Evaluation of M6 Leukemia Progression with the XE-2100
–Progression in Erythroleukemia–

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Quantitation of nucleated red blood cells (NRBCs) is easily performed with application of the Sysmex XE-2100 hematology analyzer. In a case study of a subject with M6 erythroblast leukemia, the diagnosis and progression of disease activity was monitored until the final stage.

An obvious shift from orthochromatic erythroblasts towards immature pro-erythroblasts was depicted in sequential scattergrams. Results demonstrate that Sysmex XE-2100 test methodology can be used for diagnosis and establishing the effect of therapy.

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Key Words Polymethine Dye, Automated Hematology Analyzer, XE-2100, Erythroblast

INTRODUCTION

Detection of hematological malignancies on hematology analyzers can be difficult. Erythroleukemia makes up about 5 percent of cases of acute myelocytic leukemia (AML) and is referred to as M6 in the FAB classification. Anemia and thrombocytopenia are present in nearly all cases. In the early stage of the disease granulopoiesis and thrombopoiesis may be only mildly abnormal and only a few nucleated red blood cells (NRBCs) are present in the blood. Progression of the disease results in development of severe neutropenia and thrombocytopenia. The disease may evolve further into polyblastic AML[1].

Hitherto, it has been difficult to discriminate NRBCs by application of automated methods. Since 1997 quantitative estimation of NRBCs can be performed by a sophisticated hematology analyzer, the Sysmex XE-2100[2]. NRBCs may be classified into three stages based on changes in cell biology and structure.

Polymethine dyes are used for detection of mitochondria and endoplasmatic reticulum[3]. Polymethine dyes are structurally characterized by the binding affinity of an heterocyclic nucleus (cyclic compound containing nitrogen, sulphur and oxygen) and a methine chain (–CH2=).

In the first pretreatment step of the blood sample, complete hemolysis of red blood cells (RBCs) is performed by the addition of a specific lysing reagent, which simultaneously denucleates, shrinks and slightly stains nuclei of NRBCs[4,5].

Longitudinal monitoring of progression in a patient with M6 erythroleukemia was performed to yield insight into the efficacy of Sysmex XE-2100.

SUBJECTS, MATERIALS AND METHODS

Blood samples

Blood samples were collected after venepuncture in sample tubes containing K_3EDTA anticoagulant (Vacutainer®, Becton Dickinson, Rutherford, New Jersey, USA). Samples were processed within 4 hours.

XE-2100 automated hematology analyzer

For investigation of the complete blood count (CBC) including NRBCs the XE-2100 hematology analyzer (Sysmex Corporation, Kobe, Japan) was used. Fluorescence flow cytometry technology is used in this instrument for quantifying the five part white blood cell (WBC) differential count, characterising the immature granulocytes (metamyelocytes, myelocytes and promyelocytes), counting NRBCs, reticulocytes and immature reticulocyte fraction. A fluorescence (optical) platelet count is generated in addition to the standard impedance count.

Combined measurement of side scatter light intensity (a measure reflecting inner cell complexity), forward light scatter intensity (a measure of cell volume) and fluorescence intensity of nucleated cells in addition to direct current (DC) and radio frequency (RF) signals results in separation of clusters. Abnormal and immature cells with large nuclear sizes will yield higher fluorescence intensity compared to normal cells.

STROMATOLYSER-NR lytic and staining agent

Erythrocytes are lysed by the addition of surfactant contained in the STROMATOLYSER-NR lytic agent. This pre-treatment results in an increased permeability of the leukocyte membrane. Subsequently, the polymethine dye is able to enter the cell and will stain nucleic acids (nuclear membranes). Red fluorescence intensity is measured using a semiconductor laser at 633 nm oscillation wavelength.
Because basophilic erythoblasts appear in zones of higher fluorescence intensities than orthochromatic erythoblasts, the polymethine dye is considered to stain nucleic acids (nuclear membrane) as well as cytoplasmic organelles.
As a result of this treatment erythroblasts are clearly distinguished from the population of lymphocytes[3)].

Microscopic examination

Microscopic examination of peripheral blood smears and bone marrow aspirate was performed after Wright and May-Grünwald/Giemsa staining respectively.

Subject

For evaluation in the case study, a subject with erythroleukemia was selected.
Blood samples were drawn from a 62 year old female subject suspected of having an hematological malignancy after admission to hospital. Clinical features consisted of petechiae and icterus. Additional investigations resulted in the diagnosis of M6 leukemia. Subsequently, additional samples were obtained once a week.

For comparison and identification of aberrant morphology of cells, scattergrams were generated from an apparently healthy subject, a subject with orthochromatic erythroblasts and a subject with orthochromatic and polychromatic erythroblasts.

RESULTS

Evaluation of XE-2100 results

Progression of disease activity is demonstrated in ***Figs. 1 and 2***.
Quantitative hematological results are shown in ***Table 1***. The severity of the dyserythropoietic phase is suggested from the low reticulocyte count and the tendency to decreasing HGB and RBC results. Progressive thrombocytopenia occurred. In the category OTHER in ***Table 1*** erythroid precursors were identified. Quantitative evaluation revealed a progressive increase in case of erythroid precursors.

Peripheral blood smear

According to morphological criteria, the majority of erythroblasts were classified as pro-erythroblasts in the final stage of disease (***Fig. 3***).

Immunophenotyping

In the mononuclear cell fraction, isolated from a peripheral blood sample (date 04/26), an immature cell population amounting to 60% was detected.

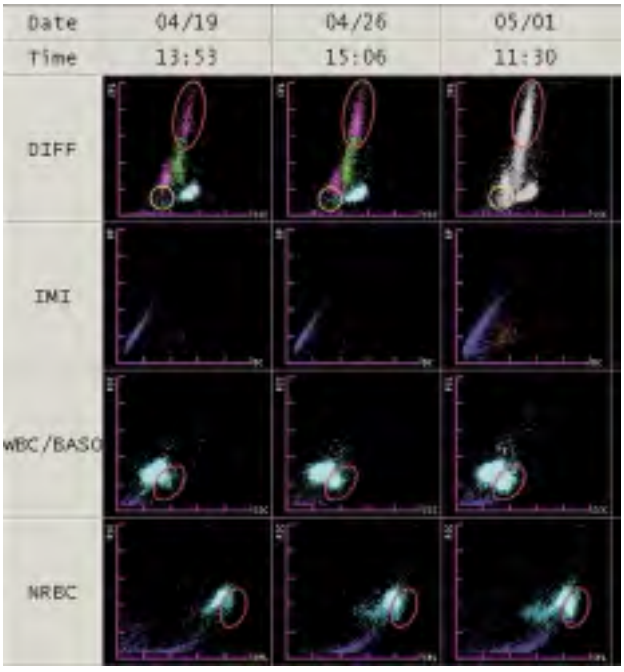

Fig. 1 *DIFF, IMI, WBC/BASO and NRBCs scattergrams indicating progression of disease in a subject with M6 leukemia. Blood samples are drawn at weekly intervals (dates 04/19, 04/26 and 05/01 respectively).*

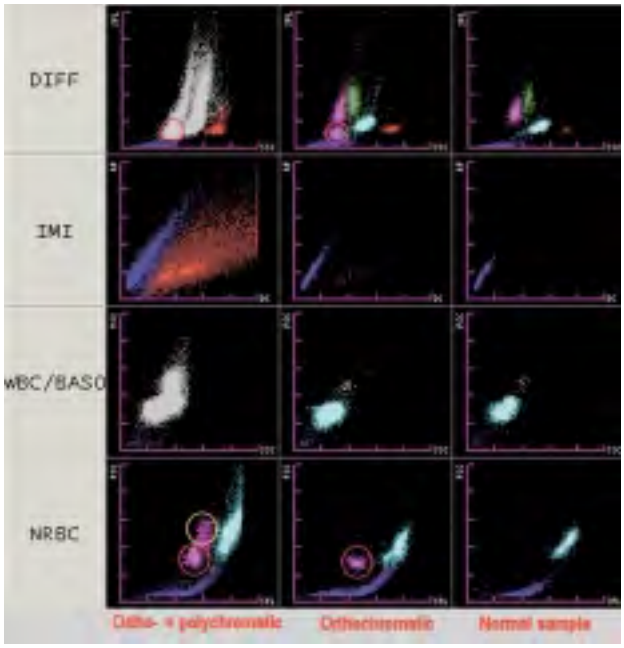

Fig. 2 *DIFF, IMI, WBC/BASO and NRBCs scattergrams in an apparently healthy subject, a subject with orthochromatic erythroblasts and a subject with orthochromatic and polychromatic erythroblasts.*

Table 1 *Test results during progression of M6 leukemia*

Parameter	Date		
	04/19	04/26	05/01
WBC (x10^9/L)	8.2	10.8	22.4
RBC (x10^{12}/L)	4.32	4.15	3.31
HGB (g/L)	126.8	122.0	96.3
MCV (fL)	84	82	85
MCH (pg)	29.36	29.38	29.10
PLT (x10^9/L)	29	19	7
RDW-SD (fL)	40.7	43.0	48.2
RDW-CV (%)	13.1	14.1	15.5
RET (x10^9/L)	6	8	3
IG (%)	0.2	0.2	0.5
NEUT (%)	60.1	60.4	42.1
LYMPH (%)	13.9	9.0	10.5
MONO (%)	16.9	16.0	20.3
EO (%)	0.1	0.0	0.0
BASO (%)	0.2	0.2	0.4
OTHER (%)	8.6	14.2	26.2
NRBC % (/100 WBC)	10	0	0

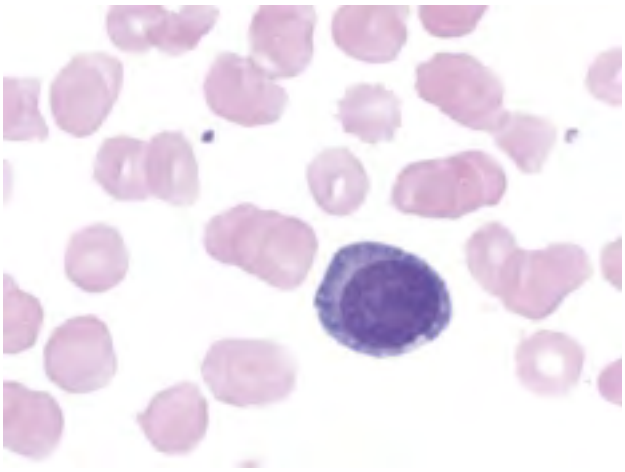

Fig. 3 *Images of a leukemic cells in a peripheral blood smear after Wright staining*

The immature cells showed expression of the following relevant immunologic markers:

CD117 (precursormarker): positive
CD33 : weak
CD36 : strong
Glycophorin A : positive

Bone marrow

At the date of admission microscopic examination of bone marrow revealed erythroid dysplasia. Differential counting revealed 13% erythroblasts.
An initial diagnosis of Refractory Anemia with Ring Sideroblasts (RARS) was made.

DISCUSSION

The frequency of detection of erythroblastic leukemia is increased if methods of detecting erythroid differentation more sensitive than light microscopy are used for the characterisation of erythroid progenitors. The incremental advance in automated extended RBC measurements, the reticulocyte count and the NRBC count, add a dynamic dimension to the diagnostic value of traditional RBC indices. By means of multivariate measurement combinations the additional state of the art parameters are able to enhance diagnostic capability and patient monitoring during therapy.
Detection, identification and enumeration of NRBCs have significant clinical importance[6]. In conjunction with a low platelet count the presence of NRBCs in peripheral blood samples may suggest thrombotic thrombocytopenic purpura or myeloproliferative disorder. Even low numbers of NRBCs may indicate abnormal erythropoiesis.
For clinical diagnosis and evaluation of therapy it is important that NRBCs are identified appropriately. The manual microscopic evaluation of a peripheral blood smear has only poor sensitivity and reproducibility because small numbers of cells only are counted.
If NRBCs in blood films are not identified appropriately abnormalities can be even missed completely.
In terms of appropriate patient care and cost effectiveness, it should be the ultimate goal of automated hematology analyzers to maximise clinical information obtained from a single EDTA blood sample. Requirements for bone marrow assessment both for diagnostic and particularly for treatment monitoring purposes continue to increase. The role of automated routine blood cell counters instead of laborious cytological bone marrow examination by means of microscopy should be considered and further evaluated.

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