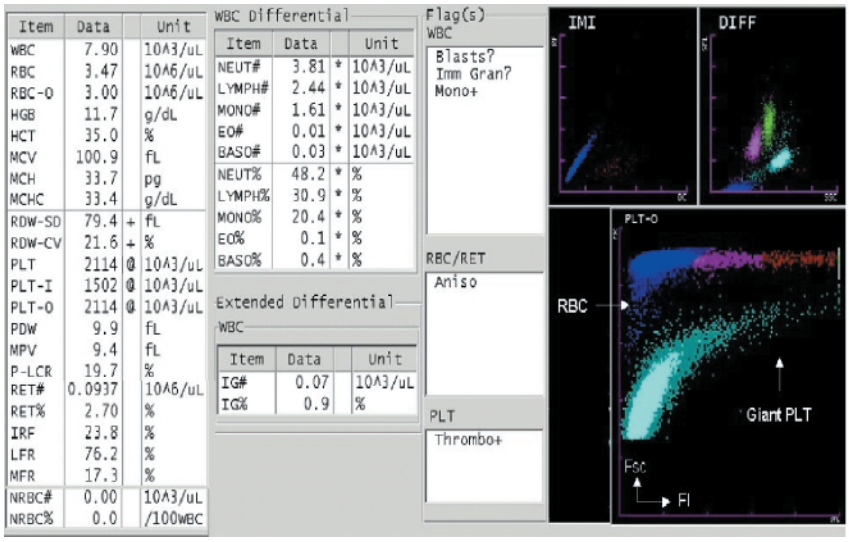

Fig. 23 *XE-2100 haemogram (RET mode)*
Note the difference between the impedance and optical platelet counts.

MULTIPLE MYELOMA

The patient is a 67-year-old male suffering from Bence Jones (λ) multiple myeloma with extensive infiltration of the bone marrow and osteoporosis. Immunophenotypic analysis showed expression of CD38, CD138, CD56, cyIgM and cy λ but no expression of CD10 and CD34/CD33. This indicates that the malignant cells are mature with B-cell activation (CD38, see ***Fig.24***) and L-chain λ (Bence-Jones protein).

Further laboratory data shows elevated levels for serum calcium (13.1mg/dL) and serum creatinine (2.9mg/dL). Bence-Jones protein excretion was grossly elevated (14g/24h).

The presenting XE-2100 haemogram is illustrated in ***Fig. 25*** and reveals a pancytopenia (Hb 89g/L, total leukocyte count 1.86×10^9 /L, PLT-I 19×10^9 /L). The reticulocyte count is 0.6% and the HFR (high fluorescence reticulocytes) fraction is 0% indicating failure of erythropoiesis presumably due to bone marrow infiltration by myeloma cells.

The DIFF scattergram of the XE-2100 depicted in ***Fig. 25*** shows essentially a single population of cells restricted to the lymphocyte region but exhibiting very high fluorescence activity. (Total lymphocytes are Lymph% 45.4 + "other" 46.8% = 92.2% see enlarged DIFF scattergram in ***Fig. 26***).

Examination of the peripheral blood smear shows a single population (90%) consisting mainly of mature small plasma cells (confirmed by immunophenotyping see ***Fig. 24***). This suggests that the fluorescence staining does not only give information about the size of the nucleus (DNA), but also about activity in the cell cytoplasm (RNA in the ribosomes).

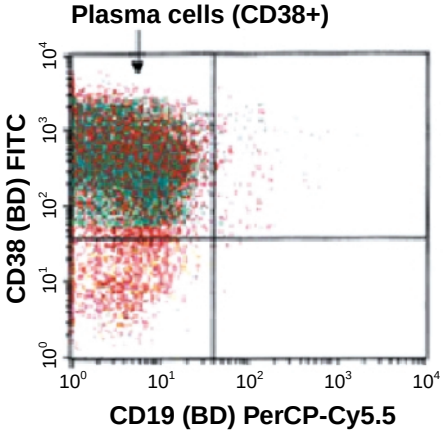

Fig. 24 *Immunophenotypic analysis (Becton Dickinson)*

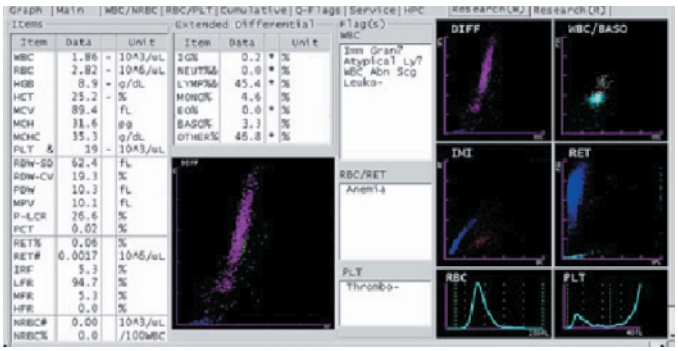

Fig. 25 *Pancytopenia in a patient with multiple myeloma*

The IMI (***Fig. 27***) and DIFF scattergrams show < 1% immature cells confirmed on peripheral blood smear examination (1% metamyelocyte). The combined interpretation of the DIFF and IMI scattergrams depicted in ***Figs 26 and 27*** shows that the population in the DIFF-channel are lymphoid cells with a high DNA/RNA content but that such cells are not represented in the IMI channel (all mature cells).

The peripheral blood smear shows RBC rouleaux (***Fig. 28***) caused by the high protein concentration and mature small plasma cells (***Fig. 29***).

An important criterion for the WHO classification of Bence-Jones myeloma is a plasma cell bone marrow infiltration greater than 30%. ***Fig. 30*** shows an XE-2100 profile obtained from an essentially pure culture of plasma cells. The DIFF scattergram is very similar to that of the patient's peripheral blood.

This was confirmed microscopically on the bone marrow smear (***Fig. 31***), which showed > 80% mature plasma cells.

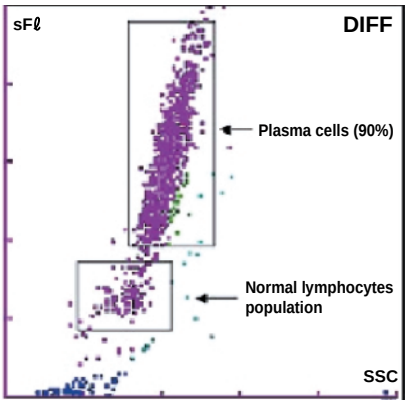

Fig. 26 *Enlarged DIFF scattergram*

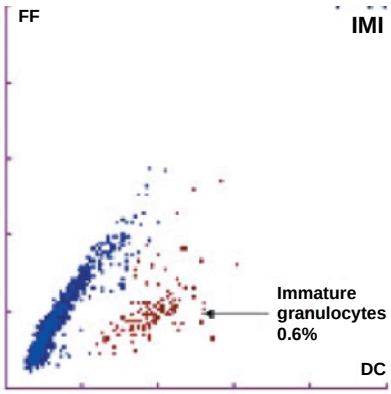

Fig. 27 *Enlarged IMI scattergram*

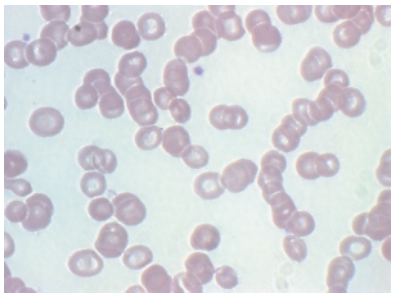

Fig. 28 *RBC rouleaux*

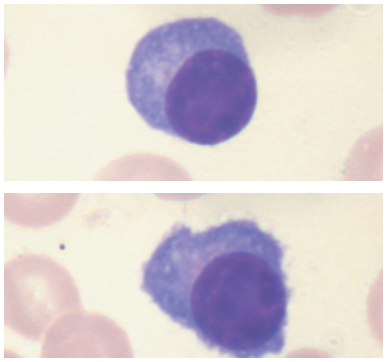

Fig. 29 *Plasma cells*

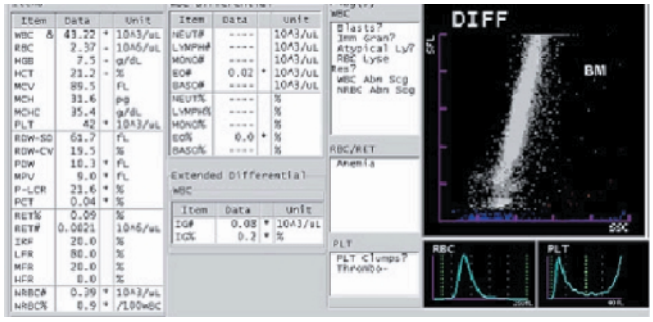

Fig. 30 *XE-2100 DIFF scattergram of plasma cell culture showing a monomorphic population similar to that of the patient's peripheral blood*

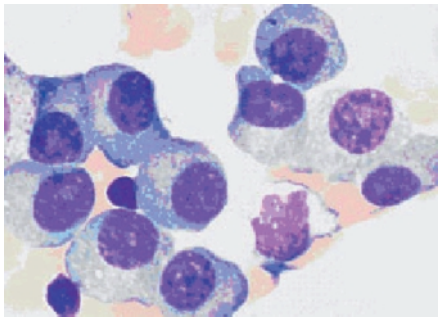

Fig. 31 *Bone marrow infiltration by mature plasma cells*

HAEMOGLOBIN H DISEASE

This section shows that the XE-2100 provides reliable NRBC and reticulocyte counts in α-thalassaemia intermedia (haemoglobin H disease). α-thalassaemia is the result of defective production of the alpha globin chain of haemoglobin. In haemoglobin H disease α chain synthesis is severely suppressed so that unstable tetramers of excess β globin chains (Hb H) are formed. Clinically haemoglobin H disease resembles β-thalassaemia intermedia.

The patient is a 6-year-old child with α-thalassaemia intermedia (deletion of three α globin genes (genotype --/-α)). The haemogram (***Fig. 32***) shows a haemoglobin concentration of 80g/L, an increased reticulocyte count at 329,000/μL (10.94 %), an increased immature reticulocyte fraction (IRF) at 41.4% and a high concentration of NRBC at 1,580/μL (13.8/100WBC) when measured on the XE-2100.

Punctate basophilia (***Fig. 33***) and Cabot rings (***Fig. 34***) were found in the peripheral blood smear. Hb H inclusions (***Fig. 35***) were found and the presence of Hb H was confirmed on electrophoresis.

In haemoglobin H disease patients, the abnormal haemoglobin has a high oxygen affinity and is unstable, precipitating to form intracellular inclusions (demonstrable on exposure to supravital staining (***Fig. 35***). This damages the red cells during their passage through the microcirculation. The result is a haemolytic anaemia, manifest, in the present patient, by an increased serum bilirubin, decreased haptoglobin level and an increased reticulocyte count with extremely elevated immature reticulocytes.

The DIFF scattergram is abnormal showing a cell cluster in the NRBC area (***Fig. 36***). The NRBC scattergram (***Fig. 37***) shows a high concentration of orthochromatic, polychromatic and even basophilic erythroblasts with excellent separation from the WBC. The RET scattergram illustrated in ***Fig. 38*** shows a high concentration of reticulocytes, IRF and the presence of microcytic erythrocytes.

The smear confirmed the pleomorphic nature of the NRBCs (orthochromatic erythroblast in ***Fig. 39*** and polychromatic erythroblast ***Fig. 40***).

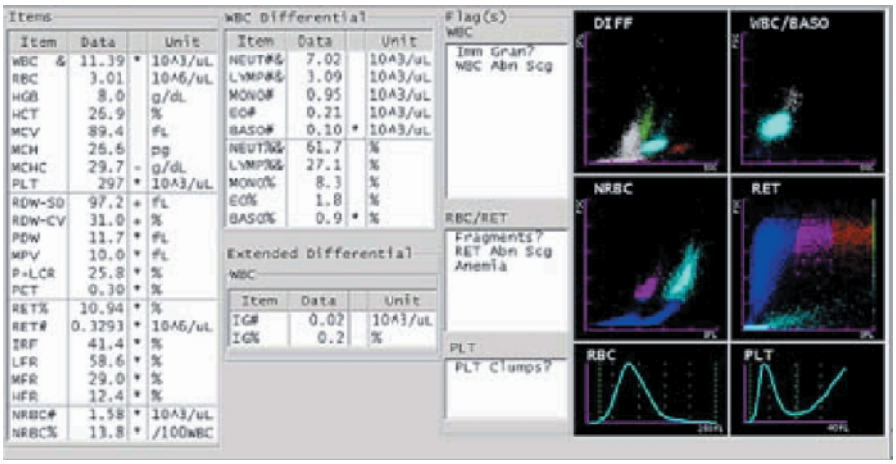

Fig. 32 *Presenting haemogram*

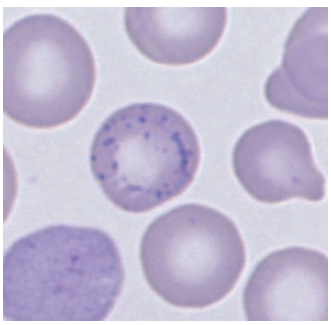

Fig. 33 *Punctate basophilia*

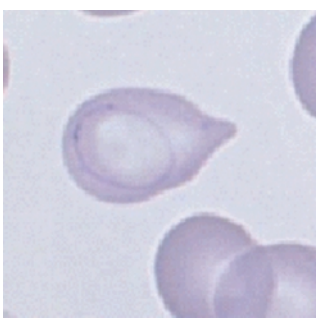

Fig. 34 *RBC containing Cabot ring*

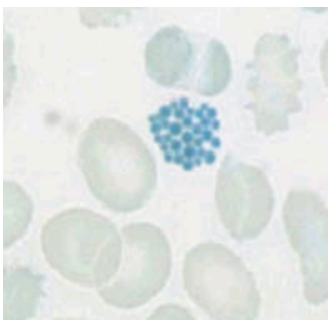

Fig. 35 *Hb H inclusions on supravital staining*

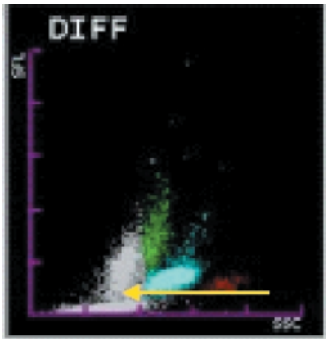

Fig. 36 *NRBC location indicated by "* ← *"*

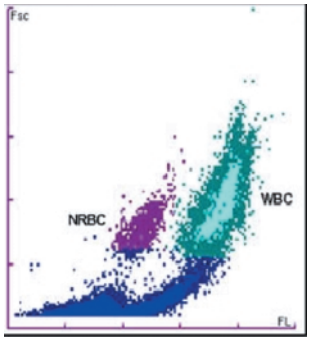

Fig. 37 *NRBC scattergram showing clear separation*

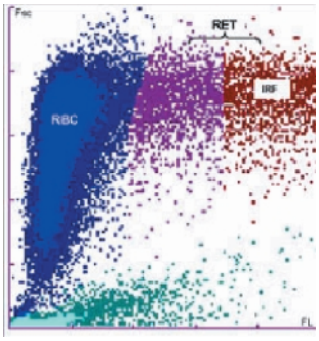

Fig. 38 *Reticulocyte scattergram*

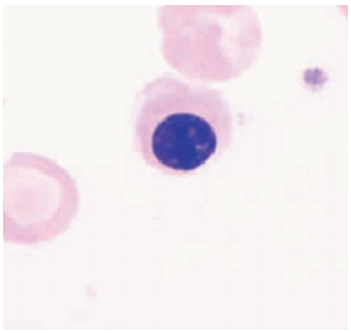

Fig. 39 *Orthochromatic NRBC*

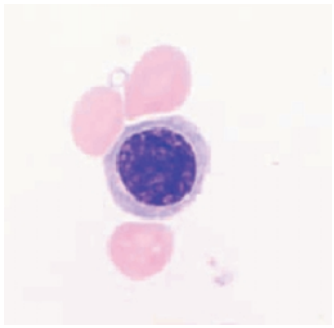

Fig. 40 *Polychromatic NRBC*

XE-2100 AND FALCIPARUM MALARIA

Typical XE-2100 DIFF scattergram appearances occur in heavy infestation with falciparum malaria, a phenomenon first reported by Briggs and Machin in 2001[7] (***Fig. 41***). This shows a tail of increased side fluorescence particles (purple dots) extending upwards from the monocyte cluster. A possible explanation of this high fluorescence activity could be the increased phagocytic activity of the monocytes ingesting malaria inclusions.

Reticulocyte parameters may also be of interest in untreated malaria. It has been noted that the absolute reticulocyte count may be increased but at the same time the immature reticulocyte fraction (IRF) is decreased. This is theoretically and practically impossible as these findings contradict each other in terms of reticulocyte physiology. It rather suggests, however, that the fluorescence activity of the cells counted represents inclusions which possess RNA/DNA, but which are not reticulocytes. These could be malarial parasites. The reticulocyte count is therefore falsely elevated due to this interference. A high absolute reticulocyte count and a low IRF may be a good indicator for malaria inclusions in the red blood cells however further confirmation of this observation is required.

This illustraitve patient is a 32-year-old male who presented with classic attacks of fever followed by identification of malaria parasites in a thick peripheral blood smear leading to a diagnosis of falciparum malaria (***Figs 42 and 43***). Lariam therapy was started immediately.

Figs 42 and 43 illustrate the morphological characteristics of falciparum malaria prior to Larium therapy. Note that the parasitised red cells are not enlarged, ring forms are delicate and there can be several in one cell; there may be two chromatin dots, but no developing forms are present and no gametocytes are found. The initial XE-2100 haemogram is illustrated in ***Fig. 44***.

There is thrombocytopenia but no anaemia and the leucocyte count is normal. The DIFF channel scattergram shows an abnormal population extending from the upper monocyte area (see 'a', ***Fig. 44***), indicating a higher fluorescence signal (RNA/DNA) than normal or even that produced by activated monocytes. The appearance is similar to that illustrated in ***Fig. 1***. The manual mono-

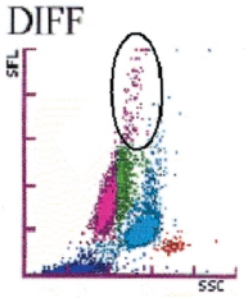

Fig. 41 *DIFF scattergram from a patient with 4.7% malaria parasitaemia (Briggs and Machin)*

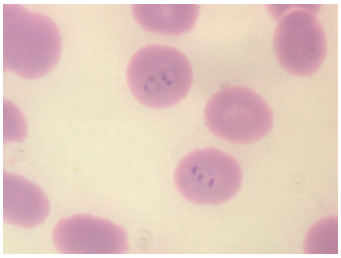

Fig. 42 *Malaria parasites in RBC*

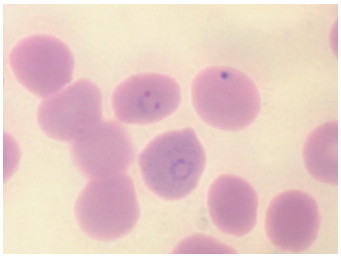

Fig. 43 *Malaria parasites in RBC*

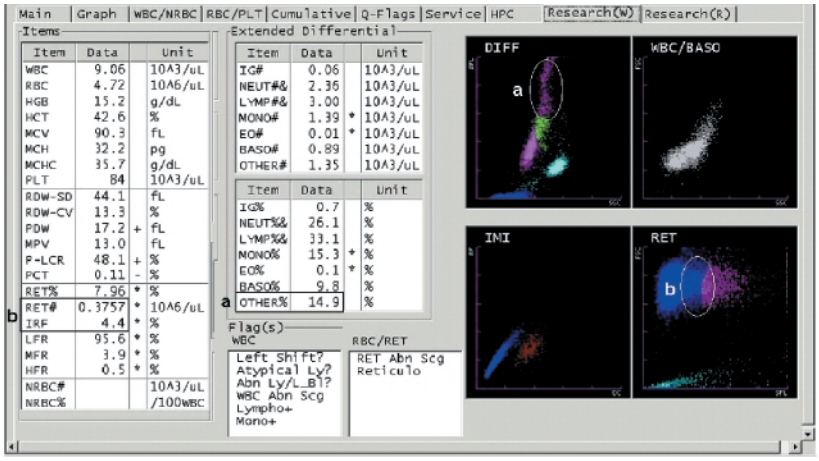

Fig. 44 *Initial XE-2100 haemogram*

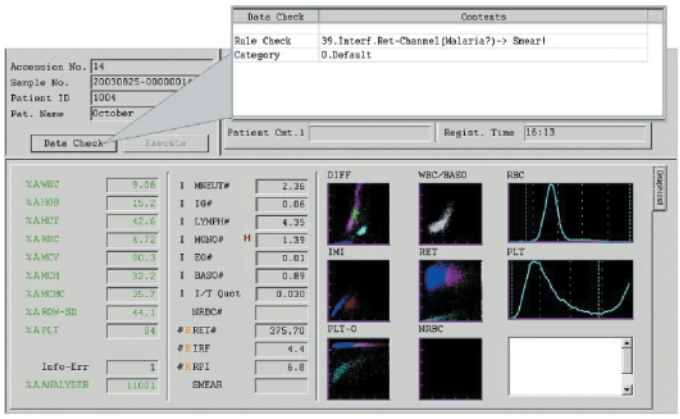

Fig. 45 *SIS result from this patient with an additional retest order (#R) for RET (Manual smear). See Rule 39*

Table 1 *Reticulocyte parameters after 12h of Larium therapy*

Registr. Time (h)	16:00	22:00	04:00
RET# (10^3 / μL)	375,7	244,3	116,2
IRF (%)	4,4	9,2	17,5
RPI (index)	6,8	4,8	1,4

cyte count is increased (26%) but this is not reflected by the instrument monocyte count (15.3%). However, if the 'OTHER' count (14.9%) is added to this, the resulting 30.2% is a closer approximation to the manual count. The reticulocyte count and RET-scattergram also show some interesting anomalies. The RET scattergram (see 'b', ***Fig. 44***) is abnormal between the RBCs (blue) and the reticulocytes (purple). The absolute reticulocyte count is increased, however the immature reticulocyte fraction (IRF) is decreased, the physiological contradiction described above and which may be a good indicator of malaria inclusions in the red blood cells. Although the elevated reticulocyte count is due to interference by malarial parasites, its serial measurement may indicate response to treatment. This hypothesis was tested in the present patient and appears to be valid as shown in ***Table 1***, the reticulocyte count (including RBCs with malaria inclusions) decreasing by a factor of 3 over a 12-hour period.

The reticulocyte abnormalities are included in the rule based Sysmex Information System (SIS) interface, shown in ***Fig. 45***. The rule guide, a combination of increased reticulocytes, decreased IRF and the flag "RET abn Scg" (see ***Fig. 44***), recommends and opens an additional order for a manual smear and reticulocyte count with the text "Rule 39: Interference RET Channel (Malaria?) - > Smear".

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