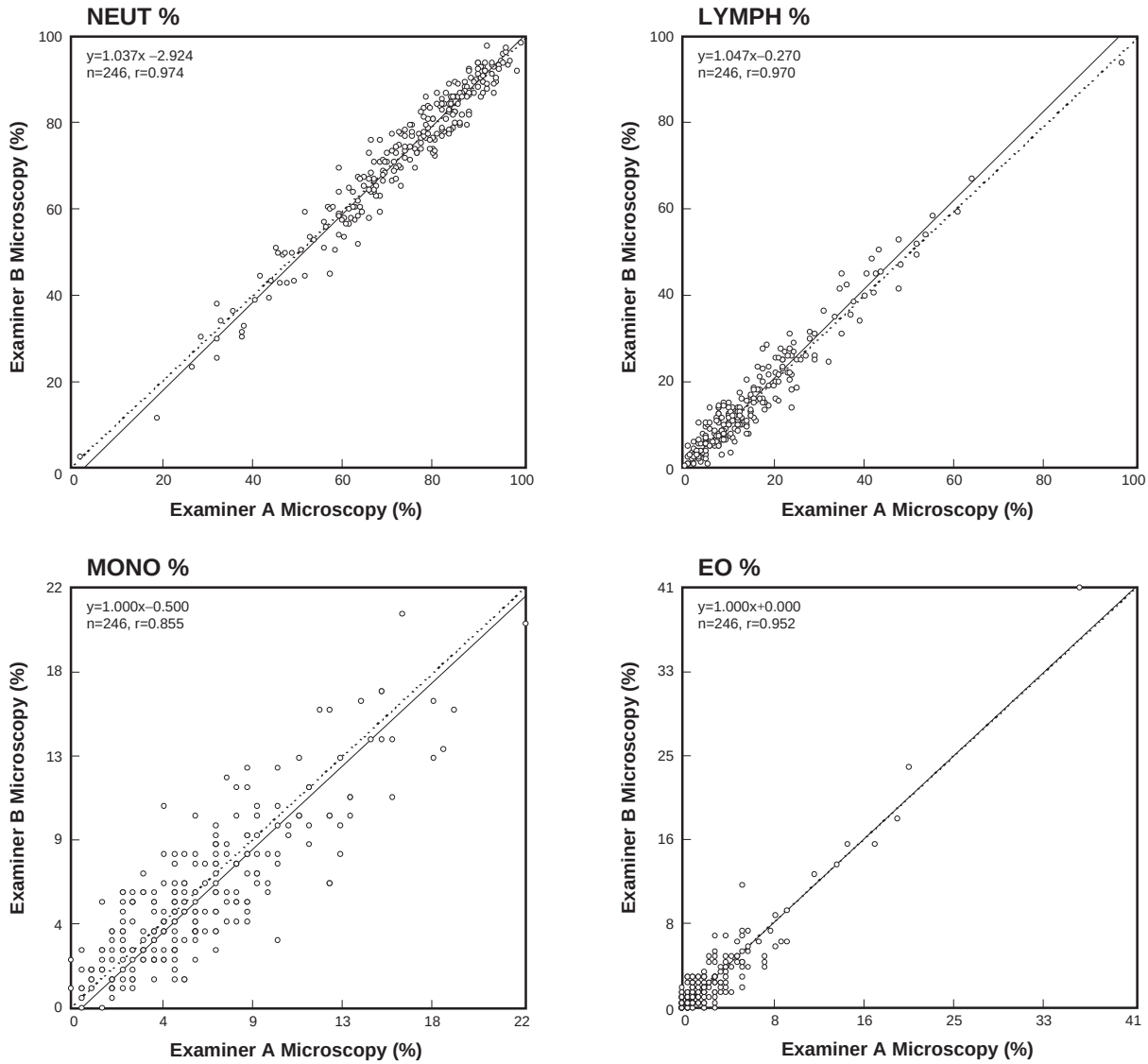

Fig. 2a *Microscopic relative count (%) examiners A vs. B*

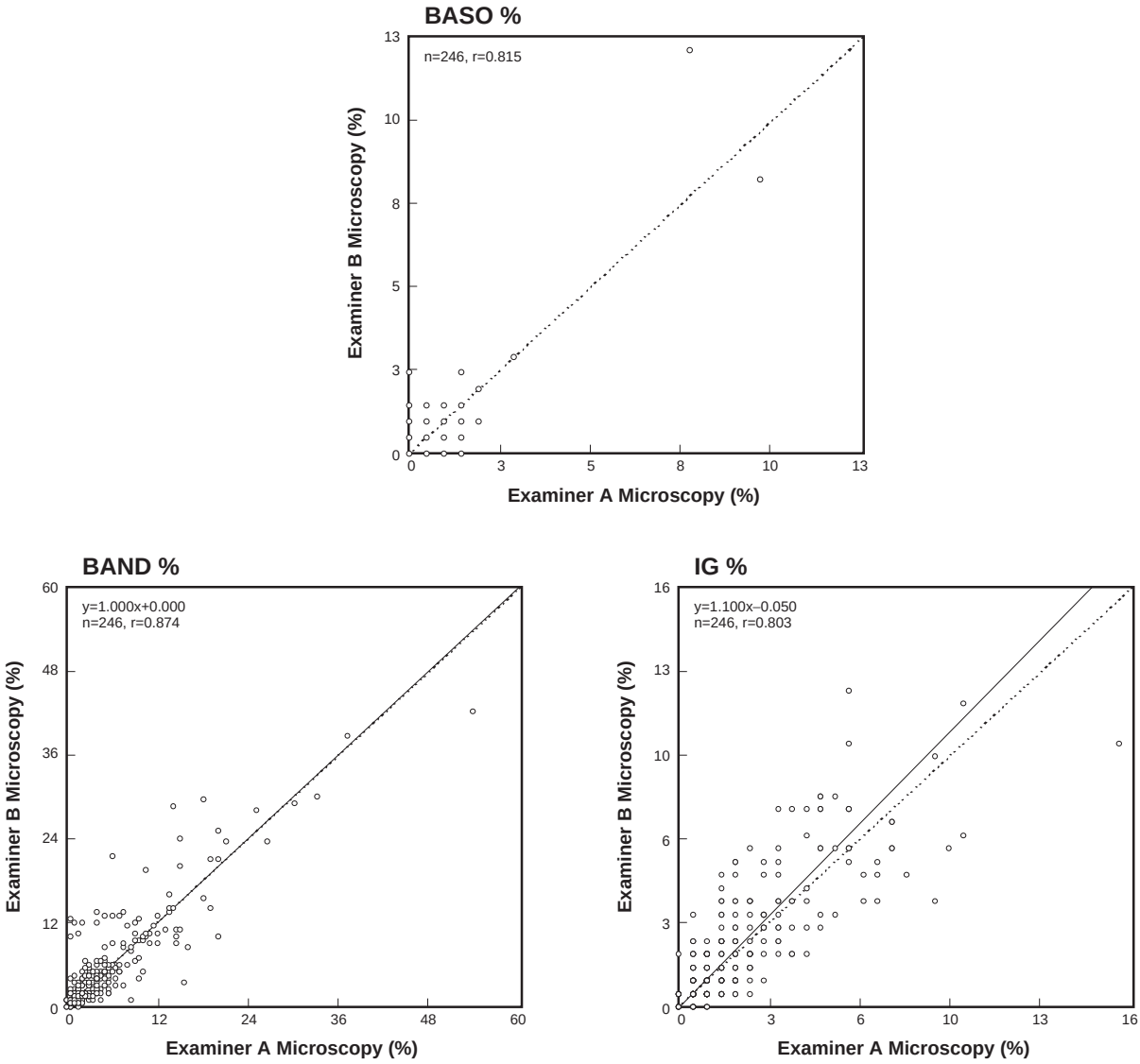

Fig. 2b *Microscopic relative count (%) examiners A vs. B*

(IG) includes metamyelocytes, myelocytes and promyelocytes, thus excluding band forms and blasts. XE-2100 and microscopic values showed good correlation both when comparing IG absolute counts (r=0.900) and IG percentages (r=0.871) (***Fig. 4***). IG counts reported by the XE-2100 tended to be slightly higher than the corresponding microscopic values as suggested by a slope of b=1.153.

Of the 246 reported IG counts 72 had been marked with an asterisk mark by the IG Master software. Asterisk marks denote reduced reliability of a reported value due to special circumstances in the specimen, *e.g.* an abnormal scattergram, extremely high or low WBC counts, or morphological aberrations. On separate analysis of samples whose XE-2100 IG counts were marked by an asterisk and those not marked (***Fig. 5***) no significant difference in correlation with visual microscopy could be demonstrated (r=0.881 vs. r=0.868). Significantly more samples containing high IG concentrations were marked by an asterisk (mean IG count of samples with asterisk: 0.612×10^3 /μL vs. without asterisk 0.265×10^3 /μL).

Correlation of XE-2100 IG count and visual microscopy in pre-term infants and neonates

Pre-term and neonatal blood samples commonly contain immature blood cells of particularly variable morphology and thus frequently need to be differentiated manually. Analysis of the small subset of neonatal study samples (n=24; aged 0-33 days) showed a markedly decreased inter-examiner correlation on visual examination of IG (r=0.529) but good correlation of combined visual differentials with the XE-2100 IG results (r=0.884) (***Figs. 6 and 7***).

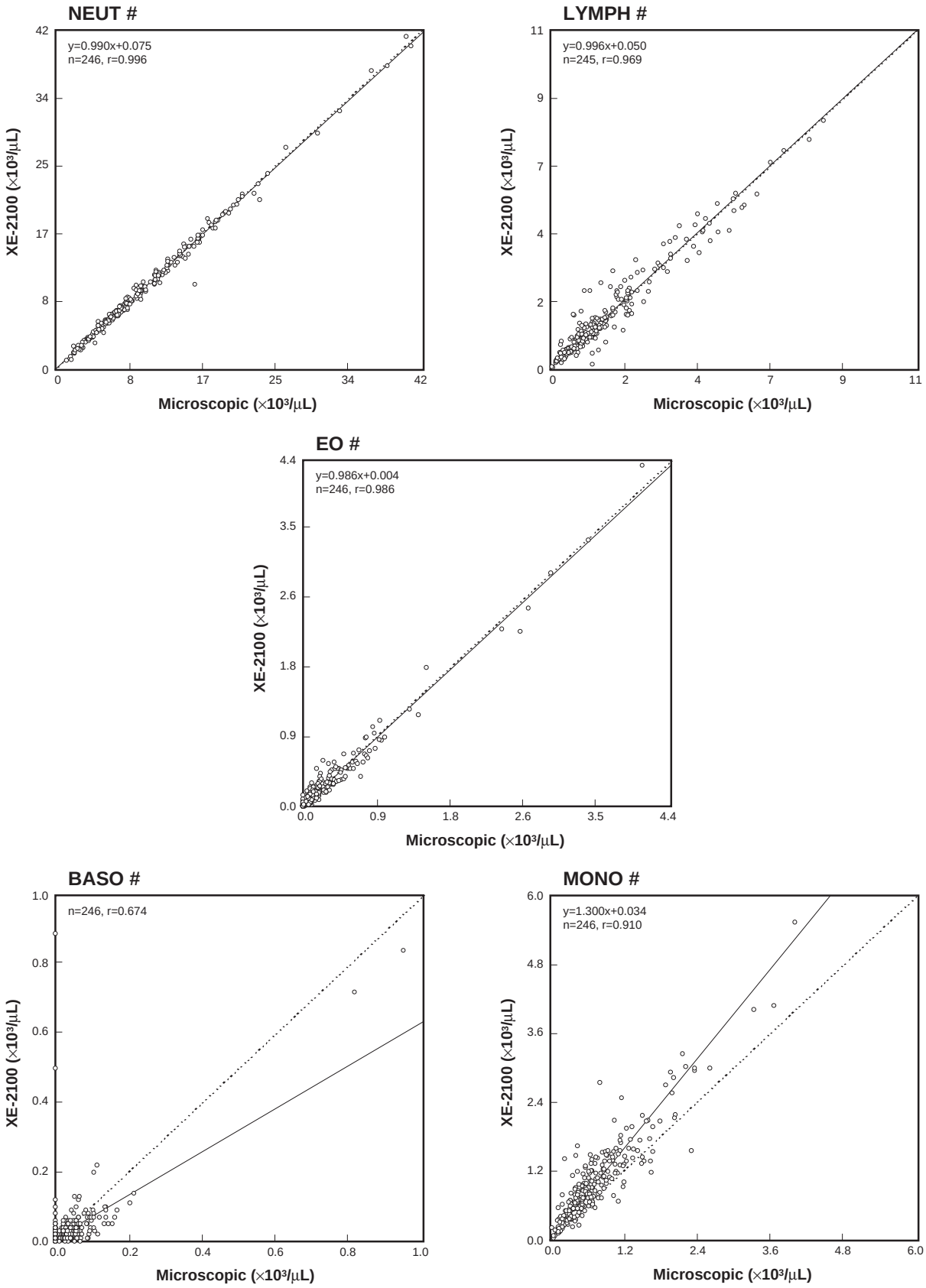

Fig. 3 *Comparison with visual microscopy (absolute count)*

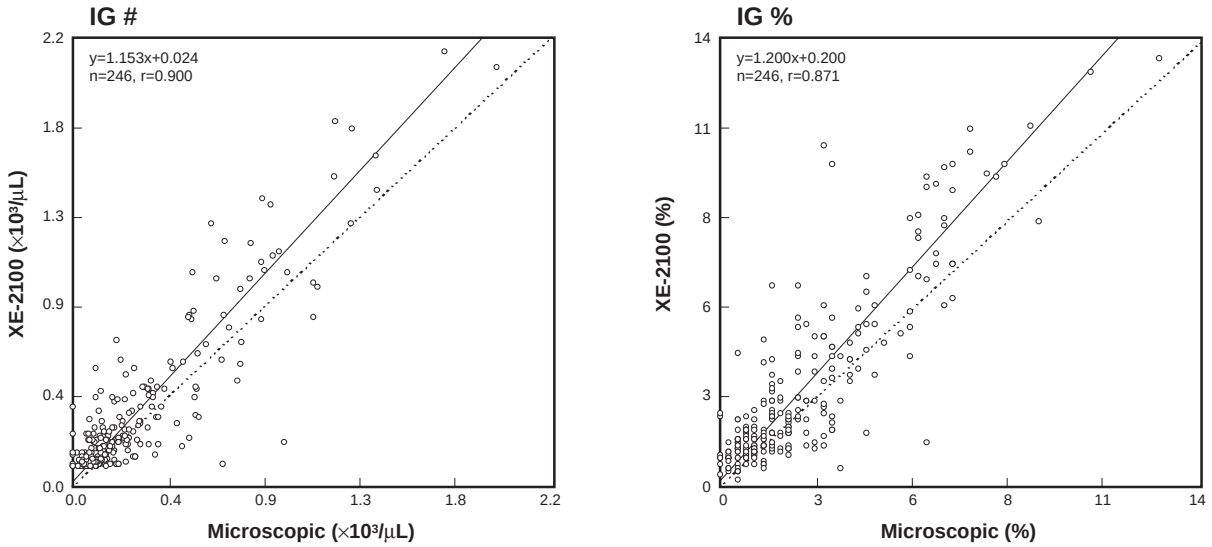

Fig. 4 *Comparison with visual microscopy (absolute IG count)*

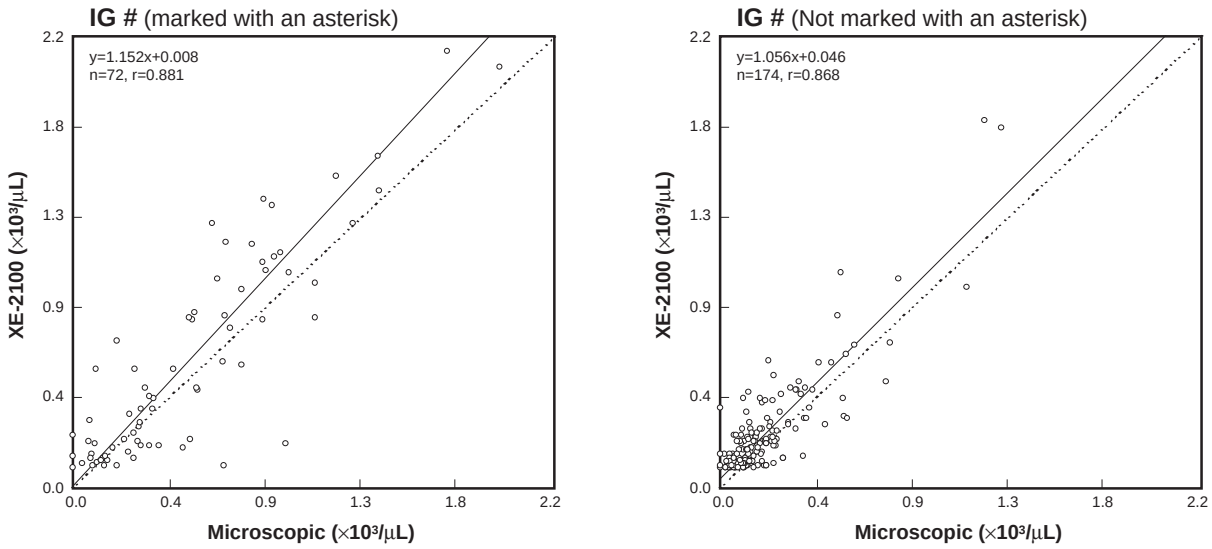

Fig. 5 *Comparison with visual microscopy (absolute IG count marked/could not marked) with an asterisk*

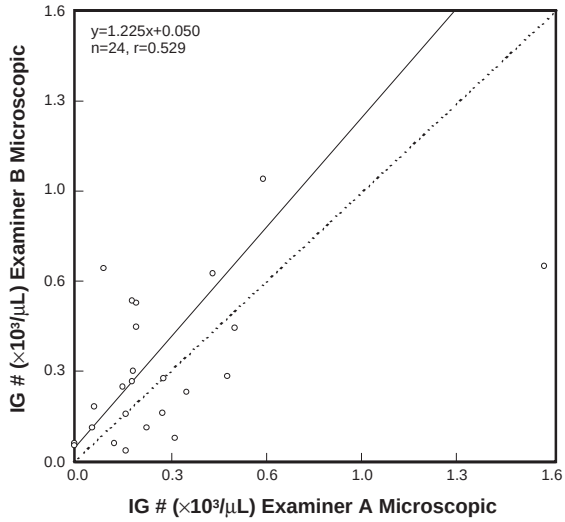

Fig. 6 *Neonatal samples: microscopic IG counts examiners A vs. B*

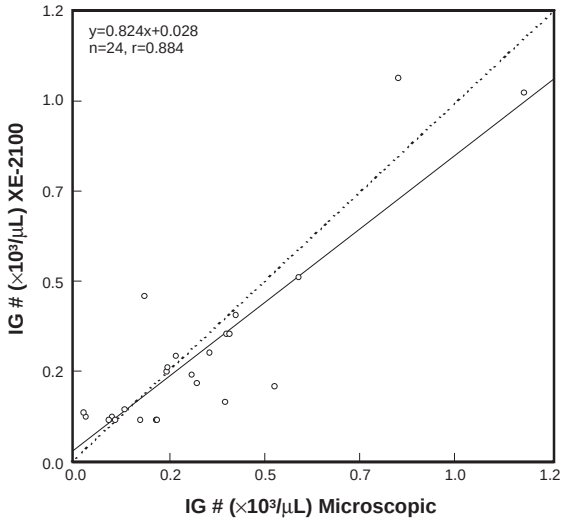

Fig. 7 *Neonatal samples: absolute IG count – comparison with visual microscopy*

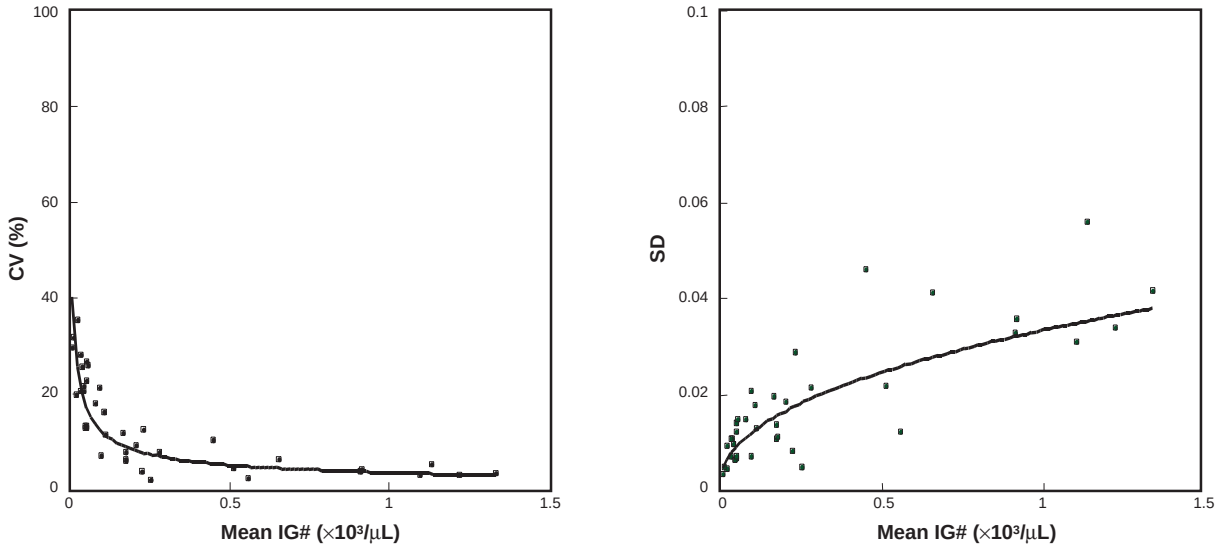

Fig. 8 *Precision profile of IG count (CV and SD)*

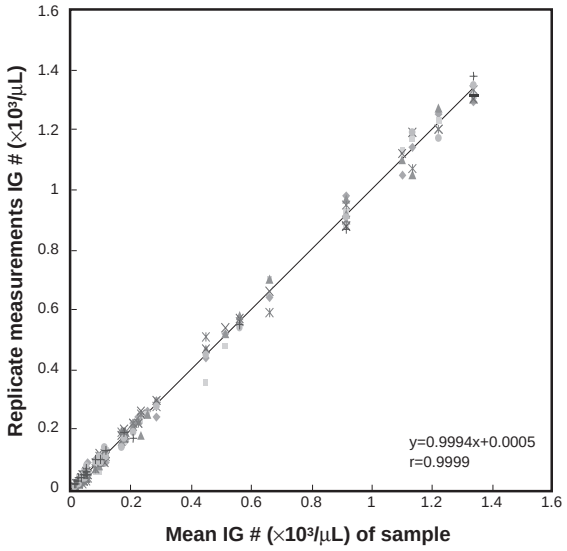

Fig. 9 *Reproducibility of IG counts*
(IG values of replicate measurements vs. IG means of specimen)

Reproducibility

Measurements for the precision profiles were done on an analyser that has been in routine use for one year without special adjustments or calibrations. Annual maintenance of the analyser was done one week prior to these measurements.

Specimens containing different concentrations of IG were measured repeatedly (but not consecutively) within 2 hours. As analyses were done with clinical samples the volume available allowed for 3 to 10 replicate tests. Results are summarized in ***Fig. 8***. Considering the low absolute values of the IG count , CV and SD show very reasonable variability. Results of replicate measurements are plotted against their means in ***Fig. 9***.

DISCUSSION

Quantification of immature myeloid cells beyond band forms in peripheral blood provides valuable clinical information in patients with inflammatory disease. So far, this information can only be obtained by visual microscopy and suffers from wide inter-examiner variation and time and labour consumption. The aim of this study was to assess the performance of the newly available Sysmex XE-2100 quantitative IG count compared to traditional visual microscopy.

Visual microscopy has been done according to NCCLS H20-A to minimize examiner bias and provide a statistically sufficient number of reference cells. Analysis of the two separate visual counts from each sample showed minimal inter-examiner variation for NEUT, LYMPH, EO, and BASO. Not unexpectedly, correlation coefficients for the morphologically more diverse cell populations like IG, MONO and BAND were lower, but still very good. This indicates good reliability and stability of the technicians' performance of the microscopic reference counts.

The XE-2100 conventional five-part differential compared excellently to microscopic results (see ***Fig. 3***). The slightly higher number of MONO detected by most automated analysers results in separate reference ranges for automated and microscopic monocytes in some institutions. Comparison of BASO regularly suffers from poor statistical performance due to small absolute and relative values, but qualitative correlation was satisfactory.

Our results confirm previous studies [1,3] in that there is excellent performance of the automated five-part white blood cell differential by the Sysmex XE-2100 automated haematology analyser.

Table 1 shows a summary of linear regression statistics for all cell populations tested.

Table 1 *Comparison of XE-2100 WBC differential and visual microscopy 400-cell differential*

Absolute counts			
Cell type	Correlation coefficient	Slope	y-axis Intercept
NEUT#	0.996	0.990	0.075
LYMPH#	0.969	0.996	0.050
EO#	0.986	0.986	0.004
BASO#	0.674	-	-
MONO#	0.910	1.300	0.034
IG#	0.900	1.153	0.024

Relative counts			
Cell type	Correlation coefficient	Slope	y-axis Intercept
NEUT%	0.978	0.984	1.241
LYMPH%	0.976	0.977	0.685
EO%	0.983	1.000	0.000
BASO%	0.895	-	-
MONO%	0.875	1.273	0.580
IG%	0.871	1.200	0.200

The correlation of XE-2100 IG count and the visual 400-cell diff (defined as promyelocytes, myelocytes and metamyelocytes for the microscopic differentials) was impressive with r=0.900 (agreement between visual combined 400-cell count and automated relative counts was better than that between the two microscopic examiners A and B (r=0.871 vs. r=0.803)). The slope of the P/B regression line b=1.153 and the intercept of a=0.024 indicated a slightly elevated automated IG count compared to the calculated microscopic values. Since the morphologic differences between band forms and metamyelocytes are not always readily distinguishable, a limited number of cells still being identified as "bands" in the microscope but already regarded as "metamyelocytes" by the XE-2100 is a possible explanation. Compared to former evaluations of the then research parameter "IG count" using the XE standard software package (Briggs, et al. 1999; Herklotz and Huber 1999) the agreement between microscopy and XE-2100 has improved considerably in our study using "XE Pro" and "IG Master". Herklotz (1999) had reported an initially unacceptably steep slope in the IG regression line, which had been corrected by readjustments in the software. Now, the algorithms in the IG Master software obviously improved the performance of the reportable IG count even further.

XE-2100 software uses sophisticated flagging algorithms to detect situations in which the results of the automated differential might not be reliable (see user's manual for a complete overview). Some of these rules lead to a flagging of the IG results with an asterisk mark, indicating that the IG count of that sample should not be reported without further investigation. Samples with an IG asterisk tended to have a high band and/or IG count. Separate analysis of marked and unmarked samples showed only minor differences in agreement between automated and visual IG counts (***Fig. 5***), so that at least some flagging mechanisms seem to have no influence on actual IG count reliability. Many of these marked IG results could well be reported and safely used clinically. Sysmex Corporation is currently undertaking a revision of the asterisk mark rules with respect to IG counts.

The clinical significance of an isolated increase of band forms (which is widely referred to as "left shift" in Germany) is currently under debate. Band forms are not reported separately by XE-2100. Band forms are largely reported as NEUT, as the excellent agreement between the NEUT count and the microscopic counts of NEUT + BAND proves. Microscopic band counts are notoriously prone to inter-examiner and inter-laboratory variation even when using the same morphologic definition (here: the "one-third-rule" which is widely common in Germany), hence a more reliable and stable automated parameter like the IG count is desirable.

Since the technical evaluation of the IG Master / XE Pro software on Sysmex XE-2100 analyser showed very good overall reliability and reproducibility, we are looking forward to studies on the usefulness of the IG count in the assessment of patients' clinical courses. To our knowledge, generally accepted reference values have yet to be established.

Once the automated IG count has been established in clinical medicine and is accepted by physicians in clinical routine, re-evaluation by visual microscopy will not be necessary for blood samples containing immature granulocytes (in the absence of IP messages or other particularities denoting decreased reliability). This will further reduce workload in the haematology laboratory and provide time for more rewarding tasks than routine microscopy. Particularly in laboratories providing service to neonatal care units this might prove economically beneficial.

ACKNOWLEDGEMENTS

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Visual microscopy was done by Kristina Begemann, Julia Kulin, Birte Walter, Andrea Schmidt, Ulrike Pohl, Sabine Zörner, and Brigitte Heitmann-Thiede.

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