Evaluation of the Sysmex SE-9500 WBC Morphological Flag "Blasts" in Pregnancy

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

[*1] *Laboratory of Clinical Pathology, Azienda Ospedaliera O.I.R.M.-S.Anna, Torino, Italy.*
[*2] *Laboratory of Clinical Pathology, Ospedale S. Agostino, Modena, Italy.*

There is a tendency for the leukocyte count to increase during normal pregnancy. This is due mainly to an increase in segmented neutrophils and the count may, in fact, double. Immature myeloid cells can also be found, especially during the second and third trimesters of pregnancy. When a complete blood count (CBC) is performed on EDTA anticoagulated specimens from pregnant women, the presence of these immature myeloid cells generates morphology flags. The aim of this study is to evaluate the correlation between the occurrence of the WBC morphology flag 'Blasts' and the presence of morphological abnormalities on microscopy of the peripheral blood during pregnancy.

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INTRODUCTION

Normal pregnancy involves extensive adjustments in maternal physiology that are often reflected in characteristic alterations in a number of laboratory indices. The effect of normal gestation on the blood cell count is to produce a leukocytosis and sequential changes in the various types of leukocytes[1)]. The cell type mainly responsible for this is the segmented neutrophil whose absolute count may double during the gestational period. This increase in count, however, may not reflect a true increase in granulocytopoiesis but rather a demargination of circulating neutrophils[2)]. The lymphocyte count has been noted previously to be lower in pregnant women. The absolute monocyte count has been reported to rise during the second and third trimesters, while the absolute eosinophil and basophil counts show slight but significant progressive decreases[3)].

The effect of normal gestation on blood cell counts is still unclear and sequential changes of the various types of leukocytes during gestation have not been precisely defined. Two mechanisms may explain these alterations:

1) Increased plasma free cortisol levels are found in pregnancy and in other states characterised by elevation of corticosteroid levels, e.g. exogenous glucocorticoid administration and Cushing's disease. These are known to be associated with increased circulating neutrophil counts and decreased lymphocyte, eosinophil and basophil counts[4)].
2) A second feature involved might be the oestrogen level: normally, women present slightly but statistically significantly higher neutrophil counts than men[5)].

Finally, the presence of various circulating immature myeloid cells is well documented usually during the second half of pregnancy.

Morphology flags are an important feature of modern haematology analysers. The Sysmex SE-9500 (Sysmex Corporation, Kobe, Japan) provides morphology flags for red cell, white cell and platelet abnormalities. The WBC related flags, due to the presence of immature cells of the granulocyte series are generated in the Immature Myeloid Information (IMI) channel and are designated as Left Shift (LS), Immature Granulocytes (IG) and Blasts. These flags may be present singly or in clusters of two and three (e.g. LS/IG, Blasts/IG, Blasts/LS or Blasts/IG/LS). The presence of flags indicates those abnormal samples requiring review by microscopy. The 'Blast' flag is very important because of its sinister connotations in haematological malignancy.

This study was designed to evaluate the correlation between the SE-9500 morphology flag 'Blasts' in normal pregnant women and the microscopic differential count on their blood smear and to determine the exact clinical association.

MATERIAL AND METHODS

EDTA-anticoagulated blood specimens were obtained from 210 normal pregnant women in the three trimesters of pregnancy (70 subjects in each trimester). All specimens (analysed in duplicate) were processed within 1 hour of venesection.

Instrument

The Sysmex SE-9500 dedicates five channels to the identification of WBC. The technology has been fully described previously[6-8)].

Manual differential leukocyte counts

Manual differential counts were performed on all single and multiple flagged specimens. The reference differential counts were performed by microscopy examination of 400 cell counts on May-Grünwald-Giemsa stained preparations performed by two qualified senior technologists each counting 200 cells on separate smears according to the NCCLS H20-A protocol[9]. In this evaluation of WBC differential count clinical sensitivity and specificity were studied as described previously[10,11].

RESULTS

In the study, 13 of the 210 samples analysed showed the 'Blast' cell flag (6%). These results analysed by trimester of pregnancy are shown in ***Table 1***. No flagged samples were found in the first trimester; 1 flagged sample occurred during the second trimester; 12 samples were flagged during the third trimester. In the 13 positive samples, the 'Blast' flag was always accompanied by at least one other flag as illustrated in ***Fig. 1***. The IMI scattergram appearances are shown in ***Fig. 2. (2a*** showing : a negative sample, ***2b*** : a typical positive sample.
The microscopic evaluation of these 13 flagged samples is shown in ***Table 2***. It is noteworthy that by microscopy (2×200 cell counts) no blast cells were found where the instrument generated a blast cell flag. The other cell types were identified, mainly myelocytes and metamyelocytes but occasional promyelocytes (3 cases) and cells showing asynchrony in nuclear maturation (2 cases). In all 13 samples, therefore, immature cells were found although not necessarily in the categories defined by the instrument but blast cells were not a feature. Flagging of an immature category of granulocytic cells in pregnancy is therefore achieved with a sensitivity of 100% and a specificity of 94%.

Table 1 *Number of WBC morphological flags in the three trimesters of normal pregnancy (210 women)*

Trimester of pregnancy	Blasts	IG	LS
I	0	0	0
II	1	1	0
III	12	14	6

Table 2 *Microscopic evaluation of the 13 samples positive for "Blasts" flag and related cell finding*

Cells	Number of cells	Mean
Blasts	0	0
Myelocytes	1 to 4	2.5
Metamyelocytes	1 to 7	3.4
Promyelocytes (3 cases)	1	1
Asynchrony (2 cases)	present	–

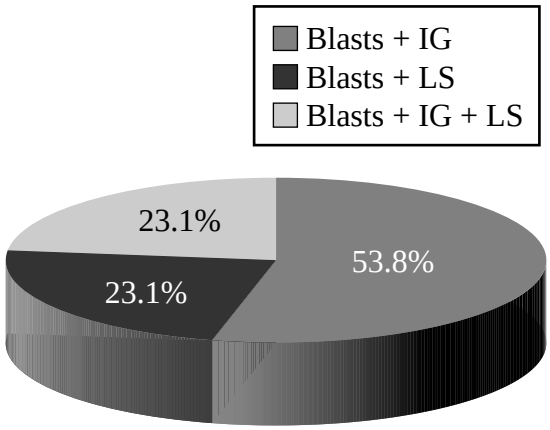

Fig. 1 *Percentage (%) incidence of the Sysmex SE-9500 WBC morphology flags cluster (IG, LS, Blasts) in the 13 positive samples*

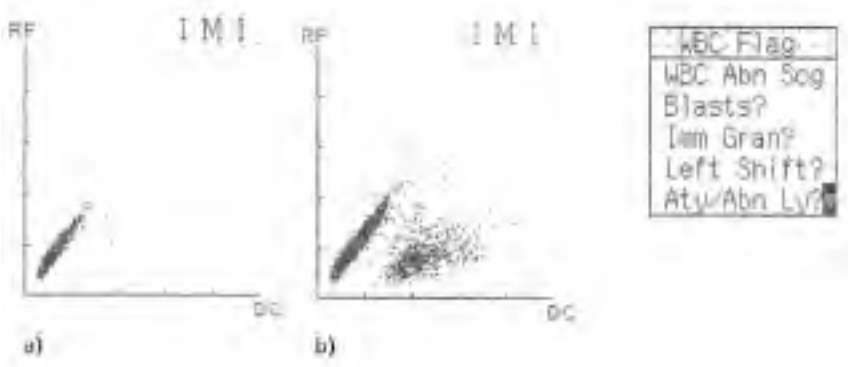

Fig. 2 *WBC distribution in IMI scattergram*
a) negative sample
b) positive sample from pregnant women (3rd trimester). The more immature cells generate the smaller RF signal strength. The Blasts cluster appears in the lower area on the IMI scattergram under LS and IG ones.

DISCUSSION

The presence of flags generated by the SE-9500 usually indicates abnormalities which must be resolved by microscopy. It is recognised that immature granulocytic cells can appear in the peripheral blood in normal pregnancy and the puerperium. This is confirmed in the present study using the SE-9500 where morphology flags (Blasts, IG and LS) were generated, mainly in the third trimester. The appearance of the instrument flags correlated completely with the microscopy finding of immature granulocytic cells including metamyelocyte, myelocyte and promyelocyte categories but not blast cells in every case. This phenomenon resembles that seen, for example, during treatment with growth factor in which there appears to be an accelerated but asynchronous maturation in the granulocytic series. In our opinion a similar phenomenon may be occurring in pregnancy as a result of which the instrument may be recognising a functional rather than a morphological entity but still remaining part of the granulocytic maturation continuum. If this theory is correct, the instrument is not misclassifying but simply exposing a further intermediate segment of the granulocytic continuum.

In conclusion, when immature granulocytic cells are flagged by the SE-9500 as can occur during the third trimester of normal pregnancy, and the other haematological parameters remain within appropriate reference ranges, this represents a paraphysiological status and has no sinister connotation.

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