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Cell Age Characterization of Erythrocytes in the Buffy Coat

The removal of the buffy coat from red cell concentrates is generally considered beneficial because it reduces the frequency of febrile transfusion reactions [1], the formation of microaggregates [2], and hemolysis during prolonged storage [3]. However, those advantages entail the wastage of about 16% of the red blood cells (RBC) [4]. Moreover, the removal is performed under conditions so similar to those successfully used to collect young RBC [5, 6] that we suspected that the buffy coat would be significantly enriched with young RBC. Therefore, we measured two age-related parameters, pyruvate kinase (PK) activity [7] and reticulocyte count, in the whole blood before processing the buffy coat and the red cell concentrate. PK activity was measured with a commercial UV method (Boehringer Mannheim, FRG) and the reticulocyte count by cytofluorimetry with a thiazole orange reagent (Retic-Count, Becton Dickinson, Mountain View,

Calif., USA). The leukocyte content of the samples for PK activity was reduced to less than one leukocyte per 10^5 RBC prior to measurement [8]. Buffy coats were removed after centrifugation at 22°C in a Beckman J6BP centrifuge, using special blood packs and an automated device (Optipac and Optipress, respectively, both from Baxter, Deerfield, Ill., USA) [4]. We studied the effects of three types of centrifugation: a 'hard' (5 min at 4,200 rpm, 5,000 g), a 'soft' (3 min at 2,400 rpm, 1,650 g), and a 'two-step' [9] (9 min at 1,900 rpm, 1,000 g, plus 5 min at 4,000 rpm, 4,550 g) spin. The results are shown in table 1.

The reticulocyte count in the whole blood was 10.5 ± 4.8 per 10^3 RBC. The buffy coat contained 10–12% of the initial amount of RBC, but 28–37% of the reticulocytes, depending on the spin mode. PK activity in the buffy coat was significantly higher than in the whole blood ($p < 0.03$ for all spin modes). In

the red cell concentrate, PK activity was significantly decreased after the 'hard' spin ($p = 0.03$) and approached the significance level after the 'two-step' spin ($p = 0.06$). Approximate calculations performed using the known relationship between PK ratio (PK_r) and age [10], as derived from the PK rate of decline [7], yield a mean age of 46 days for the erythrocytes in the buffy coat prepared after the 'hard' and the 'two-step' spin.

These results demonstrate that young RBC are selectively removed with the buffy coat. In this study, we kept the volume and the RBC content of the buffy coat at a minimum. This notwithstanding, PK activity was significantly, albeit slightly, reduced in the red cell concentrates prepared after the 'hard' and the 'two-step' spin. The young RBC are concentrated in the buffy coat to a lesser degree by the 'soft' spin, but, unfortunately, the 'soft' spin is also less efficient in removing the leukocytes, and the yield of

Table 1. Erythrocyte age-related parameters (retics and PK_r) and leukocyte (WBC) and platelet content (PLT) of buffy coats (BC) and red cell concentrates (RCC; mean \pm 1 SD)

Spin mode	Blood component	n	WBC $\times 10^9$	PLT $\times 10^9$	Hb g	Retics / 10^3 RBC	PK _r
Hard	BC	10	2.0 \pm 0.5	90 \pm 32	7 \pm 1	35 \pm 13	1.352 \pm 0.120
	RCC	10	0.6 \pm 0.4	2 \pm 2	51 \pm 7	7 \pm 4	0.965 \pm 0.061
Soft	BC	5	1.4 \pm 0.5	18 \pm 20	6 \pm 1	27 \pm 22	1.201 \pm 0.053
	RCC	5	0.9 \pm 0.5	4 \pm 1	53 \pm 5	8 \pm 4	1.061 \pm 0.064
Two-step	BC	5	1.7 \pm 0.7	92 \pm 17	7 \pm 1	35 \pm 18	1.373 \pm 0.172
	RCC	5	0.3 \pm 0.2	0.4 \pm 0.1	53 \pm 3	7 \pm 4	0.911 \pm 0.094

Hb = Hemoglobin; PK_r = Ratio between the PK activities (IU/g Hb) in the component and in the whole blood.

plasma is smaller [4]. In fact, given the similar sedimentation characteristics of leukocytes and young RBC, it is expected that they will sediment together during centrifugation.

However, our data do not necessarily imply that this relative loss of young RBC is

clinically significant. Actually, we expect it to be negligible for the vast majority of patients. However, in default of pertinent clinical data, we would caution against the use of buffy-coat-poor red cell concentrates in chronic anemia patients with transfusion-induced

iron overload. In any case, removing the buffy coat would be of little use for those patients because they are commonly treated with leukocyte-poor blood prepared by more efficient methods [11].

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