Automated Bone Marrow Analysis : Dream or Reality?

Gina ZINI

Research Center for the Development and Clinical Evaluation of Automated Methods in Hematology, Hematology Department, Catholic University of Sacred Heart, Rome, Italy.

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BACKGROUND AND RATIONALE

Since 1997, the availability, in haematology laboratories of automated analyzers capable of performing an extended differential blood cell count, including the nucleated red blood cell (NRBC) count, allowed their experimental use to estimate the cell composition of bone marrow fluid. In 1999 we evaluated the Sysmex XE-2100 to detect the clinical usefulness of immature granulocyte (IG) and NRBC counts on more than 300 specimens, using the NCCLS-H20A protocol as reference. This first trial showed for both cell types a predictive negative value > 95 % and a predictive positive value > 90 %. During 2000 we continued evaluation of XE-2100 bone marrow analysis, focusing on:1) a reproducibility study on 85 bone marrow samples assessed by duplicate analysis, which showed CVs of < 4.5 % for cellularity (total nucleated cell count [TNCC]), granulocytes, platelets and RBC parameters (*Table 1*); 2) a comparison study of XE-2100

with manual microscopy, which showed good agreement for granulocytes (r^2 = 0.657; mean difference - 0.9 %) and a slightly lower agreement for erythroblasts (r^2 = 0.444; mean difference - 13.4 %), with a tendency of the XE-2100 to underestimate erythroblast count.

The aims of the latest study have been to extend the above-mentioned second study to include more patients with a view to:
1. Obtaining reproducible data on bone marrow cellularity
2. Detecting reproducible quantitative data from comparison of:
   - peripheral blood (PB) and bone marrow fluid (BMF) automated analysis
   - BMF automated analysis and microscope count (Micro)
3. Discriminating samples:
   - Evaluable Samples (ES) versus Non Evaluable (NEv)
   - Normal samples (Nor) versus Pathological (Path).

Table 1 *Duplicate analyses of 85 bone marrow specimens.*
MCV = mean corpuscular volume; IRF = immature reticulocyte fraction; TNCC = total nucleated cell count.

Parameter	Mean	CV%
Haemoglobin	10.6 g/dL	1.9
MCV	85.6 fl	0.5
Platelets	130×10^9/L	4.5
Reticulocytes	71.3×10^9/L	7.3
IRF	0.43	12.2
TNCC	28.1×10^9/L	4.2
Neut	49.7 %	6.6
Lymph	25.4 %	15.8
NRBC	12.8 %	18.5

MATERIAL AND METHODS

In our laboratory, during a 3-month period, we analysed 362 specimens of BMF and PB from patients submitted for diagnostic and/or follow-up purposes in our Haematology Day Hospital. These comprised acute leukaemia (63), chronic leukaemia (78), MDS (42), aplasia (16), BMT follow-up 57, lymphoma (72) and others (34). Bone marrow was obtained from the superior posterior iliac crest. In routine practice about 90% of diagnoses concerned suspected or confirmed haemopathies, while 10% included other pathologies (i.e., infectious diseases or suspected metastatic bone marrow involvement).

The method is very simple. The first 0.5 mL of specimen was used for smear preparation, while the next 1-2 mL, collected into K_3-EDTA anticoagulant, was analysed within 2 hours after gentle mixing, simultaneously with the patient's PB specimens, without filtration or other treatment, using XE-2100 reticulocyte and NRBC mode analysis.

As the reference method, morphological examination and count of May-Grünwald-Giemsa stained BM films was performed using the standard routine practice in our laboratory: 500 cells were counted on at least two slides for specimens with normal or reduced cellularity and 1,000 cells for specimens with increased cellularity by two independent haematologists. Additional cytochemistry stains were performed where necessary. A third expert reviewer was available to resolve discrepancies. The microscopy cellularity assessment was semi-quantitative and included two categories (2 and 3) for normal plus two extreme categories (1 and 4) for specimens with absent or hugely increased cellularity respectively. Morphological review of May-Grünwald-Giemsa stained PB films was performed only on flagged specimens, according to the routine guidelines in our laboratory. All data were analysed using Descriptive Statistics methods. Two working guidelines were applied for statistical data analysis:

- Elimination of all BMF specimens with an incomplete instrument report due to high WBC count (n = 89 [24.6 %]) to avoid bias due to sample dilution,
- Only those parameters with good reproducibility (CV < 10 %) used for statistical purposes.

According to the results of the microscope reference method we have subdivided the BMF samples with complete instrument report into three groups:

- 26 Non Evaluable (NEv)
- 50 Normal (Nor)
- 197 Pathological (Path)

Microscopically NEv have been defined as smears without particles and with marked contamination by peripheral blood: the final report in these cases contains only a qualitative description, without myelogram. Surprisingly, all 26 NEv have revealed pathological cells, so we have considered them as pathological. The Nor samples have been defined as bone marrow aspirates containing cell distributions within normal quantitative and qualitative ranges, and with no pathological cells, while the Path were those falling out of such ranges.

RESULTS

1. Bone Marrow Cellularity:
We found a very strict correlation between the microscope semi-quantitative evaluation and instrument total nucleated cell count (TNCC = NRBC + WBC): this allows a very precise numerical quantitation of cellularity. The median value of the normal cellularity classes 2 and 3 was 50×10^9 /L and for the increased cellularity the median value was 100×10^9 /L or more.

2. Correlation between PB and BMF parameters:
In this particular study, we have confirmed our previous observation, made in 1999, that RBC, HGB and MCV in peripheral blood (PB) and bone marrow fluid (BMF) correlate well in all three sets of specimens; normal, pathological and non-evaluable (***Table 2***).

Table 2 *Correlation of PB (y-axis) and BMF (x-axis) for RBC, HGB and MCV.*
Instrument groups are Non Evaluable (NEv), normal (Nor) and pathological (Path).

Parameter	Instrument group	y =	r^2
RBC	All	0.9493x + 12.2	0.8862
HGB	All	0.9744x + 2.2971	0.8807
MCV	All	0.9828x + 9.8533	0.9578

When comparing platelet and reticulocyte counts in PB and BMF, the behaviour is rather different. For both platelets and reticulocytes the correlations are very good in normal specimens being $r^2 = 0.85$ and $r^2 = 0.97$ respectively (***Table 3***). In pathological and non-evaluable specimens, however, a greater dispersion of points is noted for both platelets and reticulocytes (***Table 3***).

The correlation of granulocytes from PB and BMF in the non-evaluable specimens is very good ($r^2 = 0.94$) confirming the same cellular composition of the two biological materials (***Fig. 1***). In evaluable specimens, both normal and pathological a much less good correlation exists between PB and BMF granulocyte counts (***Fig. 2***). This is confirmed on examining the granulocyte frequency distribution curves for the same data (***Fig. 3***). In normal specimens no correlation was observed for eosinophils between PB and BMF. In normal specimens the ratio of PB to BMF lymphocytes is always > 1, confirming already reported observations. Turning now to the immature

Table 3 *Correlation of PB (y-axis) and BMF (x-axis) for platelets and reticulocytes. Instrument groups are non-evaluable (NEv), normal (Nor) and pathological (Path).*

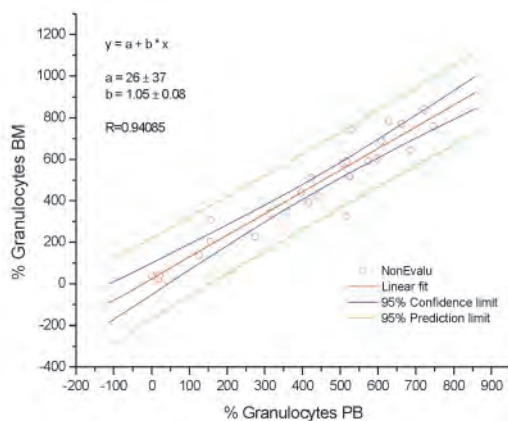| Parameter	Instrument group	y =	r^2
Platelets	NEv	0.4031x + 41.012	0.5094
Platelets	Path	0.8554x + 4.9965	0.7323
Platelets	Nor	0.8478x + 12.884	0.8581
Reticulocytes	NEv	1.0258x + 31.403	0.8405
Reticulocytes	Path	1.0041x + 21.805	0.9293
Reticulocytes	Nor	0.99x + 3.0785	0.9735

Fig. 1 *Comparison of BMF granulocytes (y-axis) with PB granulocytes (x-axis) for non-evaluable specimens.*
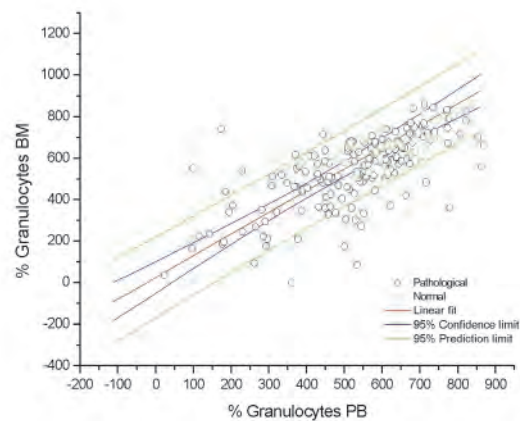

Fig. 2 *Comparison of BMF granulocytes (y-axis) with PB granulocytes (x-axis) for normal and pathological specimens.*
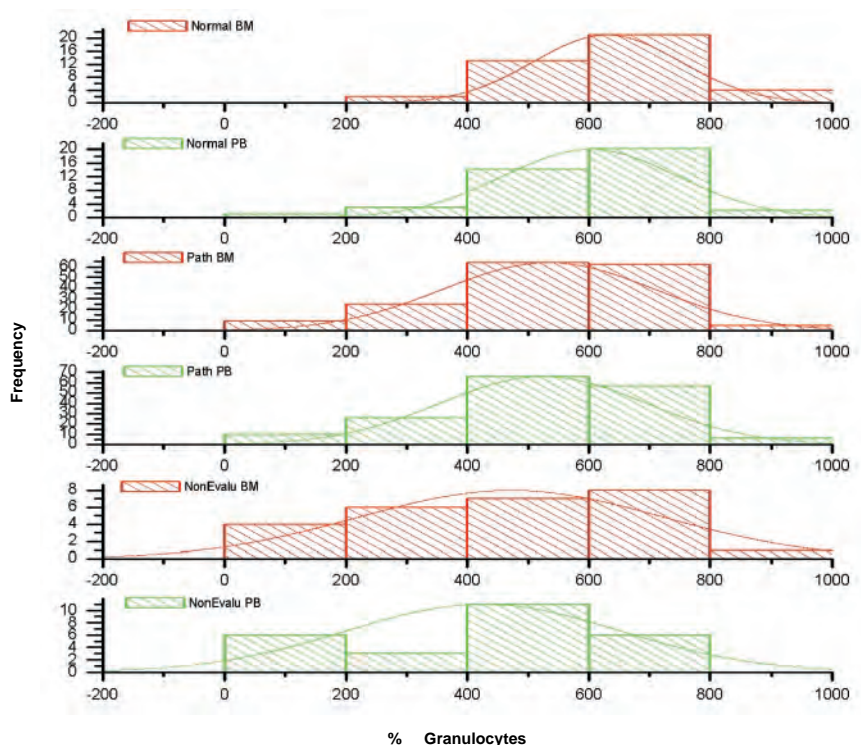
Fig. 3 *Granulocyte frequency curves for different patient groups.*

reticulocyte fraction (IRF) the PB/BMF ratio for IRF is always < 1 in normal specimens. The correlation between BMF and PB for the NRBC count is always zero in normal specimens but must always be > 0 in the other two pathological groups. In our patients the NRBC ratio BMF/PB is equal to or < 4.5 in pathological non-evaluable specimens while it is > 4.5 in pathological evaluable specimens. Finally we have analysed the TNCC, which, on the XE-2100, is the sum of the WBC plus NRBC. In all specimens, the ratio of TNCC BMF/PB is always > 1 with a wide spread of values in pathological specimens both evaluable and non-evaluable.

PRELIMINARY CONCLUSIONS ON SIMULTANEOUS ANALYSIS OF PB AND BMF

1. In normal specimens, PB and BMF measurements of HGB, RBC, MCV, platelets and reticulocytes correlate strictly. In pathological samples platelets and % reticulocytes exhibit a different behaviour.

2. Very strict correlation of PB and BMF granulocyte counts occurs in non-evaluable specimens. There is no correlation in evaluable specimens be these normal or pathological.

3. In this study it is confirmed that in normal BMF the lymphocyte count is lower than in PB while the IRF is higher.

4. NRBC in PB is always zero in normal specimens. By using the cut-off level of 4.5 for the ratio BMF/PB it is possible to distinguish evaluable from non-evaluable specimens.

5. In this study a cut-off value for the ratio BMF/PB for TNCC of </= 3 permits discrimination of 100 % of evaluable and 75 % of non-evaluable specimens.

Using these five preliminary conclusions it becomes possible to propose algorithms to discriminate (1) evaluable from non-evaluable BMF specimens and (2) normal from pathological BMF specimens.

(1) The proposed algorithm for automated discrimination between evaluable and non-evaluable BMF takes into account the following instrumental data: TNCC of BMF and WBC, lymphocyte and eosinophil counts in PB. The proposed algorithm is:

$$TNCC_{BMF} / WBC_{PB} - (\#Eos_{PB} + \#Lym_{PB})$$

In this study the discriminating values were:

Evaluable BMF specimens > 7
Non-evaluable BMF specimens < 5

In the study all evaluable and non-evaluable specimens followed the proposed algorithms.

(2) The proposed algorithms for discriminating normal from pathological BMF specimens use the following instrument parameters: from BMF - TNNC, #NRBC, #(HFR+MFR); from PB - WBC, #NRBC, #(HFR+MFR). The proposed algorithms are:

$$TNCC_{BMF} / WBC_{PB} - (\#Eos_{PB} + \#Lym_{PB}) > 7 < 20$$
$$\#NRBC_{BMF} / \#NRB_{CPB} = 0$$
$$\#(HFR + MFR)_{BMF} / \#(HFR + MFR)_{PB} > 1.5$$

Combining these data it was possible to identify all normal BMF data.

Moreover, since there is a reasonable correlation between the instrument proportional granulocyte count and microscopy, the former, in addition to the bone marrow TNCC can be used in these normal specimens.

PATIENT EXAMPLES

It is our belief that automated screening of bone marrow fluid in conjunction with the peripheral blood is very useful for both patients and clinicians particularly when applying scattergram review. To demonstrate this, three patients with peripheral blood cytopenia who were submitted to bone marrow aspiration, have been selected. In each case, analysis of the XE-2100 BMF data provided immediate feedback to the clinician for optimum patient management.

Patient 1: The patient has non-Hodgkin's lymphoma and has been submitted to autologous bone marrow transplantation. Recovery is very slow and requires strict follow-up. At the present consultation peripheral blood WBC count is 1.85×10^9 /L with a neutrophil count of 0.83×10^9 /L (*Fig. 4*). Bone marrow aspiration performed 10 minutes later provided evidence of bone marrow cellular recovery, the clinician receiving the useful clinical information that the BMF neutrophil count was 3.59×10^9 /L (*Fig. 5*).
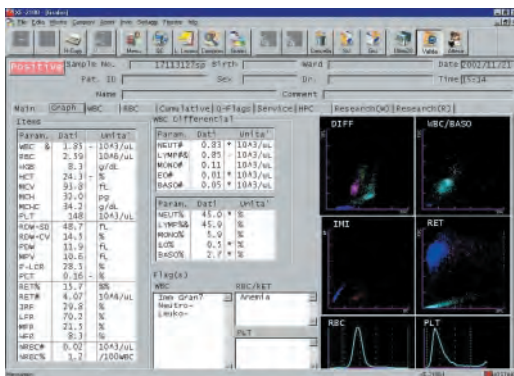

Fig. 4 *The peripheral blood haemogram of Patient 1 generated by the XE-2100. Note the low WBC and neutrophil counts.*
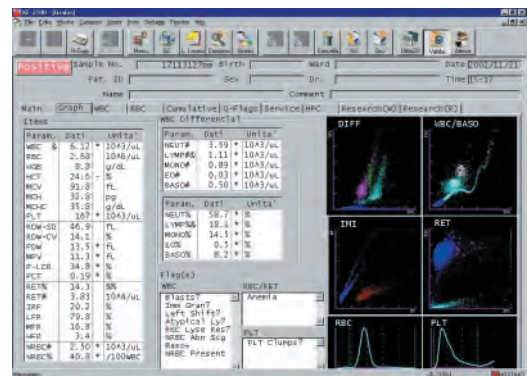

Fig. 5 *Bone marrow fluid haemogram from patient 1 generated at the same time as the peripheral blood data. Note the total WBC and neutrophil counts confirming recovery.*
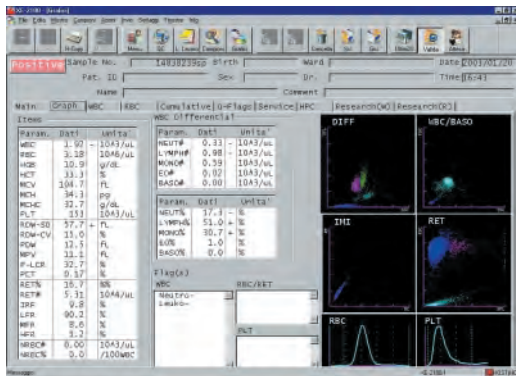
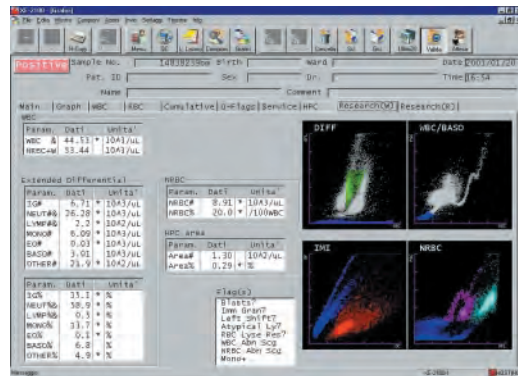**Fig. 6** *Peripheral blood haemogram from patient 2 showing leukopenia and neutropenia.*

Fig. 7 *Bone marrow fluid haemogram from patient 2 indicating increased cellularity with abnormal cell clusters. Patient has RAEB.*
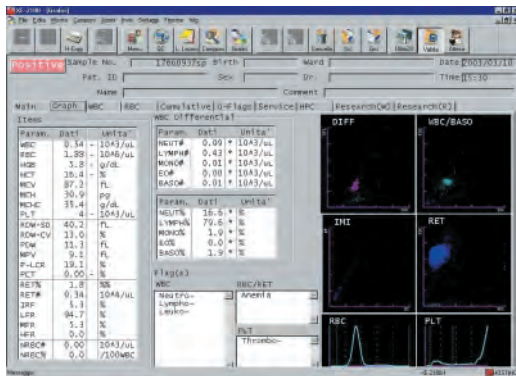

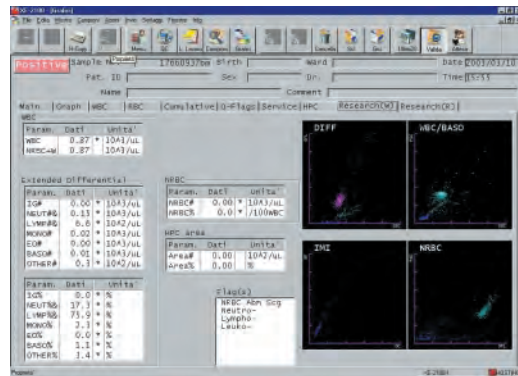**Fig. 8** *Peripheral blood haemogram from patient 3. Note the severe pancytopenia.*

Fig. 9 *Bone marrow fluid haemogram from patient 3 clearly demonstrating the reduced bone marrow cellularity. This patient has aplastic anaemia.*

Patient 2: This patient when first seen had a total WBC count of 1.92×10^9 /L with a neutrophil count of 0.33×10^9 /L (*Fig. 6*). A bone marrow aspirate was performed and the automated analysis of the BMF revealed increased cellularity with an abnormal cell cluster (*Fig. 7*). The clinician was informed and immediately admitted the patient for diagnostic work up. A diagnosis of refractory anaemia with excess of blasts (RAEB) was made.

Patient 3: This is a young patient seen for the first time with peripheral blood pancytopenia (*Fig. 8*). Automated analysis of the BMF revealed poor bone marrow cellularity (*Fig. 9*). The patient was immediately admitted and a diagnosis of aplastic anaemia confirmed.

CONCLUSIONS

This study shows that it is possible to quantify bone marrow cellularity using the automated total nucleated cell count (TNCC = NRBC + WBC) due to the very close correlation, which exists with the microscopy assessment. By simultaneously using the XE-2100 analysis of PB and BMF there exists a very rapid and reliable method to discriminate evaluable from non-evaluable specimens. Using the cytogram pattern it is also possible to discriminate normal from certain pathological samples.

Using this routine of automated BMF analysis, there is now an opportunity, prior to conventional microscopy examination, to comment on certain specimens quickly, allowing clinical decision more or less immediately to repeat the bone marrow aspirate or to perform a marrow biopsy if necessary. This way it is possible to improve the efficiency of patient management.

Reference

Zini G, Mistretta G, Giordano G et al (2001) Automated analysis of bone marrow fluid with the Sysmex XE-2100 blood cell counter. Infus Ther Transfus Med 28:277-279.