GALLERY ARTICLE

A Picture is Worth a Thousand Words

R. M. ROWAN and J. LINSSEN

Sysmex Europe GmbH, Bornbarch 1, 22848 Norderstedt, Germany.

INTRODUCTION

During the past forty years blood cell analysers have developed from comparatively simple semi-automated single parameter devices producing only numerical data to fully automated complex multiparameter devices frequently employing multiple technologies and producing a bewildering array of numerical and graphical parameters. The history of these developments is well described in a text by Groner and Simson[1] in 1995. In spite of these developments, however, many laboratories continue to ignore all but the numerical output of analyzers, which they report to the clinician frequently as uninterpreted data. Raw data often has minimal or no information content. For many, routine analytical haematology remains locked into a time frame defined by Wintrobe in the 1930s[2]. Modern cell counters, however, provide significant additional information, some of which overlaps that obtained at the microscope and some that is undoubtedly new. The latter possesses the capability to improve patient care both in a diagnostic and in a therapy monitoring sense. Ignoring these new parameters including the graphics is tantamount to 'throwing the baby out with the bath water', which seems to be a bad practice. At least six companies now produce multiparameter haematology analysers but employ various often subtly different methods and reagents for identifying and classifying cells. As a result a series of instrument specific parameters is produced and for correct clinical interpretation the particular method used must be known. The principles of measurement employed in haematology analysers manufactured by the Sysmex Corporation have recently been reviewed by Fujimoto[3].

This is the first of a series of articles describing the graphical output of the Sysmex XE-2100 and illustrating examples of clinical benefit following the scrutiny and analysis of scattergrams. The clinical cases illustrated come from a variety of sources within Europe and Sysmex is very grateful to the many clinicians who have given permission for their publication. A detailed description of these cases together with others is available and can be found on the Sysmex Europe homepage.
http://www.sysmex-europe.com/caseforum/
A new case contribution is submitted every month.

EXAMPLES OF NORMAL AND ABNORMAL SCATTERGRAMS

The XE-2100 generates six different scattergrams including DIFF scattergram, WBC/BASO (white blood cell/basophil) scattergram, IMI (immature information) scattergram, NRBC (nucleated red blood cell) scattergram, RET (reticulocyte) scattergram, PLT-O (optical platelet) scattergram. In addition there are RBC and PLT-I (impedance platelet) histograms[4].

For the WBC 4-part differential the reagent subsystem lyses RBCs and acts on WBC membranes to allow dye entry to stain DNA and RNA within the cells. The sample is then analysed by flow cytometry using a semiconductor laser to detect side fluorescence and side scattered light from which the DIFF scattergram is generated (***Figs 1 and 2***).

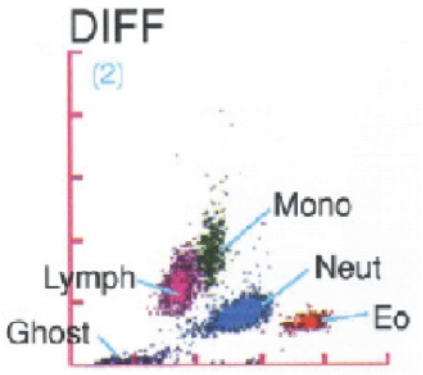


Fig. 1 *Normal DIFF scattergram*

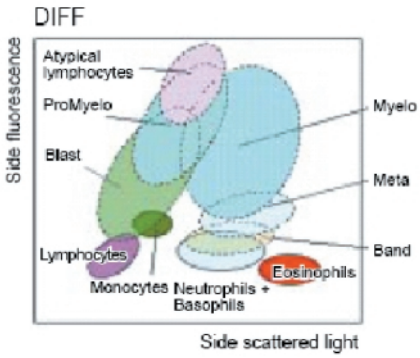


Fig. 2 *Abnormal DIFF patterns (schematic)*

For measurement of WBC/BASO the reagent subsystem lyses the RBCs and selectively suppresses degranulation of basophils resulting in their separation from other forms of WBC. After this reaction the sample is analysed by flow cytometry using a semiconductor laser to detect forward and side scattered light information (***Figs 3 and 4***) from which the WBC/BASO scattergram is generated.

For measurement in the IMI channel a polyoxyethylene series non-ionic surfactant and a sulphur-containing amino acid lyses mature WBCs. Immature cells of the granulocyte series are protected by the amino acid against lysing. The IMI scattergram is based on DC and RF impedance signals (***Figs 5 and 6***). Further qualitative differentiation of immature cells of the granulocyte series in the IMI channel is possible. The effect of the lysing reagent differs with each type of immature cell. Mature WBCs are, however, completely shrunken and located in the ghost area of the scattergram.

The XE-2100 counts reticulocytes and platelets using forward scattered light and side fluorescence (***Figs 7 and 8***). The fluorescent dye contains two stains, polymethine and oxazine. These two dyes penetrate the cell membranes staining RNA in reticulocytes and DNA/RNA in nucleated cells. Reticulocytes are separated from mature red cells by the differences in RNA content and from nucleated cells by differences in DNA/RNA content. By analysing the RET scattergram, reticulocyte counts, reticulocyte ratios for individual reticulocyte fluorescence intensity zones (LFR, MFR, HFR), immature reticulocyte fraction (IRF) [***Figs 7 and 8***] and optical platelet count [***Figs 9 and 10***] are determined.

The XE-2100 counts NRBC using side fluorescence and forward scattered light (***Figs 11 and 12***). The reagent subsystem causes lysis of mature RBCs and exposes NRBC nuclei. The specific dye stains WBC intracytoplasmic organelles and nuclei quite strongly while the staining of the NRBC nuclei is comparatively weak allowing clear discrimination of the two types and making NRBC counting possible.

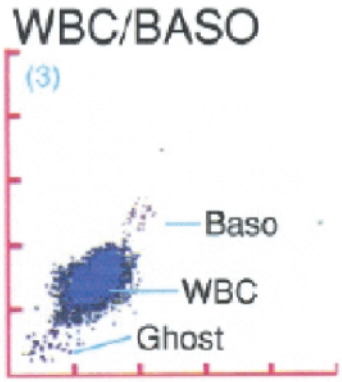


Fig. 3 *Normal WBC/BASO scattergram*

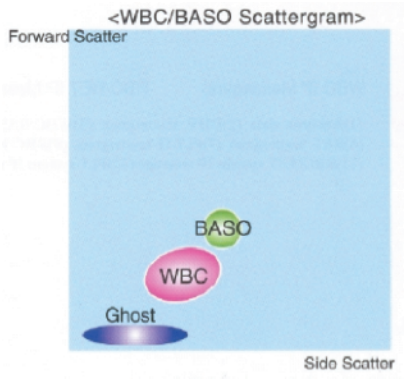


Fig. 4 *WBC/BASO patterns (schematic)*

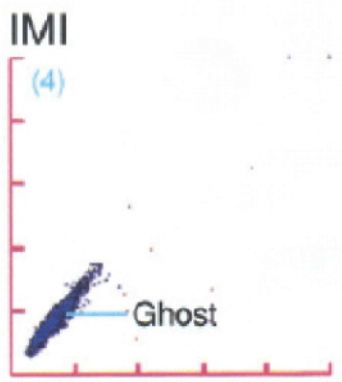


Fig. 5 *Normal IMI scattergram*

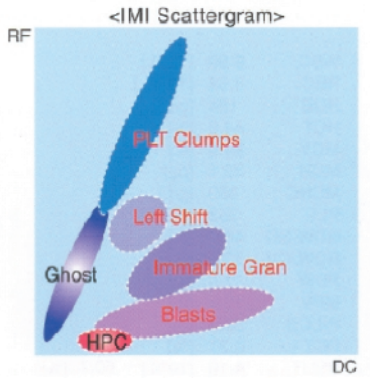


Fig. 6 *Abnormal IMI patterns (schematic)*

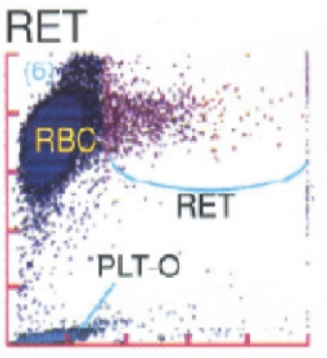


Fig. 7 *Normal reticulocyte scattergram*

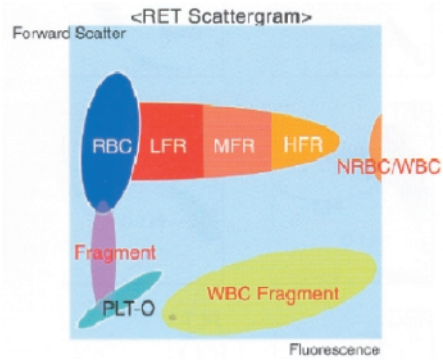


Fig. 8 *Abnormal reticulocyte patterns (schematic)*

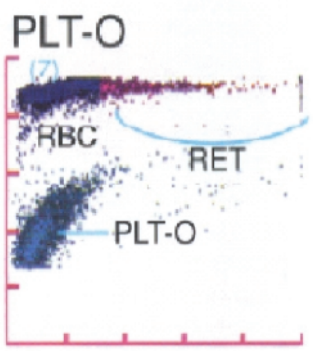


Fig. 9 *Optical platelet (PLT-O) scattergram*

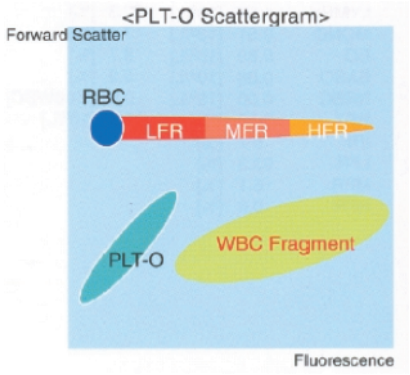


Fig. 10 *Abnormal PLT-O patterns (schematic)*

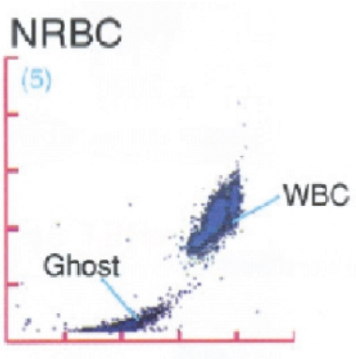


Fig. 11 *Normal NRBC scattergram*

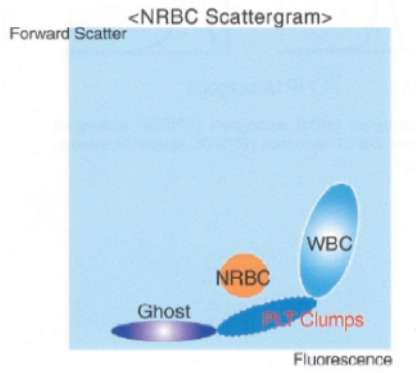


Fig. 12 *Abnormal NRBC patterns (schematic)*

THE SYSMEX INFORMATION SYSTEM (SIS)

Those interested in learning more about the Sysmex Information System (SIS) are referred to the following article in this issue of the Sysmex Journal International pp. 7-12 "Comparision of Technical Validation before and after Implementation of the Work Area Manager SIS 2.0 with Standard Rule Package" by A. Jenny, F. Senn, J. Wey, M. Tschopp, W. A. Wuillemin, J. Linssen / Switzerland, Germany.

ACUTE MONOCYTIC LEUKAEMIA (FAB-M5b)

This case illustrates the value of the XE-2100 in detecting acute monocytic leukaemia (AM_OL) and in differentiating between mature and immature monocytic cells.

The patient is a 68-year-old female who presented with anaemia, thrombocytopenia and an extremly elevated WBC count (***Fig. 13***).

The peripheral blood smear showed a spectrum of cells with monocytoid features ranging from large undifferentiated blast cells with abundant poorly granular vacuolated basophilic cytoplasm (22%) to more differentiated cells with the features of promonocytes and monocytes (58%). The monoblasts are characterized by large nuclei with delicate lacy chromatin, and up to three large vesicular nucleoli (***Fig. 14***). The cytoplasm is basophilic and voluminous and often shows one or more pseudopodia. The promonocyte illustrated in ***Fig. 15*** is similar to the monoblast but has a large nucleus with a cerebriform appearance; nucleoli are present, but the cytoplasm is less basophilic, has a greyish ground-glass appearance, and often has fine azurophilic granules (but not in the present example). Mature monocytes are illustrated in ***Fig. 16***.

These microscopy appearances suggested a diagnosis of acute monocytic leukaemia type M5b in the FAB classification, which was subsequently confirmed by cytochemical staining (myeloperoxidase (MPO) and non-specific esterase (NSE) stain were positive) and immunophenotypic analysis (CD13+; CD33+, CD65+; CD15+; CD14+; CD4dim+; CD64+; CD56+; CD11a+; CD2+ and HLA-DR+ [CD117; CD34 only 7% positive]).

Returning to the initial haemogram (***Fig. 13***), note that the "Blasts?" flag is generated. This is because cells are present in the blast cell areas of both the DIFF scattergram and the IMI scattergram indicating that these are of myeloblast/monoblast lineage rather than lymphoblast. The DIFF scattergram shows a large abnormal monocyte population, while the IMI scattergram shows that the majority of these abnormal cells are immature precusor cells with large volume (DC) and high nuclear/cytoplasmic ratio (RF). The latter are monoblasts and perhaps promonocytes although this is difficult to establish unequivocally.

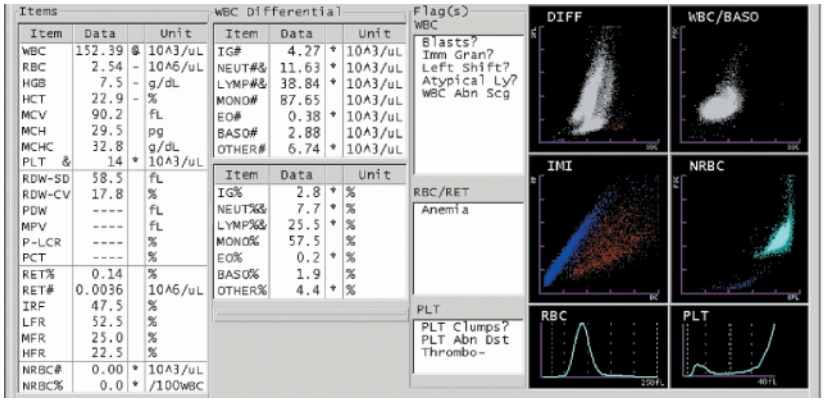


Fig. 13 *Presenting haemogram showing anaemia, thrombocytopenia and leukocytosis*

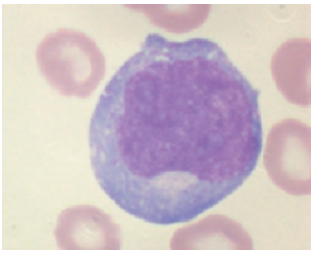


Fig. 14 *Monoblast*

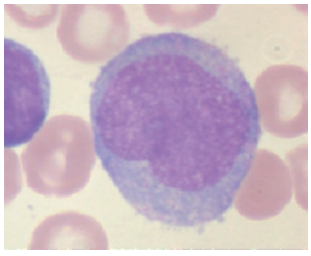


Fig. 15 *Promonocyte*

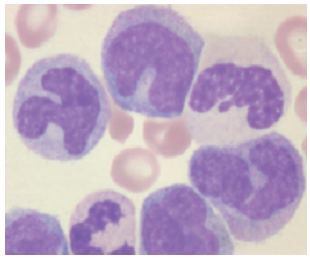


Fig. 16 *Mature monocytes*

The presence and positioning of the red dots in the IMI scattergram may be an important differential diagnostic characteristic. It has already been established that lymphoblasts do not appear in the IMI scattergram. Three IMI patterns are illustrated below (***Figs 17, 18 and 19***).

The IMI scattergram depicted in ***Fig. 17*** shows an immature population (red dots) with a proportion of high volume cells (DC x-axis) and a high nuclear/cytoplasmic ratio (RF y-axis) suggesting differentiation characteristics of promonocytes. ***Fig. 18*** is from a patient with chronic myelomonocytic leukaemia with a differential leukocyte count containing 60% mature monocytes and a virtually empty IMI channel. ***Fig. 19*** is from a patient with AML-M2 whose peripheral blood contained more then 20% small blasts and only a few differentiation characteristics.

Classified as monocytic or M5 in the FAB system, two subtypes exist namely M5a (acute monoblastic leukaemia without maturation) and M5b (acute monoblastic leukaemia with differentiation or acute monocytic leukaemia). In the new WHO classification these disorders fall into subgroup 4-AML not otherwise categorised, therefore the diagnostic characteristics of the FAB system are retained. Subtyping depends mainly on morphologic examination of the marrow and estimation of the % of monoblasts versus more mature monocytic cells, namely promonocytes and monocytes. The present case illustrates yet another differential diagnostic capability of the XE-2100.

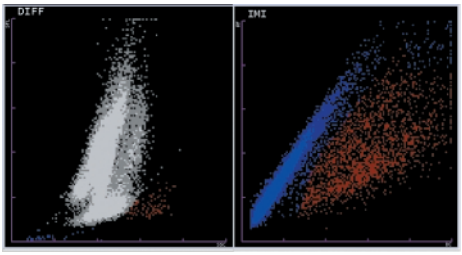


Fig. 17 *Present case. Acute monocytic leukaemia (AML-M5b)*

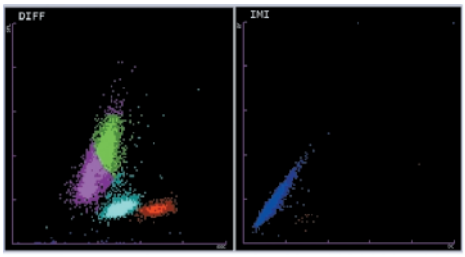


Fig. 18 *Chronic myelomonocytic leukaemia (CMM_OL)*

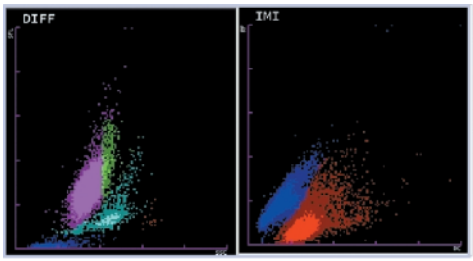


Fig. 19 *Acute myeloblastic leukaemia (AML-M2)*

XE-2100 : PLATELET COUNTING

The XE-2100 incorporates two state-of-the-art platelet methodologies, impedance (PLT-I) and fluorescent optical (PLT-O), to assure accurate and reliable platelet counts. The ability to perform platelet counts by these two different technologies presents the opportunity to provide more accurate platelet information. The analyser always provides an impedance count but there is an optical fluorescent count available when run in reticulocyte mode. RBCs and PLT-I are counted in the RBC/PLT channel using the hydrodynamically focused direct current (DC) detection method. The DC detection method of platelet counting allows increased accuracy because of the large number of particles that can be counted. However, it is well known that in specimens where small RBCs or large platelets are present, accuracy may be decreased because of overlapping populations. In such specimens, the PLT-O measurement can provide a more accurate platelet count because overlapping of populations is avoided. Reticulocytes and PLT-O are counted by flow cytometry in the RET channel using a semiconductor laser and a nucleic acid fluorescent (polymethine) dye and analyzing both forward-scattered light and side fluorescence information. A switching algorithm is incorporated within the analyser software to report the most 'correct' platelet count, either optical or impedance. In samples containing extremely microcytic or fragmented erythrocytes or giant platelets, the platelet distribution curve is abnormal and the optical count is reported. When white cell fragments are present (not uncommon in patients receiving chemotherapy) these are included in the optical platelet count, a "platelet abnormal scattergram" flag is generated and the impedance count is reported.

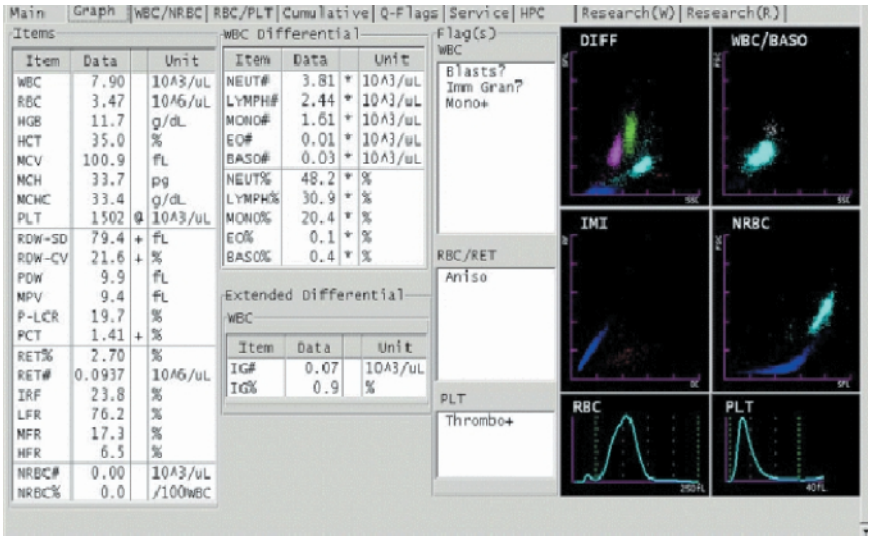
In many routine laboratories it has been assumed that the optical method gives the most accurate platelet count at low levels and for this reason the switching algorithm has been over-ridden. A study reported by Briggs, Kunka and Machin (2003)[5] strongly refutes this practice. In this study, more than 500 cytotoxic chemotherapy samples from patients with haematological malignancies and platelet counts less than 20×10^9 /L in reticulocyte mode were selected and impedance, optical and reported (selected by the switching algorithm) counts were compared to the international reference flow cytometric method (Harrison, *et al*. 2001)[6]. For chemotherapy samples in the majority of cases the impedance count proved to be the most accurate and was the most frequently reported result by the analyser. Of the remaining samples when the switching algorithm selected the optical count then this count was the most accurate. Overriding the switching algorithm and always reporting the optical count will clearly not give the best platelet count. In a number of cases the counts would be falsely high and could result in patients being denied platelet transfusions when indicated.

ESSENTIAL THROMBOCYTHAEMIA

- an example of platelet under-counting by impedance

The patient is a 52-year-old female admitted to hospital with pulmonary embolism. Splenomegaly was noted. The initial XE-2100 haemogram (***Fig. 20***) showed an elevated impedance PLT count of 1,502 × 10^9 /L with mild normochromic, normocytic anaemia, a normal leukocyte count, but with 1% immature granulocytes. Peripheral blood film examination confirmed the thrombocytosis with many giant platelets (15%) and some (< 1%) megakaryocyte fragments (***Fig. 21***). Numerous Howell-Jolly bodies were present resulting from splenic dysfunction. The bone marrow showed marked megakaryocytic hyperplasia (***Fig. 22***). A diagnosis of essential thrombocythaemia was made.

The presence of giant platelets is the reason for the difference between the hydrodynamically focused underestimated impedance platelet count (PLT-I = 1,502 × 10^9/L) and the correct optical fluorescence stained platelet count (PLT-O =2,114 × 10^9/L) on the XE-2100 (***Fig. 23***). There is no overlap between giant PLT and RBC of similar size following RNA staining.



Item	Data		Unit
WBC	7.90		10A3/uL
RBC	3.47		10A6/uL
HGB	11.7		g/dL
HCT	35.0		%
MCV	100.9		fL
MCH	33.7		pg
MCHC	33.4		g/dL
PLT	1502	@	10A3/uL
RDW-SD	79.4	+	fL
RDW-CV	21.6	+	%
PDW	9.9		fL
MPV	9.4		fL
P-LCR	19.7		%
PCT	1.41	+	%
RET%	2.70		%
RET#	0.0937		10A6/uL
IRF	23.8		%
LFR	76.2		%
MFR	17.3		%
HFR	6.5		%
NRBC#	0.00		10A3/uL
NRBC%	0.0		/100WBC

Item	Data		Unit
NEUT#	3.81	*	10A3/uL
LYMPH#	2.44	*	10A3/uL
MONO#	1.61	*	10A3/uL
EO#	0.01	*	10A3/uL
BASO#	0.03	*	10A3/uL
NEUT%	48.2	*	%
LYMPH%	30.9	*	%
MONO%	20.4	*	%
EO%	0.1	*	%
BASO%	0.4	*	%

Item	Data	Unit
IG#	0.07	10A3/uL
IG%	0.9	%

Fig. 20 *Presenting haemogram. Note the impedance platelet count is 1,502×10^9 /L*

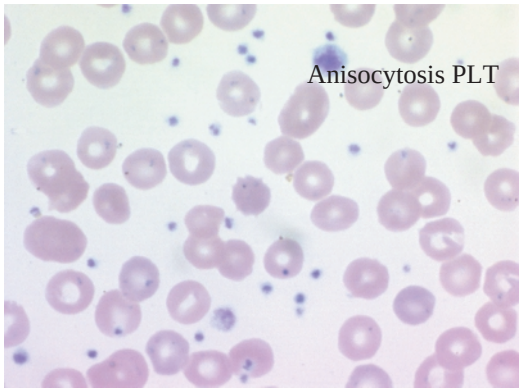


Fig. 21 *Peripheral blood film with thrombocytosis and giant platelets*

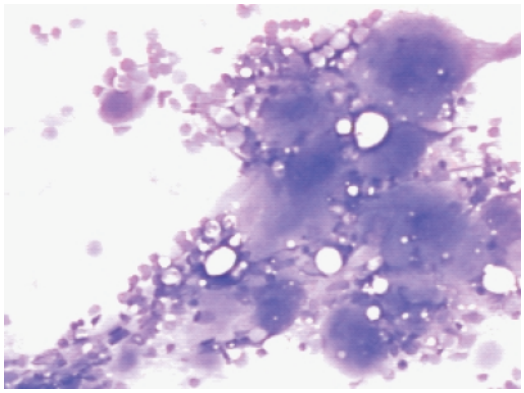


Fig. 22 *Bone marrow showing megakaryocytic hyperplasia*