**NEW RED CELL PARAMETERS ON THE SYSMEX XE-2100 AS POTENTIAL MARKERS OF FUNCTIONAL IRON DEFICIENCY.**

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**INTRODUCTION**

- Early detection of iron deficiency is vital to optimise erythropoietin treatment of chronic anaemia associated with renal failure and malignancy. Failure to supply iron at sufficient rate adversely affects patient outcome. Standard biochemical measurement of iron status in these patients may be misleading. Ferritin is an indirect marker of storage iron but not iron supply. It is also an acute phase reactant so may be normal or high despite evolving iron deficiency.

- Impedance blood cell counters simply provide the traditional red cell parameters and indices that have little bearing on iron availability to erythroid cells.

- The Technicon series of cell counters (Bayer Diagnostics) measure the number of cells with a concentration of less than 28g/dl as being hypochromic (% Hypo). On the Advia 120 (Bayer Diagnostics) in addition to % Hypo a more acute evaluation of bone marrow activity is achieved by measuring the reticulocyte haemoglobin content (CHr).

- In the reticulocyte channel of the Sysmex XE-2100 two new parameters are measured by tracerepochrome flow cytometry (Fig 1). These are the mean value of the forward scatter light histograms of the red cells (RBC-Y) and reticulocytes (RET-Y). These values seem to equate with the red cell haemoglobin content.

- These new parameters could potentially provide additional information on iron status and erythropoiesis response following recombinant erythropoietin (rHuEPO) therapy in chronic renal failure.

- The aim of this pilot study was to compare biochemical markers of iron status, ferritin and soluble transferrin receptor levels, with % Hypo, CHr and the two new parameters, RBC-Y and RET-Y and assess their potential predictive value in diagnosing iron deficiency.

**MATERIALS AND METHODS**

- 240 peripheral blood samples collected into K$_{2}$EDTA (Becton Dickinson) were analysed at University College Hospital London using the XE-2100 for RBC-Y and RET-Y. The same samples were analysed within 4 hours on the Advia 120 for % Hypo and CHr.

- The samples studied were from 40 normal healthy males, 50 untreated iron deficient patients (selected by indices), 90 from patients receiving long term peritoneal dialysis and 60 from patients receiving long term haemodialysis. 128 of the renal patients were on rHuEPO therapy and 64 on regular iron therapy.

- An additional 70 samples, 20 from normal healthy males and 50 chronic renal dialysis patients were collected at the Royal Berkshire hospital and analysed on the XE-2100 and the Technicon H2 for % Hypo. There is no reticulocyte analysis on the H2. CHr was unavailable.

- In total 315 samples were analysed. After blood count analysis the plasma was frozen for the soluble transferrin assay and ferritin levels.

- The soluble transferrin assay (sTfR) was performed using the Bio-stat diagnostics kit. This is a particle enhanced immunometric assay based on the detection of an immunobinding between sTfR and TfR- specific antibodies. This has been shown to be a more sensitive and less variable index of iron status than the conventional measurement of ferritin.

- Ferritin was measured by an immunoassay technique using an Abbott Architect analyser and Abbott reagents in the routine chemical pathology service.

**RESULTS**

- Clearly defined ranges for CHr, % Hypo, RBC-Y and RET-Y have been defined in health and untreated simple iron deficiency (Table 1).

- With sTfR levels and ferritin there is some overlap between the normal and iron deficient range. 10 out of 70 apparently iron deficient patients had a normal sTfR (8 of these also had a normal ferritin) however all the samples had microcytic and hypochromic indices, low CHr, RBC-Y and RET-Y and high % Hypo so it was decided to include this data. 22 samples from the iron deficient group had normal or in one case high ferritin levels (6 had a normal sTfR). Again these all had microcytic and hypochromic indices, low CHr, RBC-Y and RET-Y and high % Hypo. One sample in this group had a ferritin level of 804 µg/L with an iron deficient sTfR of 4.85 mg/L.

- For all samples RET-Y and RBC-Y are closely correlated to CHr, r = 0.84 and 0.81 respectively.

- % Hypo correlated to a better extent with RBC-Y and RET-Y (r = 0.84 and 0.83) than CHr (r = 0.75).

- Figure 2 demonstrates the correlation of % Hypo with RBC-Y andfigure 3  CHr with RET-Y. The normal range cut off values for all parameters are marked on the graphs. This enables truth tables of agreement between each method to be visualised.

- When comparing % Hypo to RBC-Y there are a significant number of samples with a normal RBC-Y and abnormal % Hypo, these are all from renal patients, either pre dialysis or haemodialysis. There are also some normal % Hypo with an abnormal RBC-Y.

- There are only 3 samples with a normal CHr and abnormal RET-Y. Two of these renal patients had other haematological disease, one myeloma and the other acute myeloid leukaemia. There is only one sample with an abnormal CHr and normal RET-Y.

- There was very weak correlation between sTfR and any of the red cell or reticulocyte parameters.

- There was no correlation between sTfR and ferritin. In patients with renal disease the mean ferritin level was high at 54g/ml in contrast to a level of 2.49 mg/L for the sTfR which would indicate developing iron deficiency.

- There was a reasonable correlation between CHr and RBC-Y and RET-Y (both r = 0.80) and sTfR and % Hypo (r = 0.84). There was slightly less good correlation with CHr (r = 0.85).

**CONCLUSIONS**

- We have clearly defined ranges for CHr, % Hypo, RBC-Y and RET-Y in health and untreated simple iron deficiency.

- The primary purpose of this study has been achieved. Despite the fact that CHr is a measurement of mean reticulocyte haemoglobin content and RET-Y a measurement of reticulocyte size the correlation between the two in all patient groups was excellent and effectively interchangeable for clinical diagnostic purposes.

- There is reasonably good correlation between % Hypo and RBC-Y.

- No correlation was found between sTfR levels and ferritin and only weak correlation between ferritin and the red cell and reticulocyte parameters. This was not unexpected as ferritin is an acute phase reactant.

- Reasonable correlation was found between sTfR and CHr, RET-Y and % Hypo, slightly less good correlation with CHr.

- Where CHr may be used for monitoring functional iron deficiency RET-Y can be used in the same way.

- Future studies are needed whereby iron therapy is regularly monitored by standard haematological parameters in comparison with the new parameters, RBC-Y and RET-Y, to determine their true predictive value for clinical diagnostic purposes.