


# Impact of the preparation method of red cell concentrates on transfusion indices in thalassemia patients: A randomized crossover clinical trial

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## Abstract

**Background:** The average hemoglobin content of red cell concentrates (RCC) varies depending on the method of preparation. Surprisingly less data are available concerning the clinical impact of those differences.

**Study Design and Methods:** The effects of two types of RCC (RCC-A, RCC-B) on transfusion regime were compared in a non-blinded, prospective, randomized, two-period, and crossover clinical trial. RCC-A was obtained by whole blood leukoreduction and subsequent plasma removal, RCC-B removing plasma and buffy coat first, followed by leukoreduction. Eligible patients were adult, with transfusion-dependent thalassemia (TDT).

**Results:** RCC-A contained 63.9 (60.3–67.8) grams of hemoglobin per unit (median with 1<sup>st</sup> and 3<sup>rd</sup> quartile), RCC-B 54.5 (51.0–58.2) g/unit. Fifty-one patients completed the study. With RCC-B, the average pre-transfusion hemoglobin concentration was  $9.3 \pm 0.5$  g/dl (mean  $\pm$  SD), the average transfusion interval 14.2 (13.7–16.3) days, the number of RCC units transfused per year 39.3 (35.4–47.3), and the transfusion power index (a composite index)  $258 \pm 49$ . With RCC-A, the average pre-transfusion hemoglobin concentration was  $9.6 \pm 0.5$  g/dl (+2.7%, effect size 0.792), the average transfusion interval 14.8 (14.0–18.5) days (+4.1%, effect size 0.800), the number of RCC units transfused per year 34.8 (32.1–42.5) (–11.4%, effect size –1.609), and the transfusion power index  $272 \pm 61$  (+14.1%, effect size 0.997). All differences were statistically highly significant ( $p < .00001$ ). The frequency of transfusion reactions was 0.59% with RCC-A and 0.56% with RCC-B ( $p = 1.000$ ).

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**Conclusion:** To reduce the number of RCC units consumed per year and the number of transfusion episodes, TDT patients should receive RCC with the highest average hemoglobin content.

**KEYWORDS**

RBC transfusion

## 1 | INTRODUCTION

Red cell concentrates (RCC) can be produced from whole blood according to many different methods. Those methods entail differences in the average total hemoglobin content and other aspects,<sup>1</sup> which are expected to impact on the clinical outcome. The average hemoglobin content depends particularly on such procedural variables as removal of the buffy coat and filtration of whole blood or RCC. However, the differences in the hemoglobin content of individual RCC units within a single preparation method are quite large<sup>2</sup> and greater than those between average values of different preparation methods.

The Italian guidelines on transfusion treatment of hemoglobinopathies recommend RCC with the highest possible hemoglobin content. Among RCC obtained from whole blood donations, the Italian guidelines consider those prepared by pre-storage filtration of whole blood, without removing the buffy coat, as preferable for this reason.<sup>3</sup> However, the only clinical support for this opinion comes from a retrospective observational study.<sup>4</sup>

Supplying patients with hemoglobinopathy (and possibly all transfusion-dependent patients) with RCC specifically designed for their use is not without undesirable consequences. In Europe, platelets are usually prepared by the buffy coat method. Therefore, Transfusion Services should produce at least two kinds of RCC and maintain two separate inventories, which is obviously a complication.

Owing to the scarcity of objective data and the organizational impact of producing a blood component specifically designed for transfusion-dependent patients, we decided to perform a prospective crossover clinical trial to compare the clinical effects of two RCC, one obtained from whole blood filtration (RCC-A) and the other from filtration of red cells after buffy coat removal (RCC-B).

## 2 | MATERIALS AND METHODS

### 2.1 | Study design

This is a single-center, prospective, randomized, two-period, crossover clinical trial. After inclusion, patients

were randomized into two groups. The first group was to receive RCC-A for a period of 6 months and RCC-B for the next 6 months. The second group was to receive the blood components in the inverted order. Groups were formed numbering patients in alphabetical order and randomizing the list using Research Randomizer (<https://www.randomizer.org>). Beforehand, we decided with the toss of a coin to include the first half of the list in the first group and, in case of an odd number, the smaller number of patients in the first group.

The crossover design was chosen owing to the efficiency and increased statistical power.<sup>5</sup> Common drawbacks of crossover design were considered improbable in our clinical setting, as thalassemia major is a chronic, stable disease. Causes of increased blood consumption were monitored throughout the study. The two RCC being compared are akin to two (slightly) different dosages of the same drug. Therefore, a washout period was not deemed necessary.

The patients were not informed of the sequence of blood components that they were going to receive. However, being the exterior appearance of the two types of unit different, patients and care providers were able to notice it. Therefore, this is not a blinded study.

This study was approved by the local Institutional Ethical Committee and was registered with ClinicalTrials.gov (identifier NCT03992001).

### 2.2 | Patients

Eligible patients were adults (age  $\geq 18$  years) with transfusion-dependent beta-thalassemia major, exclusively transfused at the Day Hospital for Thalassemia and Hemoglobinopathies of the Azienda Ospedaliera-Universitaria S. Anna of Ferrara from at least 5 years. The following exclusion criteria were considered: hemolytic red cell auto-antibodies, severe splenomegaly ( $>18$  cm on echography), elevated blood consumption ( $>200$  ml/kg of pure red blood cells in the last year), any significant clinical pulmonary, cardiovascular, endocrine, hepatic, gastrointestinal, renal, infectious, or immunological disease, not adequately controlled prior to the study; patients transfused with washed RCC or treated with red cell exchange

or receiving hemoglobin inducers currently or in the last 6 months; pregnancy. Patients with an average transfusion interval less than 9 days were also excluded because we feared that it would have been difficult to obtain RCC-A on time, as this blood component was not prepared by our usual blood supplier. Once enrolled, patients were withdrawn if they presented one of the exclusion criteria (except that regarding the transfusion interval) or if they were transfused outside our Institution. Written informed consent was obtained from all patients.

### 2.3 | Blood components

RCC-B was prepared by the Blood Component Production Center of Bologna (Polo Trasfusionale di Qualificazione biologica e Lavorazione dell'Area Vasta Emilia Centrale), using the TACSI system (Terumo). It was obtained from whole blood (450 ml collection) after separation of plasma, buffy coat, and red cells and successive leukoreduction of the red cells. The Production Center of Bologna was the usual supplier of our Institution. At the time of this study, it only produced RCC-B. Therefore, we solicited the help of other Production Centers of our region (Modena, Romagna, Parma, Reggio Emilia, Piacenza), which kindly agreed to supply us with RCC obtained by leukoreduction of whole blood (450 ml collection in Fresenius bags), and successive separation (Compomat, Fresenius) of plasma and red cells (RCC-A). All RCC were stored in SAGM for a maximum of 14 days, as appropriate for transfusion to thalassemic patients.<sup>6</sup>

### 2.4 | Treatment protocol and measurements

No change in the usual transfusion treatment was caused by the present study, apart from the choice between the two RCC.

Transfusion episodes were discarded in case of violation of protocol. The following violations were considered: incorrect blood component (RCC-A instead of RCC-B or vice versa); transfusion not completed (because of a reaction or for any other reason); transfusion in another department of our hospital. Transfusion episodes were also discarded in case of hemolytic transfusion reaction. A non-hemolytic transfusion reaction was considered as a sufficient cause, only when transfusion had not been completed. Fever is known to impact on the post-transfusion hemoglobin increment.<sup>7</sup> Therefore, we also discarded the transfusion episodes where fever higher than 38°C occurred during the transfusion interval and lasted for at least 3 days.

Samples were collected from the patients before transfusion for compatibility testing and the measurement of the pre-transfusion hemoglobin concentration. A representative sample was obtained aseptically from the RCC units for the measurement of hemoglobin concentration and hematocrit. The RCC units were weighed together with the transfusion set before and after transfusion in order precisely to calculate the volume of transfusion. The volume of the RCC unit was calculated dividing the net weight by the density (D). D was calculated according to the following formula:  $D = S + ([R - S] * H)$ , where S is the density of the supernatant (consisting of SAGM plus a small amount of plasma), estimated to be 1.01, R is the density of the red blood cells (1.095, according to literature),<sup>8</sup> and H is the hematocrit in decimal form.

### 2.5 | Outcomes

Main outcomes were the following transfusion indices: average pre-transfusion hemoglobin concentration, calculated starting from the second transfusion of the period to the first transfusion of the following period (the pre-transfusion hemoglobin concentration is influenced by the previous transfusion and transfusion interval); average transfusion interval: sum of the transfusion intervals in days, divided by the number of transfusion episodes (excluding any discarded transfusion episodes); total number of RCC transfused per year, calculated dividing the number of RCC transfused during the period by the sum of the transfusion intervals in days, and multiplying by 365; frequency of transfusion reactions: number of transfusion reactions divided by the number of transfusion episodes. Transfusion reactions were classified according to recommendations.<sup>9</sup>

In addition to the above indices, we also considered a combined index, calculated as the ratio between two simple indices, the average pre-transfusion hemoglobin concentration, and the total number of RCC transfused per year, multiplied by 1000 to avoid decimals. The rationale for the combined index comes from the expected response to a change in the average hemoglobin content of the RCC. Transfusion-dependent patients tend to adopt a transfusion regime, which allows them a comfortable pre-transfusion hemoglobin concentration and does not interfere too much with work and life in general. When the pre-transfusion hemoglobin concentration deviates from the usual values, for example, in response to a different RCC, a compensation becomes necessary, in the form of a change in either the number of RCC units per transfusion or the transfusion interval, or both. As a result, the pre-transfusion hemoglobin concentration and the total number of RCC transfused per year will

certainly vary, while the transfusion interval may or may not be altered. The first two indices, however, are not independent: the extent of change in the total number of RCC transfused per year depends on how much deviation from the usual values is allowed in the pre-transfusion hemoglobin concentration. This is likely to vary between different patients, making any comparison between different RCC difficult. In our opinion, the combined index is a suitable solution for this problem, as its value changes in the appropriate direction when any or both of the single indices change. We call it transfusion power index.

## 2.6 | Statistical analysis

We calculated the sample size on the basis of the results of an observational study (unpublished), which compared RCC-B with a RCC containing on average 59 gHb/unit. At least 32 patients were needed to provide a power of 0.9 to detect the differences in the outcomes observed in that study (transfusion power index, mean  $\pm$  SD of the difference:  $23.8 \pm 29.9$ ; RCC transfused/year:  $-2.7 \pm 4.5$ ), at a two-sided 0.05 significance level. We expanded the target for enrollment to approximately 52 patients, as we had a sufficient workforce to support a study with that numerosity.

The two-period crossover design allows the analysis of the treatment effect together with the period and the sequence effects. In this case, the treatment effect is the comparison between the transfusion indices obtained by the two RCC. The period effect is the comparison between the indices calculated in the two successive periods of study. This can be statistically significant when a number of patients change in time in the same direction independently of the RCC they receive, for example, because their weight increases/decreases or owing to seasonal variations. The sequence effect is the comparison between the indices calculated for the sequence RCC-A–RCC-B and for the sequence RCC-B–RCC-A.

The treatment effect was also assessed separately, dividing the patients in two subgroups according to the splenectomy status, as it is well known that the spleen is a major determinant of the annual blood consumption in thalassemia major patients.<sup>10</sup>

The comparison between the two RCC was the main aim of the present study. Therefore, results were only analyzed per protocol. Transfusion episodes where protocol violations occurred were discarded (see above).

Results were reported as means with standard deviations (normal distributions) or medians with the first and third quartiles (non-parametric distributions). Normality was checked by the Shapiro–Wilk test. Transfusion

indices obtained by RCC-A and RCC-B were compared using the paired samples *t*-test (normal distribution of differences) or Wilcoxon signed ranks test (non-parametric distribution). The effect size was reported as Cohen's *d* (normal distribution) or as the matched rank biserial correlation (non-parametric distribution). The sequence effect was checked using the *t*-test (normal distribution) or Mann–Whitney test (non-parametric distribution). Mann–Whitney test was also used to compare the hemoglobin content and the storage length of RCC-A and RCC-B. The proportion of two-unit transfusions with RCC-A and RCC-B was compared using Yates corrected chi-square test.

Sensitivity analyses included an intention to treat analysis, which considered also the discarded transfusion episodes, and alternative, more comprehensive statistical tests (mixed design analysis of variance [ANOVA]), when data met their assumptions. A mixed design model was chosen because treatment and period are within subjects factors, while sequence and other variables of interest, such as splenectomy, are between subjects factors. This model also allows testing interactions between the variables.

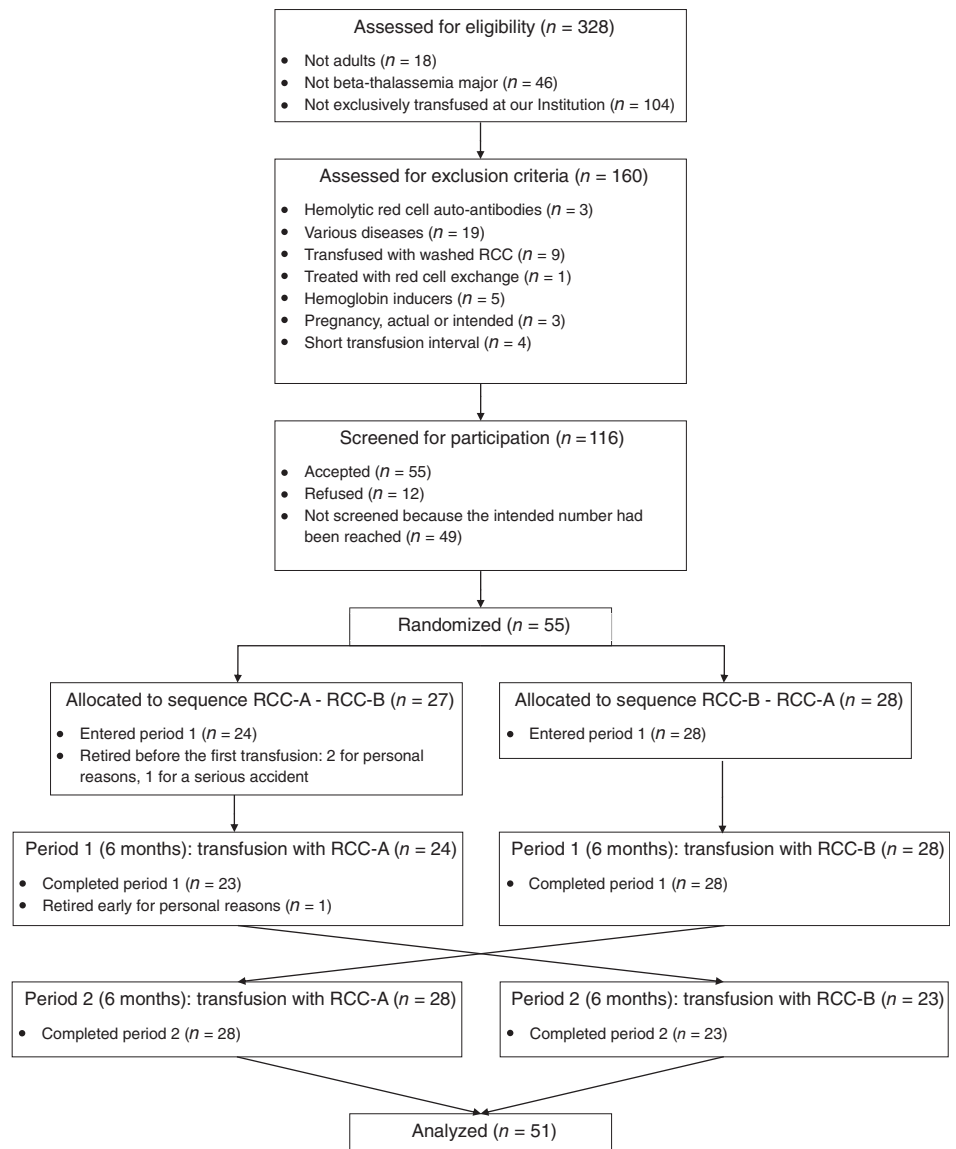
A *p* value less than .05 was considered statistically significant but values were adjusted with Hommel's procedure in case of multiple comparisons.<sup>11</sup> All analyses were performed using SPSS version 19, Jasp version 0.12.2, and G\*Power version 3.1.9.

## 3 | RESULTS

### 3.1 | Patients

Out of a total of 328 patients with hemoglobinopathy, 160 met eligibility criteria. They were screened for the presence of exclusion criteria and those remaining ( $N = 116$ ) were offered to participate in the trial. When 55 patients had accepted, we stopped enrollment (Figure 1). Two of them retired from the study for personal reasons before beginning it; one after a few transfusions. Shortly after the start of the study, a fourth patient was excluded because of a serious accident, with hospitalization in another hospital for several weeks. Fifty-one patients, 24 females and 27 males, median age 46 years (41–50), completed the study and were available for analysis. Their body weight was measured at least once in each 6-month period in which they received one of the two blood components. Their median weight was 59.0 kg (55.1–64.8) when they received RCC-A and 58.6 kg (54.0–65.4) when they received RCC-B. The difference is not statistically significant ( $N = 51$ , paired samples *t*-test,  $p = .343$ ). Thirty-two patients (63%) were splenectomized. In

**FIGURE 1** Patient inclusion and design of the study



the 19 non-splenectomized patients, splenic length was measured by ultrasound at the beginning of the study and at the end of each period. Splenic length was 13.5 cm (12.0–15.0) at the end of the period in which they received RCC-A and 14.0 cm (12.3–15.2) after they received RCC-B (Wilcoxon signed ranks test,  $p = .065$ ). In period 1 (from May to November 2018), 23 patients received RCC-A and 28 RCC-B. The opposite occurred in period 2 (from December 2018 to June 2019). The difference was caused by randomization and by the loss of 4 patients after randomization (Figure 1).

### 3.2 | Transfusions and blood components

A few transfusion episodes were discarded for the following reasons:

- Violation of protocol: RCC-B was transfused instead of RCC-A (7 cases) or vice versa (1 case)
- The transfusion was not completed because of a non-hemolytic transfusion reaction (2 cases for each blood component); in a further case (RCC-A) the patient interrupted the transfusion for personal reasons
- Infectious episode with fever during the transfusion interval, deemed sufficiently serious potentially to impact on the transfusion indices (2 cases for each blood component)
- Transfusion in another department of our hospital (2 cases of RCC-A and 1 case of RCC-B).

Transfusion episodes available for analysis were 672 (RCC-A) and 713 (RCC-B), during which 1039 RCC-A units and 1144 RCC-B units were transfused. No more than 2 units were transfused per transfusion

TABLE 1 Hemoglobin content and length of storage of RCC-A ( $N = 966$ ) and RCC-B ( $N = 1103$ )

	RCC-A	RCC-B	%	$p^a$
Hemoglobin content/unit (g) <sup>b</sup>	63.9 (60.3–67.8)	54.5 (51.0–58.2)	17.3	<.00001
Hemoglobin actually transfused/unit (g)	60.5 (56.9–64.4)	51.1 (47.6–54.5)	18.6	<.00001
Length of storage (days)	8 (5–10)	3 (3–4)		<.00001

Abbreviation: RCC, red cell concentrate.

<sup>a</sup>Mann–Whitney test.  $p$  values reported in this and the following Tables were adjusted with Hommel's procedure.<sup>11</sup>

<sup>b</sup>Data shown are medians with the first and third quartile in parentheses.

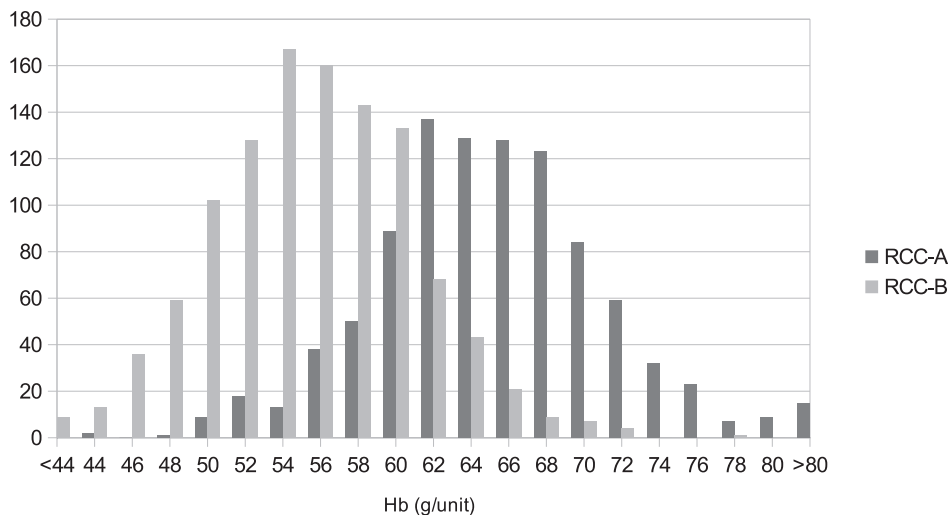


FIGURE 2 Hemoglobin content (g/unit) of individual RCC units (RCC-A:  $N = 966$ ; RCC-B:  $N = 1103$ )

episode. Two-unit transfusions were more common with RCC-B: 431 (60%) versus 367 (55%). The difference is statistically significant (Yates corrected chi-square:  $p = .032$ ). The period of treatment with each of the two blood components, namely, the sum of all transfusion intervals of a single patient, was similar for both RCC-A and RCC-B: RCC-A, 205 days (196–210); RCC-B, 203 (196–212). The difference is not significant ( $N = 51$ , Wilcoxon signed ranks test,  $p = .992$ ).

The total hemoglobin content and the amount actually transfused was available for most RCC units administered during the present study: 966 units of RCC-A (93%) and 1103 units of RCC-B (96%). Data are shown in Table 1 and Figure 2. RCC-A contained 9.4 grams of hemoglobin (17.3%) more than RCC-B. The difference is statistically highly significant (Mann–Whitney test,  $p < .00001$ ). The same difference in absolute value was also found in the amount actually transfused ( $p < .00001$ ). In percentage terms, it was even slightly greater (18.6%). We calculate that, in the average patient of the present study, a difference of 9.4 g of hemoglobin would result in a difference of about 0.3 g/dl of transfusion increment. About 3.4 g of hemoglobin (approximately 16 ml of RCC) remained in the blood bag and the tubings at the end of transfusion.

The length of storage was available for all the RCC transfused. Before transfusion, RCC-A was stored for longer than RCC-B (8 days vs. 3 days; Mann–Whitney test,  $p < .00001$ ; Table 1). Unlike RCC-B, RCC-A was not prepared by our usual Production Center. It was obtained from Centers in other provinces of our region (see above). It had to be shipped from the production site to our usual Production Center and then to our Institution. However, only 5 RCC-A units (0.5%) were older than 14 days and the maximum length of storage was 18 days.

### 3.3 | Treatment effect

All four transfusion indices considered showed statistically highly significant differences between RCC-A and B (paired samples  $t$ -test or Wilcoxon signed ranks test,  $p < .00001$ ; Table 2). All effect sizes can be described as large, as their absolute value is 0.79 or greater. In 44/51 patients (86%), the average pre-transfusion hemoglobin concentration was higher when transfused with RCC-A. However, the difference was greater than 1 g/dl just in two cases and in percentage terms this index showed minor changes only (2.7% on average). The median transfusion interval was greater with RCC-A in 39/51 patients (76%) and was identical to RCC-B in 3 of the other

TABLE 2 Comparison of transfusion indices obtained using RCC-A and RCC-B: All patients,  $N = 51$ 

Transfusion index	RCC-A	RCC-B	%	Effect size <sup>a</sup>	$p^b$
Pre-transfusion [Hb] (g/dl) <sup>c</sup>	9.6 ± 0.5	9.3 ± 0.5	2.7	0.792 (0.474–1.104)	<.00001
Transfusion interval (days)	14.8 (14.0–18.5)	14.2 (13.7–16.3)	4.1	0.800 (0.481–1.112)	<.00001
RCC units/year	34.8 (32.1–42.5)	39.3 (35.4–47.3)	–11.4	–1.609 (–2.023–1.188)	<.0001
Transfusion power index <sup>d</sup>	272 ± 61	239 ± 49	14.1	0.997 (0.994–0.998)	<.00001

Abbreviation: RCC, red cell concentrate.

<sup>a</sup>Effect sizes, with 95% confidence intervals in parentheses, are reported as Cohen's  $d$  for pre-transfusion hemoglobin concentration, transfusion interval, and RCC units/year, and matched rank biserial correlation for the transfusion power index.

<sup>b</sup>Paired samples  $t$ -test for pre-transfusion hemoglobin concentration, transfusion interval, and RCC units/year; Wilcoxon signed rank test for the transfusion power index.

<sup>c</sup>Data shown as mean ± SD or median with first and third quartile in parentheses.

<sup>d</sup>The transfusion power index is a combined index, obtained dividing the pre-transfusion hemoglobin concentration by the number of RCC per year and multiplying by 1000.

TABLE 3 Transfusion indices in splenectomized and non-splenectomized patients

	Pre-transfusion [Hb] (g/dl) <sup>a</sup>	Transfusion interval (days)	RCC units/year	Transfusion power index
<b>Splenectomized patients (<math>N = 32</math>)</b>				
RCC-A	9.7 ± 0.5	15.1 (14.0–19.3)	33.5 (28.0–37.7)	296 ± 60
RCC-B	9.4 ± 0.5	14.5 (13.2–18.8)	37.1 (32.6–42.2)	258 ± 49
%	3	4.2	–9.7	14.8
$p^b$	.00001	.00001	<.00001	<.00001
<b>Non-splenectomized patients (<math>N = 19</math>)</b>				
RCC-A	9.3 ± 0.5	14.5 (13.9–15.4)	40.4 (33.7–46.8)	233 ± 39
RCC-B	9.1 ± 0.5	14.0 (13.8–14.6)	44.7 (39.2–52.1)	207 ± 31
%	2.1	3.4	–9.7	12.7
$p^b$	.03	.03	<.00001	<.00001

Abbreviation: RCC, red cell concentrate.

<sup>a</sup>Data shown as mean ± SD or median with first and third quartile in parentheses.

<sup>b</sup>Comparison between RCC-A and RCC-B, paired samples  $t$ -test.

12 cases. In 26 patients (51%), the difference was equal to or greater than 1 day. We calculate that using RCC-A each patient would avoid at least one transfusion episode per year, on average. The difference in the number of blood units transfused (calculated per year) is more marked. It was 34.8 (32.1–42.5) with RCC-A and 39.3 (35.4–47.3) with RCC-B (–11.4%). In 46/51 patients (90%), the number of blood units/year was lower with RCC-A and it was equal to RCC-B in 2 of the other 5 cases. Using the measured blood consumption of each patient, we calculate that these 51 thalassemic patients need 2062 RCC-B units per year but would consume 195 less units if transfused with RCC-A. Finally, the transfusion power index was higher with RCC-A in 50/51 patients (98%). This combined index also showed the greatest difference between the two blood components: 14.1%.

Splenectomy did not influence the response to the two RCC compared in the present study. Table 3 shows

the results obtained in splenectomized ( $N = 32$ ) and non-splenectomized ( $N = 19$ ) patients separately. The similarity with the data shown in Table 2 is evident. However, comparing the two groups of patients within each RCC separately, the RCC units transfused per year and the transfusion power index were significantly different (Mann–Whitney test or  $t$ -test,  $p < .01$  in all cases). On the whole, splenectomized patients had a slightly higher pre-transfusion hemoglobin concentration (+3%), consumed fewer RCC units per year (–19%) and had a higher transfusion power index (+26%).

### 3.4 | Period effect

Transfusion indices in periods 1 and 2 were not significantly different, with the exception of the transfusion interval: pre-transfusion hemoglobin concentration

(period 1 vs. period 2), 9.5 versus 9.4 g/dl,  $p = .080$  (paired samples  $t$ -test); transfusion interval, 14.2 versus 14.5 days,  $p = .008$  (paired samples  $t$ -test); RCC units/year, 38.4 versus 35.5,  $p = .060$  (Wilcoxon signed ranks test); transfusion power index, 237 versus 258,  $p = .313$  (paired samples  $t$ -test). If periods were compared within each RCC separately, the differences in the transfusion indices were not significant ( $p > .05$  in all cases).

### 3.5 | Sequence effect

The order of administration of the two RCC did not influence the transfusion indices significantly: pre-transfusion hemoglobin concentration (sequence RCC-A–RCC-B versus sequence RCC-B–RCC-A), 9.5 versus 9.4 g/dl,  $p = .706$  ( $t$ -test); transfusion interval, 14.1 versus 14.9 days,  $p = .156$  (Mann–Whitney test); RCC units/year, 37.1 versus 37.4,  $p = .706$  (Mann–Whitney test); transfusion power index, 251 versus 249,  $p = .706$  (Mann–Whitney test).

### 3.6 | Transfusion reactions

We observed 4 non-hemolytic transfusion reactions (fever/chills, 2 cases; urticaria, 1 case; dizziness and headache, 1 case) in 4 patients when they were transfused with RCC-A and 4 reactions (fever/chills, 2 cases; urticaria, 2 cases) in 3 patients with RCC-B. The reaction rate (per transfusion episode) was 0.59% with RCC-A and 0.56% with RCC-B (Yates corrected chi-square test:  $p = 1.000$ ). In 2 cases for each RCC the reaction occurred during transfusion and impeded its completion. Those transfusion episodes were not considered for the calculation of the transfusion indices (see above). No hemolytic transfusion reaction occurred during this study.

### 3.7 | Sensitivity analyses

We excluded from analysis only 20 transfusion episodes out of 1385 (1.4%), for the reasons mentioned in the section Transfusions and Blood Components, above. In an intention to treat analysis we considered them, too, but their inclusion did not change the results in an appreciable way (data not shown).

Of the transfusion indices, which are the outcomes of this study, only the pre-transfusion hemoglobin concentration and the transfusion power index met the assumptions of the mixed design ANOVA completely. The total number of RCC transfused per year deviated somewhat

from normality ( $p = .030$  for RCC-A,  $p = .103$  for RCC-B) but it could be considered suitable, as the assumptions only require an approximately normal distribution. The transfusion interval, however, is distinctly bimodal, with many patients centering around 2 weeks and a minority around 3 weeks. Consequently, we applied the more powerful mixed-design ANOVA, with RCC type as within subjects variable and splenectomy and sequence as between subjects variables, to the three suitable transfusion indices only. Mixed-design ANOVA showed that both RCC type ( $p < .00001$ ) and splenectomy ( $p < .0002$ ), but not sequence ( $p = .835$ ), influenced the transfusion power index. There was no significant interaction between the independent variables. An interaction between RCC type and sequence was found for the pre-transfusion hemoglobin concentration ( $p = .003$ ) and the total number of RCC transfused per year ( $p < .001$ ). However, the analysis of the simple main effects confirmed that RCC type and splenectomy, but not sequence, influenced the indices. We investigated the nature of this interaction and found that the pre-transfusion hemoglobin concentration of patients receiving RCC-A in sequence A-B was slightly greater than that in sequence B-A but the opposite occurred for patients receiving RCC-B. Similarly, the patients receiving RCC-A in sequence A-B consumed more RCC units per year than those in sequence B-A, while those receiving RCC-B showed the opposite tendency. The origin of this difference lies most probably in the behavior of the physicians ordering the transfusion: in period 1 (RCC-A of sequence A-B and RCC-B of sequence B-A) they kept the pre-transfusion hemoglobin concentration at a slightly higher level than in period 2 (RCC-A of sequence B-A and RCC-B of sequence A-B), possibly because of the relative unfamiliarity with the characteristics of the two blood components. The transfusion power index was not influenced because it remains stable when pre-transfusion hemoglobin concentration and RCC consumption concurrently increases or decreases. In any case, the interaction with the sequence does not confuse the interpretation of the difference between the two RCC, which is fully confirmed by this supplementary analysis.

In theory, results similar to those observed in this study could be produced if patients in sequence A-B increased their blood consumption during the study, while those in sequence B-A decreased it, for reasons extraneous to the study itself, that is, not depending on any difference between the RCC types. In order to verify if blood consumption changed during the study, we measured the actual amount of hemoglobin transfused. This information is available for 614/672 (91%) transfusions of RCC-A and 682/713 (96%) transfusions of RCC-B. In this way, we calculated the total amount of hemoglobin



transfused to each patient in each period and from those values we were able to estimate the annual hemoglobin consumption. When receiving RCC-A, the patients consumed  $2227 \pm 450$  g/Hb per year; with RCC-B, they consumed  $2067 \pm 412$  g/Hb per year. The difference (8%) is statistically significant (paired samples *t*-test,  $p < .00001$ ). In other terms, the patients consumed more hemoglobin when they were transfused with RCC-A. Therefore, the lower number of RCC-A units consumed per year was due to the higher hemoglobin content of RCC-A, not to a hidden and unexpected decrease in blood consumption on the part of the patient.

A greater hemoglobin consumption implies a higher iron loading. Serum ferritin was not included among the measurements collected as part of this study. However, it is regularly measured every few months as part of the usual transfusion protocol. We checked if our patients had at least one ferritin measurement performed during the second half of each 6-month period and took those nearest to the end of the period. Forty-four patients (86%) had suitable measurements. They were taken after a median of 181 days (167–193) of transfusion with RCC-A and 179 days (155–195) of transfusion with RCC-B (paired samples *t*-test,  $p = .374$ , not significant). Median ferritin values were 528 ng/ml (366–818) with RCC-A and 434 ng/ml (272–666) with RCC-B. The difference is statistically not significant (Wilcoxon signed ranks test,  $p = .129$ ).

## 4 | DISCUSSION

Many variables influence the post-transfusion hemoglobin increment, some obvious, some less so.<sup>12</sup> Perhaps the most obvious characteristic of the blood component is the amount of red blood cells it contains. The two RCC compared in the present study had, on average, a difference of 9.4 grams of hemoglobin (17.3%). This value closely corresponds to what was observed by others, comparing preparation methods similar to ours.<sup>1,13,14</sup> It should therefore be considered representative of their expected performance.

It is all too natural to presume that the transfusion of RCC-A should result in a greater hemoglobin increment than RCC-B. However, in practice this effect would easily be obscured by a host of confounding factors: recipient characteristics, such as blood volume, bleeding, splenomegaly or splenectomy, hemolytic auto-antibodies; RCC characteristics, such as storage solution and storage length. Moreover, the difference between single units of the same RCC is often greater than that between the average values of RCC-A and B, as it is apparent looking at Figure 2. More precisely, about 40% of RCC-A and 37% of RCC-B lie outside a 9.4 g range around the average. In

order reliably to predict the post-transfusion hemoglobin increment, both the total hemoglobin content of the transfused unit and the recipient's blood volume must be taken into account.<sup>15</sup>

In our experience, thalassemia major patients adjust their pre-transfusion hemoglobin concentration around a threshold below which they know that symptoms of anemia will appear. The threshold is generally between 9 and 10.5 g/dl. In each patient, it is quite constant in time. When they receive a new RCC with an average hemoglobin content significantly different from the previous one, their attending physician will try to manage transfusions so as to minimize the change in the pre-transfusion hemoglobin concentration. In principle, there are two ways, not mutually exclusive: either occasionally increasing/decreasing the number of units transfused per transfusion episode or adjusting the transfusion interval. These two ways are not available for every patient: some of them prefer to maintain a fixed transfusion schedule, for example, for work needs. Others would not tolerate an increased volume of transfusion. A further possibility is to adapt to the new RCC, at least in part, accepting a change in the pre-transfusion hemoglobin concentration. In practice, our results show that all these options were adopted to varying degrees. Apart from the latter, they entail a change in the number of RCC units consumed per year. Therefore, in order to document the therapeutic power of a type of RCC, both the pre-transfusion hemoglobin concentration and the number of RCC units consumed per year must be considered. This is not ideal when comparing different RCC because the extent of change in one transfusion index may vary and may influence the change in the other one. To obviate this problem, we propose a combined index, which is the ratio between the two. We call it transfusion power index. Its most useful property is that a change in any of the two simple indices provokes a corresponding change in the combined index. For example, adjusting from RCC-B to RCC-A, any increase in the pre-transfusion hemoglobin concentration increases the numerator of the fraction and hence its value; any decrease in the number of RCC units transfused per year decreases the denominator and hence increases the result of the fraction. In both cases, the transfusion power index would become greater, as expected.

The transfusion power index does not incorporate a correction for the storage duration. In the present study, all transfused units were relatively fresh but RCC-A units were stored for longer than RCC-B. A recent study compared the hemoglobin increments of RCC with different length of storage. It showed that the transfusion of RCC with a storage duration similar to RCC-A resulted in a modestly but perceptibly smaller increment than RCC stored as long as RCC-B.<sup>16</sup> Whether that difference in the

length of storage influenced the transfusion indices in our study is not known. In any case, if it did, it would have reduced any difference between the results of the two RCC.

We calculated that the average difference in the hemoglobin content between RCC-A and -B would result in a difference of 0.3 g/dl of transfusion increment (one-unit transfusion) for the average patient of the present study. This is negligible for many transfused patients. However, the present study shows that for transfusion-dependent patients it is clinically significant. The effect on the annual RCC consumption is particularly important. Annually, about 200 beta-thalassemia major patients are transfused in our Institution, many regularly, some occasionally, during control visits only. They consume definitely more than 6000 RCC units per year. Projecting the results of the present study on all our thalassemia major patients, we calculate that with RCC-A we would save more than 700 RCC units per year.

With RCC-A the patients received less RCC units per year but consumed more red cells. In terms of hemoglobin, approximately 8% more. In our relatively short period of study, we did not observe a change in ferritin values but in the long term an adjustment of the chelation therapy may be necessary. The increased consumption is an unavoidable consequence of the higher average pre-transfusion hemoglobin concentration and transfusion interval that we observed with RCC-A. Both indicate a higher average hemoglobin concentration during the transfusion interval. It is generally believed that the normal removal rate of red cells from circulation is a constant fraction of the circulating amount. Therefore, the absolute value of the daily consumption should change in proportion to the hemoglobin concentration. In our case, it is also possible that it depends in part on the longer storage age of RCC-A compared with RCC-B (Table 1). However, the transfusion of RCC-A does not necessarily lead to an increase in the average pre-transfusion hemoglobin concentration or the average transfusion interval or both: this would be unavoidable for the few patients who always receive one RCC unit per transfusion only (12% in the present study). In the other patients, the transfusion of RCC-A could be managed, at least in principle, so as to decrease the number of two-unit transfusions, avoiding those consequences. In this way, it is conceivable that red cell consumption and iron load would not change, too.

Thalassemia major patients are transfusion-dependent for all their life. As such, they are an extreme example of transfusion dependence but many other diseases cause dependence for long periods: myelodysplasia, hematological malignancies, other hemoglobinopathies. The results of our study should also apply to them. It is known that among medical patients, 3% of them account for 80% of

transfusion costs. Myeloproliferative diseases, other hematologic disorders, and bone marrow transplantation represent by far the medical conditions associated with the highest transfusion burden.<sup>17</sup> Choosing for them the RCC with the highest average hemoglobin content seems both clinically and economically preferable. This can be accomplished preparing a blood component specific for those patients. A negative consequence would be the need to manage both the production and the inventory of at least two different RCC. Inventory problems, however, should not be critical: patients would not be harmed by an occasional transfusion with another RCC. A more serious drawback is that the production of RCC with the maximum hemoglobin content precludes the production of platelets, at least using the buffy coat method: from a donation of whole blood, only plasma and red cells can be obtained in this way. Transfusion Services should assess how much the needs of their transfusion-dependent recipients of RCC can be satisfied without harming the recipients of platelets. An alternative possibility is to choose the RCC units at the right tail of the distribution of the hemoglobin content. As Figure 2 clearly shows, the values of individual units of RCC-A and -B overlap to a considerable extent. The hemoglobin content of a RCC unit can be estimated with sufficient precision from its weight.<sup>18</sup> This approach would be successful as long as the number of available units with the required characteristics was sufficient to satisfy the needs of the patients.

This study is not blind. It is conceivable that unconscious biases might have influenced the decisions of the attending physicians. However, our study outcomes are based on objective measurements and are not susceptible to subjective biases. Almost all enrolled patients who began the study completed it (51/52). Just a few of the transfusion episodes were discarded, according to pre-specified criteria (20/1385).

The presence of a period effect and, particularly, of a sequence effect may complicate the analysis of a cross-over trial or even render it impossible.<sup>5</sup> In our case, there was no sequence effect but a period effect was apparently displayed by the average transfusion interval. However, there was no significant difference when the period effect was analyzed within each RCC. We believe that the apparent period effect was in fact due to the unequal distribution of the transfusions of the two RCC: owing to randomization and the loss of 4 patients after randomization, in period 1 there were 23 patients receiving RCC-A but 28 patients receiving RCC-B. The opposite occurred in period 2. The marked differences in the transfusion indices obtained by the two RCC were sufficient to provoke an apparent period effect.

Research in the transfusion field has long been dominated by the effort to define and avoid side effects. Issues

of therapeutic power are equally important, we believe. This study shows that research on these aspects may result in significant clinical and organizational improvements and emphasizes the importance of donor and manufacturing variables in the management of patients on chronic transfusions. The use of RCC with a higher hemoglobin content in transfusion-dependent patients reduced by 11% the number of RCC units consumed per year. For the patient, this means less donor exposure. For health organizations, less waste of time, clerical work and money, and a welcome availability of more RCC units for other patients.

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## CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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