Evaluation of the Diagnostic Performance of the Sysmex XT-2000*i* Automated Hematology Analyzer in the Detection of Immature Granulocytes

A. M. CENCI[*1], M. MACONI[*2], and B. CASOLARI[*1]

[*1] *Laboratory of Clinical Pathology, Ospedale S. Agostino, Modena, Italy.*
[*2] *Laboratory of Clinical Pathology, Azienda Ospedaliera Arcispedale Santa Maria Nuova, Reggio Emilia, Italy.*

Immature Granulocytes (IG) are normally not present in peripheral blood. Their presence is an important indicator of enhanced bone marrow activation. The present study evaluated the diagnostic performance of the Sysmex XT-2000i automated hematology analyzer in the detection of myelocytes and/or metamyelocytes in patients affected by non-haematological disease, in comparison with manual microscopy review (NCCLS H20-A) and also in comparison with Sysmex XE-2100, used as a predicate device.

Sysmex XT-2000i shows good specificity and sensitivity in the detection of low, medium and high concentrations of immature myeloid cells. The diagnostic performance of the XT-2000i IG and LS flags is very high and comparable to the results obtained with Sysmex XE-2100.

These results suggest that Sysmex XT-2000i would be an excellent second analyser for larger laboratories concerned with harmonization of instrumentation (in particular as a back-up for Sysmex XE-2100), or as a primary instrument for medium-sized routine or STAT laboratories.

(Sysmex J Int 15 : 1-6, 2005)

Key Words Automated Hematology Analyzer, XT-2000*i*, Immature Granulocytes, NCCLS H20-A

Received 13 Jan, 2005; Accepted 20 Jan, 2005

INTRODUCTION

The presence of immature granulocytes (IG) in peripheral blood is potentially important information indicating enhanced bone marrow activation. The promyelocyte, myelocyte and metamyelocyte stages of myeloid maturation may be correlated with systemic inflammatory stress or leukaemic reactions. The detection of IG is also important in distinguishing patients with haematological diseases [chronic myeloproliferative disorders (CMPD) and myelodysplastic syndromes (MDS)] from patients with infection. Early detection of bacteraemia and sepsis facilitates timely initiation of antimicrobial therapy and reduces morbidity and mortality in these situations. While there is no true "gold standard" for the detection of sepsis and blood culture has some problems resulting from poor collection technique[1,2] and in vitro bacterial growth, clinicians have tried to use other laboratory parameters to predict bacteraemia and sepsis. So far, quantitation of leucocytes, including neutrophils, is important in patients who might have an infection, because of the acute inflammatory response to bacterial infection[3-5]. Preterm and neonatal blood samples commonly contain immature blood cells of particularly variable morphology and thus frequently need to be differentiated.

During pregnancy the absolute count of segmented neutrophils approximately doubles, reflecting not a true increase in granulopoiesis, but rather increased demargination of the circulating neutrophils[6]. The precise automated detection and enumeration of immature granulocytes would be a useful parameter to predict the presence of these physiological and pathological states.

Sysmex XT-2000*i* (Sysmex Corporation, Kobe, Japan) is a new fully automated haematology analyser with a throughput of 80 samples per hour. The analyser can provide up to 30 parameters including CBC, a complete 5-part leucocyte differential, reticulocyte parameters and optical (fluorescence) platelet counts[7]. These measurements are performed with the same technologies and reagents used by Sysmex XE-2100, a 'top of the range' haematology analyser with a throughput of 150 samples per hour that, in addition, performs nucleated red blood cell (NRBC) and the haematopoietic progenitor cell (HPC) counts in two additional channels: the NRBC and the IMI (Immature Information) channel[8]. Both analysers detect the presence of abnormal or immature WBCs providing a list of suspect messages: Blasts?, Immature Gran?, Left Shift?, Atypical Lympho?, Abn Lympho/Blasts?. These suspect messages are generated from abnormal cell locations on the WBC/DIFF scattergram. Sysmex XE-2100 also combines pattern abnormalities from the IMI scattergram (not present on XT-2000*i*).

Our study focusses on the diagnostic performance of the "Immature Gran?" (IG) and "Left Shift?" (LS) flags gen-

erated by Sysmex XT-2000*i* in the detection of myelocytes and/or metamyelocytes, with normal morphology, in patients affected by non-haematological disease (e.g. infection, inflammation, post-surgery/bleeding, pregnancy). The results, in terms of sensitivity, specificity and efficiency of the IG and LS flags, are presented in comparison with those from Sysmex XE-2100, used as a predicate device.

MATERIALS AND METHODS

Haematology analysers

Sysmex XT-2000*i* performs the WBC count and the 5-part differential in two different channels by flow cytometry using a semiconductor laser. In the WBC/BASO channel a specific reagent lyses the red cells and selectively suppresses degranulation of basophils, resulting in their separation from other forms of WBCs.
The combination of the side-scattered light (inner complexity of the cells) and the forward-scattered light (size) are presented in the WBC/BASO scattergram (***Fig. 1***) where the WBC and basophil counts are estimated.
In the WBC/DIFF channel, after lysis of the red cells, a specific polymethine fluorescence dye enters the WBCs and stains the DNA and RNA therein. Lymphocytes, monocytes, eosinophils and neutrophils + basophils are then classified and counted in the DIFF scattergram (***Fig. 2***) according to their side-scattered light (inner complexity of the cells) and side fluorescence intensity (proportional to their content of nucleic acid). Abnormal and immature WBCs are located in specific areas of the DIFF scattergram[9] (***Fig. 3***), flagged by the following suspect messages: Blasts?, Immature Gran? (IG), Left Shift? (LS), Atypical Lympho? and Abn Lympho/Blasts?. The immature granulocytes are classified as cells with higher fluorescence intensity than neutrophils, because their DNA content is greater than the latter. The IG flag of XT-2000*i* is mainly related to the presence of metamyelocytes and myelocytes. The LS flag is mainly related to the presence of band cells and metamyelocytes.

Sysmex XE-2100 performs the leucocyte count and 5-part differential with the same technology and the same reagents as Sysmex XT-2000*i*[10]. XE-2100 is, however, equipped with an additional specific channel (IMI) that provides information on the presence of immature granulocytes, blasts and haematopoietic progenitor cells by a combination of radio frequency and direct current (RF/DC) technologies. This channel uses a haemolytic reagent that selectively protects immature cells.
Both Sysmex XT-2000*i* and Sysmex XE-2100 offer immature granulocyte counts (IG % and IG #) as research parameters. With a new software release "XE-PRO" and "XE-IG Master" the IG count can now be reported with Sysmex XE-2100 both in absolute terms and as a percentage of total WBC[11].
In this article we do not present any comparison between the automated IG count and manual microscopy, because the main focus of this study was to assess the predictive value of the myeloid flags IG and LS of XT-2000*i* in detecting the presence of paraphysiological immature granulocytes in patients without haematological malignancy.

Blood specimen selection and processing

822 samples were included in this study. All venous blood specimens were collected into K_2-EDTA anticoagulant, kept at room temperature, and analysed on both instruments within 1 hour of their arrival in the laboratory. The blood specimens were divided into three different groups using the following criteria:
1st group - 178 samples from outpatients and inpatients (e.g. cardiology, orthopaedics, surgery, internal medicine, pregnancy), taken during a preliminary evaluation of Sysmex XT-2000*i* at the time of its installation
2nd group - 600 samples, from outpatients and inpatients, randomly chosen from the routine workload on six different days (100 samples each day) excluding those from onco-haematology wards.
3rd group - 44 samples, selected from patients with normal WBC count (lower than 10.0×10^9/L) and minimal haematological abnormalities on manual microscopy review for the presence of 2.0% or less of metamyelocytes and/or myelocytes and/or more than 5% of band cells.

Manual microscopy review

The clinical sensitivity and specificity of the IG and LS

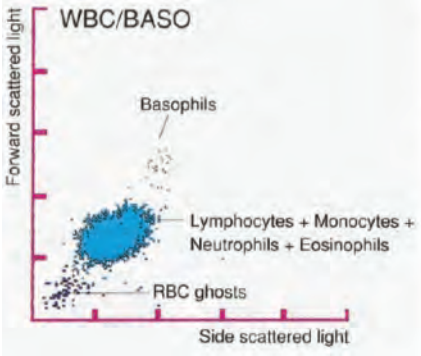

Fig. 1 *WBC/BASO scattergram*

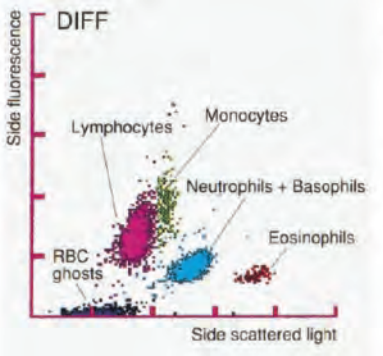

Fig. 2 *WBC/DIFF scattergram*

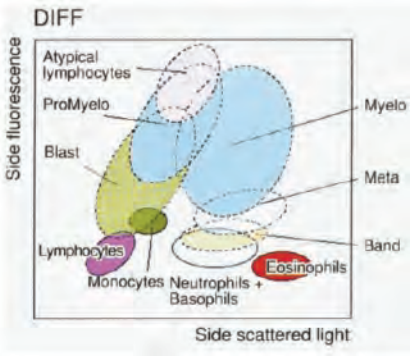

Fig. 3 *Abnormal cell location*

suspect flags of Sysmex XT-2000*i* were assessed by comparison with microscopy WBC differential counts as described previously[12-14] and the performance of both analysers was compared. The reference differential WBC counts were performed by microscopy examination of 400 cells on May-Grünwald-Giemsa stained preparations by two qualified medical doctors each counting 200 cells on separate smears according to the NCCLS H20-A protocol[15].

For the 1st and the 2nd group of specimens, the samples were considered positive by microscopy review if they showed more than 2% metamyelocytes and/or myelocytes and/or more than 10% band cells.

For the 3rd group of specimens, as stated previously, all samples were selected and considered positive by microscopy review if they showed from 0.5% to 2% metamyelocytes and/or myelocytes and/or more than 5% band cells.

None of the positive samples selected in this study were positive by microscopy review for the presence of promyelocytes or blasts.

STATISTICAL ANALYSIS

To compare the results from XT-2000*i*, XE-2100 and the reference procedure, truth tables were constructed to determine:

- True Positive [TP] (samples positive at microscopy review flagged by the instrument with the IG and / or the LS flag)
- True Negative [TN] (samples negative at microscopy review and not flagged by the instrument with the IG and / or the LS flag)
- False Positive [FP] (samples negative at microscopy review but flagged by the instrument with the IG and/or the LS flag, or samples positive at the microscopy review but flagged by the instrument with other flags such as "Blasts?" in addition to IG and/or LS flag)
- False Negative [FN] (samples positive at microscopy review but negative or flagged by the instrument with flags other than IG or LS)

The predictive values of IG and LS flags for the presence of immature granulocytes after microscope review were determined as follows:

Sensitivity (%) = [TP / (TP + FN)]×100

Specificity (%) = [TN / (TN + FP)]×100

Efficiency (%) = [(TP + TN) / (TP + TN + FP + FN)]×100

Comparison between XT-2000*i* and XE-2100 for the IG and LS flags generated was determined as follows:

- Number of negative samples in agreement (concordance): number of samples without IG or LS on both analysers
- Number of positive samples in agreement (concordance): number of positive samples flagged with only IG and/or LS on both analysers
- Number of negative samples in disagreement (discordance): number of negative samples flagged with IG and/or LS only by one analyser
- Number of positive samples in disagreement (discordance): number of positive samples flagged with IG and/or LS only by one analyser.

RESULTS

In a preliminary study of Sysmex XT-2000*i*, 178 samples were compared to Sysmex XE-2100 to assess the sensitivity and specificity of the IG and LS suspect flags to the presence of more than 2% myelocytes and/or metamyelocytes on microscopy examination. Only 6 out of 178 samples (3.37%) had a WBC count higher than normal (10.0×10^9/L to 15.0×10^9/L).

The comparison of IG and LS flags generated by the two instruments is shown in ***Table 1***.

The discrepancies that were present in the 11 discordant samples are described in ***Table 2***.

The results of sensitivity, specificity and efficiency for both analysers compared with reference microscopy review are shown in ***Table 3***.

In the 2nd group we reviewed the occurrence of the IG and LS (alone or combined) in our normal routine workload. In 600 samples, randomly chosen from the routine workload of six different days, only 11 (1.83 %) had WBC counts higher than normal (from 10.0×10^9/L to 15.0×10^9/L).

With both analysers we found 18 out of 600 samples flagged with IG and/or LS. By microscopy review we confirmed that 9 samples were true positives for minimal myeloid abnormalities and 7 samples were true positive for the presence of 4% or more myeloid immature cells.

2 samples were false positive on both analysers. In both these samples we found platelet clumps.

The 582 samples not flagged with IG and/or LS on both analysers were negative by microscopy review. Thus, we found a full agreement between XT-2000*i* and XE-2100 in this group of patients. The results of the diagnostic performance of both analysers are described in ***Table 4***.

Finally, the sensitivity of the IG and LS suspect flags was studied on a 3rd group of 44 samples, selected from patients with a normal WBC count (less than 10.0×10^9/L) and presenting minimal haematological abnormalities on manual microscopy review (0.5% to 2.0% metamyelocytes and/or myelocytes and/or more than 5% of band cells).

Examples of scattergrams obtained by Sysmex XT-2000*i* in two samples from this patient group are shown in ***Figs 4 and 5***.

The comparisons of IG and LS flags generated by the two analysers in this group of 44 samples are shown in ***Table 5***.

The results of the diagnostic performances of both instruments in this group of patients are shown in ***Table 6***.

Despite the number of false positive and false negative results for the IG and LS flags obtained in this group of patients, the high sensitivity of both analysers should be underlined in the detection of very low concentrations of myelocytes and metamyelocytes when all suspect flags are considered (40 of 44 for XT-2000*i*, 37 of 44 samples for XE-2100).

Grouping all 822 samples together, the overall diagnostic performance of the IG and LS flags in the detection of immature myeloid cells is shown in ***Table 7***.

Table 1 *XT-2000i vs XE-2100 : comparison of IG and LS flags on the 1st group of 178 samples*

Concordance in negative samples	130	(73.03 %)
Concordance in positive samples	37	(20.79 %)
Discordance in negative samples	2	(1.12 %)
Discordance in positive samples	9	(5.06 %)

Table 2 *Discordance in the 1st group of 178 samples*

TP XT-2000*i* ; FP on XE-2100	6	- 4 samples flagged with Blasts+IG on XE-2100 - 2 samples flagged with Blasts+IG+At. Lymph on XE-2100
FP XT-2000*i* ; TN on XE-2100	2	- 2 samples flagged with IG on XT-2000*i*
TP XT-2000*i* ; FN on XE-2100	1	- 1 sample without IG / LS on XE-2100
FN XT-2000*i* ; TP on XE-2100	2	- 2 samples flagged with “Blasts?” on XT-2000*i*

Table 3 *Diagnostic performance of IG and LS flags on the 1st group of 178 samples*

	XT-2000*i*	**XE-2100**
True Positive (TP)	44	39
True Negative (TN)	130	132
False Positive (FP)	2	6
False Negative (FN)	2	1
Sensitivity	95.6%	97.5%
Specificity	98.5%	95.7%
Efficiency	97.8%	96.1%

Table 4 *Diagnostic performance of IG and LS flags on the 2nd group of 600 samples*

	XT-2000*i*	**XE-2100**
True Positive (TP)	16	16
True Negative (TN)	582	582
False Positive (FP)	2	2
False Negative (FN)	0	0
Sensitivity	100%	100%
Specificity	99.6%	99.6%
Efficiency	99.6%	99.6%

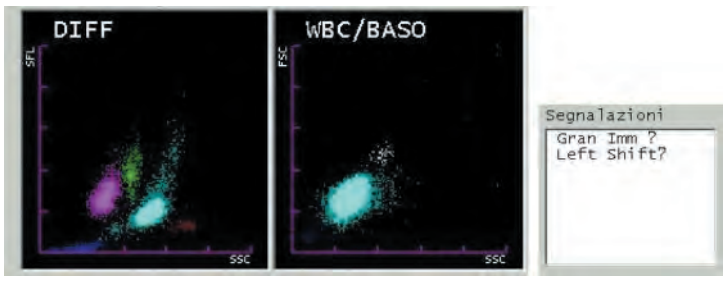

Fig. 4 *WBC/BASO and DIFF scattergrams from XT-2000i*
WBC = 9.8×10⁹/L. Microscopy review: 1 myelocyte; 1 metamyelocyte /100 WBC

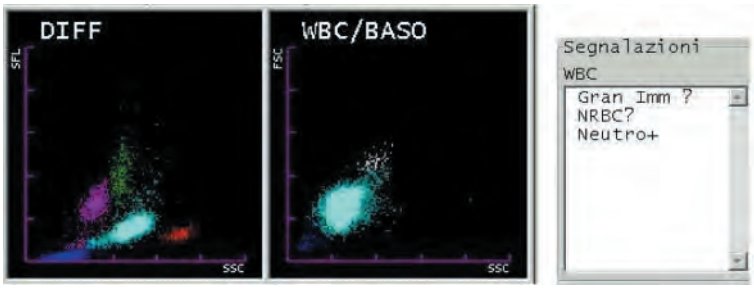

Fig. 5 *WBC/BASO and DIFF scattergrams from XT-2000i*
WBC = 7.4×10⁹/L. Microscopy review: 1 myelocyte /100 WBC

Table 5 *XT-2000i vs XE-2100 : comparison of IG and LS flags on the 3rd group of 44 samples*

	XT-2000*i*	**XE-2100**
IG	14	5
IG + LS	4	2
LS	4	1
IG / LS + other flags	13	20
Negative for IG / LS	9 (5 samples flagged with Blast?, 4 samples without any flag)	16 (9 samples flagged with Blasts?, 7 samples without any flag)

Table 6 *Diagnostic performance of IG and LS flags on the 3rd group of 44 samples*

	XT-2000*i*	**XE-2100**
True Positive (TP)	22	8
True Negative (TN)	0	0
False Positive (FP)	13	20
False Negative (FN)	9	16
Sensitivity	71%	33.3%
Specificity	N.A.	N.A.
Efficiency	50%	18.2%

Table 7 *Diagnostic performance of IG and LS flags on the 822 total samples*

	XT-2000*i*	**XE-2100**
True Positive (TP)	82	63
True Negative (TN)	712	714
False Positive (FP)	17	28
False Negative (FN)	11	17
Sensitivity	88.2%	78.8%
Specificity	97.7%	96.2%
Efficiency	96.6%	94.5%

DISCUSSION

Quantitative analysis of immature granulocytes is useful for good clinical management of patients affected by haematologic diseases (chronic myelogenous leukaemia, chronic myeloproliferative disorders, myelodysplastic syndromes) or patients presenting with infection. The IG compartment includes promyelocytes, myelocytes and metamyelocytes. Several studies have assessed the clinical usefulness of quantitative and qualitative changes in IG for predicting infection in paediatric and adult populations associated, or not, with other laboratory data (total WBC count, C-reactive protein (CRP), etc). Myeloid progenitor cells were significantly higher in infected than in uninfected patients[16].

Sysmex XT-2000*i* shows good specificity and sensitivity in the detection of low, medium and high concentrations of immature myeloid cells. The diagnostic performance of the XT-2000*i* IG and LS flags in the detection of immature myeloid cells is very high and comparable to the results obtained with Sysmex XE-2100, even without the immature information (IMI) channel.

The use of fluorescence flow cytometry for the 5-part differential analysis may be the reason for this performance and for the very good agreement with the manual reference method.

These results suggest that Sysmex XT-2000*i* would be an excellent second analyser for larger laboratories concerned with harmonization of instrumentation (in particular as a back-up for Sysmex XE-2100 since the same technology and reagents are used), or as a primary instrument for medium-sized routine or STAT laboratories.

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