The Prediction of Autologous Red Cell Survival*

B. A. MYHRE, M.D., Ph.D., C. S. MARCUS, M.D., and N. C. WHEELER, M.D.

Habor-UCLA Medical Center,
Department of Pathology and Division of Nuclear Medicine,
UCLA-School of Medicine,
Department of Biomathematics
Torrance, CA 90509

ABSTRACT

Nine donors predicted from earlier studies to have increased autologous red cell survival and four predicted to have lesser survival were selected from a pool of previously studied donors. Blood was drawn from these donors, and stored in Neutracel (R-Cutter Lab) preservative for eight hours at room temperature. The platelet rich plasma was removed, the additive solution was introduced, and the red cells were stored for 42 days in an approved refrigerator. At the end of the storage period, a 24 hour Cr-51 viability analysis was performed on each donor. Predicted red cell survival scores correlated reasonably well with the observed percent of red cell viability (r = 0.648). This study suggests that it may be possible to predict increased viability of autologous red cells in certain donors.

Introduction

Several authors have stated that there are significant differences in the survival of red cells following storage and that these differences are at least partially dependent on the donors. De Gowin1 in 1949 noted that the amount of hemolysis in blood units at the end of the storage time varied considerably. Mollison6 and Dern4, when studying the post transfusion survival of stored red cells, found considerable individual differences in the viability of red cells measured by 24 hour Cr-51 survival in the autologous donor or a patient. Dern attributed this variability to a characteristic of the donor and showed some evidence that it might be an inheritable characteristic.3,5

As a result of a series of autologous red cell survival studies with various red cell preservative media, the authors were able to develop a pool of 110 donors of whom 34 had been studied in at least two sets of determinations. These studies were primarily designed to establish acceptable storage times for blood in preservative media and containers, and these times were determined by autologous post-infusion viability measured with Cr-51 after a 24 hour sampling period. This procedure has been stan-
standardized by Moroff et al and is generally regarded as the acceptable method of determining post transfusion survival. As would be expected, the survival varied depending on the anticoagulant, the bag, and the storage time. However, from an examination of the records it appeared that, independent of the study being performed, some donors consistently had good red cell viability when compared with other donors in the same study, while some consistently displayed lower viability.

To test this assumption, an experiment was designed to determine if the survival of red cells from these known donors in a new anticoagulant system could be predicted prospectively.

Materials and Methods

Blood Donor Pool

A history and physical examination based on the requirements of the Standards of the American Association of Blood Banks was administered to 110 donors. Selection criteria for the donors were as follows: The donors were selected so that approximately half were female, and all were adults within the age range of 29 to 56 years. The donors were of white, black, hispanic, and oriental lineage. Preliminary laboratory studies were performed that included a complete blood count, selected blood chemistries, a urinalysis, and a pregnancy test for all women in the childbearing period. This examination and all subsequent studies were performed under the surveillance of the Human Subjects Committee of the Research and Education Institute of Harbor-UCLA Medical Center.

Donor Selection

The percentage of red cells surviving 24 hours as determined by Cr-51 measurement had been recorded for 110 donors in ten previous studies which had used various anticoagulants, isotopes, holding times, and plastic containers. Donors were in varying numbers of studies. Measured raw survival percentages ranged from a high of 95.0 percent to a low 42.2 percent. The mean average percent was 72.1 percent. Several individuals had even lower results (in the 20s); however, these were felt to be outliers owing to poor handling of the specimens, and the results were rejected. Using a linear model on the raw 24 hour survival data, a least squares survival percentage was estimated for each donor. This least squares survival percentage, or score, is an estimate of the donor's average red cell survival percentage that would be expected if each donor had been in each study. The 110 least squares survival scores were then ordered from the highest to the lowest. From this list, 34 donors were marked who had been studied in at least two separate studies. Ten donors were recruited from this group, subject to availability, starting with the highest ranked survival scores and proceeding down the list in rank order. Similarly, four donors were recruited starting from the bottom of the list.

During the study one higher ranked donor was eliminated from the study owing to difficulty reinjecting her red cells.

Blood Drawing and Storage

A unit of blood (450 ml) was drawn from each of the 13 donors into a CL-3000 plastic bag containing Nutracel anticoagulant* and stored at 25°C (room temperature) for eight hours. Nine of these donors had been selected from the upper group and four from the lower. After the completion of the room temperature storage period, the platelet rich plasma was removed from all the units and AS003 additive solution was added.

---

* Cutter Laboratories, Inc.
to the red cells. The unit was then placed into an approved blood bank refrigerator and stored for 42 days at 2 to 4°C. At the end of this time an aliquot of blood was removed from each bag and labeled with Cr-51. Human serum albumin labeled with I-125 was also added to the cells. The blood sample was then reinjected into the original donor and its 24 hour viability determined.

**Labeling of Red Cells**

The method for labeling RBCs with Cr-51 and performing the viability calculations was taken from the report of Moroff et al\(^7\) employing several slight modifications. Owing to the small amount of Cr-51 used, the sample injected was larger, the first two samples were not taken, and the counting period was increased. Eighty ml of the stored cells were transferred aseptically into a plastic bag, and 20 ml of sterile saline was added. Fifteen μCi of Cr-51 were added to the bag, which was incubated for one hour at 37°C with gentle agitation. At the end of this time, 100 mg of ascorbic acid were added. Twelve ml of these labeled RBCs were removed and retained as a counting standard. Fifty ml were removed for injection into the subject. Just prior to injection, labeled human serum albumin (HSA) containing 4 μCi of I-125 was added to the red cell sample. A catheter was placed in the vein of the other arm and eight ml samples were drawn at 10, 12.5, 15, 20, and 60 minutes. A subsequent sample was taken at 24 hours. All counting was performed on an automated double channel NaI(Tl) well type scintillation counter set at the peaks of Chromium-51 and I-125.

**Viability Calculations**

These were performed according to the "double isotope method."\(^7\) The I-125-HSA data at 10, 15, 20, and 60 minutes were averaged to obtain an independent plasma volume. The average of the hematocrit determinations was measured at 10, 15, 20, and 60 minutes and was used to calculate red cell volume, using a factor of 0.9 to correct large vein hematocrit to total body hematocrit. The "100 percent" level of Cr-51, the circulating red cell Cr-51 activity at 24 hours, and the 24 hour red cell survival (in percent) were calculated using equations 6(e), 6(f) and 6(g), respectively, of reference 7. As a rough check only, the 10, 15, and 20 minute values of Cr-51 were subjected to least squares analysis, extrapolated to t = 0, and a "100 percent" level of Cr-51 was calculated also using an abbreviated "single isotope method."\(^7\) The 24 hour survival calculated in this manner was within six percent of the survival calculated using the double isotope method.

**Results**

The results of these studies are listed in table I. The donors have been arranged in ascending order for the estimated survival scores, and the measured viability percentages are listed in column three.

To investigate whether or not it is possible to predict the viability of the red cells, three methods of statistical analysis were used. First the viability percentages were plotted against the estimated survival scores. If the survival scores predict red cell viability well, then the plotted points should fall close to a line. The plots show that the higher ranked donors tended to have higher percentages of red cell viability while the lower ranked donors tended to have lower viability. Although the sample sizes are small (n = 13), the plots suggest reasonably good correlations r = 0.648 (p = 0.01) of the scores.

Another approach was to compare the higher and lower donors' mean red cell viability percentages. The null hypothesis that both donor groups had the same viability was tested. The alternative
TABLE I

Percent Viability of Donor Red Cells

<table>
<thead>
<tr>
<th>Donor Number</th>
<th>Estimated Viability</th>
<th>Measured Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>66.2</td>
<td>74.0</td>
</tr>
<tr>
<td>108</td>
<td>66.3</td>
<td>66.8</td>
</tr>
<tr>
<td>109</td>
<td>67.3</td>
<td>77.9</td>
</tr>
<tr>
<td>101</td>
<td>68.6</td>
<td>84.1</td>
</tr>
<tr>
<td>26</td>
<td>72.3</td>
<td>75.6</td>
</tr>
<tr>
<td>21</td>
<td>84.0</td>
<td>76.8</td>
</tr>
<tr>
<td>24</td>
<td>95.5</td>
<td>90.6</td>
</tr>
<tr>
<td>68</td>
<td>90.3</td>
<td>82.8</td>
</tr>
<tr>
<td>88</td>
<td>90.6</td>
<td>71.5</td>
</tr>
<tr>
<td>59</td>
<td>91.2</td>
<td>91.9</td>
</tr>
<tr>
<td>14</td>
<td>91.5</td>
<td>87.5</td>
</tr>
<tr>
<td>96</td>
<td>95.0</td>
<td>95.4</td>
</tr>
<tr>
<td>102</td>
<td>95.0</td>
<td>88.8</td>
</tr>
</tbody>
</table>

x = 81.8

1 S.D. = 8.77

(one-sided) hypothesis was that the higher donors had a higher viability. Results (shown in table II) again limited owing to small sample size, suggest the higher ranked donors, defined by their survival scores, may have a higher viability mean than the lower ranked donors (p = 0.047).

In a third approach a cutpoint of 70 percent was used on viability to classify donors into low and high viability groups. Thus, by definition, all the lower ranked donors had estimated viability scores of less than 70 percent, and all the higher ranked donors had more than 70 percent estimated viability. When measured in the eight hour hold study, all nine higher donors had more than 70 percent viability. Thus the higher ranked donors showed consistently high viability rates, while the lower donors did not. Again, although only a small sample has been studied, the authors are cautiously optimistic about being able to identify good donors who can be expected to produce consistently high viability rates.

Discussion

These studies suggest it may be possible to predict transfused autologous red cell viability as measured by Cr-51 survival. Dern showed in his work that a similar variability existed and that it was consistent when applied to the same donors. Thus, if a donor has red cells that survive well when stored with several different anticoagulants, the donor’s cells will probably survive well when tested with another anticoagulant. Dern also believed that this survival was correlated with pre- and post-storage adenine triphosphate (ATP) values. Dern and Wiorowski finally reported that the tendency to have high or low ATP values may be inherited by offspring from the parent.

There are several variables that must be considered in this work that might influence the results. First, the tested sample is a small group and may not be typical of a larger series. The study needs to be repeated and enlarged. Secondly, the estimated survival score was derived from the pooling of 10 different viability experiments, each involving a different anticoagulant or different plastic bag formulation and each with a different mean survival. Although the experimental method of determining viability in each study was the same, some unknown variable in one or two experiments could have influenced the results. Nevertheless, it is believed the

TABLE II

Descriptive Statistics and T-tests on Percent of Viability of Higher Ranked and Lower Ranked Donor Groups

<table>
<thead>
<tr>
<th>Donor Group</th>
<th>T-test (one-sided)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higher Ranked</td>
<td>Lower Ranked</td>
</tr>
<tr>
<td>Percent</td>
<td>Percent</td>
</tr>
</tbody>
</table>

| Mean       | 84.5          | 75.7          | 0.047  |
| S.D.       | 8.3           | 7.2           |       |
| Maximum    | 95.4          | 84.1          |       |
| Minimum    | 71.5          | 66.8          |       |
| Number     | 9             | 4             |       |
survival score for each donor is a reasonable estimate of his or her expected red blood cell viability.

A major concern with these studies is whether viability, as measured with Cr-51 survival, actually correlates with red cell survival in the transfused patient or simply measures the binding of chromium to the red cell. Dern\textsuperscript{a} showed that red cell viability in autologous donors and in stabilized anemic patients were comparable, but survival was measured only by Cr-51 counting in both cases. Szymanski and Valerie\textsuperscript{8,10} developed an automated differential agglutination technique employing the an analyzer* to determine the survival of transfused red cells. In a subsequent paper, Szymanski and Valerie\textsuperscript{11} compared the results with this method and with the Cr-51 analysis. They found that the survival values were similar as long as a correction is made for the elution of Chromium from the red cell. This elution occurs over a period of time and, therefore, would not greatly affect the 24 hour viability data.

From these results the present authors feel cautiously optimistic that it is possible to predict the increased viability of transfused red cells. However, subsequent larger studies on patients transfused with blood from donors of known viability should confirm whether or not this is true. This variability may create problems in determining red cell viability. If so, it will be necessary to design the survival studies so that each donor serves as his/her own control, and the study of the unknown anticoagulant is compared with another known example.

There is also a clinical implication to this study. If established, the use of donors with extended red cell viability would be certainly indicated for transfusing patients with long term transfusion needs, such as those with aplastic anemia, sickle cell anemia, and thalassemia. A metabolic indicator for this increased viability should be sought so that this type of donor could be easily located.

References