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The Nucleated Red Blood Cell (NRBC) Count in Thalassaemia Syndromes

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Introduction

The purpose of this study was to evaluate the performance of the automated NRBC count generated by the SYSMEX XE-2100 in a large series of thalassaemia patients under the care of the Division of Pediatric Haematology and Oncology in the Haematology Department of an Italian hospital.

The principle of the automated NRBC count on the XE-2100 by now is well known being accomplished by flow cytometry using a semiconductor red diode laser and a polymethine based fluorescent dye. Mature RBCs are completely lysed. WBC membranes become perforated but the cells retain their original shape. NRBCs are denucleated and shrunken. The polymethine dye stains the intracytoplasmic organelles and the nucleus of the WBCs quite strongly while the staining of the NRBCs is comparatively weak. These different staining characteristics allow clear discrimination between the cell counts (**figure 1**) and therefore make NRBC counting possible. Imprecision studies and comparison with manual microscopy counts have been reported by Zini *et al* [**1**].

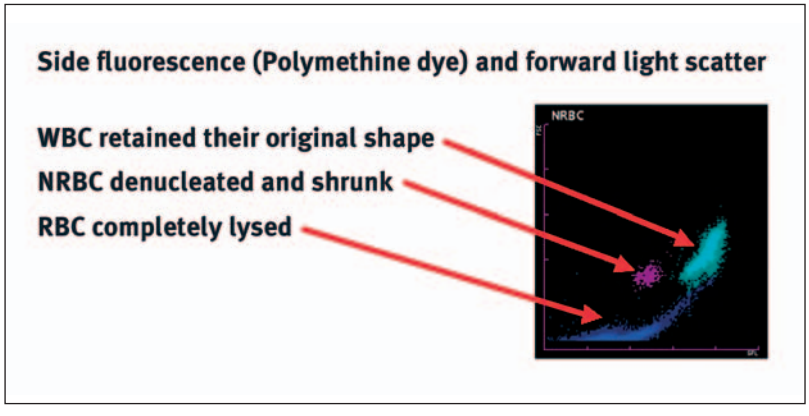

Figure 1
NRBC count method by the SYSMEX XE-2100.

The thalassaemias are the most common monogenic diseases in man. Both historical and recent transmigrations have made the thalassaemias a world wide problem. There are many unresolved issues particularly in relation to population screening, the differential diagnosis between β-thalassaemia trait and mild thalassaemia intermedia and the management of therapy of thalassaemia major patients. Why specifically count NRBC in the thalassaemia syndromes? A number of reasons have been advanced. The first is to improve the accuracy of the WBC count but, since thalassaemia patients are usually very well known to the clinicians, this is not a very important reason. The second reason is to improve the differential diagnosis between β-thalassaemia trait and mild thalassaemia intermedia and this may be a rather controversial point. The third reason is to improve the transfusion regimen in transfusion-dependent patients.

β-thalassaemia trait

In the β-thalassaemia trait patients the finding of NRBC was previously considered unusual, but how often were adequate routine NRBC counts carried out in such patients? In the authors' opinion, the search for NRBCs could improve the accuracy of the diagnosis

β-thalassaemia intermedia

The clinical phenotype of patients designated to have thalassaemia intermedia is more severe than the usual asymptomatic thalassaemia trait but milder than transfusion-dependent thalassaemia major [**2, 3, 4**]. There exists a wide spectrum of disability. In the worst cases it is possible to observe patients with haemoglobin levels around 6 g/dL, retarded growth and development, marked skeletal deformities, splenomegaly, leg ulceration. In the least severe cases it is possible to observe completely asymptomatic patients with haemoglobin levels within the 10–12 g/dL range. However, it is possible to observe the entire spectrum of intermediate severities.

Cooley's Anaemia

In Cooley's anaemia there is a marked imbalance of globin chain synthesis with an excess of α-chains and their precipitation in the red cell precursors. This α-chain precipitation results in the formation of large inclusion bodies that are responsible for the intramedullary destruction of red cell precursors and therefore the ineffective erythropoiesis, which characterises all β-thalassaemia syndromes and, particularly, Cooley's anaemia. The anaemia is determined substantially by three different mechanisms: [**1**] ineffective erythropoiesis, [**2**] haemolysis due to the destruction of mature red cells containing α-chain inclusions and [**3**] microcytosis and hypochromia due to the reduction in haemoglobin synthesis.

Ineffective erythropoiesis leads to many of the disabling complications associated with thalassaemia: bone disease (cosmetic deformities, pathological fractures, osteoporosis); increased gastro-intestinal iron absorption; hepatosplenomegaly (hypersplenism); hypercatabolic state (increased oxygen and caloric requirement); and growth failure.

Transfusion therapy for patients with Cooley's anaemia was introduced by Wolman [**5**] in 1964. It was soon clear that the therapy had two main objectives: [**1**] to prevent anaemia and [**2**] to suppress the highly ineffective endogenous erythropoiesis. The problem of severe β-thalassaemia is not only one of anaemia but the combined effect of anaemia with the massive enlargement of the erythroid marrow. Wolman proposed a pre-transfusion haemoglobin level of 8.5 g/dL. In 1969 Piomelli *et al* [**6**] proposed the maintenance of the haemoglobin level at over 10 g/dL (hypertransfusion regimen). Propper *et al* [**7**] in 1980 proposed a pre-transfusion haemoglobin level of 12 g/dL (supertransfusion programme) but this

soon appeared to require a high quantity of transfused blood with consequent high iron overload. By the 1990s a pretransfusion haemoglobin level of › 9 g/dL was proposed by Olivieri *et al* [**8**] or ‹ 9 g/dL in individual patients by Brittenham *et al* [**9**]. Erythropoietic suppression by transfusion therapy can produce negative effects because of iron overload, thus an appropriate regimen of transfusion therapy should be prescribed for each patient. Cazzola *et al* [**10**] in a major study on the relationship between transfusion regimen and suppression of erythropoiesis in ß-thalassaemia major concluded that pretransfusion values of ≤ 9 g/dL should be adopted with caution, because these levels can be associated with an insufficient inhibition of erythroid marrow expansion. Soluble transferrin receptor was the single parameter most closely related to erythroid marrow activity. Since fixed haemoglobin levels may not be the best target for transfusion treatment in all thalassaemic patients, assay of soluble transferrin receptor may be helpful for individualising the transfusion regimens. As measured through soluble transferrin receptor level, erythroid activity was 1–2 times normal for pretransfusion Hb levels between 10 and 11 g/dL, 1–4 times normal for levels of 9–10 g/dL and 2–6 times normal for levels of 8.6–9 g/dL. In this study the relationship of the pretransfusion haemoglobin level to NRBC count (by manual microscopy) was weak. This study, however, has not considered any difference between splenectomized and non-splenectomized patients nor has any evaluation been made of parameter variation in the individual patient.

Since 1969, Piomelli *et al* [**6**] proposed evaluation of ineffective erythropoiesis by using the NRBC count. This approach was confirmed by Fosburg and Nathan [**11**] in 1990 who defined an NRBC count level lower than 5/100 WBC as indicating an adequate transfusion programme.

The present study

Our study populations included:

(A) 120 patients with β-thalassaemia trait
(B) 20 thalassaemia intermedia patients (age 7–62 years)
(C) 10 transfusion dependent thalassaemia patients (age 4 mo–59 years)

(A) The authors have studied a group of 120 β-trait patients with HbA2 levels ranging from 3.5–6.9 % using the automated NRBC counting facility on the XE-2100. No NRBC were found in this group of patients, taking into account that the instrument has a lower limit of detection of 20/mm^3. This can be illustrated by the case of a 44 year-old woman with β-thalassaemia trait and iron deficiency, the latter being due to polymenorrhoea. The Hb concentration was 7.5 g/dL and the peripheral blood showed hypochromia, severe microcytosis and anisopoikilocytosis, and severe platelet anisocytosis with frequent giant platelets (**figure 2**). The patient had been referred to the Haematology Department as a case of thrombocythaemia in the belief that the platelet count was 845 x 10^9/L. It was, in fact only 169 x 10^9/L, the additional counts being produced by the extreme degree of

microcytosis. No NRBC were present in the peripheral blood in spite of two causes of defective erythropoiesis.

(B) The study population of thalassaemia intermedia patients covers a very broad and shifting clinical spectrum. Mean haemoglobin level is 9.4 ±1 g/dL; half of them have sometimes required transfusions, seven have been splenectomized, one is on chelation therapy. The mean NRBC is 1113/mm^3. Therefore NRBC count could provide an easy discrimination between β-thalassaemia trait and thalassaemia intermedia.

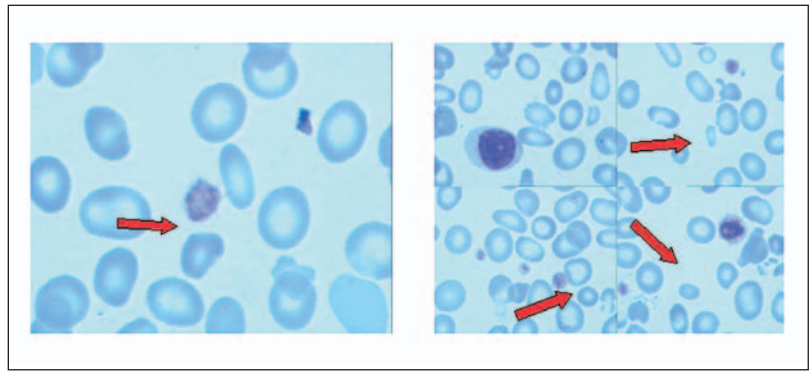

Figure 2
Note the extreme microcytosis which contributed to a spuriously elevated impedance platelet count.

(C) In Cooley's anaemia patients the haemoglobin concentration is compared with soluble transferrin receptor levels. The number of comparisons thus far is small and a statistical approach has not been employed but it seems clear that no correlation exists (**figure 3**). The same conclusion is reached when comparing the NRBC count with soluble transferrin receptor levels (**figure 4**). However there would appear to be an inverse relationship between the haemoglobin concentration and the NRBC count in transfusion-dependent non-splenectomised patients (**figure 5**). These series will be expanded and statistical analysis undertaken.

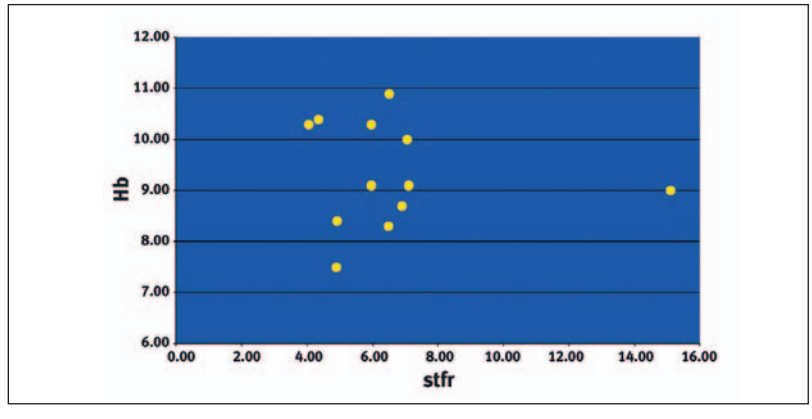

Figure 3
Lack of correlation between Hb level and soluble transferrin receptor level.

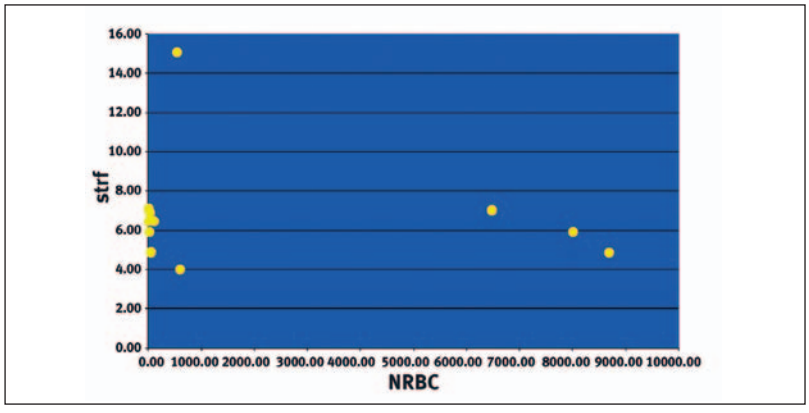

Figure 4
Lack of correlation between soluble transferrin receptor level and NRBC count.

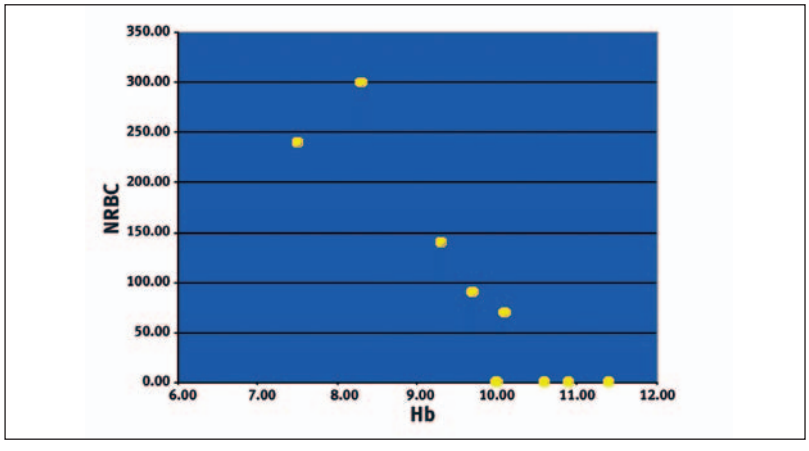

Figure 5
Transfusion-dependent non-splenectomized subjects. Inverse correlation between pre-transfusion Hb level and NRBC.

When we start to analyse individual patients some interesting features emerge. **Figure 6** illustrates the results of haemoglobin concentration, reticulocyte count and NRBC count on two different occasions from a patient with transfusion dependent β-thalassaemia ($\beta^+ - 92C \rightarrow T/\beta^+ + 745$). Note the inverse relationship of haemoglobin concentration with both reticulocyte and NRBC counts. **Figure 7** shows similar behaviour from her elder sister with the same genotype but not transfusion dependent. In **figure 8** the data of these two sisters are shown. The elder sister (green line) has good control over ineffective erythropoiesis at a haemoglobin concentration of just below 9 g/dL. The soluble transferrin receptor level is at the upper limit of the healthy reference range and the NRBC count is zero. The second patient (red line) has an elevated soluble transferrin receptor level and a persistently increased NRBC, even if she is on a chronic transfusion regimen.

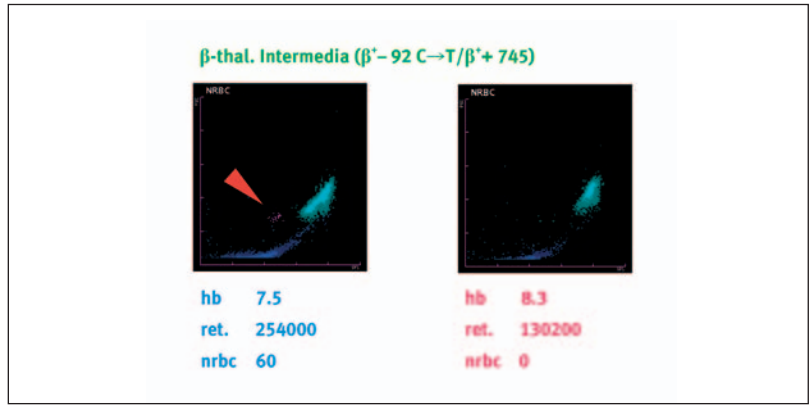

Figure 6
Transfusion dependent β-thalassaemia (β+ – 92C ➞ T/β+ + 745). Note the inverse relationship between Hb and NRBC count.

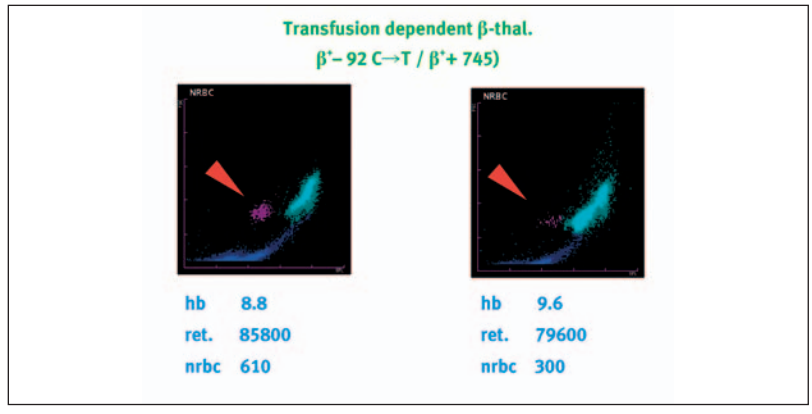

Figure 7
Sister of patient illustrated in figure 6 but non-transfusion dependent (β+–92C➞T/β++745). Again note the inverse relationship between Hb and NRBC count.

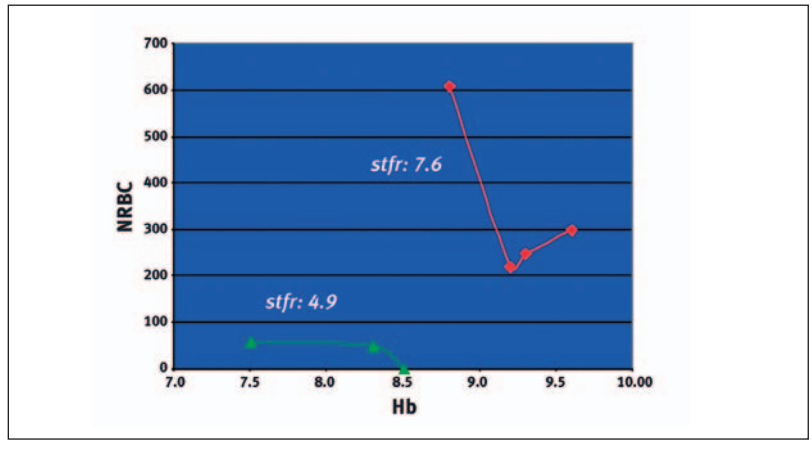

Figure 8
Data from the two siblings shown in figures 6 and 7. The elder sister (green line) is 12 years of age with thalassaemia intermedia but without clinical symptoms. The younger sister (red line) is 10 years of age with transfusion dependent thalassaemia with growth failure, bone deformities and gallstones.

The 10 year old transfusion dependent patient shown in **figure 9** with a haemoglobin level concentration ranging between 10 g/dL and 11.4 g/dL has normal soluble transferrin receptor levels (2.54, 1.77 mg/L) and no circulating NRBC and therefore does not show any evidence of ineffective erythropoiesis.

In **figure 10** two transfusion dependent patients are shown: the first (red line) consistently shows evidence of ineffective erythropoiesis with persistent elevation of both NRBC count and soluble transferrin receptor levels (2.54, 1.77 mg/L), thus needing a higher pretransfusional haemoglobin level; the second (green line) shows good control of ineffective erythropoiesis on a lower pretransfusional haemoglobin level.

The final illustration (**figure 11**) is of a 59-year-old transfusion-dependent patient showing an increasing NRBC count with an increasing pretransfusion haemoglobin level. Although very interesting there is no immediate explanation of this.

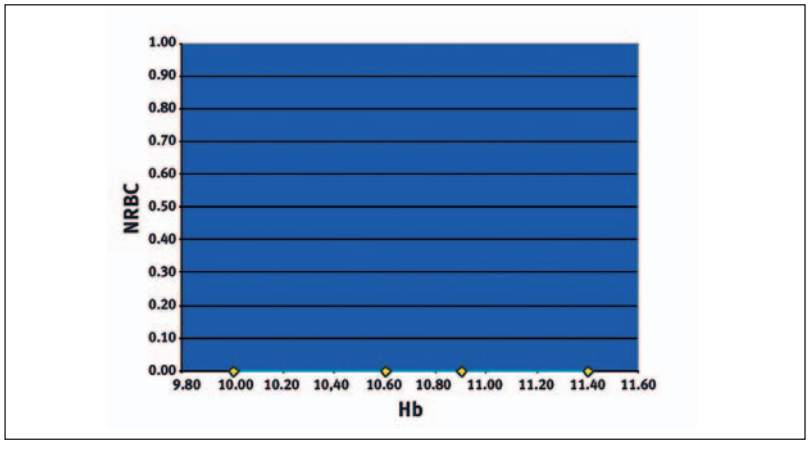

Figure 9
Ten year old homozygous IVS 1-1 transfusion dependent patient.

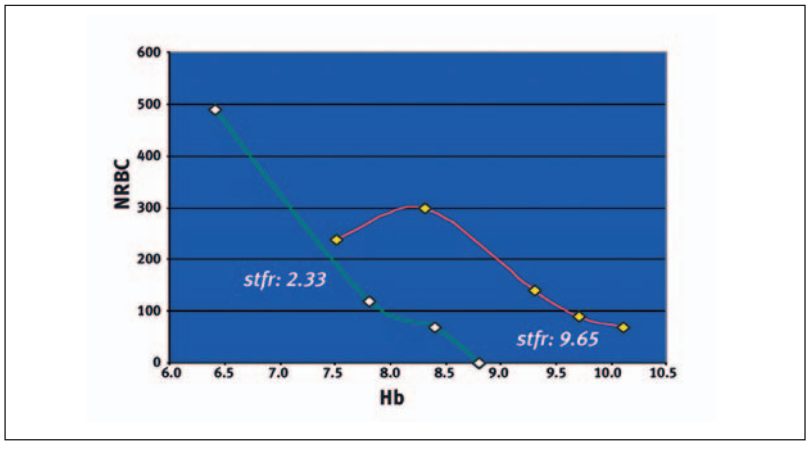

Figure 10
Ten year old (red line), β°IVS 1-1 /β°IVS 2-1 transfusion-dependent patient. One year old (green line), β°39 /β°IVS 1-1, transfusion dependent patient.

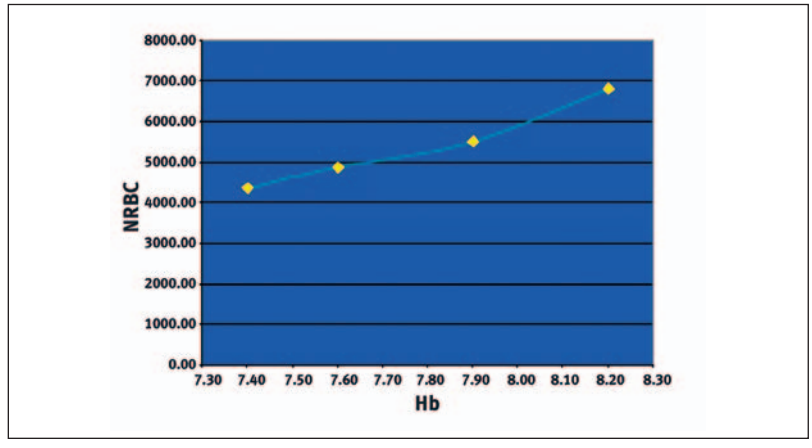

Figure 11
Fifty-nine year old splenectomized transfusion-dependent patient. Note the increase in NRBC count with increase of pretransfusion level.

Conclusions

The NRBC count is a reliable, inexpensive haematological parameter, immediately available with other parameters of the complete blood count. The presence of NRBC in the peripheral blood excludes the diagnosis of simple β-thalassemia trait; therefore the NRBC count is a useful parameter in the differential diagnosis between β-trait and thalassemia intermedia. The NRBC count, like the sTfR, could be useful in the choice of the best pretransfusional Hb level, mostly in the young non-splenectomized transfusion-dependent patients. In conclusion, the automated NRBC count could be used for a more effective control of transfusion therapy in transfusion-dependent thalassaemic patients, thus permitting selection of the most appropriate pretransfusion haemoglobin levels to suppress ineffective erythropoiesis.

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