5.
Clinical Utility of the Automated IG-Count

Xavier Troussard

Introduction

Quantitative analysis of immature granulocytes (IG) is useful for the optimal clinical and biological management of patients with certain haematologic diseases (chronic myelogenous leukaemia, chronic myeloproliferative disorders, myelodysplastic syndromes) or patients with infection. The IG compartment includes promyelocytes, myelocytes and metamyelocytes (**figure 1**). The interpretation of the IG can be difficult in a few cases. From a clinical point of view, four major questions require to be asked:

1. How many patients have IG?
2. Is it useful to detect IG?
3. Is there any correlation between automated blood cell counter (the XE-2100) and microscopic analysis?
4. Is it relevant to interpret IG as IG/N ratio?

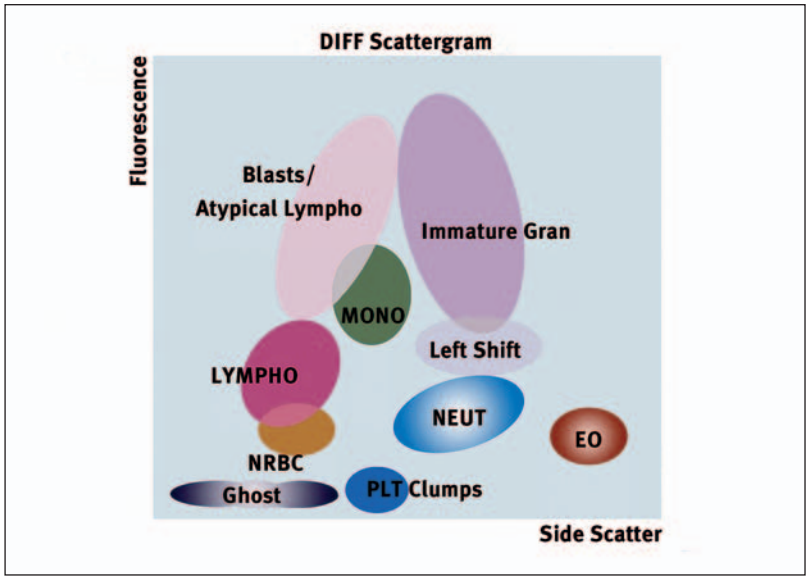


Figure 1
The DIFF scattergram of the XR-2100 showing the locations of the different leukocyte types. Fluorescence is depicted on the y-axis and side scattered light on the x-axis. The IG count is derived from the DIFF channel.

Frequency of patients with IG

The frequency of patients with IG was assessed by analysis of 860 samples (**figure 2**). The study cohort came from an academic hospital and consisted of patients with infection and malignant disease. For 20% of patients, no immature granulocytes were detected. Interestingly, 70% of hospitalised patients presented with an IG count ‹ 1% and 80% with an IG count ‹ 2%. In the laboratory when the IG count is › 2% (20% of patients), the policy is to examine a peripheral blood smear by microscopy. However, the level of this cut off (2%) remains questionable. This high frequency is probably very artificial since it depends on the hospital and the

presence of intensive care and haematology units and on the ratio of in-patients to out-patients. It also depends on the frequency of infections and cancer and particularly on the use of haemopoietic growth factor treatment for the latter.

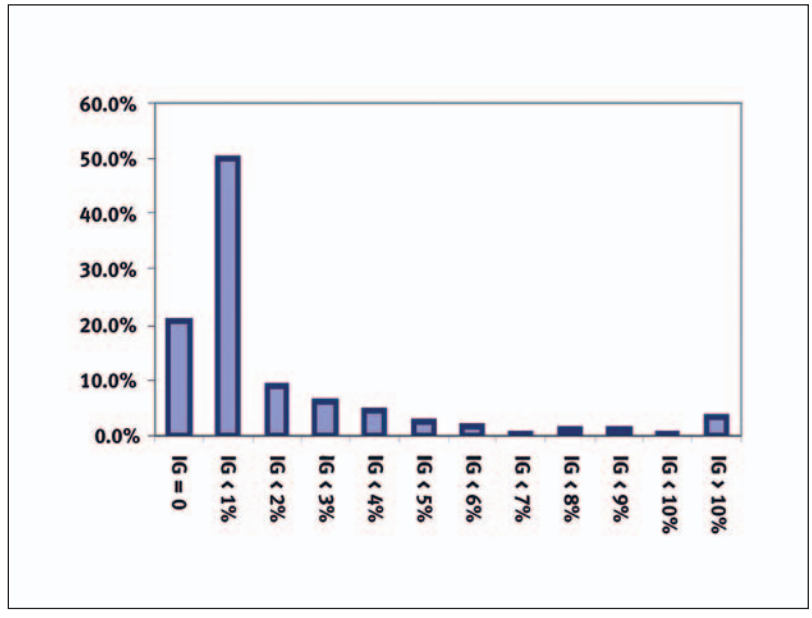


Figure 2
Frequency of patients with IG (n=860).

Is it useful to detect IG?

The answer to this second question is, of course, affirmative. The detection of IG is important for distinguishing patients with chronic myeloproliferative disorders (CMPD) and myelodysplastic syndromes (MDS) from patients with infection. Since other presentations in this Symposium concentrate on the usefulness of IG measurement in infections, this article will concentrate on CMPD and MDS. CMPD include chronic myelogenous leukemia (CML), polycythaemia vera, essential thrombocythaemia, agnogenic myeloid metaplasia, atypical chronic myeloproliferative disorder (8p11-p12) and chronic neutrophilic leukaemia. The detection of IG in the group of myelodysplastic syndromes (MDS) may also be useful. The MDS group includes refractory anaemia (RA), refractory anaemia with excess of blasts (RAEB), refractory anaemia with ring sideroblasts (RARS), chronic myelomonocytic leukaemia (CMML) and refractory anaemia with excess of blasts in transition (RAEBT). The distribution of haematological malignancies in the North Cotentin region of France is shown in **figure 3**. CMPD accounts for 11% of all haematological malignant disorders in the region and this frequency is identical to that of MDS.

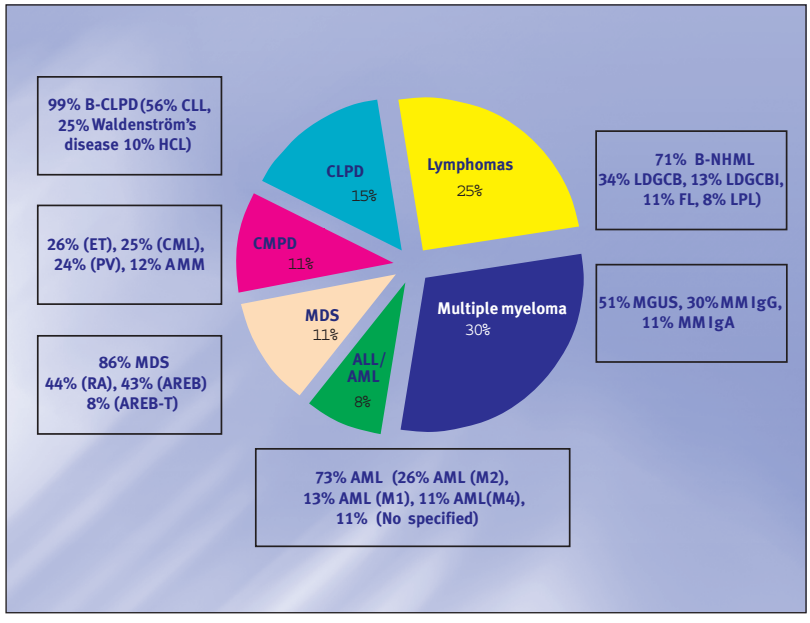


Figure 3
North Cotentin Region, France: register of haematological malignancies and their distribution.
CLPD = chronic lymphoproliferative disease,
AMM = agnogenic myeloid metaplasia,
NHML = Non-Hodgkin's malignant lymphoma,
LDGCB = large cell lymphoma,
MGUS = monoclonal gammopathy of unknown significance,
PV = polycythaemia vera,
CMPD = chronic myeloproliferative diseases,
MDS = myelodysplastic syndromes,
ALL/AML = acute leukaemia.

A review of the peripheral blood cell characteristics of certain CMPDs and MDSs reveals a role for the IG count (**Table 1**)

Cell type	CML	aCML	CMML
Basophils	› 2%	‹ 2 %	‹ 2 %
Monocytes	‹ 3 %	› 3 – 10 %	Usually › 10 %
Gran. dysplasia	–	++	+
Immature Gran (IG)	› 20 %	10 – 20 %	‹ 10 %
Blasts	‹ 2 %	› 2 %	‹ 2 %

Table 1
Differential leukocyte count features in CMPD and MDS.

Is there a correlation between the XE-2100 and microscopic analysis?

Examination by microscopy is time consuming. The automated quantitative analysis of IG is rapid, specific and accurate. For both IG (XE-2100) and the sum of metamyelocytes, myelocytes and promyelocytes by microscopy a very good correlation (**figure 4**) was found ($y = 0.8476x + 0.3103$ $r^2 = 0.7087$). The microscopy sum represents the ratio of these immature granulocytes to the total leukocyte count.

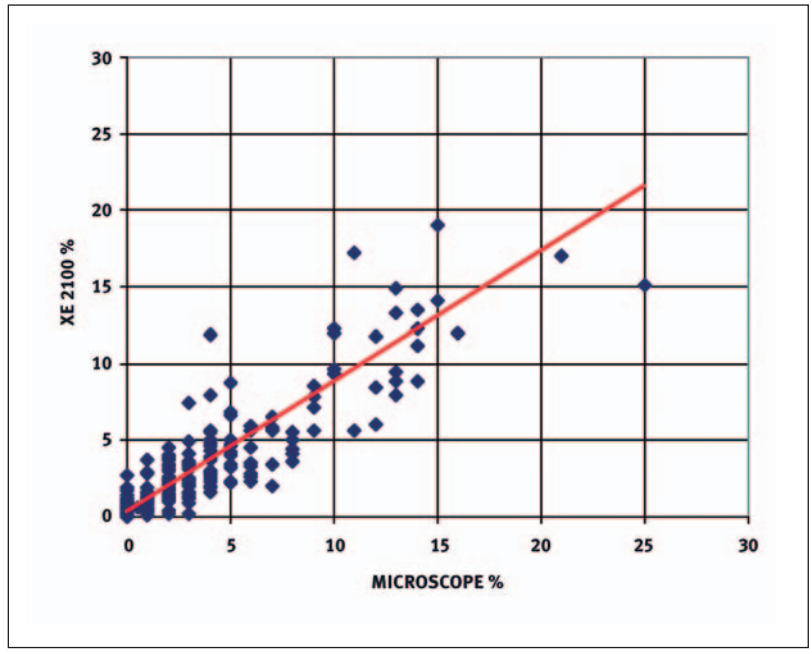


Figure 4
Comparison of IG count (%) by XE-2100 (y-axis) with microscopy counts (x-axis).

Is it relevant to interpret IG as a new parameter defined by the IG/N ratio?

For interpreting the IG count, the immature granulocyte/total leukocyte ratio is commonly used. However, because this ratio can be difficult to interpret in patients with neutropenia, we have tested the value of using a new parameter defined as the IG/N (Immature granulocyte/Neutrophil) ratio. The comparison of IG/N by instrument with IG/N by microscopy is shown in **figure 5**. The results clearly show that the best correlation is observed for the IG/N ratio rather than the usual IG/total leukocyte ratio ($r^2 = 0.8837$ versus $r^2 = 0.7087$). Frequency distribution histograms for both IG and IG/N are displayed in **figure 6** each panel representing distribution when the neutrophil count is $< 2.5 \times 10^9$/L (neutropenia [PN-]), $2.5 - 8.0 \times 10^9$/L (reference range in health [PN=]) and $> 8.0 \times 10^9$/L (neutrophil leukocytosis [PN+]). Taking 2% as the decision point some 10% of the IG results will be considered abnormal whereas only 5% of the IG/N results would be considered abnormal.

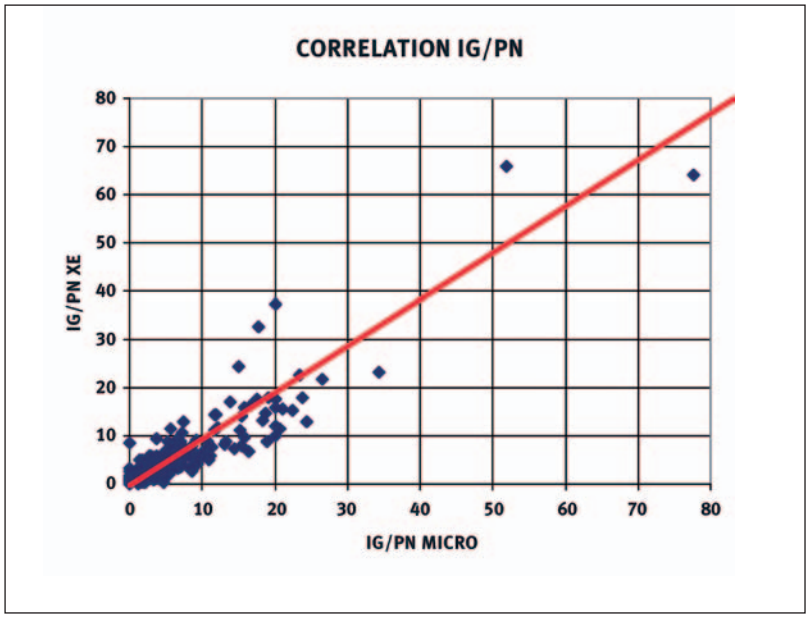
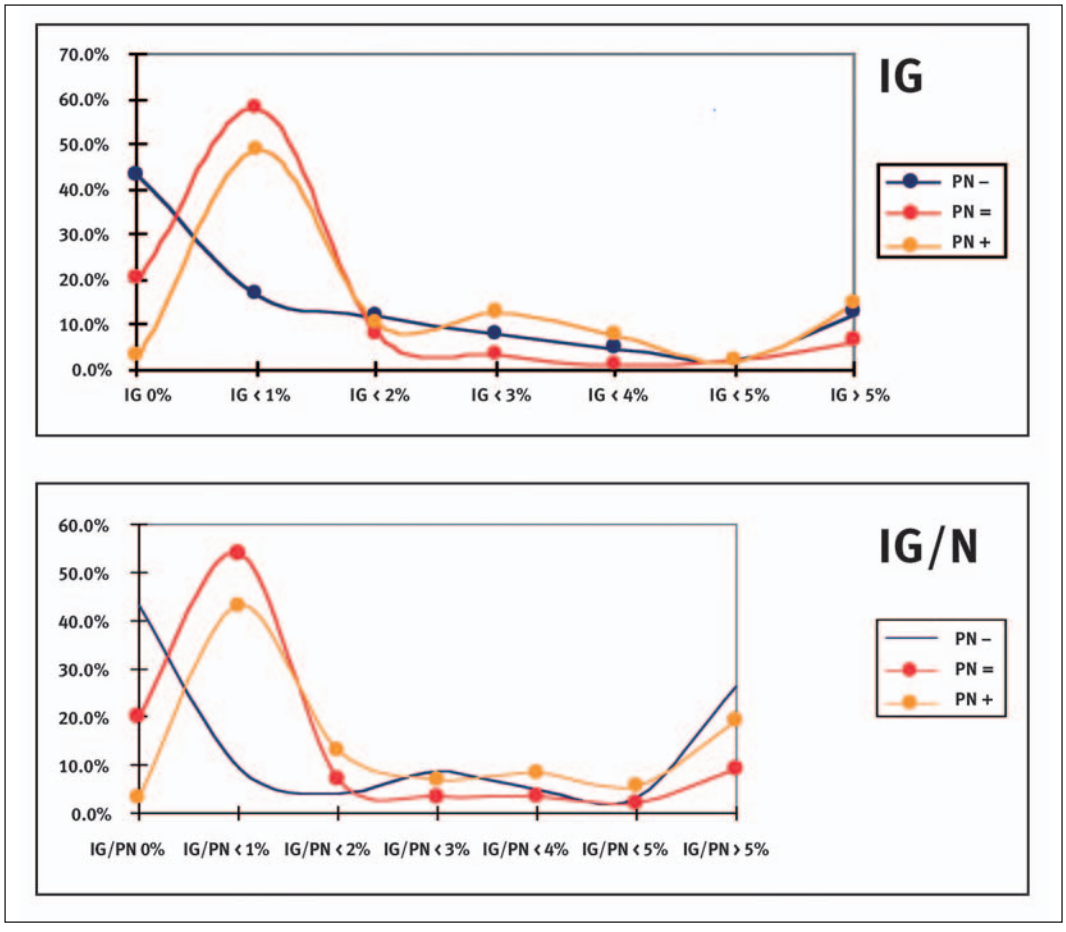
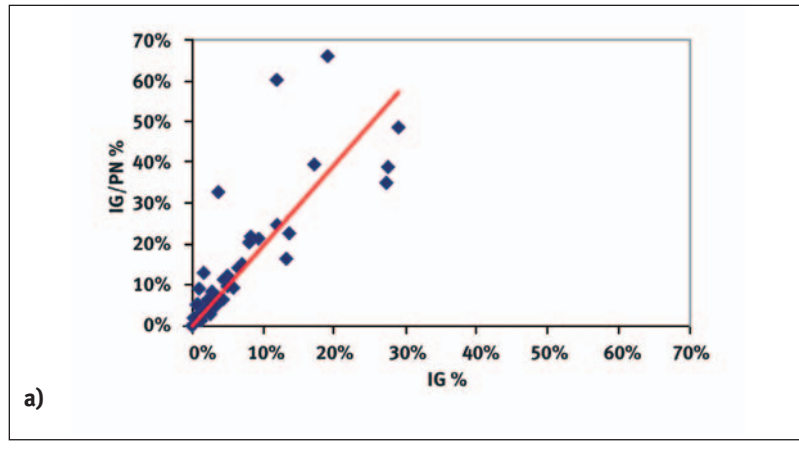


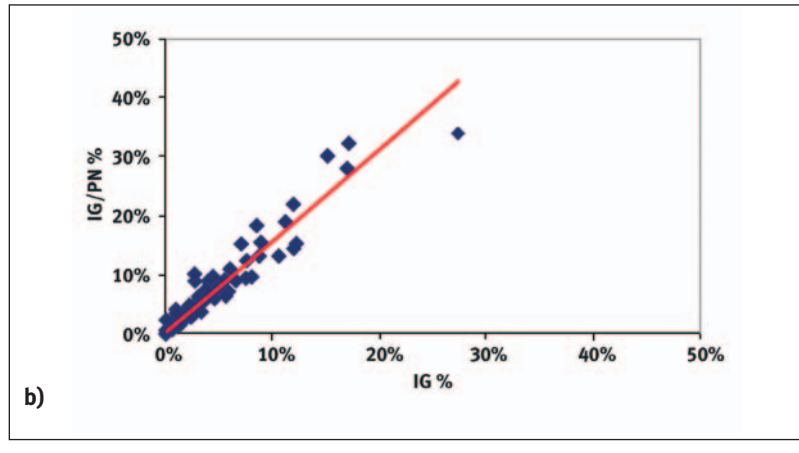
Figure 5
Comparison of IG/N ratio by XE-2100 (y-axis) with microscopy counts (x-axis).

Figure 6
The upper panel is a frequency distribution histogram of the IG counts. The lower panel is a frequency distribution histogram of the IG/N ratios from the same patients. 'PN' = neutropenia, 'PN=' = normal neutrophil count, 'PN+' = neutrophil leukocytosis.



The correlation is good for patients with normal (**figure 7b**) or high neutrophil (**figure 7c**) counts but is clearly insufficient for patients with neutropenia (**figure 7a**). Finally, the use of IG/N ratio could be relevant. It could be more sensitive: we detected 52 samples with IG/N › 2% but with IG/total ratio ‹ 2% (**figure 8**).





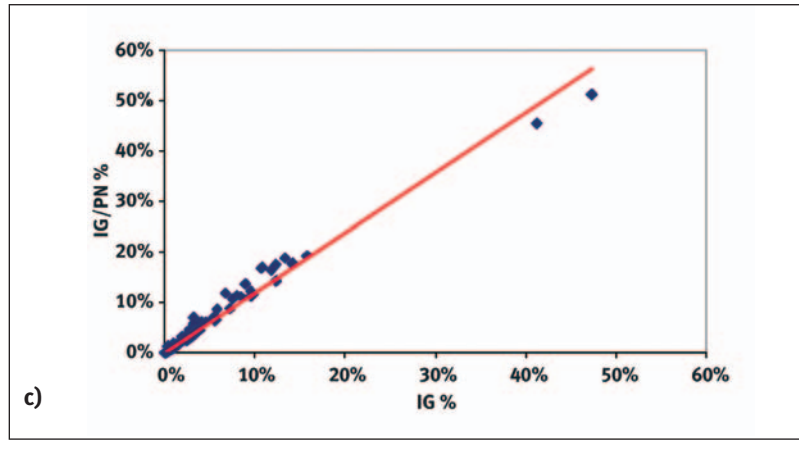
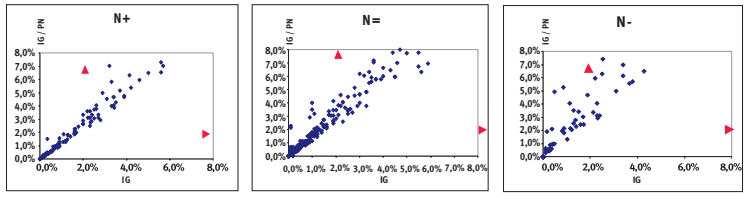


Figure 7
*Comparison of IG/N (y-axis) with IG %. In **7a** the comparison is made for neutropenic specimens (r = 0.762, slope = 1.961); in **7b** comparison is made for specimens with normal neutrophil counts (r = 0.948, slope = 1.554); in **7c** comparison is made for specimens with neutrophil leukocytosis (r = 0.978, slope = 1.184).*



IG %		IG/N>2	IG/N>3	IG/N>4	Total
N-	< 2	19	9	6	34
	> 2	1	3	30	
N=	< 2	28	10	2	40
	> 2	2	12	60	
N+	< 2	5	0	0	5
	> 2	6	13	39	
	Total	52			

Figure 8
Interpretative differences when IG/N is used in neutropenic, normal and neutrophilic specimens.

Conclusion

The following conclusions were reached from this study:

- The detection of IG is useful for the diagnosis of CMPD
- The correlation of IG between automated cell analysis and microscopy is excellent,
- The new parameter determined by the ratio IG/N could be useful for improving the diagnosis of infection and bone marrow recovery.

Extensive studies are required, however, for validating these preliminary results.

Acknowledgements

I would like to thank Dr. F. Schillinger and J.P. Perol for their scientific help in this work, Maude Marchiset and all the technicians in the laboratory for their technical help and all the patients who participated in the study.