The Preliminary Study of Nucleated Red Blood Cell Counting by Automated Hematology Analyzer

Jianying LI

Clinical Laboratory, Peking Union Medical College Hospital, Beijing, 100730, China.

Objectives : To evaluate the performance of the nucleated red blood cell (NRBC) counting by automated hematology analyzer and to research its clinical implications.

Methods: NRBC in the peripheral blood of 115 patients suffering with hematological diseases or other diseases were counted by the Sysmex XE-2100, ADVIA 120 automated hematology analyzer and manual microscopy.

Results: There was very good correlation between the manual method and XE-2100 automated method. The average within-run CV of NRBC count using high, medium and low samples was below 6.5%. The NRBC count had good linearity in the range of 0~15 ($\times 10^9$/L) (r=0.9998). Both the sensitivity and the negative prediction value were 100%. When the peripheral NRBC increases, the WBC count was significantly higher than the value obtained when the NRBC was deducted (P=0.0168).

Conclusion: The NRBC counting by the XE-2100 was sensitive, accurate and reliable. The XE-2100 could also measure the WBC in samples with increased NRBC. Besides patients with hematological diseases, such as leukemia, anemia etc, the NRBC could also be observed in other patients with some other non-hematological diseases; e.g. cancer patients under chemotherapy. Therefore, the automated quantification of peripheral NRBC may be helpful to the screening and diagnosis of some hematological and non-hematological diseases.

(Sysmex J Int 14 : 13-17, 2004)

Key Words Hematology Analyzer, Nucleated Red Blood Cell, Blood Cell Counting

Received 2 July, 2004; Accepted 7 July, 2004

INTRODUCTION

The observation of nucleated red blood cell (NRBC) in the peripheral blood is due to the release of premature erythrocyte from bone marrow. The NRBC may be found in the peripheral blood of infants or young children, but rarely observed in adults[1]. Most of the NRBC are polychromatic and orthochromatic erythroblast, however basophilic erythroblast or even more premature erythrocyte may be observed in certain situations; e.g. erythroleukemia. The presence of NRBC in peripheral blood suggests pathological conditions in most cases, such as hemolytic anemia, autoimmune hemolytic anemia, the acute blood loss, tissue ischemia, malignant diseases and marrow fibrosis, etc. Therefore, it is important to measure the NRBC accurately in clinical practice.

The counting of NRBC number in 100 nucleated cells under microscope, so-called NRBC%, is the classic means of NRBC estimation. However, only a small number of cells are counted by the manual method, so the accuracy and/or the precision depends in large measure on a technician's subjectivity. Wang and his group developed a method combining fluorescence staining and certain protocol of hemolysis and applied it in the NRBC counted of XE-2100 hematology analyzer[2]. We adopted the automated counting of NRBC in the clinical diagnosis in the outpatient department and compared it with traditional manual method in order to study the clinical implications of automated method.

MATERIALS AND METHODS

Instruments and reagents

1. Sysmex XE-2100 automated hematology analyzer and the original reagents (Sysmex Corporation, Japan).
2. ADVIA 120 automated hematology analyzer and the original reagents (Bayer Diagnostics, US)
3. Vacutainer with anticoagulant of EDTA-K2 (Becton Dickinson and Company, US)
4. Optical microscope (Olympus Corporation, Japan)

Sample collection

2mL blood samples were collected by EDTA-K2 anticoagulated tube from randomly selected patients in outpatient department, 61 with hematological diseases and 54 with other diseases.

The NRBC counting

1. Automated protocol

The Sysmex XE-2100 and ADVIA 120 under good quality control performed the assays within 4 hours after sample collection according to operator manuals . When the sample, which suggests the existence of NRBC, is examined, the Sysmex XE-2100 shows the flag of NRBC and gives out the absolute value of NRBC ($\times 10^9$/L), the NRBC% and real number of leukocyte without NRBC.

The ADVIA 120 could detect the NRBC, however the results will be expressed as "+", "++", "+++" depending on the quantity of NRBC.

2. Manual microscopy protocol

The percentage of NRBC was obtained by counting 100-200 leukocytes under oil immersion lens of microscope and the assay were performed by experienced technician.

RESULTS

The scattergram features of a whole blood cell analysis with elevated NRBC

The existence of NRBC in peripheral blood sample was detected by Sysmex XE-2100 and further analysis was done. The accurate count was performed and the abnormality were reported in the scattergram. ***Fig. 1*** is an example of NRBC counting and scattergram of a leukemia patient. ***Fig. 2*** shows the dynamic change of NRBC of the patient within 6 months. The fluorescence dye (STROMATOLYSER-NR) is used by XE-2100 to stain white blood cells and NRBC. In the two dimensional scattergram, the X and Y axis stands for the side fluorescence (SFL) and the forward scatter (FSC) respectively. The completely isolated NRBC lies at the left side of WBC in the scattergram and could be observed clearly even when the NRBC is as low as 0.12×10^9/L.

The correlation between the microscopy and XE-2100 counting of NRBC

The 46 samples were positive in the screening and absolute counting of NRBC by XE-2100. There was good correlation of these samples' NRBC% between XE-2100 and manual microscopy. The correlation coefficient

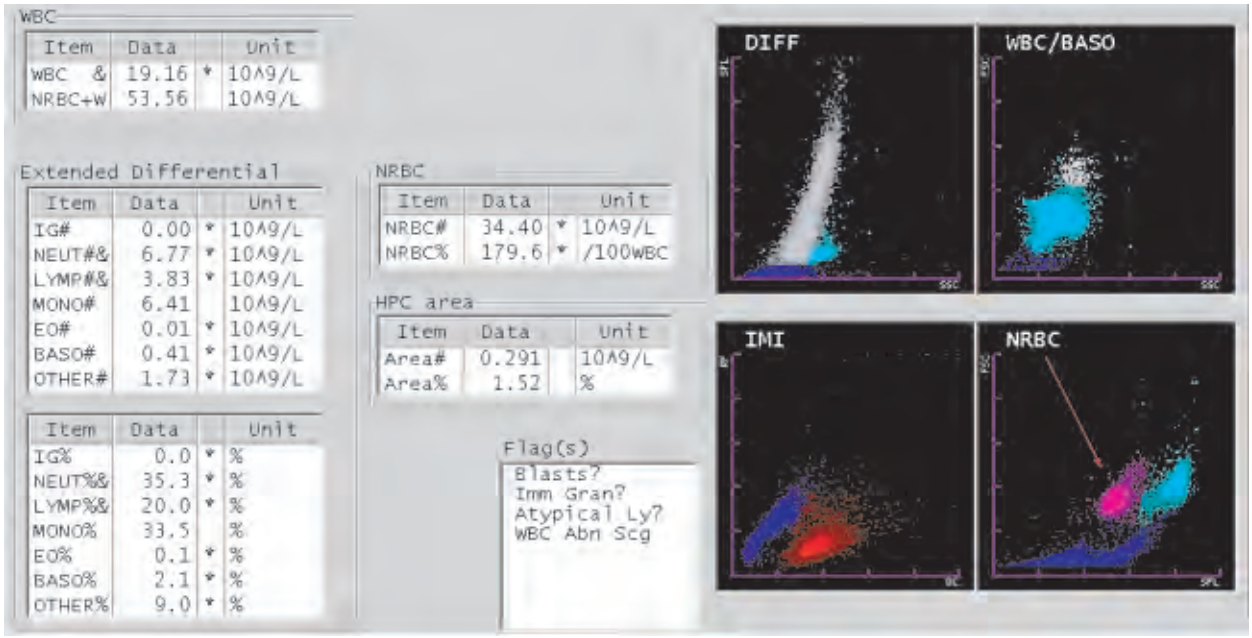

Fig. 1 *The cell counts and the scattergram of WBC and NRBC in a leukemia patient by XE-2100*
WBC: The white blood cell counting after deduction of NRBC; NRBC+W: The sum of NRBC and WBC; DIFF: White cell sorting; WBC/BASO: Basophilic granulocyte counting; IMI: Immature cell counting; NRBC#: Absolute NRBC counting; NRBC%: NRBC percentage.

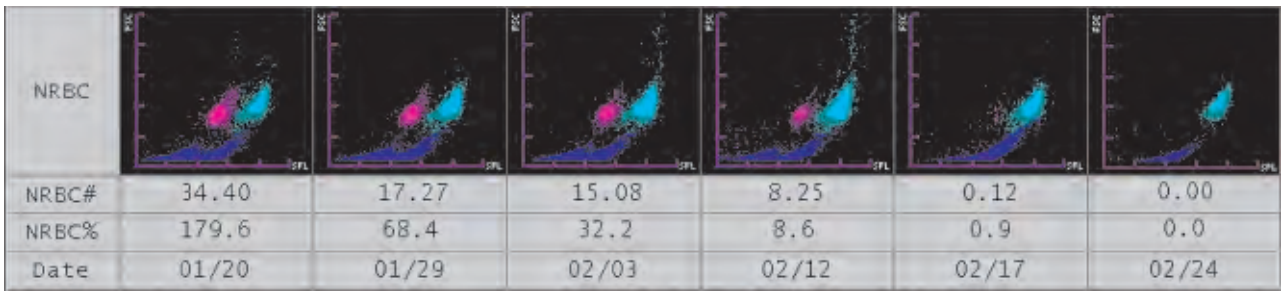

Fig. 2 *The dynamic change of the NRBC counting and scattergram of a leukemia patient*

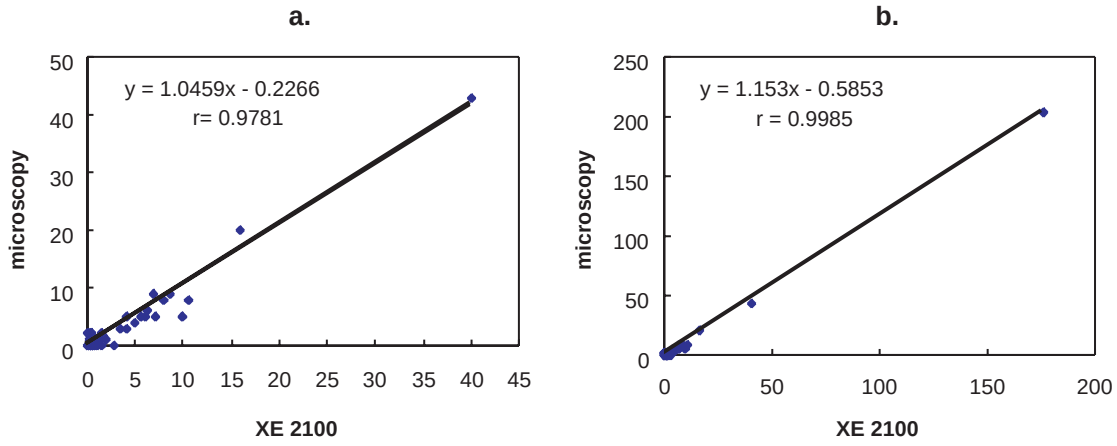

Fig. 3 *The correlation between XE-2100 and microscopy*

Table 1 *The reproducibility of NRBC counting by XE-2100*

Sample	NRBC($\times10^9$/L)		NRBC%		NRBC+W($\times10^9$/L)		WBC($\times10^9$/L)	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV
High	34.65	1.27	181.34	2.30	53.79	0.42	19.22	1.20
Medium	3.09	2.31	40.00	2.30	10.84	1.46	7.77	2.19
Low	0.19	14.82	1.90	14.88	10.01	2.14	9.94	2.17

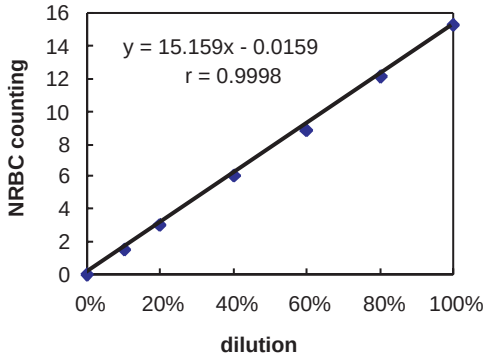

Fig. 4 *The linearity of NRBC counting*

was 0.9781 in the range of 0~50% (***Fig. 3a***), and was 0.9985 in the range of 0~220% (***Fig. 3b***). The paired t-test of NRBC% obtained by two methods on the 46 samples showed that there was no significant difference between the two methods (P=0.2018).

The methodological comparison of NRBC counting between automated hematology analyzer and manual microscopy

1. The within-run reproducibility of NRBC counting by XE-2100

3 samples, the low, medium and high NRBC counts, were repeatedly measured 5-7 times within 30 minutes. The absolute NRBC counts, NRBC%, the sum of NRBC and WBC and WBC counts without NRBC were obtained and CVs were calculated (***Table 1***). The average CV of the absolute NRBC counts of the three samples was 6.13% and the average CV of NRBC% was 6.49%

2. The linearity of NRBC counting by XE-2100

A sample with high NRBC (15.27×10^9/L) was diluted into different concentration and measured by XE-2100. ***Fig. 4*** shows the correlative coefficient, the regressive curve and formula.

3. The comparison of the positive and negative samples determined by automated hematology analyzer and manual microscopy

The XE-2100 has both the function of the screening and the counting of NRBC. XE-2100 screening suspects NRBC existence by Diff channel, and displays the message of "NRBC?" when NRBC exists. XE-2100 counting measures NRBC by NRBC channel, and displays the number of NRBC and NRBC% numerically. The ADVIA 120 can detect the NRBC through the cell nucleus granule analysis and certain protocol of hemolysis. The results is expressed as “+”, “++”, “+++” depending on the quantity of NRBC, however, the ADVIA 120 cannot count the number of NRBC accurately. The samples of the study were all tested by the two instruments and were regarded as the positive ones when the NRBC was detected. The NRBC detection results of the four methods were listed in ***Table 2***.

The XE-2100 screening detected 89 positive samples while the XE-2100 counting detected 46 samples, indicting that the positive rate of the counting to the screening was 51.69%. Among the 46 positive samples determined by XE-2100 counting, 32 were positive also in microscopy and the positive rate is 69.57%; the remaining 14 samples has a low NRBC%, an average of 0.71%

by XE-2100. One of XE-2100 negative screened samples showed NRBC% as low as 5% by XE-2100 counting and manual method (***Table 2****[*1]*). It proves that XE-2100 Counting is more sensitive and accurate than general NRBC flag. Another XE-2100 negative screened sample collected from a patient with anemia appeared to be positive by XE-2100 counting; however, the manual microscopy method did not validate the existence of the NRBC (***Table 2*** *[*2]*).
The ADVIA 120 revealed 19 positive samples and 16 of them were positive by manual microscopy. The XE-2100 screening and counting are also positive for these samples.

4. The diagnostic efficiency of three NRBC counting methods

The manual microscopy counting of NRBC as the golden standard, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of XE-2100 screening, counting and ADVIA 120 screening were calculated (***Table 3***).

The effect of NRBC on the WBC counting

The WBC counts without NRBC is lower than those with NRBC in the 46 NRBC positive samples determined by XE-2100. The paired t-test showed significant difference between these two groups (P=0.0168). The WBC counts of 10 samples were lower (more than 1.0×10^9/L) compared with the WBC counts with NRBC. The largest difference of the samples was 34.15×10^9/L.

DISCUSSION

The peripheral NRBC counting by automated hematology analyzers were developed in recent years and has been applied in clinical practice gradually; e.g. both the Sysmex XE-2100 and Cell-Dyn 4000 hematology analyzer (Abbott Laboratories, US) adopt the NRBC screening and accurate counting function[1]. The ADVIA 120 hematology analyzer also has specific semi-quantitative NRBC screening function. All these analyzers facilitate the convenient and fast screening of NRBC in clinical laboratories. Since the technology of automated NRBC detection has been applied recently, its feature, diagnostic efficiency and application fields need to be understood further. Our study provides some information about these.

Table 2 *The comparison of the NRBC positive and negative results of automated and manual methods*

The number of cases	XE-2100 screening	XE-2100 counting	ADVIA 120 screening	microscopy counting
16	+	+	+	+
15	+	+	–	+
12	+	+	–	–
1	+	+	+	–
1	+	–	+	–
44	+	–	–	–
23	–	–	–	–
1	–	–	+	–
1[*1]	–	+	–	+
1[*2]	–	+	–	–
Total of positive cases	89	46	19	32

+: NRBC screening or counting positive; – : NRBC screening or counting negative; "[*1]": XE-2100 counting 5%, microscopy counting 4%.; "[*2]": XE-2100 counting 0.2%, microscopy counting 0%.

Table 3 *The comparison of the diagnostic efficiency of three NRBC counting methods*

Counting methods	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
XE-2100 screening	96.88	30.12	34.83	96.15
XE-2100 counting	100	83.13	69.57	100
ADVIA 120 screening	50.00	96.39	84.21	83.33

The methodological characteristics of automated NRBC counting by XE-2100

The XE-2100 hematology analyzer can accurately measure the peripheral NRBC as both the absolute counts and the percentage. Besides differential and counts of the whole blood cell including NRBC, the XE-2100 hematology analyzer can also display the distribution of NRBC in scattergram (***Fig. 1***) which facilitates the discovery of abnormal samples with NRBC. The manual microscopy method, as the golden standard, and XE-2100 counting showed a good linear correlation in 46 NRBC positive samples determined by XE-2100 (***Fig. 3a,3b***), and no significant difference between these two methods (P=0.2018). It is suggested that the XE-2100 not only can work as well as manual microscopy method, but is much faster and more convenient. Although there are 14 samples with discrepant results obtained from the two methods, it is not absolutely that the results of the XE-2100 are false positive. It is rational to presume that the NRBC could not be observed among the 100~200 nucleated cells under microscope due to the nature of cell distribution on the blood smear and the low NRBC% of these samples (average of 0.71% by XE-2100). Since XE-2100 counts many cells more than the manual microscopy method, and does not have the influence by the subjectivity of technician or the nature of cell distribution on the blood smear, it shows excellent sensitivity.

The within-run reproducibility of XE-2100 counting in three samples from which the NRBC concentration differs was very good (***Table 1***). In the high NRBC sample, CV was very small. And it was 15% or less also in the low NRBC sample, and was smaller than microscopy. In the range of 0-15 ($\times 10^9$/L), XE-2100 counting and the manual microscopy method showed good correlation (r= 0.9998). Walter et al. also reported that good correlation was shown in the range of 0-50 ($\times 10^9$/L)[3]. These observations suggest that XE-2100 counting satisfies the clinical demands well enough.

The diagnostic efficiency of NRBC counting by the automated hematology analyzers

The XE-2100 screening, XE-2100 counting and ADVIA 120 screening have the different sensitivity, specificity, PPV and NPV.

(1) The XE-2100 screening has high sensitivity and NPV but low specificity and PPV, so it is suitable to be applied in clinical screening. However, the NRBC counting channel may be needed for further accurate NRBC estimation.

(2) The sensitivity and NPV of the XE-2100 counting are both 100%, while the specificity is 83.13%. It can be adopted in clinical accurate NRBC counting. If the counting result is 0, there is little possibility that the sample contains NRBC and the manual microscopy counting is generally not necessary.

(3) The ADVIA 120 has relative high specificity but low sensitivity. If the ADVIA 120 screening is positive, the NRBC may be present in sample, but it is necessary to validate the results by manual methods.

The effect of NRBC on the WBC counting

The NRBC is included in the WBC counts obtained by hematology analyzer, so the presence of NRBC may affect the WBC counting greatly and lead to the false elevation of the WBC counts. The XE-2100 hematology analyzer can measure the NRBC automatically and report the actual WBC counts after deduction of NRBC. Generally, the low level of NRBC ($<0.5\times10^9$/L) does not produce significant effect over the WBC counts, but it is better to report the WBC counts which deducted NRBC for a sample with significantly elevated NRBC ($>1.0\times10^9$/L).

The analysis of NRBC counting positive cases

Among the 46 NRBC positive cases determined by XE-2100 hematology analyzer, 31 patients were with hematological diseases, and includes 10 with anemia, 13 with acute/chronic leukemia, 2 with marrow fibrosis and 6 with other hematological diseases; the other 15 patients were with non-hematological diseases. The prevalence of NRBC in these patients showed that the NRBC is prone to present in the peripheral blood of patients with leukemia, anemia and marrow fibrosis, so it is proper to perform NRBC counting in patients who are suspected or diagnosed with hematological diseases to obviate misdiagnosis. In non-hematological diseases, it is easier to observe the NRBC in patients with cancer taking chemotherapy; however, the mechanism is not clear yet. It has been reported that NRBC may present in various pathological conditions such as heart failure, pulmonary diseases, burn, sepsis, uremia, collagen disease, diabetic acidosis, myocardial infarction, liver diseases, adenomegaly, etc. Besides, the NRBC may also appear in the peripheral blood of the newborn, pregnant woman and the patients who are treated with cytokines such as hematopoietic factors. The presence of NRBC in the peripheral blood may be the pathologic signs of the various diseases, and the NRBC counting has the extensive clinical implications. If the peripheral NRBC is observed in any patients, further analysis is recommended to obviate misdiagnosis.

References

1) *De Keijer MH, Van Der Meer W : Automated counting of nucleated red blood cells in blood samples of newborns. Clin Lab Haem, 24:343-345, 2002.*
2) *Wang FS, et al. : Development and clinical application of nucleated red blood cell counting and staging on the automated haematology analyzer XE-2100. Clin Lab Haem, 25:17-23, 2003.*
3) *Walter J, Garrity P : Performance evaluation of the Sysmex XE-2100 hematology analyzer. Lab Hemat, 6:83-92, 2000.*