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COMMENTARY ON THE STERILITY OF BLOOD PRODUCTS AND THE MEASUREMENT OF THE 24-HOUR POSTTRANSFUSION SURVIVAL OF PRESERVED RBC USING 51CR SINGLE LABEL AND 51CR DOUBLE LABEL PROCEDURES

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Abstract: -

This commentary is written to respond to recent publications on the storage of whole blood at room temperature for 24 hours prior to leukoreduction and storage of plasma at less than -18 C for one year and red blood cells stored at 4 C in Additive Solution 7 for 42 and 56 days and blood stored at room temperature for less than 2 hours and 6 to 8 hours prior to storage of RBC in the Additive Solution 7 for 42 and 56 days. The authors of these 3 papers provided no data on the sterility of these blood products. In addition, the 24-hour posttransfusion survival values of autologous red blood cell stored at 4 C in the additive solution AS-7 for 42 and 56 days were performed using a 51Cr single label procedure.

Keywords: - *red blood cell (RBC) preservation, RBC posttransfusion surviva, RBC sterility*

INTRODUCTION

Plasma and red blood cells prepared from whole blood held overnight at room temperature have been studied by Dumont LJ et al (1, 2). Blood stored at room temperature for less than 2 hours and 6 to 8 hours and then the RBCs stored in the additive solution 7 (AS-7) at 4 C for 42 to 56 days have been studied by Cancelas JA et al (3). In the three studies, the testing of the plasma and red blood cells isolated from whole blood stored at room temperature for 24 hours and then leukoreduced prior to storage did not provide data on the sterility of the plasma stored at less than -18 C for one year and the red blood cells stored in the additive solution 7 (AS-7) at 4 C for 42 and 56 days and RBC isolated from whole blood stored at room temperature for less than 2 hours and for 6 to 8 hours and then stored at 4 C in the solution AS-7 for 42 and 56 days. The 51Cr single label procedure was used to measure the 24 hour posttransfusion survival and the lifespan of the autologous RBC stored in AS-7 at 4 C for 42 and 56 days (2,3). In 1965, Dr. Max Strumia, the Chairman of the National Research Council reported the need to ensure the sterility of blood products and the need to measure the 24-hour posttransfusion survival of preserved red blood cells using 51Cr double label procedures which measure the red blood cell volume of the recipient at the time of the transfusion of the 51Cr labeled autologous preserved RBC. In reference 2, Dumont LJ et al reported "To our knowledge there are no published in vivo recoveries in healthy subjects for 6 week AS-1 RBCs". This statement is not correct. The FDA approved the storage of ADSOL (AS1) preserved RBCs for 49 days based on data reported by Heaton A and associates (4). The results of the Heaton and associates study showed a mean 24-hour posttransfusion survival of 76 percent for ten autottransfusions stored for 49 days and a mean 24 hour posttransfusion survival of 72 percent for ten autotransfusions of RBC preserved in ADSOL at 4 degrees C for 56 days. In the Heaton A and associates study, the 24 hour posttransfusion survival values for the autologous RBC stored at 4 C for 49 days and 56 days were measured using a 51Cr single label procedure.

In 1980 the American Red Cross was utilizing the additive solution AS-1 (ADSOL) to store RBC in CPD/AS-1 at 4C for 49 days which was approved by the Food and Drug Administration (FDA). The American Red Cross Northeast was interested in biochemical treatment of outdated group O Rh positive and group O Rh negative RBC stored in CPD/AS-1 at 4C for 49 days. NBRL was requested by ARC Northeast to study whether or not RBC stored in CPD/AS-1 at 4C for 49 days could be biochemically modified and frozen. Studies done in 1980 showed that autologous RBC stored in CPD/AS-1 for 49 days, biochemically modified to increase the RBC ATP, DPG, and p50 values, frozen with 40% W/V glycerol and stored at -80C, thawed and deglycerolized and stored at 4C in sodium chloride glucose phosphate solution for 24 hours had 24-hour posttransfusion survival of 60%. These observations required that control studies were needed to assess the 24-hour posttransfusion survival of autologous and allogeneic RBC stored in CPD/AS-1 for 49 days. The 24-hour posttransfusion survival for autologous RBC was measured using the double radioisotope procedure: 125I albumin to measure the plasma volume and the total body hematocrit (peripheral venous hematocrit multiplied by 0.89) to measure the RBC volume of the healthy male volunteer and 51Cr labeling of the autologous liquid preserved RBC. At the same time, the 24-hour posttransfusion survival values of allogeneic compatible identifiable RBC stored at 4C in CPD/AS-1 for 49 days transfused into stable anemic patients were measured using the automated differential agglutination procedure using the Technicon Autoanalyzer and the RBC volume of the patient was measured using 51Cr-labeled autologous RBC. The method utilized to assess the allogeneic compatible identifiable RBC stored at 4C in CPD/AS-1 for 49 days produced 24-hour posttransfusion survival values of 53% which was similar to the 24-hour posttransfusion survival values observed with autologous RBC stored in AS-1 (ADSOL) for 49 days measured by the 51Cr/125I-albumin double radioisotope procedure (5).

The questions raised by the American Red Cross Northeast stimulated studies by the NBRL to assess the survival and function of autologous and allogeneic RBC stored in CPDA/AS-1 for as long as 49 days. The results of the NBRL studies demonstrated that 24-hour posttransfusion survival of autologous and allogeneic RBC preserved in CPD/AS-1 could be stored at 4C for only 35 days with a 24hour posttransfusion survival of 75% and the RBC function was preserved at 4C for only 2 weeks. These findings were in contrast to Heaton and associates who reported that the autologous RBC stored in CPD/AS-1 at 4C for 49 days had 24-hour posttransfusion survival of 76% and the FDA had approved storage of the RBC stored in CPD/AS-1 at 4C for 49 days (4).

Dr. Joseph Bove, who was the Chairman of the Standards Committee of the AABB, suggested that our observations should be reported as a letter to the editor of the New England Journal of Medicine (7). In 1985, the FDA conducted a meeting to review the length of storage of human RBC in CPD/AS-1 at 4C and the information reported resulted in reducing the length of storage of CPD/AS-1 RBC from 49 days to 42 days but not to 35 days (5).

The question raised by the American Red Cross Northeast in 1980 whether CPD/AS-1 RBC stored at 4C for 49 days could be biochemically modified to increase the RBC ATP, DPG and p50 values prior to freezing stimulated the controversy regarding the measurement of nonviable RBC in preserved RBC products. Dr. M. Strumia in 1965 had reported that a double label procedure was needed to detect the quantity of nonviable RBC in the preserved RBC product. Two radioisotopes were utilized, one radioisotope to measure the RBC volume of the normal recipient and the other radioisotope to measure the preserved 51Cr labeled autologous RBC. At the NBRL, the 125I albumin and 51Cr method, the 99mTC and 51Cr method, and the 111In-oxine and 51Cr method and the double 51Cr method were evaluated in normal volunteers and in baboons to assess 24-hour posttransfusion survival and lifespan of preserved autologous RBC. Alternatively, methods were used in patients to measure the survival of compatible but identifiable allogeneic RBC using anti-A, Anti-B, and Anti-M antibodies using the Technicon Autoanalyzer and the Coulter Counter and 51Cr to measure the RBC volume of the patient (6). If RBC are well preserved, then the single method utilized using 51Cr to label the preserved RBC without independent measurement of the RBC volume of the recipient will provide similar data to the double isotope methods which measures independently the red cell volume of the recipient. If the preserved RBC contain a quantity of nonviable RBC the single 51Cr method will not detect these irreversibly damaged RBC that are rapidly

removed from the circulation during the 5, 10, 15 and 30 minute postinfusion period. The single 51Cr method has been reported in the majority of the published reports because it is a simple procedure compared to the double radioisotope procedure to measure the survival of autologous preserved RBC. Unfortunately, the single 51Cr method to measure the 24-hour posttransfusion survival values overestimates the 24-hour posttransfusion survival when nonviable RBC are rapidly removed during the mixing time of the small aliquot in the recipient's blood volume.

In 1980, the request by the American Red Cross Northeast that the NBRL investigate the salvaging of universal donor O positive and O negative RBC stored in CPD/AS-1 for 49 days resurrected the controversy discussed by Dr. M. Strumia in 1965 as to the method to determine the quantity of nonviable RBC in the preserved RBC products which contain a quantity of irreversibly damaged preserved RBC.

At the NBRL, Boston, MA 24-hour posttransfusion survival of autologous RBC stored in ADSOL (AS-1) at 4 C for 49 days was measured using a 51Cr double label procedure which measured the red blood cell volume of the recipient at the time of the transfusion of the 51Cr labeled autologous preserved red blood cells (7,8). The NBRL measured the 24-hour posttransfusion of autologous RBC stored at 4 C in ADSOL for 35, 42 and 49 days using two 51Cr single label procedures and a 51Cr double label procedure (8). At the same time the 24 hour posttransfusion survival of units of compatible allogeneic identifiable RBC were transfused into stable anemic patients. In the patient the compatible allogeneic identifiable preserved RBC were measured by an automated differential agglutination procedure using the Technicon Autoanalyzer and the red blood cell volume of the recipient was measured using 51Cr labeled autologous red blood cells (8).

The data obtained at the NBRL reported that 8 units of autologous RBC stored at 4 C in ADSOL (AS-1) for 35 days had 24 hour posttransfusion survival of 75 ± 5%, 15 units of autologous RBC stored in ADSOL for 42 days had a 24 hour posttransfusion survival of 71 ± 5%, and 10 units of autologous RBC stored in ADSOL for 49 days had 24-hour posttransfusion of 57 ± 9% using a 51Cr double label procedures (Method1 in Table 1, Reference 8). These data were published in a letter to the editor of the New England Journal of Medicine (7). The paper: A clinical experience with ADSOL preserved erythrocytes: was published in Surgery, Gynecology and Obstetrics 166:33-46, 1988 (8).

The data obtained at the NBRL reported that allogeneic compatible RBC stored in ADSOL at 4 C for 34 to 36 days had 24-hour posttransfusion survival of 79 + 4% in six patients. Allogeneic compatible RBC stored in ADSOL for 37 to 43 days had 24-hour posttransfusion survival of 65 ± 9% in nine patients and allogeneic compatible RBC stored in ADSOL for 45 to 49 days had 24-hour posttransfusion survival of 53 ± 11% in ten patients (Table 2) (8).

In a collaborative study in 1998, three sites measured the 51Cr survival of autologous deglycerolized RBC processed in the Haemonetics 215 stored in AS-3 (Nutricel) at 4 C for 15 days using three different 51Cr double label procedures. At the NBRL the 51Cr/I125 albumin procedure was used, at Walter Reed Army Institute of Research (WRAIR) the 51Cr/99mTc procedure was used, and at UMass the double 51Cr procedure was used. The 24 hour posttransfusion survival values were similar (77 ± 9%) for the three 51Cr double isotope procedures in 36 normal volunteers (Table 3) (9). In addition, in Table3, the 24-hour posttransfusion survival of ten autologous RBC stored in AS-1 (ADSOL) at 4 C for 42 days was measured using the 51Cr/125I albumin double label procedure which produced a value of 72 ± 5% (9).

The studies published by Dumont LJ et al (2) and Cancelas JA (3) reported the 24-hour posttransfusion and lifespan of autologous RBC stored at 4 C in solution AS-7 for 42 and 56 days using a 51Cr single label method. The 51Cr labeled autologous RBC were transfused and the 100% survival value estimated from the 51Cr radioactivity in the blood during the 30 minute posttransfusion period. The NBRL data reported in reference 8 on autologous RBC stored in AS-1 (ADSOL) at 4 C for 35, 42 and 49 days show that the 51Cr double label procedure reported as Method 1 in Table 1 had significantly reduced 24-hour posttransfusion survival values compared to the 51Cr single label procedure reported as Method 2 in Table 1 which was the procedure used by Dumont LJ et al and Cancelas JA et al to measure the 24-hour posttransfusion survival of autologous RBC stored in solution AS-7 at 4 C for 42 and 56 days (2,3).

The NBRL experience with RBC stored in AS-1 (ADSOL) at 4 C for 49 days demonstrated that the 24 hour posttransfusion survival values using 51Cr single label procedures provided significantly higher values compared to a 51Cr double label procedure.

Bacterial contamination of blood and blood products is a major complication of blood transfusion and the sterility of blood and blood products stored at room temperature for 24 hours prior to leukoreduction and storage must be ensured (10). In addition, the need to measure the red blood cell volume of the recipient at the time of the transfusion of preserved autologous red blood cells labeled with 51Cr must be done to detect the quantity of nonviable compatible preserved red blood cells that are transfused. A 51Cr double label procedure and not a 51Cr single label procedure is needed to measure the quantity of nonviable compatible RBC stored at 4 C in solution AS-7 for 42 and 56 days.

In 1980, the Food and Drug Administration (FDA) approved the storage of autologous red blood cells stored at 4 C in AS-1 (ADSOL) for 49 days predicated on the 24-hour posttransfusion survival measured by the 51Cr single label procedure of 76% reported by Heaton A and associates (4). Whereas in 1985 the 24-hour posttransfusion survival of autologous RBC preserved at 4 C in AS-1 (ADSOL) for 49 days measured by the 51Cr double label procedure was 57% reported by Valeri, C.R. and associates (8).

Table 3 reported in reference 9 tabulates the 24-hour posttransfusion survival of autologous RBC that were deglycerolized in the Haemonetics ACP215 instrument and stored in AS-3 (Nutricel) at 4 C for 15 days. Three (3) laboratories measured the 24-hour posttransfusion survival of autologous red blood cells using three difference 51Cr double label procedures: 51Cr/125I albumin procedure by the NBRL, Boston, Mass; 51Cr/99mTc procedure by Walter Reed Army Institute of Research; and the double 51Cr procedure by University of Massachusetts. Similar 24-hour posttransfusion survival values of 77% were observed in the 36 autologous RBC transfusions that were performed (9). In May 2001, the FDA approved

the storage of deglycerolized RBC processed in the Haemonetics Blood Processor 215 and stored in the AS-3 (Nutricel) at 4 C for 2 weeks.

In 2014 the FDA approved the storage of autologous red blood cells stored in the additive solution AS-7 at 4 C for 42 days predicated on the 24-hour posttransfusion survival values measured by the 51Cr single label procedure reported by Durant LJ and associates (2) and Cancelas JA and associates (3). The red blood cell volumes of the recipients were not measured using the 51Cr single label procedure.

Over the past 50 years, the data reported by the NBRL, Boston, MA demonstrate that the 24-hour posttransfusion survival of preserved red blood cells must be measured by 51Cr double label procedures for autologous preserved red blood cells and by a differential agglutination procedure of allogeneic identifiable preserved RBC in anemic patients whose red blood cell volume is measured by 51Cr labeled autologous RBC.

The statement "Those who cannot remember the past are condemned to repeat it" is certainly applicable to procedures required to accurately measure the 24-hour posttransfusion survival of autologous and allogeneic preserved red blood cells. As the late baseball philosopher king, Lawrence "Yogi" Berra would say, the commentary on the sterility of blood products and the 24-hour posttransfusion survival of preserved red blood cells measured by the single and double label procedures are examples of "déjà vu all over again"!

1. Dumont LJ, Cancelas JA, Maes LA, Rugg N, Whitley P, Herschel I, Siegel AH, Szczepiokowski ZM, Hess JR, Zia M. The bioequivalence of frozen plasma prepared from whole blood held overnight at room temperature compared to fresh-frozen plasma prepared within eight hours of collection. Transfusion 55:476-484, 2015.
2. Dumont LJ, Cancelas JA, Maes LA, Rugg N, Whitley P, Herschel I, Siegel AH, Szczepiokowski ZM, Hess JR, Zia M. Overnight room temperature hold of whole blood followed by 42-day storage of red blood cells in additive solution 7. Transfusion 55:485-490, 2015.
3. Cancelas JA, Dumont LJ, Maes LA, Rugg N, Herschel I, Whitley PH, Szczepiokowski ZM, Siegel AH, Hess JR, Zia M. Additive solution-7 reduces the red blood cell cold storage lesion. Transfusion 55:491-495, 2015.
4. Heaton A, Miripol J, Aster R, and others. Use of ADSOL preservative solution for prolonged storage of low viscosity AS-1 red blood cells. Br J Haematol 57:467-478, 1984.
5. Valeri CR, Ragno Giorgio G. Forty-five years of research at the NBRL, Boston, Massachusetts. Chaotic observations, serendipity, and patience. Chapter 2. Lulu Publishing Services, 2013.
6. Valeri CR, Pivacek LE, Ouellet R, Gray A. A comparison of methods of determining the 100 percent survival of preserved red cells. Transfusion 24:105-108, 1984.
7. Valeri CR. Measurement of viable ADSOL-preserved human red blood cells. NEJM 312:377-378, 1985.
8. Valeri CR, Pivacek LE, Palter M, Dennis RC, Yeston N, Emerson CP, Altschule MD. A clinical experience with ADSOL® preserved erythrocytes. Surg Gynec Obstet 166:33-46, 1988.
9. Valeri CR, Ragno G, Pivacek LE, Srey R, Hess JR, Lippert LE, Mettille F, Fahie R, O'Neill EM, Szymanski IO. A multicenter study of in vitro and in vivo values in human RBCs frozen with 40-percent (wt/vol) glycerol and stored after deglycerolization for 15 days at 4 C in AS-3: assessment of RBC processing in the ACP215. Transfusion 41:933-939, 2001.
10. Lafeuillade B, Eb F, Ounnoughene N, Petermann R, Daurat G, Huyghe G, Vo Mai M-P, Caldani C, Rebido D, Weinbreck P. Residual risks and retrospective analysis of transfusion-transmitted bacterial infection reported by the French National Hemovigilance Network from 2000 to 2008. Transfusion 55:636-646, 2015.

Table 1: **The 24-hour 51Cr posttransfusion per cent survival rate of autologous ADSOL preserved erythrocytes stored at 4 degrees C for 35, 42 and 49 days (8)**.

Length of storage at 4 degrees C of RBC	Method 1 – measured labeled RBC radioactivity, recipient RBC volume estimated from the 125I plasma volume and the total body hematocrit	Method 2 – extrapolated logarithmic extrapolation to time 0 of RBC radioactivity 5 to 15 minutes after infusion	Method 3 – estimated RBC radioactivity 30 minutes after infusion
35 Days Mean:	74.8	80.1	88.3
SD:	4.9	4.5	5.7
n:	8	8	8
Range:	67-81	71-84	77-94
42 Days Mean:	71.0	79.5	83.9
SD:	4.9	5.1	5.2
n:	15	15	15
Range:	61-79	71-87	75-95
49 Days Mean:	57.0	66.6	71.6
SD:	9.4	9.9	8.0
n:	10	10	10
Range:	42-72	51-84	58-87

Table 2: The survival of compatible but identifiable ADSOL-preserved erythrocytes stored at 4 degrees C for as long as 49 days and transfused into patients with anemia who were stable (8).

Length of storage at 4 degrees C of RBC	RBC Survival in vivo, per cent	
	Immediately after completion of the transfusion "0" hour	24 hours after completion of the transfusion
34 to 36 Days Mean:		
SD:	87.2	79.0
n:	4.9	4.3
Range:	6	6
	82-95	72-84
37 to 43 Days		
Mean:	75.1	65.1
SD:	7.9	9.2
n:	9	9
Range:	59-83	47-76
45 to 49 Days		
Mean:	68.2	52.8
SD:	10.0	10.8
n:	9	10
Range:	47-79	38-71

Table 3: 24-hour posttransfusion survival, lifespan and ITE of RBCs deglycerolized in the ACP215 and stored at 4 C in AS-3 for 15 days (9).

Test Site	Label Method		24 hour posttransfusion survival value (double label procedure)	Lifespan (T50, days)	ITE* (%)
Combined		Mean	77	28	68
		SD	9	4	8
		n	36	34	36
NBRL	51Cr/125I albumin	Mean	75	29	65
		SD	7	4	6
		n	14	13	14
WRAIR	51Cr/99mTc	Mean	78	24	68
		SD	9	5	6
		n	8	7	8
UMASS	Double 51Cr	Mean	78	29	71
		SD	10	5	10
		n	14	14	14
NBRL[x]	51Cr/125I albumin	Mean	72	27	72
		SD	5	3	5
		n	10	10	10

***Product of in vitro FTW recovery and 24-hour posttransfusion survival value. [x]Control: CPD RBCs stored in AS-1 at 4 C for 42 days.**