ABSTRACT

Patients with haemolytic anaemias of different etiologies were investigated using the $^{51}$Cr red cell survival and surface counting technique. The study provided definitive information regarding the presence of a haemolytic process and was found useful in the estimation of the rate as well as in the demonstration of the principal site of red cell destruction. The study also proved valuable in predicting the response to splenectomy (JPMA 40: 283, 1990).

INTRODUCTION

The differential agglutination technique first described in 1919 by Ashby$^1$ and subsequently refined by Dacie and Mollison$^2$ was the earliest valid method for the determination of red cell survival. However, this became outmoded following the development of red cell radioisotope labelling techniques including $^{14}$C-glycine$^3$ $^{59}$Fe$^4$ $^{32}$p-diisopropyl phosphofluoridate$^5$, and $^{51}$Cr-sodium chromate$^6$. Presently $^{51}$Cr red cell survival and surface counting studies are being employed for the investigation of haemolytic anaemias and provide an insight into the rate, the site and the method of haemolysis. The results of a few representative cases studied at the Nuclear Medical Centre, AFIP, are presented and the clinical significance of the various patterns encountered is discussed.

PATIENTS AND METHODS

Patients

Five patients (2 females and 3 males) aged 10 to 35 years, diagnosed or suspected of suffering from haemolytic anaemia, who were referred to the Centre for $^{51}$Cr red cell survival (RCS) and surface counting study are included in the study. Clinical and haematological data of these patients is presented in table 1.
Case 1
A young male with hepatomegaly and pancytopenia was sent for evaluation prior to splenectomy, following failure of response to drug therapy for tropical splenomegaly and later for tuberculosis (liver biopsy having revealed a chronic granulomatous lesion).

Case 2
A young male suffering from spherocytic hereditary elliptocytosis had a past medical history of repeated haemolytic crises. He now complained of persistent jaundice and splenectomy was being contemplated.

Case 3
An adult female with a Coomb’s negative haemolytic anaemia of undiagnosed etiology, presumably with an intrinsic RBC defect, was referred for the assessment of the rate and the site of haemolysis.

Case 4
An adult male who was a recently diagnosed case of paroxysmal nocturnal haemoglobinuria (PNH) was being investigated. RCS study was requested to estimate the rate of red cell destruction and the ratio of the different populations of red cells in order to obtain relevant information for effective therapy planning.

Case 5
A female child, a case of thalassaemia under treatment for the past 5 years, now showed an increased requirement for blood transfusion - an indication for splenectomy. Compatible donor packed red cells were labelled (12 days after blood collection) with $^{51}$Cr and injected into the patient.

Procedure
Ten ml of the patient’s blood in acid citrate dextrose (ACD) solution was labelled with $^{51}$Cr sodium chromate in a dose of 1.5 uCi kg-1 body weight and reinjected 30 mm later. Blood samples were withdrawn 24 hr post injection and subsequently on alternate days, 3 days a week for a total period of 2-3 weeks. Haematocrit values and RBC counts were obtained for each sample. Accurately measured duplicate aliquots were prepared in ethylene diaminotetraacetate (EDTA) and later haemolysed with saponin. At the end of the study period the activity in each sample was measured, using a Miniassay Type 6-20 scintillation well counter system linked to scaler-timer, for a period of 200 sec each to obtain statistically acceptable results. Surface counting studies were performed on each visit using a Nuclear Enterprise DM1-2 scintillation probe fitted with a Nal (Tl) crystal and a cylindrical hole collimator. Duplicate counts were recorded for 1 minute each at fixed marked points on the thigh, heart, spleen and liver according to the International Committee for Standardization in Hematology (ICHS) protocol.

DATA ANALYSIS
Red cell survival
The averaged background subtracted sample counts were calculated as a percentage of day 1 counts and plotted on semilog or arithmetical paper to obtain the best straight line, and the value of the half-life of $^{51}$Cr RBC (T50%) was obtained. Mean cell life (MCL) calculations were made according to the ICHS recommendation using elution correction factor tables. Where the survival curve appeared to consist of two components the double population of cells was estimated using the following formula:

$$MCL_s = \% S/(100/MCL_t - \% L/MCL_i) = \% S/(100/MCL_t - \% L/MCL_e)$$

Where
- $MCL_s$ = Mean cell life of shorter lived population
- $\% S$ = Percentage population of shorter lived RBCs
- $MCL_t$ = Mean cell life of total red cell population
- $\% L$ = Percentage population of longer lived RBCs
**RESULTS**

The results of $^{51}$Cr red Cell T 50%, MCL and surface counting studies are presented in table II.

<table>
<thead>
<tr>
<th>No.</th>
<th>Diagnosis</th>
<th>T 50% Day (N=25-33)</th>
<th>MCL Day</th>
<th>Spleen/Liver on T50%</th>
<th>Excess spleen Cts (N= &lt;350)</th>
<th>Excess liver Cts (N= &lt;200)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Hypersplenism</td>
<td>18</td>
<td>42</td>
<td>2.49</td>
<td>101</td>
<td>76</td>
</tr>
<tr>
<td>2.</td>
<td>Hereditary elliptocytosis</td>
<td>11</td>
<td>25</td>
<td>2.84</td>
<td>550</td>
<td>46</td>
</tr>
<tr>
<td>3.</td>
<td>Nonimmune hemolytic anaemia</td>
<td>13</td>
<td>26</td>
<td>0.90</td>
<td>235</td>
<td>629</td>
</tr>
<tr>
<td>4.</td>
<td>Paroxysmal nocturnal haemoglobinuria</td>
<td>(i)06</td>
<td>0.91</td>
<td>-74</td>
<td>362</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Thalassaemia</td>
<td>15</td>
<td>-</td>
<td>1.44</td>
<td>313</td>
<td>194</td>
</tr>
</tbody>
</table>

N = Normal value  
MCL = Mean cell life  
Cts = Counts

**Case 1**

The studies showed a moderately reduced red cell survival (RCS), an initially high spleen/liver ratio but no subsequent rise in this or the spleen/heart ratio and no excess spleen or liver counts during the course of the study (Figure-I)
which indicated a large splenic blood pool but no active splenic sequestration of red cells.

**Case 2**

The studies showed a significantly reduced RCS, initially high and subsequently rising spleen/liver and spleen/heart ratios and moderately increasing excess of spleen counts (Figure 2).
This pattern provided evidence of a large splenic blood pool with active ongoing splenic sequestration.

Case 3

$^{51}$Cr studies showed a shortened RCS, a progressive rise in the liver/heart ratio and a significant excess of liver counts but no significant excess over the spleen (Figure 3),

Figure 2. $^{51}$Cr Red Cell Surface Counting Studies. Case 2.
indicating a significantly shortened MCL with increased intravascular haemolysis.

Case 4
The studies recorded a markedly shortened RCS. The survival curve showed two populations of cells, 72% short-lived with a MCL of 6 days and 28% longer lived with a MCL of 48 days (Figure 4).
The lives/heart ratio showed a progressive rise with a moderate excess of liver counts while excess spleen counts were of low significance (Figure 5).
This suggests a significant degree of intravascular haemolysis with non-significant splenic sequestration.

**Case 5**
The T 50% of the labelled donor cells in the patient was 15 days and a mild rise in both the spleen/heart and liver/heart ratios was recorded with a mild excess of counts over the liver but no significant excess over the spleen.
DISCUSSION

In order to establish the haemolytic aetiology in anaemias the standard investigations are directed towards seeking evidence of increased haemoglobin breakdown, of the presence of abnormal red cell morphology or red cell antibodies, and of compensatory erythroid hyperplasia. In most cases these features establish the haemolytic nature of the anaemia. However, in doubtful cases direct estimation of the red cell life span with Cr is indicated to establish the presence of a haemolytic process. The anionic hexavalent form of Na$_2$CrO$_4$ penetrates the red cell membrane and binds almost exclusively to the beta chain of the globin molecule. The major shortcomings of this label are its toxicity$^{11,12}$ and its gradual elution from the globin bond. The factor of toxicity has been overcome through the development of highly concentrated commercial preparations of radioactive sodium chromate thereby limiting the amount of chromium required to 0.25μg/ml of red cells. By correcting the data for elution, using the tables published by Donohue et al$^{13}$ the MCL can be calculated according to the recommendations of ICHS$^8$ so as to obtain a fairly accurate estimate of the true survival. In clinical practice a high degree of accuracy is rarely required and inspite of these drawbacks $^{51}$Cr RCS studies provide a reliable estimate of red cell destruction. $^{51}$Cr red cell surface counting studies provide a direct method of determination of the site of predominant red cell destruction and are useful in reaching a decision as to whether splenectomy should be undertaken. Theoretically the counts over the spleen and liver should fall at the same rate as the heart counts, unless lysis or sequestration of red cells is taking place within the organ or unless $^{51}$Cr eluted from red cells is being accumulated therein$^{14}$. Two patterns of high splenic activity were encountered in these studies. In the patient with hypersplenism (Case 1) the RCS study showed a moderately shortened MCL providing definitive evidence of a haemolytic process. The surface counting studies reflected the effect of an increased splenic blood pool. The enlarged spleen has been shown to bring about an accelerated red cell destruction resulting in a moderately shortened life span. Anaemia may become apparent when a significant fraction of the available red cells are sequestered in the large nonfunctional splenic blood pool. The dilutional contributing factor to the functional anaemia is the dilutional phenomenon of the expanded plasma volume, is a general characteristics accompanying splenic enlargement$^{15}$. Exaggerated platelet and granulocyte pooling in the hypertrophic spleen accounts for peripheral cytopenia.$^{16}$ In this particular patient the underlying disorder responsible for the picture of hypersplenism had not been conclusively defined. In view of the persistent cellular deficits, the clinical picture and a definitive evidence of an expanded splenic blood pool, a beneficial effect following splenectomy could be anticipated. The second pattern of high splenic activity was seen in case 2 suffering from spherocytic hereditary elliptocytosis (HE). A significantly reduced RCS, an enlarged splenic blood pool and splenic sequestration of red cells provided evidence of splenic destruction of red cells. Conditions in which active splenic cell destruction occurs are those associated with detectable abnormalities of the red cells e.g. hereditary spherocytosis and HE, the presence of surface antibodies e.g. autoimmune haemolytic anaemias, and haemoglobinopathies such as sickle cell anaemia and thalassaemia. In HE due to defective cell membrane proteins the RBCs are unable to regain their normal biconcave shape subsequent to deformation in the splenic vasculature and are thus rendered fragile and may fragment. A significant fraction of red cell destruction may however also occur in the extra splenic reticuloendothelial system.$^{15}$ The spleen exerts a two-fold influence on the red cell survival; firstly, a conditioning factor makes them susceptible to destruction and secondly there is an intense phagocytosis. Splenectomy is expected to relieve the haemolysis, followed by a return of the haemoglobin concentration and the reticulocyte count to normal$^{17}$. In thalassaemia, especially in hypertransfused patients, as in Case 5, in addition to the primary haemoglobinopathy, an extra-
corpuscular haemolytic component may also develop. This causes a progressive shortening of the interval between transfusions due to an accelerated destruction of both the transfused and the autologous cells. This increased destruction is partly due to a hypersplenic mechanism\textsuperscript{19} and splenectomy in such cases will reduce the transfusion requirement.\textsuperscript{19} In this patient \textsuperscript{51}Cr study indicated a moderately shortened survival of the transfused cells but did not provide conclusive evidence of a splenic contribution to the accelerated red cell destruction. Favourable results have been reported after splenectomy even in patients with negative \textsuperscript{51}Cr study\textsuperscript{20} and therefore in view of the clinical progression and haematological picture, splenectomy might still prove beneficial. Two cases (3\&4) showed increased hepatic \textsuperscript{51}Cr activity, which is generally associated with intravascular haemolysis.\textsuperscript{21} Case 4 suffered from PNH. In this condition an acquired defect in the red cell membrane renders the cells unusually sensitive to lysis by normal serum complement. Three cell population differing in their sensitivity have been identified.\textsuperscript{22} RCS study in case 4 effectively documented to distinct population of cells with different MCLs. Rapid intravascular haemolysis of the abnormal cells liberates free haemoglobin, most of which is taken up by the hepatocytes\textsuperscript{18} and accumulating globin moiety in the liver may account for the increased hepatic surface account in this patient. No demonstration of splenic contribution to the rapid red cell destruction was observed, and splenectomy would therefore be of no value. Indeed splenectomy has been associated with high mortality in such patients.\textsuperscript{23} Case 3 suffered from non-immune haemolytic anaemia of unknown etiology. The patient had shortened RCS with high hepatic uptake suggesting an intravascular haemolytic process. This was also supported by very low serum haptoglobin levels. Negative findings over the spleen apparently preclude the role of the spleen as a major site of red cell destruction and suggest that splenectomy in this patient would also not prove beneficial.

REFERENCES