# 12.

RET-Y: Its Utility in the Diagnosis and Monitoring of Post-Treatment Sideropenic Anaemia
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Introduction

The XE-2100 is the 'top of the range' haematology analyser manufactured by SYSMEX. This instrument, in addition to the traditional parameters of the complete blood count and the leukocyte differential, is able to perform counts of nucleated red blood cells, immature granulocytes and a number of reticulocyte measurements. These last include the absolute and proportional reticulocyte counts, three immaturity fractions of the developing reticulocyte (low, medium and high fluorescence ratios – LFR, MFR, HFR) and the immature reticulocyte fraction (IRF), consisting of MFR + HFR %. To differentiate reticulocytes from mature red cells, the instrument uses a fluorescent polymethine dye which stains ribonucleic acid (RNA). It is thus possible to differentiate the positive fluorescing reticulocytes from the negative mature red cells. This is displayed on the reticulocyte (RET) scattergram (**figure 1**) that has Forward Light Scatter (FSc) on the y-axis and Side Fluorescence (SFl) on the x-axis.
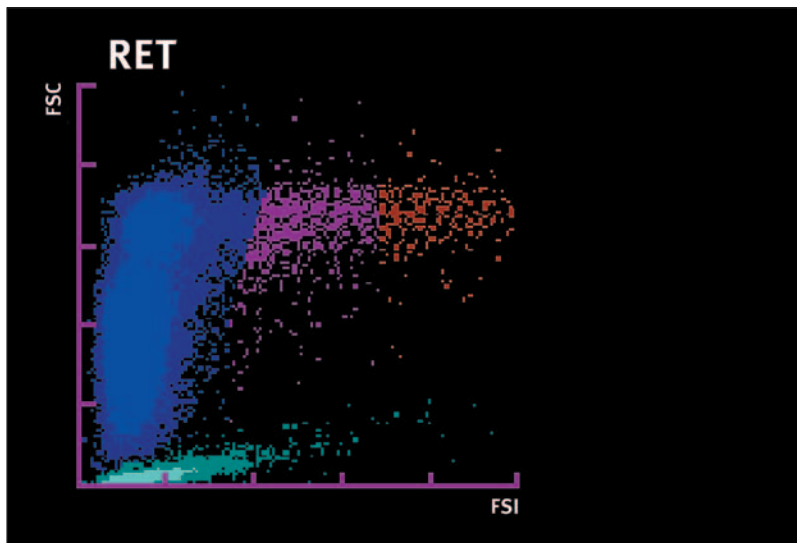
Figure 1
Reticulocyte scattergram with forward scattered light (FSc) on the y-axis and fluorescence (SFl) on the x-axis. The blue cluster represents mature RBC, the purple cluster is the LFR and the red cluster the MFR + HFR which is equivalent to the IRF. The turquoise cluster comprises the fluorescent platelets.

Using FSc, the analyser can therefore provide a raw measure of the approximate sizes of the mature red cells and reticulocytes (**figure 2**). These can be expressed as the mean channel of the FSc distribution for mature red cells (RBC-Y) and for reticulocytes (RET-Y). However, the FSc signal depends on many particle characteristics: size (volume only if the particle is spherical) [1], shape and orientation [2], refractive index (or density) [3], internal structure (complexity) [4], and staining [5].

Figure 2
Determination of RBC-Y and RET-Y from the reticulocyte channel on the XE-2100. RBC-Y is the mean channel number of the forward scattered light signal histogram within the mature RBC population and RET-Y is the corresponding channel number within the reticulocyte population.
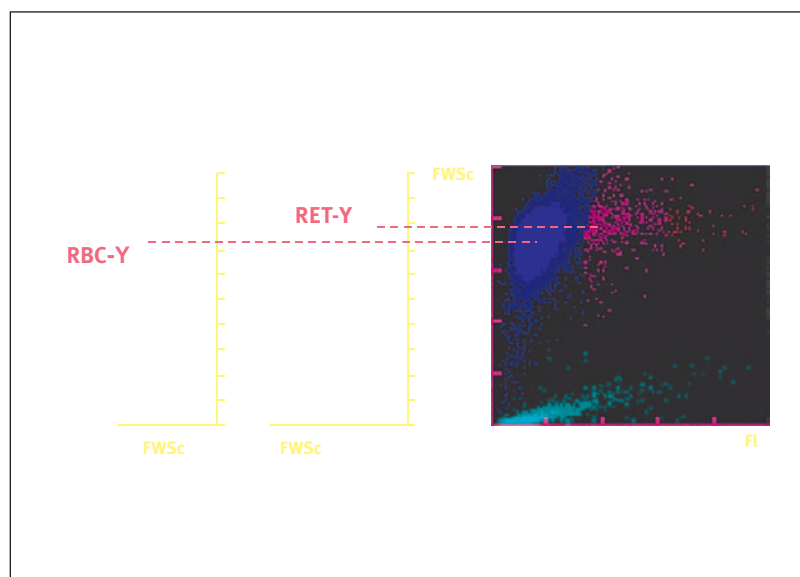
Interest in reticulocyte cellular indices is increasing. In addition to the RET-Y of the SYSMEX XE-2100 a number of indices are now available including the mean cell reticulocyte volume (MCVr), the mean reticulocyte haemoglobin content (CHr), and the mean sphered reticulocyte volume (MSRV). In the past only one analyser could measure the CHr and this is the most widely studied of the reticulocyte indices thus far. The CHr is considered useful in a number of clinical situations including the diagnosis of iron deficient erythropoiesis [6], diagnosis of functional iron deficiency [7] and monitoring response to iron supplements [8]. A major limitation of the CHr is that it is entirely manufacturer specific. Briggs *et al* [9], however, have demonstrated substantial equivalence between RET-Y and CHr. Less well known are studies concerning the clinical usefulness of the MCVr, but these include the diagnosis of iron deficiency [10], monitoring of response to iron supplements [11], monitoring of response to folic acid and cyanocobalamin [12], follow-up of bone marrow transplantation [13] and evaluation of erythropoietin abuse in sports [14]. It is possible to see how MCVr may possess clinical utility that exceeds that of CHr.

The aim of this presentation is to assess possible concordance between RET-Y, MCVr and CHr and to compare their clinical usefulness in the diagnosis and monitoring of a group of subjects with iron deficiency anaemia.

Study Design

There were four main objectives:
1. determine the reference interval for RET-Y in healthy individuals
2. evaluate the imprecision of RET-Y
3. undertake a numerical comparison of RET-Y with (i) CHr and (ii) with MCVr generated by the Bayer Advia 120
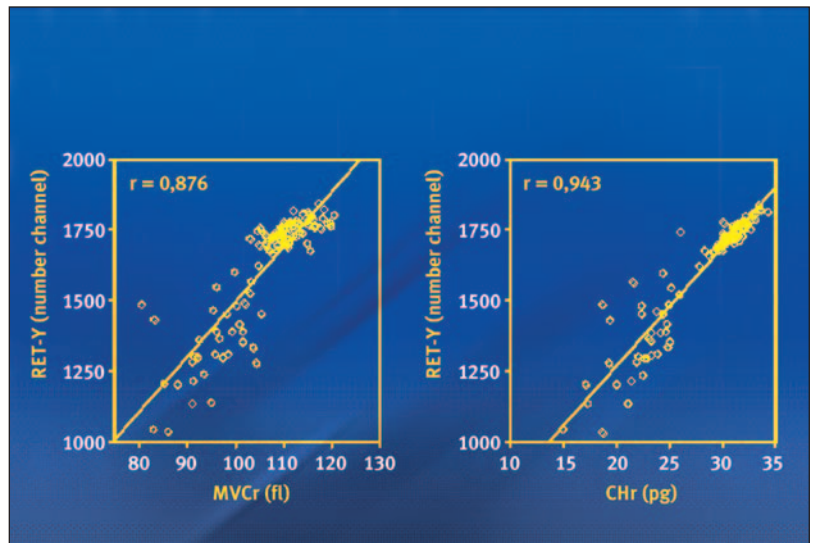
4. undertake a clinical comparison of RET-Y with CHr and with MCVr in side-ropenic anaemia.

1. *RET-Y Reference interval:* eighty healthy subjects were analysed in duplicate according to the NCCLS protocol [**15**]. The results are shown in **figure 3**.

2. *RET-Y Imprecision profile:* the coefficient of variation is close to 1% over a wide range of channel numbers and is negligible (**figure 4**).

3. *RET-Y Numerical comparison with MCVr and CHr:* the correlation (**figure 5**) is better with CHr (r = 0.943) than with MCVr (r = 0.876). From this it is clear that RET-Y is influenced by both size and, perhaps even more, by haemoglobin content of the reticulocytes.

	n° channel
mean	1741
median	1740
range	
2.5°	1661
97.5°	1820
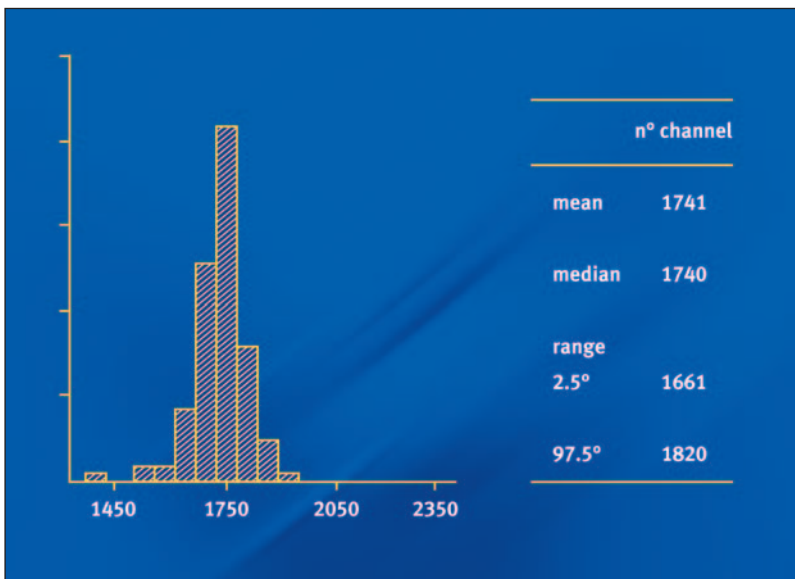
Figure 3
RET-Y Channel number reference interval based on 80 healthy subjects analysed in duplicate (NCCLS H44-A).
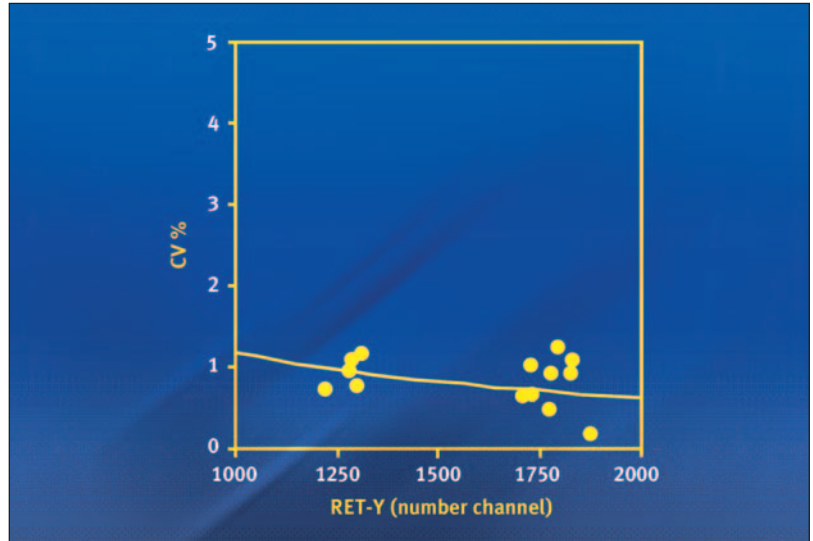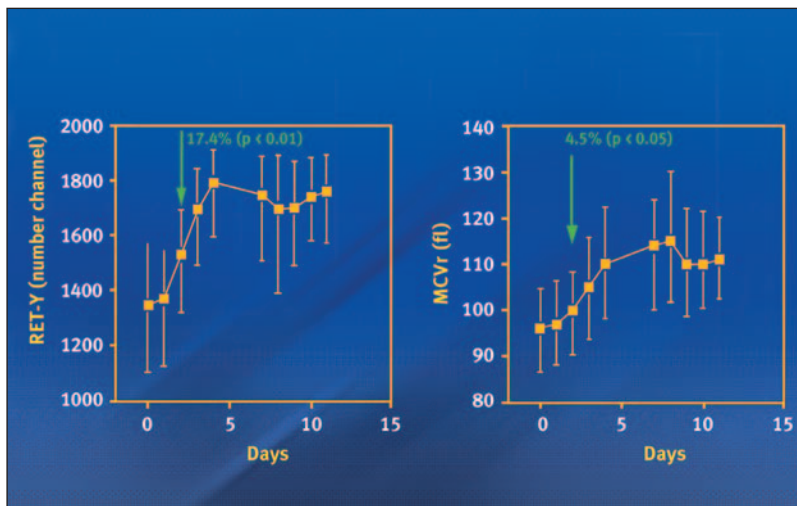
Figure 4
RET-Y imprecision profile

Figure 5
Numerical comparison of RET-Y with MCVr and with CHr.

4. *RET-Y clinical comparison with MCVr and CHr:* For the clinical study 29 patients (23 female and 6 male; age range 29–78 years) were selected from our intravenous iron clinic. Therapy consisted of slow IV administration of iron hydroxide saccharose complex, 100 mg elemental iron per day, Monday to Friday for two weeks. Pre-treatment control CBC counts were taken each morning including RET-Y, MCVr and CHr. **Table 1** shows haemoglobin concentration, MCV and reticulocyte cellular indices on Day 0 and after 11 days of treatment. It is important to note that all patients were iron deficient initially and that in each case the RET-Y values were all lower than the lower limit of the reference interval in health.

Parameter	mean		range	
	day 0	day 11	day 0	day 11
Hb (g/dl)	9.0	9.9	6.5–10.9	7.5–11.7
MCV (fl)	75.6	78.8	62.9–85	66.4–88.5
CHr (pg)	22.01	29.2	16.6–25.2	22.5–33.6
MCVr (fl)	95.25	109.9	82.4–104.5	98.7–119.4
RET-Y (n.ch.)	1352	1746	1044–1626	1502–1896

Table 1
Significant CBC parameters and reticulocyte indices 11 days after start of treatment with I.V. iron.

The average behaviour of the size parameters studied (**figure 6**) indicates a variation in percentage (+17.4% for RET-Y and +4.5% for MCVr) with respect to time zero that becomes statistically significant by the second day ($p < 0.01$ for RET-Y and $p < 0.05$ for MCVr). This behaviour of CHr, is intermediale having increased by +9.5% by day 2.
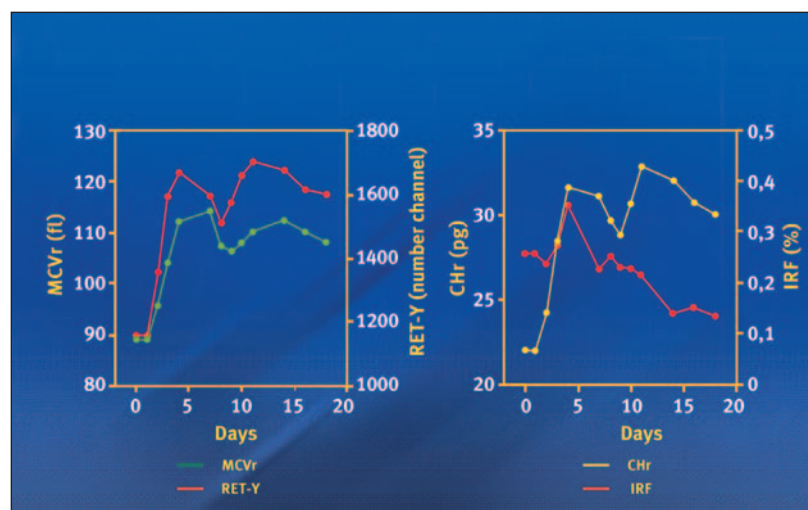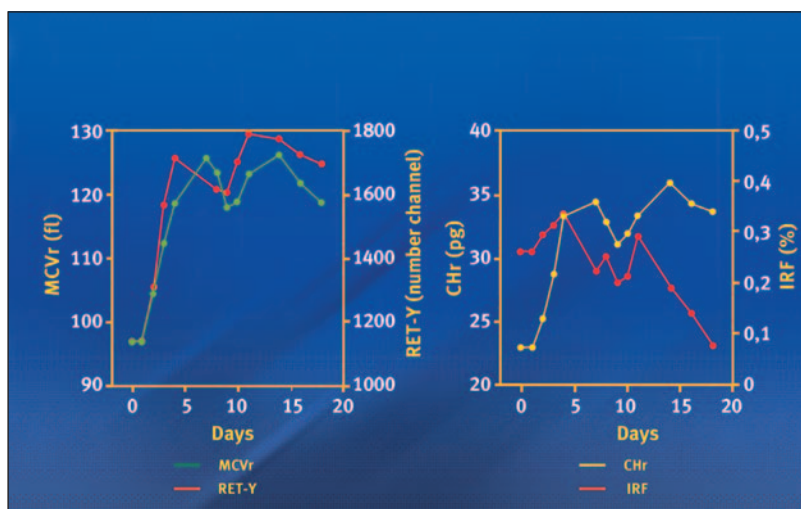

Figure 6
Change in RET-Y (left) and MCVr (right) in iron deficient patients after starting I.V. iron therapy. By day 2 (green arrow) RET-Y has increased by 17.4% and MCVr by 4.5%. During the same period the CHr increased by 9.5%.

Figures 7, 8 and **9** illustrate the behaviour of these parameters over the 11 days of treatment in three different patients. **Figure 7** illustrates the behaviour of RET-Y, MCVr and CHr in an iron deficient anaemic patient who is given treatment with 100 mg elemental intravenous iron, for five days, has a two-day break over the weekend and then recommences I.V. iron for a further five days. There is a parallel increase in all three measurements followed by a parallel decrease on days 8 and 9 followed by a further parallel increase thereafter. The patient illustrated in **figure 8** has the same interrupted therapy and behaves in an identical manner. This is the pattern observed in all patients with iron deficiency anaemia who have interrupted therapy even for only two days. The patient illustrated in **figure 9** is different in that the iron deficiency co-exists with β-thalassaemia trait. The parallel behaviour of all three reticulocyte indices persists, however, following the usual rapid increase a plateau is reached at day 5 and maintained. The values for RET-Y, MCVr and CHr fail to reach the lower limit of the respective reference intervals. This is a consistent phenomenon when iron deficiency anaemia and β-thalassaemia trait co-exist.

ROC curve analysis indicates a high diagnostic efficiency for RET-Y and MCVr for iron deficiency anaemia. From this analysis it is possible to calculate the decision levels which give the optimum balance between sensitivity and specificity which are channel No 1624 (sensitivity = 99%; specificity = 98.7%) for RET-Y on the XE-2100 and 104.5 fl (sensitivity = 96.6; specificity = 97.5%) for the MCVr on the Bayer Advia 120. These excellent results depend on the presence of anaemia. All the patients in this study had moderate to severe anaemia with haemoglobin concentrations between 6.5 and 10.9 g/dL. Further studies are necessary to evaluate patients with sideropenia but without anaemia or with only mild anaemia.

Figure 7
Patient with iron deficiency treated with daily IV iron for 5 days followed by a 2 day break followed by a further 5 days of iron. Note the parallel responses of RET-Y, MCVr, and CHr (rapid increase on starting therapy, fall when therapy discontinued and further increase on re-starting therapy.

Figure 8
This patient behaves in exactly the same way as the patient in figure 7. Both had interruption of iron therapy and both show transient fall in RET-Y, MCVr and CHr following the break in iron treatment.
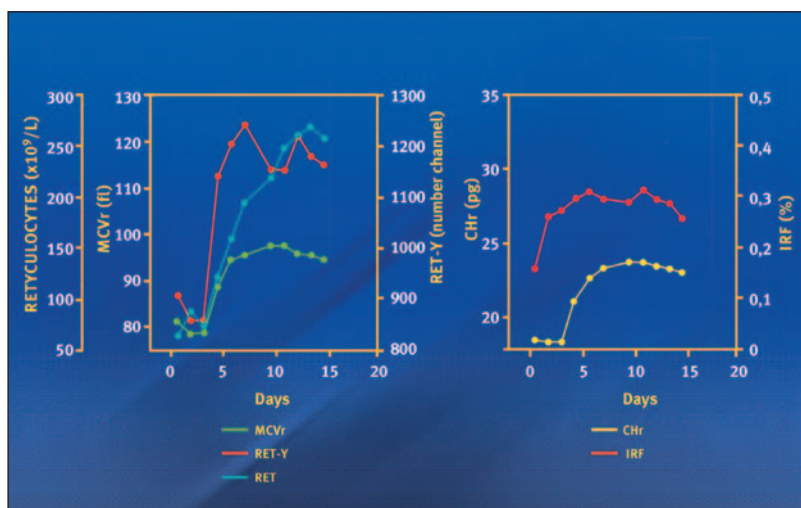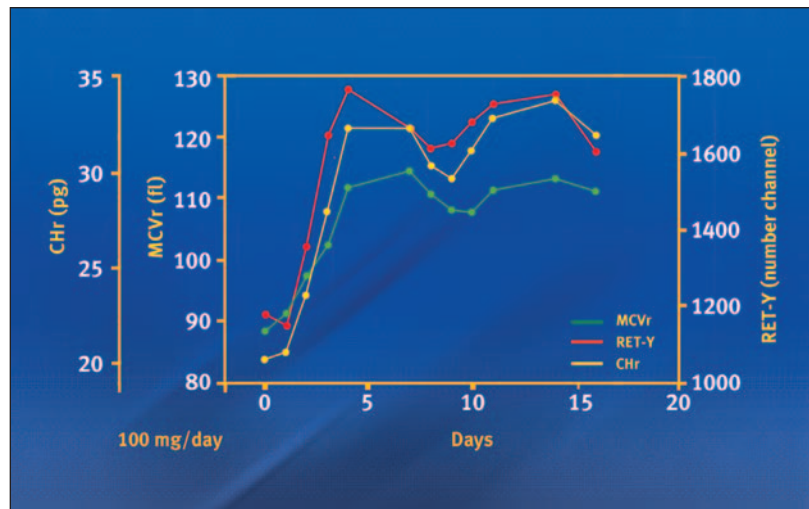

Figure 9
This third patient has iron deficiency and β-thalassaemia trait. Note that on commencing I.V. iron RET-Y, MCVr and CHr increase quickly but by day 5 reach a plateau that is always below the lower limit of the reference interval in health.

The sudden decrease (**figure 10**) of all three parameters on days 8 and 9 could result from the reappearance of iron deficient erythropoiesis due to the transient suspension of therapy. When therapy is not discontinued the decreases do not occur (**figure 11**). Increasing the dose of iron from 100 mg daily to 200 mg daily but continuing to interrupt therapy at weekends does not abolish the transient decreases of RET-Y, MCVr and CHr (**figure 12**).
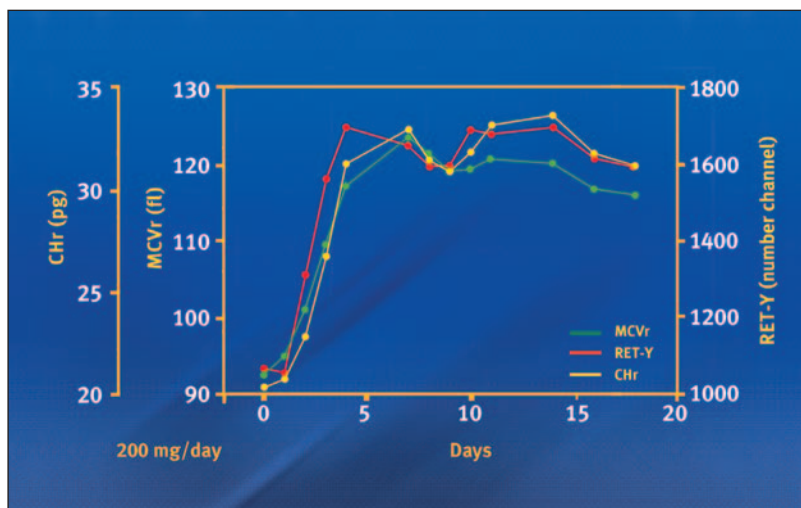
Figure 10
Patient on 100 mg elemental iron daily with suspension of therapy over the weekend. Note transient fall in RET-Y, MCVr and CHr.

Figure 11
Patient on 100 mg elemental iron daily with uninterrupted therapy. The transient falls in RET-Y, MCVr and CHr do not occur.
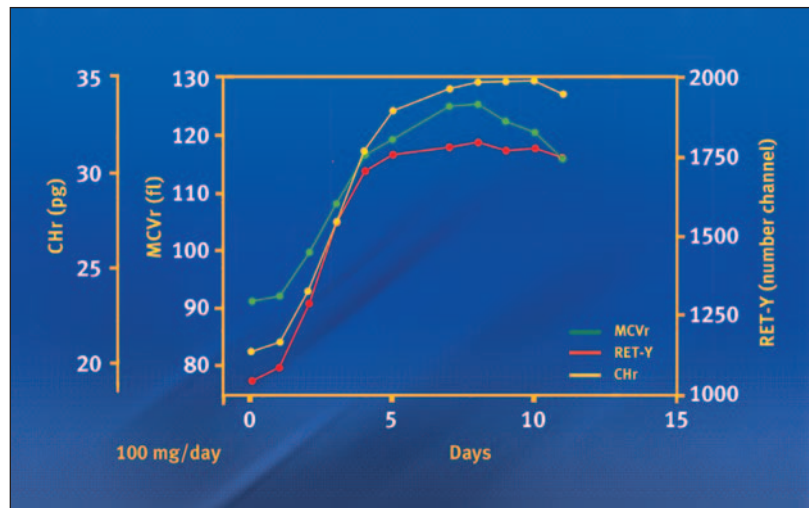
Figure 12
This patient is on 200 mg elemental iron I.V. daily but therapy is interrupted at the weekend. Note the transient falls in RET-Y, MCVr and CHr occur and are uninfluenced by the increased dose of iron.

Conclusions

A number of conclusions can be drawn from this study. First RET-Y is a sensitive and specific parameter for the recognition of sideropenic states that parallels the better-known MCVr and CHr parameters. An exception exists in co-existent β-thalassaemia trait but this applies equally to the other measurements. RET-Y is a very early indicator of response to intravenous iron therapy (second day). RET-Y is proving to be a useful parameter for the evaluation of the pathophysiology of erythropoiesis. Further studies are necessary to evaluate its usefulness in the evaluation of sideropenia in the absence of anaemia and in mild anaemia. RET-Y is not an ideal name because it is not immediately associated with a cell characteristic.

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