Preliminary Data on the Feasibility of Bone Marrow Screening on the Sysmex XE-2100 Automated Hematology Analyzer

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In the present study we investigated the potential of the Sysmex XE-2100 automated hematology analyzer as a screening device for bone marrow (BM) aspirates. We evaluated BM cellularity, white blood cell (WBC) differential count, immature granulocyte (IG) count, nucleated red blood cell (NRBC) count, granulocyte/erythroid (G/E) - and myeloid/erythroid (M/E) ratio, and potential pathology-related scattergrams ('pathology patterns'). Our data are derived from analyses of 160 BM samples of patients with hematological malignancies.

Our preliminary data indicate that the Sysmex XE-2100 opens new perspectives for use as a primary screening device for BM samples, particularly in cases of acute and chronic lymphocytic and myeloid leukemias, where distinct pathology-related scattergram patterns have the potential to be of diagnostic help. Software improvements are required for further progress.

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Key Words Born Marrow Screening, Scattergram Patterns, Automated Hematology Analyzer, XE-2100

INTRODUCTION

The idea of analyzing bone marrow (BM) samples on hematology analyzers designed for analysis of peripheral blood samples has been pursued unsuccessfully for many years. The main limiting factors have been the presence of fat droplets, microfibers and cell aggregates in the BM, as well as the lack of analytical performance of the hematology analyzer in discriminating amongst the numerous blood cell precursors[1-3].

The introduction of fluorescence as a basic analytical tool on modern instruments, as well as refinement of specific cell lysing procedures and improved performance of software for data handling, might offer new opportunities for screening BM samples on the recently introduced hematology analyzers.

The Sysmex XE-2100, as a representative for the latest generation of automated hematology analyzers, is capable of producing results for more than 25 hematologically relevant parameters at a speed of 150 samples per hour. Its analytical principles are based on the combination of fluorescence flow cytometry, electrical impedance, RNA- and DNA staining, and selective permeabilization of blood cells. In addition to the common complete blood count (CBC) and differential counts, results include immature granulocyte (IG) count, myeloid cell maturation data, reticulocyte (RET) count and maturation indices, optical measurement of platelet count using a fluorescent dye, and nucleated red blood cell (NRBC) count.

The analytical performance of this instrument for peripheral blood samples is documented in several studies[4-6].

In view of the fact that this hematology analyzer is capable of generating data for both white (WBC) and red blood cell (RBC) precursors, we investigated the potential use of this analyzer as a screening device for BM aspirates.

MATERIALS AND METHODS

We evaluated BM cellularity, WBC differential count, IG count, NRBC count, granulocyte/erythroid (G/E)- and myeloid/erythroid (M/E) ratio, and potential pathology-related scattergrams ('pathology patterns'). Our data are derived from analyses of 160 consecutive BM samples, taken on lithium heparin, of patients with hematological malignancies. Although many of these samples were taken for patient follow-up, sufficient new, as yet, untreated cases were included to cover a broad spectrum of hematological malignancies. The BM samples were analyzed as native samples and after two consecutive wash cycles with phosphate-buffered saline (PBS) in order to eliminate interference by microfibers and fat droplets. The microscopic differential count on May-Grünwald-Giemsa stained blood preparations, based on examination of 500 cells, was used as the reference method.

RESULTS

Analysis of BM aspirates is complicated by the presence of varying amounts of fat droplets. On the Sysmex XE-2100, this interference presents as typical sigmoidal patterns on the WBC/BASO- and NRBC-scattergrams, as well as additional more linear zones on the IMI scattergram, as illustrated for a non-pathological sample (***Fig.1***). Attempts to eliminate the fat droplets by two consecutive wash cycles with PBS resulted only in partial and inconsistent removal of the interferents, and was therefore not retained as an option.
The following results were obtained from the native BM samples.

BM cellularity
An acceptable Spearman rank correlation (r=0.60) was found between the instrument's total nucleated cell count and microscopy-based classification into low, intermediate and high sample cellularity.

WBC differential count
Acceptable correlation with microscopy was observed for lymphocyte (LYMP) count (r=0.72, though influenced by plasmocytes when present), and eosinophil (EO) count. The monocyte (MONO) and basophil (BASO) count was overestimated due to interference by fat droplets. A good correlation (r=0.78) was found for neutrophil (NEUT) count, provided the NEUT count was defined as the sum of band and segmented neutrophils.

Sysmex XE-2100 IG count
The best correlation was found between the instrument's IG-figures (as percentages) and the microscope count for BM myeloblasts+promyelocytes. This contrasts with previously published reports[4,6] and our own unpublished data for peripheral blood where the best correlation was found between the instrumental data and the sum of microscopic promyelocytes+myelocytes+metamyelocytes, as expected.

Sysmex XE-2100 NRBC count
As also observed on other analyzers[7], the BM NRBC count is markedly and consistently underestimated, and there is a rather poor correlation with microscopy (r=0.51), reflecting interference by considerable amounts of fat droplets for distinct samples. However the correlation improves considerably when those outliers with high fat content are removed.

Instrument-derived G/E ratio
This ratio, although overestimated by a factor of almost 2 due to the low NRBC count, correlates remarkably well with microscopy.

Pathology patterns
Using the combined scattergrams for DIFF, WBC/BASO, IMI, and NRBC, distinct nucleated cell scattergram patterns could be defined for acute and chronic lymphocytic and myeloid leukemias (***Figs. 2-5***), as well as for plasmacytosis, hyperplasia and hypoplasia.

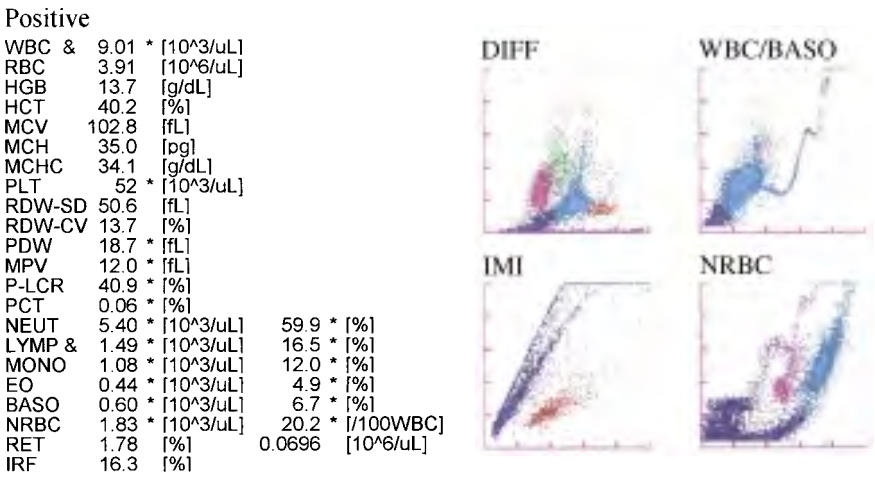

Fig. 1 *Non-pathological BM*
Note the sigmoidal patterns of fat droplets in the WBC/BASO and NRBC-scattergrams, leading to falsely increased nucleated cell- and erythroblast counts.

Positive

WBC	&	83.24	*	[10^3/uL]	
RBC		1.98	-	[10^6/uL]	
HGB		6.1	-	[g/dL]	
HCT		18.9	-	[%]	
MCV		95.5		[fL]	
MCH		30.8		[pg]	
MCHC		32.3		[g/dL]	
PLT		52	*	[10^3/uL]	
RDW-SD		63.5	+	[fL]	
RDW-CV		18.4	+	[%]	
PDW		----		[fL]	
MPV		----		[fL]	
P-LCR		----		[%]	
PCT		----		[%]	
NEUT		----		[10^3/uL]	---- [%]
LYMPH		----		[10^3/uL]	---- [%]
MONO		----		[10^3/uL]	---- [%]
EO		0.18	*	[10^3/uL]	0.2 * [%]
BASO		----		[10^3/uL]	---- [%]
NRBC		8.04	*	[10^3/uL]	9.7 * [/100WBC]
RET		2.45		[%]	0.0485 [10^6/uL]
IRF		24.9		[%]	

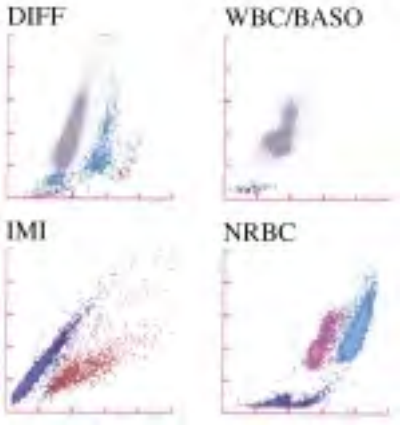

Fig. 2 *Acute lymphoblastic leukemia*
Hypercellular BM with 97% lymphoblasts, which are mainly seen as an extension of the lymphocyte cluster on the DIFF-scattergram.

Positive

WBC	&	32.01	*	[10^3/uL]	
RBC		3.25		[10^6/uL]	
HGB		9.4		[g/dL]	
HCT		27.9		[%]	
MCV		85.8	-	[fL]	
MCH		28.9		[pg]	
MCHC		33.7		[g/dL]	
PLT		63	*	[10^3/uL]	
RDW-SD		49.6		[fL]	
RDW-CV		15.8		[%]	
PDW		15.8	*	[fL]	
MPV		11.7	*	[fL]	
P-LCR		37.7	*	[%]	
PCT		0.07	*	[%]	
NEUT		----		[10^3/uL]	---- [%]
LYMPH		----		[10^3/uL]	---- [%]
MONO		----		[10^3/uL]	---- [%]
EO		----		[10^3/uL]	---- [%]
BASO		----		[10^3/uL]	---- [%]
NRBC		0.64	*	[10^3/uL]	2.0 * [/100WBC]
RET		0.16		[%]	0.0052 [10^6/uL]
IRF		11.1		[%]	

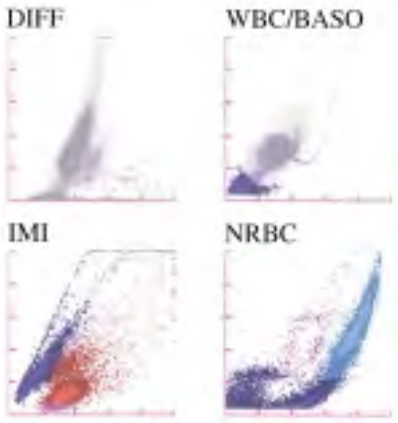

Fig. 3 *Acute myeloid leukemia*
Hypercellular BM with 49% of myeloblasts, which show a characteristic dense myeloblast cluster on the IMI-scattergram.

Positive

WBC	&209.42	@		[10^3/uL]	
RBC		4.45		[10^6/uL]	
HGB		14.5		[g/dL]	
HCT		47.3		[%]	
MCV		106.3		[fL]	
MCH		32.6		[pg]	
MCHC		30.7	-	[g/dL]	
PLT		117	*	[10^3/uL]	
RDW-SD		53.6		[fL]	
RDW-CV		15.8		[%]	
PDW		14.0	*	[fL]	
MPV		11.3	*	[fL]	
P-LCR		35.0	*	[%]	
PCT		0.13	*	[%]	
NEUT		----		[10^3/uL]	---- [%]
LYMP	&168.49	*		[10^3/uL]	80.5 * [%]
MONO		4.64	*	[10^3/uL]	2.2 * [%]
EO		0.16	*	[10^3/uL]	0.1 * [%]
BASO		----		[10^3/uL]	---- [%]
NRBC		2.11	*	[10^3/uL]	1.0 * [/100WBC]
RET		0.98		[%]	0.0436 [10^6/uL]
IRF		19.2		[%]	

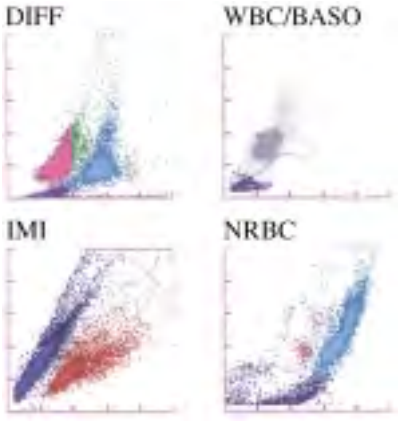

Fig. 4 *Chronic lymphocytic leukemia*
Hypercellular BM with 88% of lymphocytes, demonstrated by the predominant presence of lymphocytes on the DIFF-scattergram, without extension towards the blast area.

Positive

WBC	&163.42	@	[10^3/uL]		
RBC	3.00		[10^6/uL]		
HGB	9.9		[g/dL]		
HCT	37.9		[%]		
MCV	126.3	+	[fL]		
MCH	33.0		[pg]		
MCHC	26.1	-	[g/dL]		
PLT	194	*	[10^3/uL]		
RDW-SD	121.0	+	[fL]		
RDW-CV	27.3	+	[%]		
PDW	13.5	*	[fL]		
MPV	11.1	*	[fL]		
P-LCR	33.8	*	[%]		
PCT	0.22	*	[%]		
NEUT	----		[10^3/uL]	----	[%]
LYMPH	----		[10^3/uL]	----	[%]
MONO	----		[10^3/uL]	----	[%]
EO	----		[10^3/uL]	----	[%]
BASO	----		[10^3/uL]	----	[%]
NRBC	4.39	*	[10^3/uL]	2.7 *	[/100WBC]
RET	2.34		[%]	0.0702	[10^6/uL]
IRF	27.9		[%]		

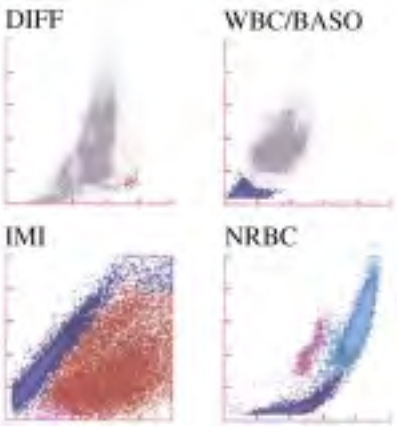

Fig. 5 *Chronic myeloid leukemia*
Hypercellular BM with myeloid hyperplasia, demonstrated by the expansion of the NEUT cluster towards the IG area on the DIFF-scattergram and the overwhelming presence of various myeloid precursors on the IMI-scattergram.

CONCLUSIONS

From these data we conclude that the Sysmex XE-2100 opens perspectives for use as a primary screening device for BM samples, particularly in cases of acute and chronic lymphatic and myeloid leukemias, where distinct pathology-related scattergram patterns have the potential to be of diagnostic help. Software improvements are required for more refined discrimination between WBC precursors and for eliminating or at least largely reducing the interference from fat droplets, which appear in a distinct pattern. At the present stage of development, although selected pathologies can already be identified, it cannot yet replace microscopic or flow cytometric BM analysis.

References

1) *Goossens W, et al.: Potential errors associated with automated cell counting in bone marrow suspensions. Proceedings 22nd Congress International Society of Haematology, Milano, pp. 413, 1988.*
2) *Goossens W, et al.: Bone marrow cytometry: feasibility of automated counts and differentials. Brit J Haematol, 87, Suppl. 1: 35, 1994.*
3) *Bentley SA, et al.: Correction of bone marrow nucleated cell counts for the presence of fat particles. Am J Clin Pathol, 104: 60, 1995.*
4) *Briggs C, et al.: Performance evaluation of the Sysmex XE-2100 automated haematology analyser. Sysmex J Int, 9: 113-119, 1999.*
5) *Briggs C, et al.: New quantitative parameters on a recently introduced automated blood cell counter- the XE-2100. Clin Lab Haematol, 22: 345-350, 2000.*
6) *Herklotz R, and Huber AR: Precision and accuracy of the leukocyte differential on the Sysmex XE-2100. Sysmex J Int, 11: 8-21, 2001.*
7) *Sakamoto C, et al.: Automated enumeration of cellular composition in bone marrow aspirate with the Cell-Dyn 4000 automated hematology analyzer. Acta Haematol, 101: 130-134, 1999.*