Survival Studies of Thalassemic Erythrocytes Transfused into Donors, into Subjects with Thalassemia Minor and into Normal and Splenectomized Subjects

By C. Vullo and A. M. Tunioli

In past years interesting results have been reported by a number of investigators on the life span of thalassemic red cells measured by the Ashby technic of differential agglutination. Kaplan and Zuelzer transfused blood from three children suffering from thalassemia major into normal subjects; the survival curves showed a marked shortening of the half life (25 days), with a straighter drop in the first days, because of prompt removal of the most abnormal red cells.

Similar results, consisting in a rapid and uniform drop of the curves with a striking survival reduction (12–19 days), have been reported by Frontali and Stegagno.

The above data speak for a corpuscular factor involved in the enhanced breakdown of thalassemic red cells, since their survival appears abnormal even in a normal environment.

Lichtmann et al. Smith et al. and McElfresh et al. found that subjects affected by Cooley’s anemia quickly destroy transfused normal red cells, and deduced that an extracorpuscular factor also plays a role in the pathogenesis of the abnormal hemolysis of thalassemia. This factor, represented by an increased hemolytic activity of the spleen, is present more frequently in patients over three years of age. The same authors noted a parallel between the improved clinical condition after splenectomy and the improvement of survival of transfused normal red cells.

If the increased hemolysis in Cooley’s anemia is due to the combined action of the two factors, intra- and extracorpuscular, it becomes obvious that a correct estimation of thalassemic erythrocyte life span is possible only by following their survival in the donor himself. This investigation, impossible with the Ashby technic, can be carried out by tagging the erythrocytes with Cr51.

By this procedure Strumia and his co-workers studied erythrocyte survival in a patient suffering from thalassemia minor who had undergone splenectomy some years before. The patient’s erythrocytes, which had been both autotransfused and injected into a normal isogroup subject, showed a reduced

From the Department of Pediatrics (Director, Professor E. Schwarz Tienc), University of Ferrara, Ferrara, Italy. Presented in part at two meetings of the Italian Society of Hematology, Ferrara Section, Ferrara, October 1956, and December 1957.

The authors wish to thank Dr. W. H. Crosby (Walter Reed Army Institute of Research, Washington, D.C.) for his helpful advice and for radioactivity measurements, and Dr. Menini (Blood Bank, Arcispedale S. Anna, Ferrara, Italy) for his help in carrying out serologic tests.

Submitted Nov. 27, 1956; accepted for publication Jan. 28, 1958.
survival much more marked in the control subject than in the patient himself. According to the same authors, the faster drop of the survival curve of the transfused red cells in the control subject must be attributed to the more rapid removal of the more abnormal erythrocytes by the normal spleen.

The above mentioned studies refer to the hemolytic activity of the spleen of normal subjects against thalassemic red cells and, vice versa, to the hemolytic activity of the spleen of children with Cooley's anemia against normal erythrocytes; no data, however, have been published on the role played by the spleen in thalassemia major in the destruction of the thalassemic red cells. The present research was intended to compare the survival curves of thalassemic erythrocytes transfused into the donors themselves, into patients with thalassemia minor, into normal subjects and, finally, into healthy splenectomized persons.

METHODS

Survival studies were performed by tagging red cells with Cr¹⁹⁵, as briefly reported: 20 ml. of blood obtained by venipuncture were put into a sterilized flask containing 6 ml of ACD solution, and immediately stirred. 1.25 μC Na₂ Cr²⁺O₄ dissolved in sterile saline was added per Kg. of body weight of the recipient; the mixture was shaken and left standing for 30 minutes at room temperature.

At the end of the incubation period 0.01 mg. ascorbic acid was added; the mixture was stirred and injected after one minute. Venous blood samples were drawn 20 minutes after the injection and later at predetermined times. Survival curves were calculated on the percentage of the radioactivity of the blood samples, referring to the first one as 100 per cent.

The following formula was used for calculation:

\[
\frac{\text{cpm WB (day x) x htc (30')} - \text{cpm WB (30')}}{\text{htc (day x) x cpm WB (30')}}
\]

where cpm WB represents the counts per minute in whole blood and htc the red cell hematocrit value.

No account was taken of the decay of Cr¹⁹⁵ radioactivity, since measurements of all the samples were done at the same time (at the end of the experiment). The Cr¹⁹⁵ elution from the red cells was overlooked, so that the results obtained show only the "apparent" survival of the transfused erythrocytes. Recipients for transfusions were compatible with the donors in respect to the factors: A, B, Rh (D), rh' (C), rh⁺ (E) and Hrₑ (C). Cross-matching tests were done by saline cell suspensions, trypsin-treated erythrocytes and the Coombs' technic. The other hematologic tests were done according to standard methods.

The diagnosis of thalassemia major was established on the basis of the clinical features and the laboratory data (table 1). Thalassemia minor was recognized on the basis of the familial incidence, together with the finding of a decreased red cell fragility in hypotonic saline.

The research was performed using erythrocytes obtained from seven children suffering from thalassemia major.

RESULTS

In each of the cases 1, 2, 6 and 7 the thalassemic blood was transfused into the donor and also into a normal isogroup subject. In the normal subject the red cell survival appeared shorter than in the children suffering from thalassemia major; the survival curves showed a quickly falling first component followed by a component roughly parallel to the one of the autotransfusion curves (figs. 1a, 1b, 4 and 5).
### Table 1.—Hematologic Data in Cooley’s Anemia

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Age</th>
<th>Hb</th>
<th>HbF</th>
<th>RBC</th>
<th>Ht</th>
<th>MCV</th>
<th>MCH</th>
<th>MCC</th>
<th>APC</th>
<th>NRBC</th>
<th>BME</th>
<th>OF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>R. Doanella</td>
<td>6</td>
<td>7.8</td>
<td>55</td>
<td>3.5</td>
<td>23</td>
<td>64</td>
<td>22</td>
<td>33</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>P. Dario</td>
<td>1</td>
<td>4.9</td>
<td>61</td>
<td>2.2</td>
<td>16</td>
<td>77</td>
<td>22</td>
<td>30</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>M. Silvana</td>
<td>4</td>
<td>5.5</td>
<td>49</td>
<td>2.2</td>
<td>15</td>
<td>70</td>
<td>25</td>
<td>36</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>F. Flavia</td>
<td>10</td>
<td>6.8</td>
<td>47</td>
<td>3.6</td>
<td>27</td>
<td>74</td>
<td>19</td>
<td>25</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>P. Enrico</td>
<td>1</td>
<td>5.6</td>
<td>64.5</td>
<td>2.2</td>
<td>17</td>
<td>78</td>
<td>24</td>
<td>32</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>A. Nadia</td>
<td>3</td>
<td>5.3</td>
<td>66.5</td>
<td>2.2</td>
<td>18</td>
<td>80</td>
<td>24</td>
<td>30</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>B. Gino</td>
<td>2</td>
<td>5.3</td>
<td>70</td>
<td>1.9</td>
<td>20</td>
<td>100</td>
<td>26</td>
<td>26</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

**Age** = in years.  
Hb = Hemoglobin, grams per cent.  
HbF = Fetal hemoglobin per cent of total.  
RBC = Red blood cells, millions per cu. mm.  
Ht = Hematocrit, venous blood.  
MCV = Mean corpuscular volume in μm².  
MCH = Mean corpuscular hemoglobin.  
MCC = Mean corpuscular hemoglobin concentration per cent.  
APC = Anisopoikilocytosis.  
NRBC = Nucleated red blood cells in peripheral film.  
BME = Bone marrow erythroblastosis.  
OF = Decreased osmotic fragility to hypotonic saline.

**Figs. 1a. and 1b.**—Disappearance of Cr⁴⁺-tagged red cells of case 1 (fig. 1a) and case 2 (fig. 1b) transfused into the donor and into a normal subject.

The blood obtained from cases 3 and 4 was transfused at the same time into the donor and into a subject with thalassemia minor; both survival curves were almost superimposing (figs. 2a and 2b).

The red cells from case 5 were transfused into the donor and into a normal
subject who had previously undergone splenectomy for traumatic rupture of the spleen (fig. 3); the blood from case 6 was transfused into a normal subject and into a woman who had undergone splenectomy for a thrombocytopenic purpura (fig. 4). The last child's red cells (case 7) were transfused into the donor, into two normal subjects and into a woman splenectomized for a thrombocytopenic purpura (fig. 5).

The survival of the thalassemic cells transfused into normal splenectomized subjects appeared similar to that observed in the donors.

In all the cases the autotransfusion curves, though varying considerably from one to the other, showed a shortening of thalassemic erythrocyte lifespan: radioactivity measurements were equal to 50 per cent of the initial value on the 22nd, 11th, 9th, 15th, 15th and 10th day, respectively. The half life of radioactivity of normal autotransfused red cells has a range of about 25 to 35 days.

**Discussion**

Our data support the knowledge that children suffering from thalassemia major show a marked shortening of the erythrocytes' average life span with a wide range of results from case to case. Since the autotransfusion curves markedly differed from the ones obtained with the same red cells in normal subjects, it is confirmed that, as far as thalassemia major is concerned, reliable
Figures 3 and 4

Fig. 3.—Disappearance of Cr$^{51}$-tagged red cells of case 5 transfused into the donor and into a normal splenectomized subject.

Fig. 4.—Disappearance of Cr$^{51}$-tagged red cells of case 6 transfused into a normal and into a splenectomized subject.

data on erythrocyte survival cannot be obtained by injecting the thalassemic red cells into normal persons.

On the other hand, the autotransfusion curves offer exact data on the seriousness of the hemolytic process in a given subject, without distinguishing, however, between the role played by the corpuscular and extracorpuscular factors.

Our results on the survival of thalassemic erythrocytes in normal subjects are in agreement with those of Strumia and co-workers and Kaplan and Zuelzer’s; we too found survival curves which are characterized by a quickly dropping first component and a reduced life span.

As mentioned above, Strumia and co-workers and Kaplan and Zuelzer suggested, to explain these findings, that the spleen of normal subjects quickly removes the most abnormal thalassemic erythrocytes from the blood stream; a comparison between the survival curves of thalassemic red cells transfused into normal and splenectomized subjects confirms the importance of the splenic factor in the hemolysis of the thalassemic red cells in healthy persons. Actually,
in those patients who had undergone splenectomy, the pathologic red cells showed a survival which was longer than in normal control subjects (cases 6 and 7; figs. 4 and 5).

From a comparison of the results obtained in children suffering from thalassemia major and in splenectomized subjects it appears that in both groups the thalassemic erythrocyte survival curves show an almost identical behavior. The evaluation of all the data from case 7 (fig. 5) allows us to compare the survival curves that we obtained with the same red cells in various conditions: we can see, on passing from the splenectomized subject to the patient suffering from thalassemia major and, thereafter, to the normal person, a progressive shortening of erythrocyte survival. Since the red cells were the same, differences in survival must of necessity be related to an extracorpuscular factor, which is the hemolytic activity of the spleen. It is therefore evident that
in thalassemia major the spleen plays a scant hemolytic role in destruction of the patient's own red cells.

The almost identical behavior of thalassemic red cells in subjects with thalassemia minor and in patients with thalassemia major lets us suppose that also the spleen of the carriers has a low hemolytic activity.

CONCLUSIONS AND SUMMARY

The analysis of the curves of thalassemic red cell survival studied in children suffering from thalassemia major, in subjects with thalassemia minor, in normal splenectomized subjects and in healthy persons shows:

(a) thalassemic erythrocytes show a marked shortening of life span, with a wide range of half life from case to case;
(b) thalassemic erythrocytes survive longer in patients with thalassemia major and minor than in normal subjects;
(c) the behavior of thalassemic erythrocyte survival curves in the anemic patients and in the normal splenectomized cases is almost similar. On the basis of these results, the authors emphasize that:

(1) valuable data on the seriousness of the hemolytic process in thalassemia major can be drawn only from autotransfusion studies;
(2) in both thalassemia major and minor the spleen has less hemolytic activity on thalassemic red cells than in normal subjects;
(3) since survival curves between anemic and normal subjects differ principally in the initial component where the most abnormal red cells are destroyed by the normal spleen, it is probable that the spleen of patients with thalassemia major is not so greatly active against these cells.

SUMMARIO IN INTERLINGUA

Le analyse del curvas de superviventia de erythrocytos thalassemic post lor transfusion in patientes pediatric con thalassemia major, in subjectos con thalassemia minor, in subjectos normal, e in normal subjectos splenectomisate supporta le sequente conclusiones.

1. Erythrocytos thalassemic manifesta un marcate reduction del periodo de superviventia. Iste reduction varia grandemente ab un caso al altere.
2. Erythrocytos thalassemic supervive plus longemente in patientes con thalassemia major e con thalassemia minor que in subjectos normal.
3. Le comportamento del curvas de superviventia de erythrocytos thalassemic in patientes anemic e in normal subjectos splenectomisate es quasi le mesme.

Super le base de iste resultatos, le autores presenta le sequente observationes.

1. Datos de alte valor relative al seriositate del processo hemolytic in thalassemia major pote esser obtenite solmente per medio de studios de autotransfusion.
2. Tanto in thalassemia major como etiam in thalassemia minor, le splen exerce minus activitate hemolytic super le erythrocytos thalassemic que in subjectos normal.
3. Viste que le curvas de superviventia in subjectos anemic e in subjectos
normal differe principalmente in le componente initial in que le erythrocytos le plus anormal es destruite per le splen normal, il es probable que le splen de patientes con thalassemia major es minus active contra tal cellulas.

REFERENCES

Survival Studies of Thalassemic Erythrocytes Transfused into Donors, into Subjects with Thalassemia Minor and into Normal and Splenectomized Subjects

C. VULLO and A. M. TUNIOLI