Use of the XE-2100 in a Patient with Cold Auto-immune Hemolytic Anemia

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The analysis of specimens, drawn from patients with cold auto-immune hemolytic anemia (caused by elevated levels of anti-I on the red blood cell (RBC) membrane) is, in general, a difficult problem for automated hematology analyzers. In such patients, because of aggregation of the RBCs, which occurs mainly at temperatures lower than 30°C, it is not possible to measure the RBC count and hematocrit accurately. During an evaluation of the XE-2100 in our laboratory we studied a patient with this disorder. During the first weeks after admission to the Haematology Clinic very marked aggregation was observed in all specimens from the patient despite pre-warming of the EDTA blood to 37°C. The patient received 13 transfusions of pre-warmed, leukocyte-poor red cells and cyclosporine A. Two weeks later the activity of anti-I decreased. The result was recovery from the hemolytic disease, detected very early by the system and accompanied by an improvement of the cell counts.

Erythropoietic activity was easily followed by the nucleated red blood cell (NRBC) counts and reticulocyte (RET) parameters generated by the XE-2100. During the first two weeks the NRBC counts were increased but the RETs remained low. During recovery the picture was completely reversed. RETs and especially their immature fractions increased very markedly and NRBCs disappeared.

EDTA associated platelet (PLT) clumping was detected by use of the IMI Channel. On using lithium heparin as anticoagulant the PLT count became normal.

In the XE-2100 newly developed principles for counting and characterization of blood cells are introduced as well as already well established methods. From our point of view, at this time, the XE-2100 is the most appropriate system for diagnosis of diseases associated with an elevated number of NRBC in the peripheral blood, e.g. the immune hemolytic anemias.

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Key Words Automated Hematology Analyzer, XE-2100, Nucleated Red Blood Cell (NRBC), Reticulocyte Count, Auto-Immune Hemolytic Anemia

INTRODUCTION

A cold auto-immune mechanism produces a special type of hemolytic anemia. IgM auto-antibodies are involved; they are complete and complement binding. They have mainly the specificity "I", rarely "i". Normally these antibodies are only active in the cold (4°C), but in severe cases with a high antibody titer their thermal amplitude is wider and results in agglutination of (RBC) and haemolysis *in vivo*[1, 2].

It is possible to differentiate primary and secondary types of the cold agglutinin disease. *In vivo* the antibody causes macrsocopically visible clumping of the RBCs at a temperature below 37°C. This phenomenon can lead to some severe problems in the laboratory diagnosis:

1) Problems in determining blood groups and in performing cross matching.
2) Measurements from photometry based assays may be influenced by the hemolysis.
3) Erythrocyte aggregation adversely affects the measurement of the RBC count, the hematocrit, the RBC indices and the reticulocyte count[2, 3].

Laboratory diagnosis of cold auto-immune hemolytic anemia[3-6]

1) Complate blood cell (CBC) including the reticulocyte count, the RBC count and hematocrit are decreased, the MCV falsely elevated and the MCH and MCHC are falsely decreased. The reticulocyte number may be increased depending on the status of the disease and its severity. Depending on the severity of the disease, many erythrocyte aggregates are found. The monospecific direct antiglobulin test (DAT) is positive (complement C3D-specifity).
2) The titer of anti-I is increased.
3) More nonspecifically increased analytes are: bilirubin, LDH, AST, serum- hemoglobin.
4) Haptoglobin is decreased.
5) Bone marrow examination is necessary for the evaluation of the erythropoiesis and to search for possible causes of the disease (*e.g.*, lymphoma). In most cases erythropoiesis is increased to compensate for vigorous hemolysis.
6) Microbiological and virological diagnostics (possible

Mycoplasma or EBV infection) Mycoplasma pneumoniae infection is sometimes accompanied by a marked increase in anti-I titer.

Treatment

Avoidance of cold is the most important treatment to prevent activation of the antibody mechanism. Transfusion of pre-warmed, washed and filtered leukocyte- poor red cells may be necessary. Successful treatment is heralded by reversal of the indicators of hemolysis and a rise in hemoglobin concentration and hematocrit[3-6].
At the time of our evaluation of the XE-2100 a patient (G.U.) was admitted to hospital. While all routine hematological measurements were made on the Sysmex SE-9500 analyzer, the patient's specimens were also analyzed on the XE-2100. The results from the latter are presented and discussed.

MATERIALS AND METHODS

Specimens

Blood anti-coagulated in K_2EDTA and pre-warmed to 37°C was used for routine blood count measurements. During the admission, EDTA associated platelet clumping was noted and subsequently lithium heparin anticoagulant was used for the CBC and reticulocyte measurements.
K_2EDTA was used for the bone marrow specimen.

Methods

For the investigation of the CBC, NRBC and reticulocytes the XE-2100 (Sysmex Corporation, Kobe, Japan) was used.
Microscopic examination of the blood smear and the bone marrow was performed after staining by the Pappenheim method (May-Grünwald/Giemsa-stain).

RESULTS

All parameters, values necessary for diagnosis were increased (***Table 1***). The monospecific DAT was positive (anti-C3D) and the anti-I titer was 1:2056.

Figs. 1-5 are examples of measurements by the XE-2100 at different stages of the illness. During the first period of the disease the RBC count and the HCT could not be used for diagnosis. Once treatment became effective the measurements became reliable.

Evaluation of the XE-2100 –results between July 26th and September 3rd

*Hemoglobin (**Fig. 6**)*
The hemoglobin concentration decreased to 38 g/L during the first days after admission because of active hemolysis. Hemolysis stopped following transfusion of pre-warmed, washed and filtered RBC-concentrates and treatment with Cyclosporine A. The patient received 13 units of leukocyte-poor red cells with the blood group O RhD-positive. Two weeks after admission the hemoglobin concentration started to show a sustained rise.

*RBC (**Fig. 7**), HCT (**Fig. 8**) (**Pictures 1-3**)*
Because of extreme RBC aggregation it was not possible to measure an accurate count. As recovery commenced two weeks after admission, the RBC count and hematocrit started to increase. Similar results were obtained from the SE-9500/RAM-1 and the XE-2100.

*MCH and MCHC (**Fig. 9**)*
The Wintrobe-Indices clearly reflect the progress of the disease. At the start the MCH and MCHC were extremely high, however, with successful treatment they returned to the normal ranges. The high reproducibility of these results is noted.

*Reticulocytes (**Fig. 10**)*
About 8-10 days after admission the number of reticulocytes increased both in relative and absolute counts (see also ***Picture 4***). Later and during regeneration both counts were very high as expected. The instrument was able to measure these counts continuously.
The different reticulocyte maturity fractions varied especially during the first days. It is not possible to give an explanation for this fact. Effects of the matrix (RBC-aggregates) may be partly responsible. Erythropoiesis may also have been variable during this time. The high fluorescence ratio (HFR) fraction was high during the first period but, as expected, it fell as recovery proceeded. Throughout the course of the disease, the immature reticulocyte fraction (IRF) maintained an inverse relationship to the low fluorescence ratio (LFR) of the reticulocyte population (***Fig. 11***).

Table 1 *Important results for diagnosis of cold auto-immune hemolytic anemia in G.U. on admission.*

Analyte	Patient result	Reference range in health
Bilirubin (Total)	135.4 μmol/L	3.4 - 22.2 μmol/L
LDH	61.2 μkat/L	5.2 - 10.3 μkat/L
AST (GPT)	1.14 μkat/L	< 0.58 μkat/L
Serum-hemoglobin	40 μmol/L	0 - 9 μmol/L

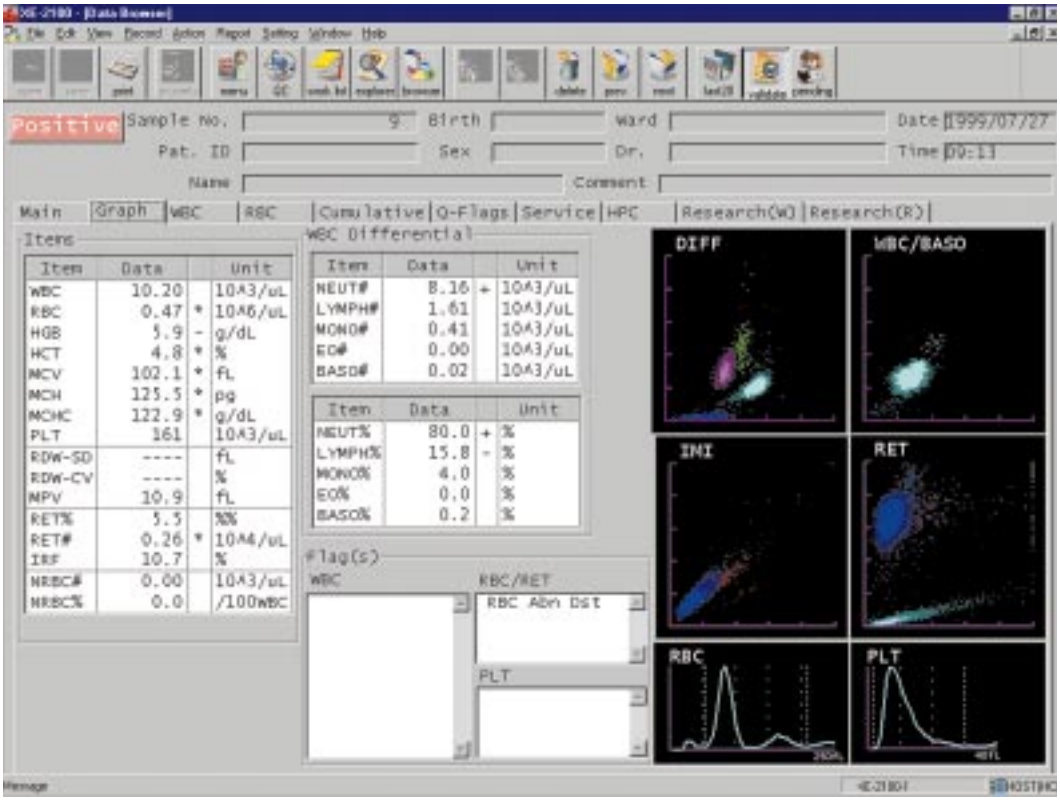


Fig. 1 *Sample 9 (27/07/99)*

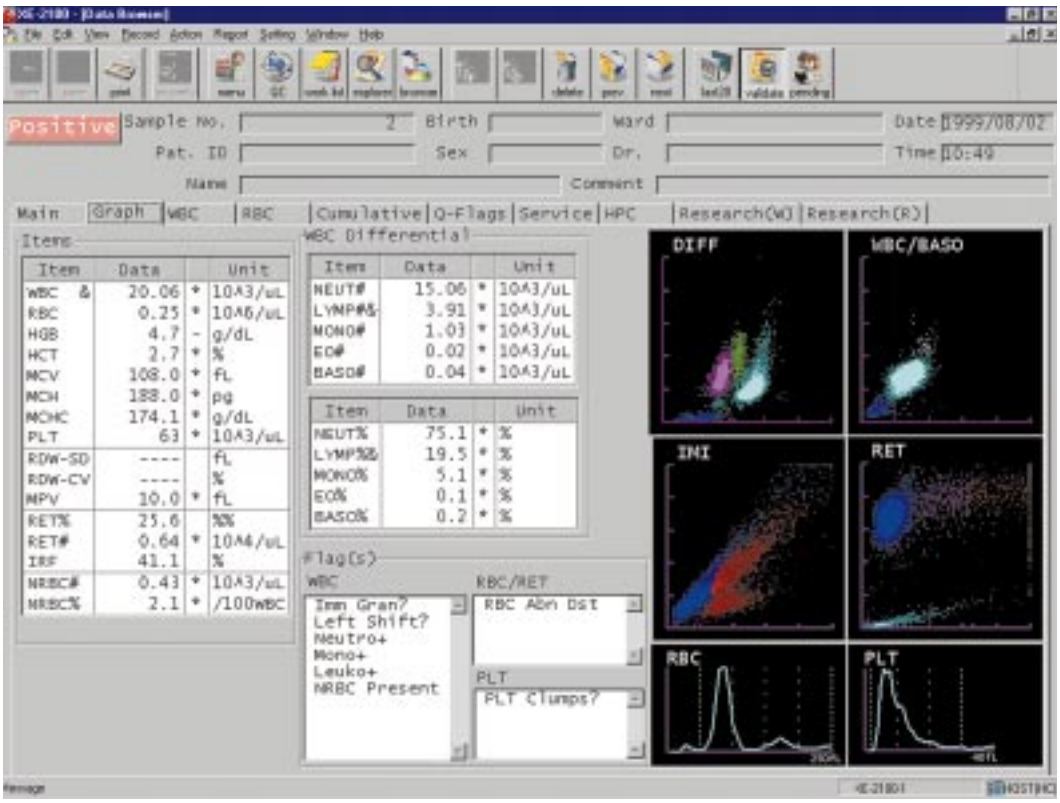


Fig. 2 *Sample 2 (02/08/99)*

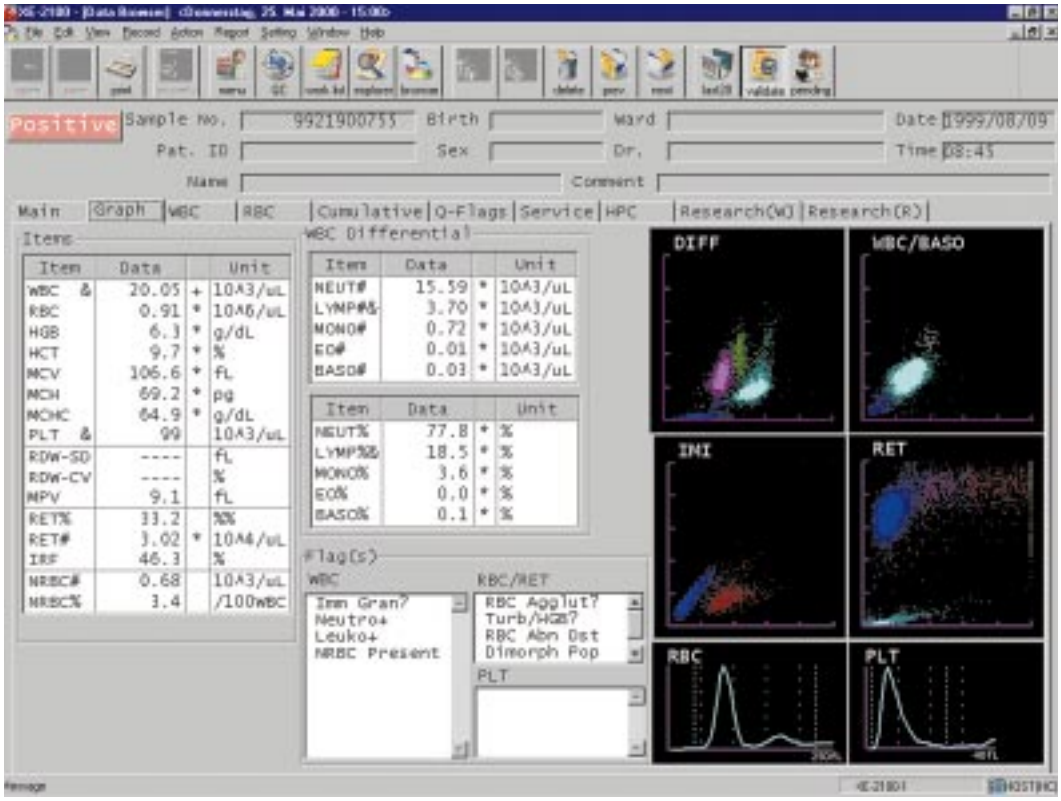


Fig. 3 *Sample 9921900755 (09/08/99)*

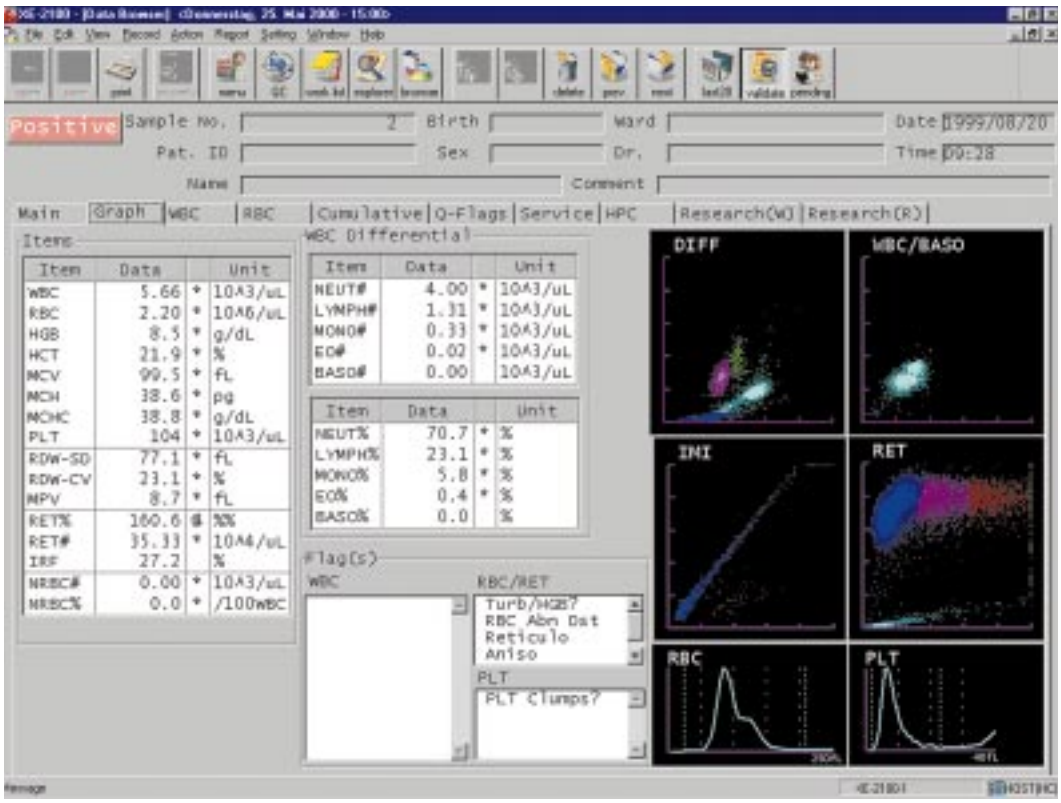


Fig. 4 *Sample 2 (20/08/99) – EDTA-blood with PLT clumps*

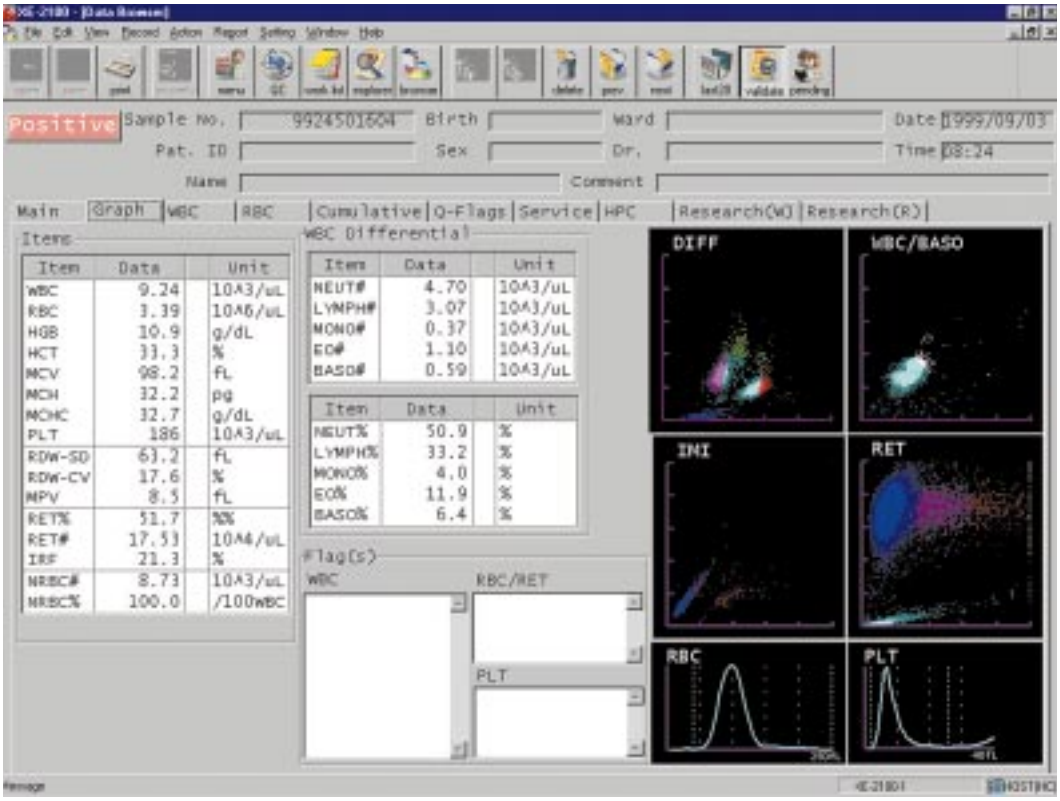


Fig. 5 *Sample 9924501604 (03/09/99)*

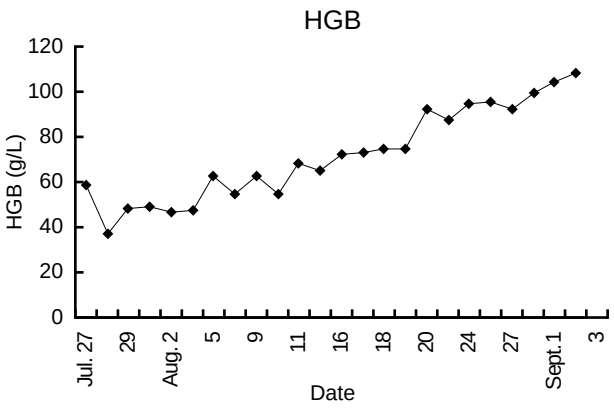


Fig. 6 *HGB on XE-2100*

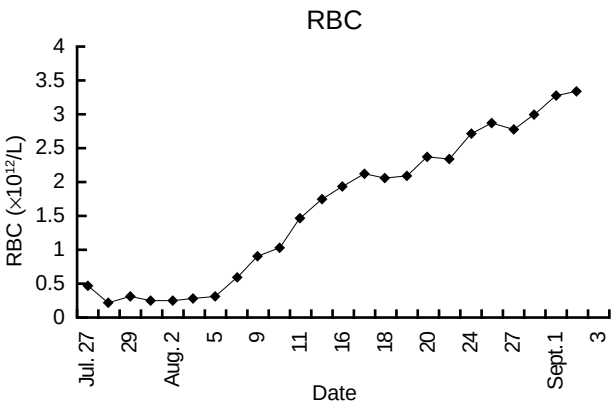


Fig. 7 *RBC on XE-2100*

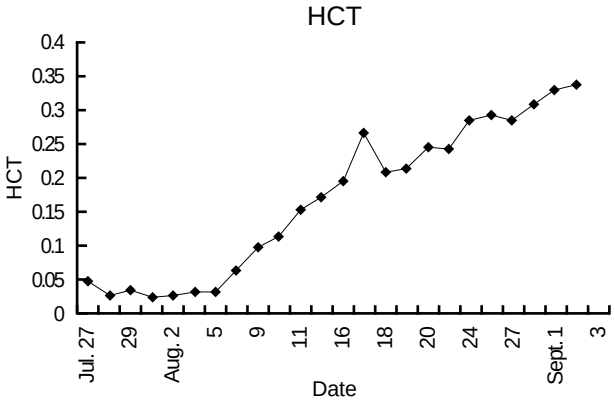


Fig. 8 *HCT on XE-2100*

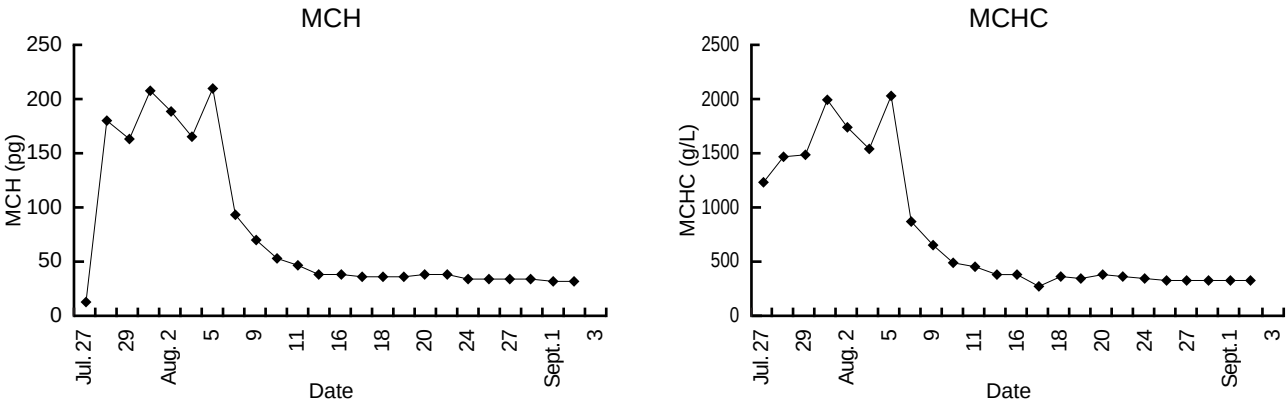


Fig. 9 *MCH and MCHC on XE-2100*

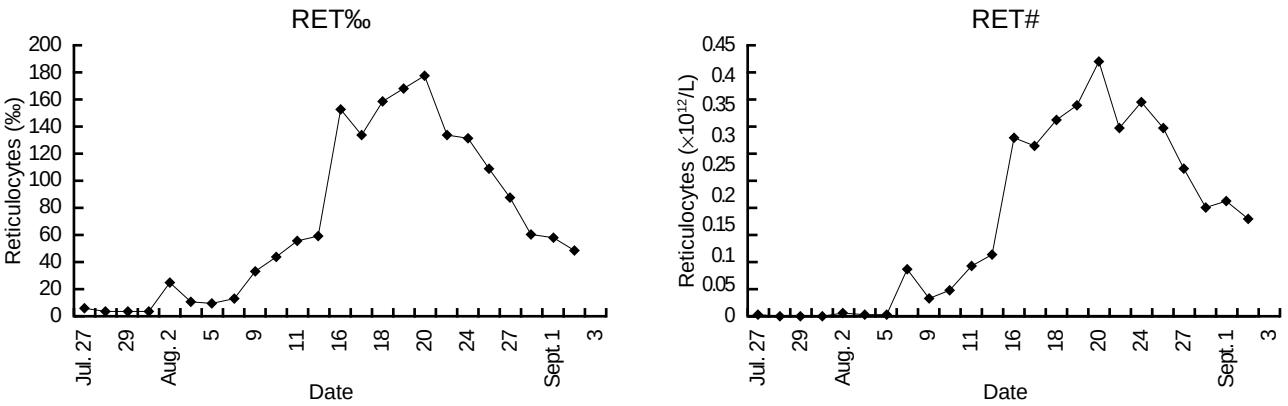


Fig. 10 *Reticulocytes on XE-2100*

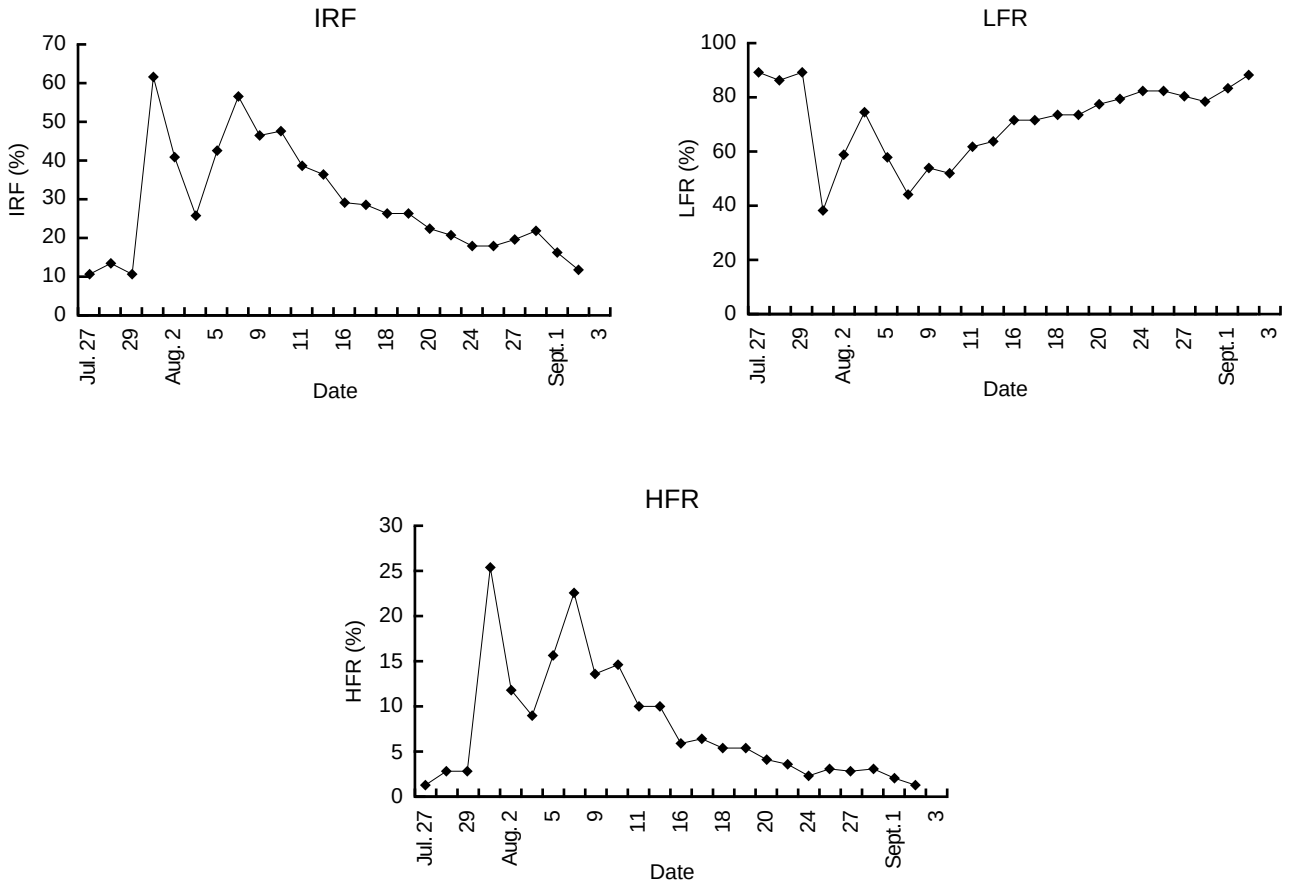
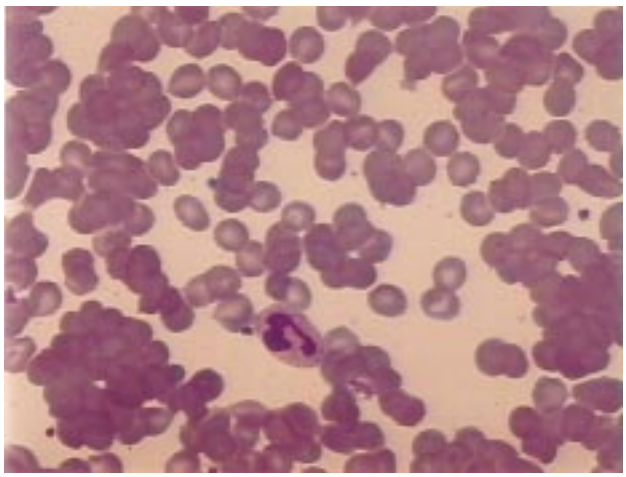
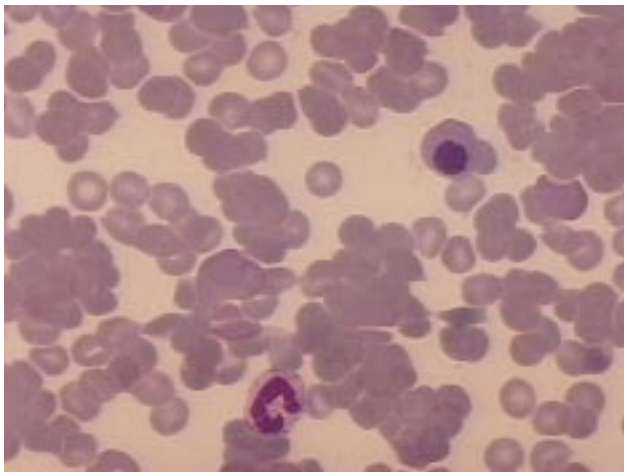


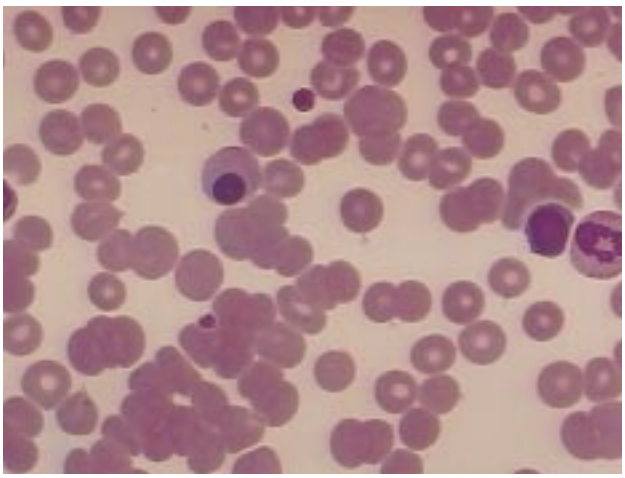
Fig. 11 *Reticulocyte fractions on XE-2100*



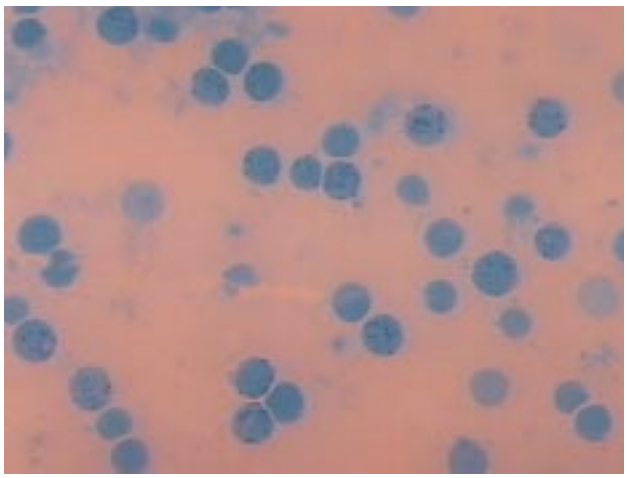
Picture 1 *EDTA-blood of G.U. on 28/07/99*



Picture 2 *EDTA-blood of G.U. on 01/08/99*



Picture 3 *EDTA-blood of G.U. on 10/08/99*



Picture 4 *Reticulocytes after Brilliant cresyl blue staining (18/08/99)*

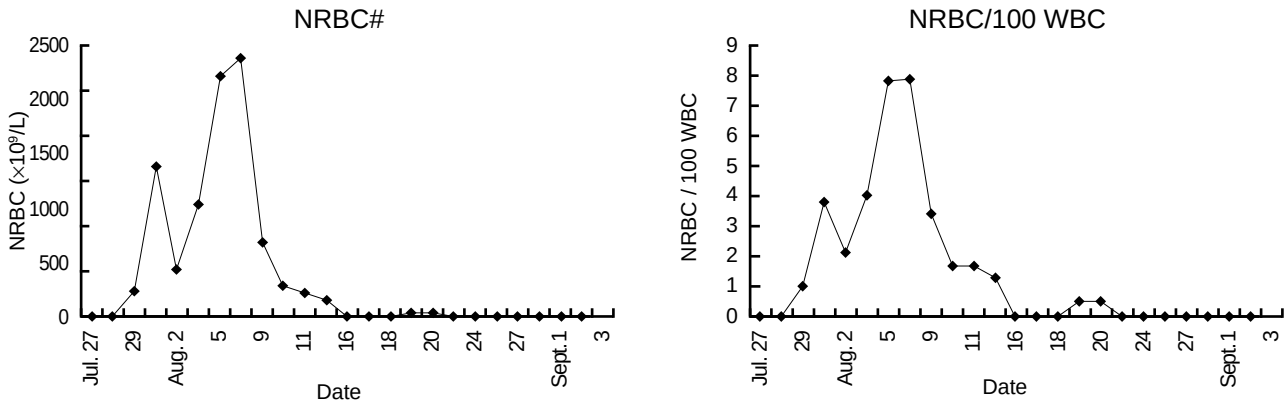


Fig. 12 *NRBC on XE-2100*

*NRBC (**Fig. 12**)*

Because of the vigorous hemolysis the entire erythropoietic system was stimulated. This was confirmed by examination of the bone marrow smear (***Picture 5***). This was reflected on the XE-2100 by both the NRBC count/100 WBC and by the absolute NRBC Count /μL. These counts decreased from August 11 and NRBC disappeared from the peripheral blood from August 24. NRBC counts from the XE-2100 correlate very well with microscopy results. This was established during a multicentre evaluation of the instrument[8].

*Platelets (**Fig. 13**)*

During the first days after admission the patient developed an hyperfibrinolysis with increased fibrinogen, D-Dimer, Thrombin anti-thrombin (TAT) complexes and a moderate decrease in platelet count. Following heparin therapy the patient's condition improved and the platelet count returned to normal.

On August 20th there occurred a further decrease in the platelet count. This proved to be EDTA-induced platelet aggregation, suspected by the appearance of the IMI scattergram and confirmed by blood film examination (***Fig. 4 and Picture 6***). Changing the anticoagulant to lithium heparin resulted in a normal platelet count.

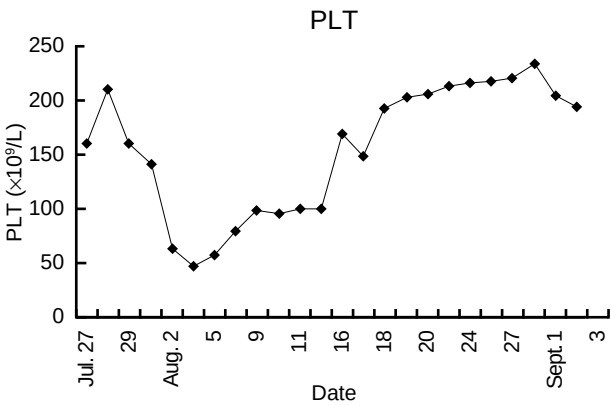
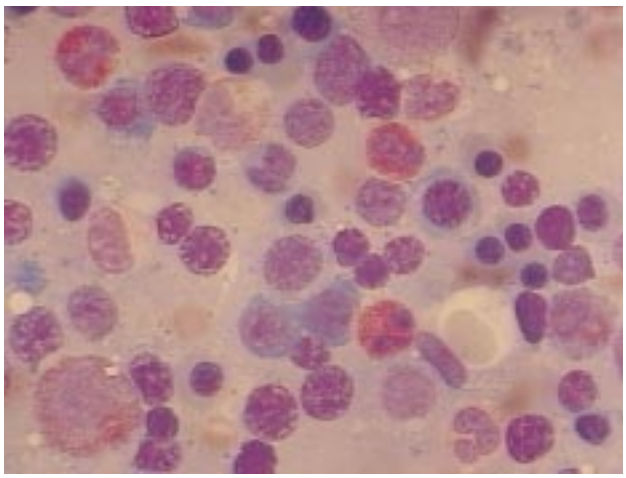
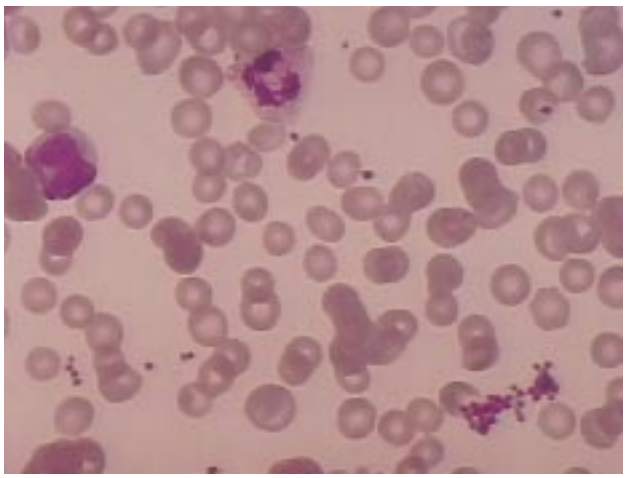


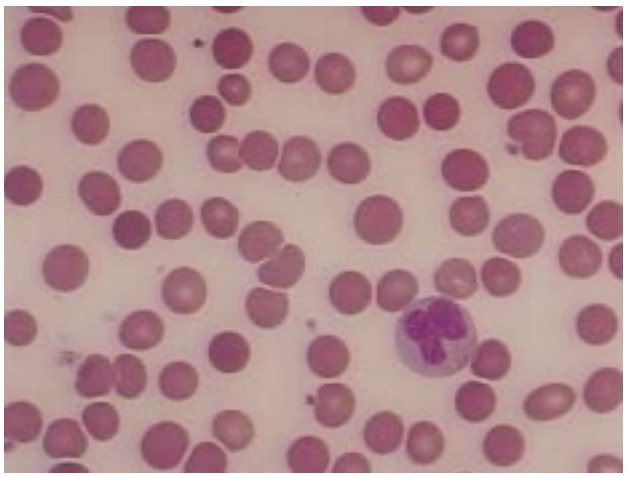
Fig. 13 *PLT on XE-2100*



Picture 5 *Bone marrow smear of G.U.*



Picture 6 *EDTA-blood of G.U. on 20/08/99*



Picture 7 *EDTA-blood of G.U. on 03/09/99*

DISCUSSION

Cold auto-antibodies (anti-I) cause a well defined type of auto-immune type of hemolytic anemia. The result is hemolysis, but only in severe cases where the antibody has high thermal amplitude[1, 2]. *In vitro* marked spontaneous agglutination of the red blood cells occurs. Several laboratory investigations can only be performed after pre-warming the blood to 37°C [1, 2]. These are CBC and automated reticulocyte counts. Difficulties also exist in immuno-hematology (blood grouping, cross matching). On the other hand it is necessary to perform accurate measurements for diagnosis and follow up and for assessing the therapeutic effect of drugs like Cyclosporine A in suppressing antibody activity[3].

The XE-2100 is a new generation hematological analyzer recently evaluated in a number of laboratories worldwide. It uses principles of measurement, which are well established in other instruments as well as newly developed technologies. These are described elsewhere in a Sysmex publication[7]. A completely new feature is the correct count of NRBC in a specific NRBC-channel. The analytical performance of the instrument was evaluated in Germany in a multicenter study in 1999 and the results are being prepared for publication. First experiences were reported in November 1999 during a Sysmex Symposium in Düsseldorf [8].

We used the XE-2100 in the diagnosis and follow up of a patient with a severe cold auto-immune hemolytic anemia. The disease was so severe (anti- I titer 1:2056) that the *in vivo* hemolysis made transfusions urgently necessary (HGB at lowest level 38 g/L). All clinical chemical markers for hemolysis such as serum bilirubin and LDH were highly increased. Although the blood was transported warm and pre-warmed to 37°C before analysis, the HGB concentration in EDTA blood was the only accurately measured red cell related analyte. The RBC agglutinates remained stable (***Pictures 1-3***). We performed measurements with the XE-2100 throughout the course of the patient's stay in hospital and were able to follow the course of results. The reproducibility of the results was impressive.

The patient was transfused 13 units of pre-warmed leukocyte-poor red cells and given prednisolone and Cyclosporine A. It was possible to detect the point in time, when agglutination of the RBC ceased and the situation improved in the patient. The HCT, MCH and MCHC returned to normal at the same time (***Picture 7***). Erythropoiesis was greatly stimulated as shown by the reticulocyte count and the levels of different maturation fractions.

During the early phase NRBC were measurable in the peripheral blood. These cells may result from a compensatory stimulation of the bone marrow or from extramedullary sources of erythropoiesis (liver, spleen). These measurement results were confirmed by microscopy findings (***Pictures 2 and 3***). The disease was accompanied in this patient by a disturbance of hemostasis, characterized by a decrease of PLT count but also in an increased fibrinogen level and high D-Dimer and TAT Complexes. Therapy with heparin was effective in returning these parameters to normal.

Subsequently the PLT decreased again but this was the result of an EDTA associated platelet clumping, which was readily detected in the IMI scattergram (***Fig. 13***). When lithium heparin was used as anticoagulant, normal PLT counts were obtained. The IMI-channel in the SE-9000 analyzer is a valuable tool for detection of platelet clumps in EDTA blood. An instrument flag is generated when platelet clumps are suspected.

In conclusion we can state that the XE-2100 is an analyzer which allows the user to analyze complex specimens as occur, for example in cold auto-immune hemolytic anemia, because of its innovative parameters, particularly the NRBC count in association with the reticulocyte count, the reticulocyte maturation measurements and the IRF. The NRBC counts correlate well with those microscopically determined. We can conclude that the value of this analyzer is much greater for the diagnosis of hemolytic diseases than preceding analyzer.

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