

# **Influence of erythrocyte storage on biophysical cell properties and primary hemostasis**

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**Thematic topic: Medicine**

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## **INTRODUCTION/BACKGROUND**

Red blood cell (RBC) storage for transfusion purposes leads to a time-dependent deterioration of certain cell properties (storage lesions). The aim of this study was to analyse the role of either hyper- or isotonic additive preservation solutions on such parameters. Since RBCs are intimately involved in the process of primary hemostasis by pushing platelets towards the vessel wall, where they adhere and aggregate, we further hypothesized that stored RBCs could also have less capacity to support primary hemostasis.

## **MATERIALS & METHODS**

RBC units were prepared from healthy blood donors using citrate-phosphate-dextrane (CPD) as an anticoagulant and either saline-adenin-glucose-mannitol (SAGM; 376 mosm/l) or phosphate-adenin-glucose-guanosin-saline-mannitol (PAGGSM; 285 mosm/l) as an additive solution and stored at 4°C for 6 weeks. At the beginning (days 0-2) and end (days 42-44) of storage, RBC properties were studied. The mean cellular volume (MCV) was calculated from the microhematocrit, morphology was assessed on fixed RBCs, aggregability was tested by the sedimentation rate (ESR) in 3% dextran 70, RBC deformability by ektacytometry, and RBC suspension viscosity with a Couette viscometer (Contraves LS-30).

For the investigation of primary hemostasis, fresh citrated blood was taken again from the same donors. Platelet-rich plasma (Platelet count  $178 \pm 40 \times 10^3/\mu\text{l}$ ) was prepared, in which RBCs were resuspended with a constant hematocrit (40%), but changing fractions of stored versus fresh autologous RBCs (0, 25, 50, 75, and 100%, respectively). A platelet function analyser PFA-100<sup>®</sup> was used to measure primary hemostasis. This instrument closely simulates in vivo conditions with blood flowing at high shear rates through a membrane pore, which is coated with collagen and either epinephrine (EPI) or ADP. Platelets, which are pushed towards the wall by RBCs, adhere, aggregate, and form an occluding plug, which stops blood flow and is measured as closure time (CT).

## **RESULTS**

The MCV increased from  $87.6 \pm 3.1$  fl in EDTA to  $100.7 \pm 4.3$  fl in CPD and PAGGSM and to  $92.2 \pm 2.5$  fl in CPD and in SAGM ( $p < 0.001$ ), after 42 days it was  $95.8 \pm 4.0$  fl and  $93.8 \pm 3.9$  fl, respectively. For both additives similar echinocytic RBC shape transformations were observed after storage and aggregability was decreased (ESR  $44 \pm 22$  and  $25 \pm 17$  mm/2h,  $p = 0.001$ ). Spontaneous hemolysis and osmotic fragility were less after storage in PAGGSM than in SAGM. Ektacytometry showed a slight decrease of RBC deformability for both additives. The viscosity of RBC units (Hct

61± 3 %) and resuspensions of RBCs in fresh plasma (Hct 42±2 %) was similarly increased after storage in both solutions.

With increasing fractions of stored blood, the PFA closure time (CT) increased, indicating less platelet aggregation. CT-EPI was 121±17s for 100% fresh and 0% stored RBCs, 129±32s for 25% stored RBCs, 164±45s for 50%, ( $p < 0.0001$  compared with 0% stored RBCs; ANOVA), 214±54s for 75% ( $p < 0.0001$ ), and 273±36s for 100% stored RBCs ( $p < 0.0001$ ). For CT-ADP the values were 91±22s, 95±12s, 101±13s, 124±44s ( $p = 0.004$ ), and 191±72s ( $p < 0.0001$ ) for 0, 25, 50, 75, and 100% stored RBCs, respectively.

#### CONCLUSIONS & OUTLOOK

The isotonic additive solution PAGGSM induced more RBC swelling in the beginning and decreased spontaneous hemolysis rate and osmotic fragility at the end of a 42-days storage than hypertonic SAGM. All other parameters such as echinocytosis, decreased RBC deformability, aggregability, increased blood viscosity and less capacity to support primary hemostasis by aggregating platelets, were similar for both additive solutions and remain a major problem of blood banking.