Journal of Anesthesia and Surgical Research

An Open Access Journal

Deformability of Erythrocytes is maintained after Autologous Cell Salvage in Orthopedic Surgery

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Received Date: 01-17-2020; **Accepted Date:** 01-27-2020; **Published Date:** 02-03-2020

Abstract

Background and objective: *Prerequisite for oxygen transport to the tissue is the ability of erythrocytes to change from a discoid into an elongated form in order to pass microvessels. Cell salvage is highly recommended to decrease blood loss and to avoid allogeneic transfusions in perioperative bleeding. The purpose of our study was to assess deformability of erythrocytes at re-transfusion.*

Materials and Methods: *After ethics committee approval and informed consent, blood was withdrawn from the autotransfusion system (Xtra, Sorin, Germany) of 24 patients undergoing joint arthroplasty 6 hours after cell salvage initiation. Deformability curves, elongation indices (EI) obtained at increasing shear stress (SS) were assessed by using Laser Optical Rotational Red Cell Analysis (LORRCA).*

Results: *Erythrocytes showed the typical sigmoid EI/SS curve with a mean maximum elongation index (EImax) of 0.593 ± 0.034 and mean half-maximal deformation (SS ½) of 1.186 ± 0.387 Pa. Irregular curve shapes and high variability in EI occurred at shear stress < 3 Pa. Recalculation of outcome parameters resulted in higher values (EImax 0.610 ± 0.035; SS ½ 1.562 ± 0.346 Pa).*

Conclusion: *Erythrocytes after autologous cell salvage are not stiff but elongate in response to shear stress. Erythrocytes showed the typical EI/SS-curve of human red cells, which indicates that the cells are able to uptake the hydrodynamic forces that act on them during the measurement. Further research is needed to investigate the effect of cell salvage processing on erythrocytes from patients with pre-existing hematological disorders.*

Keywords

Erythrocytes; Cell salvage; Deformability

Introduction

The physiological role of erythrocytes includes oxygen transport to the tissue cells and removal of metabolic waste, passing large and small vessels in series [1]. It is a typical red blood cell (RBC) characteristic that it can withstand the varying shear forces and the frequent collisions with other RBCs during its travel in the different vascularities without being damaged over a significant time span. One aspect of erythrocytes functionality is their ability to change their shape in order to align in the flow and pass microvessels [2-5]. This is important in the smallest vessels where RBC deformability is crucial for microvascular perfusion and oxygen delivery and there is the largest mismatch between RBC size and capillary diameter. Major surgery may lead to severe bleeding, anemia, hypovolemia, and allogeneic transfusion requirements. RBC availability and functionality are crucial in the clinical scenario of major surgery. Cell salvage is an important clinical tool in perioperative Patient Blood Management (PBM) not only to avoid allogeneic RBC transfusions but also to decrease loss of blood and to decrease anemia [6]. Randomized controlled trials and meta-analyses confirm that autologous cell salvage reduces the need for perioperative allogeneic RBC transfusions [7-9]. Guidelines from the European Society of Anesthesiology highly recommended the use of cell salvage, which is helpful for blood conservation in major cardiac and orthopedic surgery (GRADE 1B) [6]. In autologous cell salvage the patient's erythrocytes are collected intra- and postoperatively from the wound for up to 6 hours, dissolved in an anticoagulated crystalloid solution until centrifugation, washing, and re-transfusion [10]. While deformability of allogeneic RBC concentrates decrease despite optimized preservation fluids and storage conditions [11-15], re-transfusion of autologous salvaged blood does not impair RBC cell membrane deformability in vivo in cardiac surgery patients [13,16]. However, cardiopulmonary bypass per se stiffens the cell membranes, resulting in a loss of RBC deformability [17]. Elongation and deformability of erythrocytes in autotransfusion concentrates prior to re-transfusion remains poorly understood in non-cardiac surgery. Considering the increasing clinical use of cell salvage systems in orthopedic surgery, markers for the functionality of washed erythrocytes can be helpful in defining quality and safety of re transfused blood in this clinical setting. Moreover, in Austria in 2018, 3.974 patients underwent cardiac surgery with cardiopulmonary bypass while 37.566 patients underwent knee or hip arthroplasty (personal communication, Austrian Ministry of Labor, Social Affairs, Health and Consumer Protection). Exposure to cell salvage is highest in the orthopedic cohort, without confounding factors on deformability due to extracorporeal circulation. Accordingly, we assessed deformability of autologous erythrocytes after cell salvage for major orthopedic surgery.

Materials and Methods

Ethics committee approval was obtained from the Sigmund Freud Private University Vienna (002/2018) and informed consent was obtained from 24 adult patients scheduled for major orthopedic surgery. Inclusion criteria were primary hip or knee joint replacement with routine use of autologous cell salvage. Exclusion criteria were pregnancy, preexisting hematological or bleeding disorders. Cell salvage was performed according to our hospital-internal standard

operating procedures. The general method of autologous wound blood salvage has been summarized elsewhere [18]. We used the Xtra (Sorin, Germany) with standard equipment. Blood is collected from the wound, dissolved in an anticoagulated crystalloidal solution (unfractionated heparin 25,000 IU per 1 l sodium chloride 0.9%), and centrifugated at 820-1480 G. Performance data of the Xtra system: hematocrit of 54%, heparin removal of 99.7%, potassium removal of 95%, free hemoglobin removal of 97% [19]. In our study, cell salvage was used during and after surgery in the recovery room for a maximum of 6 hours after initiation. At that time point, patients received their re-transfusion. According to PBM, the indication for re-transfusion was salvage of patient's own blood after major orthopedic surgery [6]. Salvaged erythrocytes were re-transfused via a peripheral venous line after passing a 170-200 µm filter. From the remnant in lines and filter 4.5 mL of cell salvaged blood concentrate was withdrawn by dripping into a collection cuvette with ethylene diamine tetra acetic acid (BD Vacutainer EDTA, Becton Dickinson, France). EDTA is recommended by the manufacturer for erythrocyte deformability testing. Blood was stored before further analysis for a maximum of 3 hours. For measuring the deformability of erythrocytes, the Laser Optical Rotational Red Cell Analyzer LORRCA was used (RR Mechatronics, The Netherlands) [20]. Twenty-five microliter of blood were incubated in 5 mL LORRCA osmo-fluid (polyvinylpyrrolidone). The liquids were gently mingled until it became a homogeneous mixture. Thereafter, 800 µL of this suspension were pipetted into the cup of the LORRCA measuring system. We assessed deformability in triplicates by using new portions of the sample. LORRCA analyzes the diffraction pattern at 9 different shear stress-values of 0.30 Pa, 0.53 Pa, 0.95 Pa, 1.69 Pa, 3.00 Pa, 5.33 Pa, 9.49 Pa, 16.87 Pa, and 30.00 Pa. The integrated software automatically displays the elongation indices (EI) per applied shear stress increment (EI/SS curve) and computes the theoretical index of maximum deformation (EImax) at infinite shear stress, as well as the shear stress at half-maximal deformation (SS ½). Demographic data of patients, amount of autologous blood re transfused, complications upon re-transfusion, and allogeneic RBC requirements were documented.

Statistics

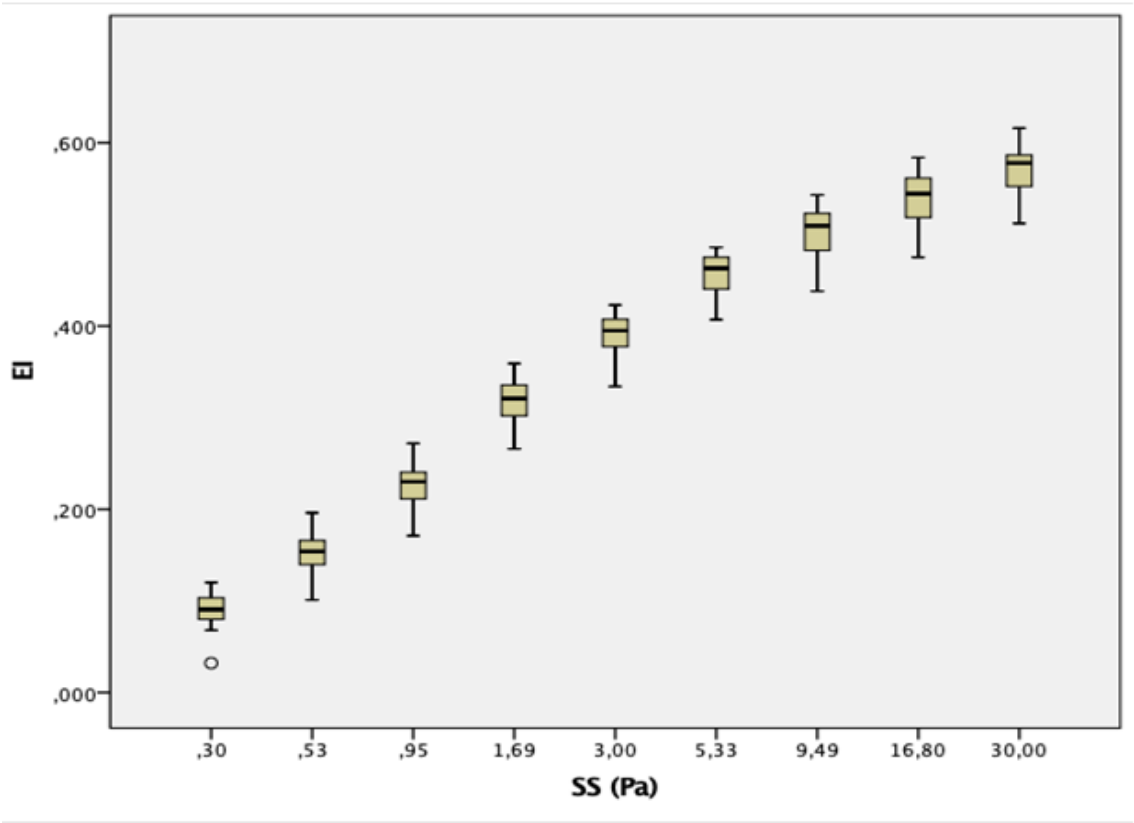
Mean values for the EI at each SS, and for EI max and SS1/2 were calculated out of the triplicate measurements. These mean values were used for further data processing. Plots to generate EI max and SS ½ were calculated by SPSS. Histograms (Lineweaver-Burke plot) revealed that the distribution of EIs of the 24 patients was nearly Gaussian for each SS. Data are, therefore, presented as mean ± standard deviation (SD). According to Baskurt [21], we re-calculated EI max and SS ½ by plotting the reciprocals of SS and EI for data linearization in addition to the values that are displayed automatically by the LORRCA software. We re-calculate EI and SS ½ at all 9 shear stress-values of the LORRCA. We obtained EImax (b) from the intersection with 1/EI=0, and SS ½(b) from the intersection with 1/SS ½=0. The difference between calculated and automated values delivered by the LORRCA was statistically analyzed by SPSS (t-test).

Results

Twelve male and 12 female patients had a mean age of 72.6 ± 8.8 years. The amount of re-transfused RBC concentrate was 114 ± 54 ml (range: 69 – 300 ml). Perioperative clinical course of patients was uneventful and no allogeneic RBC concentrates were transfused.

Deformability of salvaged erythrocytes is shown in Figure 1. The boxplot reveals a sigmoid deformability curve with a mean EImax of 0.593 ± 0.034 and mean half-maximal deformation (SS ½) of 1.186 ± 0.387 Pa.

Figure 1: Deformability of salvaged erythrocytes



In 31 out of 72 measurements we observed irregularities in the EI/SS-curve below 3 Pa with deviations from its typical sigmoid shape. The form of the curve differed in all the triplicate measurements of these blood samples. Figure 2 and 3 shows extreme examples of two types of deformability curves: The sigmoid curve was regular in 2; deviation occurred in 3 at shear stresses below 3 Pa. Re-calculations according to these irregularities (Figure 3) resulted in EImax (b) values of 0.610 ± 0.035 and SS ½(b) of 1.562 ± 0.346 Pa. The difference between automated values delivered by the LORRCA and re-calculated values was not statistically significant. Test duration was about two minutes for every single measurement. We observed no technical problems.

Discussion

Our study describes deformability of human erythrocytes after autologous cell salvage in orthopedic surgery demonstrating that recovered erythrocytes are not stiff but able to elongate in response to shear stress (Figure 1). We observed an elongation by up to 60% of the diameter of a resting red cell in the test milieu. Erythrocytes showed the typical sigmoid EI/SS-curve, which indicates that the cells are able to uptake the hydrodynamic forces that act on them during the measurement. This information is reconfirming to clinicians because deformability is a prerequisite for fulfilling erythrocytes' ultimate physiological task of delivering oxygen to the tissue [22], even after 6 hours of extracorporeal storage at room temperature, after centrifugation and washing. Our findings support the use of fresh autologous salvaged blood in major orthopedic surgery, similar to previous findings in cardiac surgery using cardiopulmonary bypass [13,16]. Re-transfusion of processed blood did not lead to a systemic reduction of RBC function [13]. Noteworthy, our EI was about 23% higher compared to cell salvage in cardiac surgery [16] when measured at shear stress of 3 and 3.89 Pa, respectively. This difference in the absolute values of EI may – at least in part – be due to the effect of hypothermic extracorporeal circulation and different methods employed: cell salvage with Brat-2 versus Xtra (Sorin, Italy) versus CATS (Fresenius, Germany), erythrocytes elongation tests with ektacytometer (Rheo Meditech, South Korea) versus LORRCA (RR Mechatronics, The Netherlands) [13,16]. Furthermore, hemodilution during cardiac surgery has been accused of inducing the drop in RBC function rather than the mechanical cell salvage procedure [16,23,24]. In our patients undergoing orthopedic surgery no major fluid shifts occurred, no major volume replacement was required, and patients were not exposed to therapeutic hypothermia. Blood transfusion of $\geq$ 5 allogeneic RBC concentrates during spinal fusion surgery reduced EI more than transfusion of up to 3 allogeneic RBC concentrates [11]. EI was lower in allogeneic RBC concentrates stored $\geq$ 21 days compared to fresher blood [11]. The mixture of older and younger allogeneic transfused cells and native patient erythrocytes that were recovered during surgery by cell salvage system showed an intermediate EI [11]. Our EI at 3 Pa of shear stress measured by LORRCA was about 23% higher compared to Frank et al. measured by a microfluidic slit-flow ektacytometer [11]. Noteworthy, our patients did not require any allogeneic blood transfusion. LORRCA is not a new technique but rarely used for clinical assessments and only for research purposes [19]. LORRCA could be used to compare and identify the quality of cell salvage devices in cardiac as well as in non-cardiac surgery [25-27]. Comparing types of centrifugation (continuous versus intermittent), various washing solutions and salvage duration, investigating time dependency the effect of cell salvage processing on erythrocyte elongation from patients with pre-existing hematological diseases would be of clinical interest. Also, in other diseases reduced RBC deformability has been observed, including diabetic nephropathy and chronic fatigue syndrome [28, 29]. Further research on autologous erythrocyte processing may permit optimizing perioperative management in patients with preexisting oxidative cell damage. In our hands, one measurement with LORRCA takes two minutes and reproducibility is satisfactory. Nevertheless, LORRCA appears not suited for perioperative or bedside testing. Newer erythrocyte function tests including chip-based devices should be investigated for their clinical applicability and compared with LORRCA

measurements [30]. Definitions of different normal ranges of the deformability parameters of whole blood samples versus erythrocytes in autotransfusion concentrates are warranted.

At shear stresses beyond 3 Pa, cell membranes homogenously maintained their physiological property (Figure 2 and 3). But at low stresses below 3 Pa we observed irregularities in the EI/SS-curves indicating difficulties of the cells to align to the shear field (Figure 3): EI/SS-curves were not smooth sigmoidal but with a notch below 3 Pa. The mode of motion can only be hypothesized, but most likely various motions may have occurred, including rolling and tumbling. Tumbling is a typical behavior of healthy erythrocytes at low shear stress and generates uneven EI/SS-curves, which are also typical for avian or camelid red blood cells [31]. Our findings (Figure 1 and 2) may indicate that deformability of erythrocytes after cell salvage may not only reflect the intrinsic functionality of erythrocytes but also the interaction with proteins of the suspension: Salvaged cells were not reconstituted in plasma containing various physiological proteins but in saline. Plasma as well as corpuscular components, such as platelets and leukocytes, is intentionally removed during washing and centrifugation in autotransfusion devices in order to remove inflammatory triggers [19]. However, in blood plasma, a film of charged molecules covers the erythrocytes' surface and possibly protects it from structural damage [32]. During cell salvage this halo is removed. Upon transfusion into the vasculature with its physiological components, improved RBC behavior in bulk and singular lines flow is expected, even at low shear stress. RBC elasticity determines a structuring of RBCs in the hydrodynamic flow fields of blood vessels [3]. A flexible RBC can migrate to the axial streamlines, which widens the marginal cell free layer and reduces the hydrodynamic effective RBC volumes [4]. Both effects reduce flow resistance. A flexible RBC deforms not only during constant shear stress, e.g. when it travels through a capillary, but also during the dynamic variation of shear stresses in larger vessels, e.g. at bifurcations or curvatures. If flow gets disturbed when RBCs cannot be structured in a vessel segment, atherosclerosis-prone vascular areas appear [11,33].

Figure 2: Deformability curve: The sigmoid curve was regular

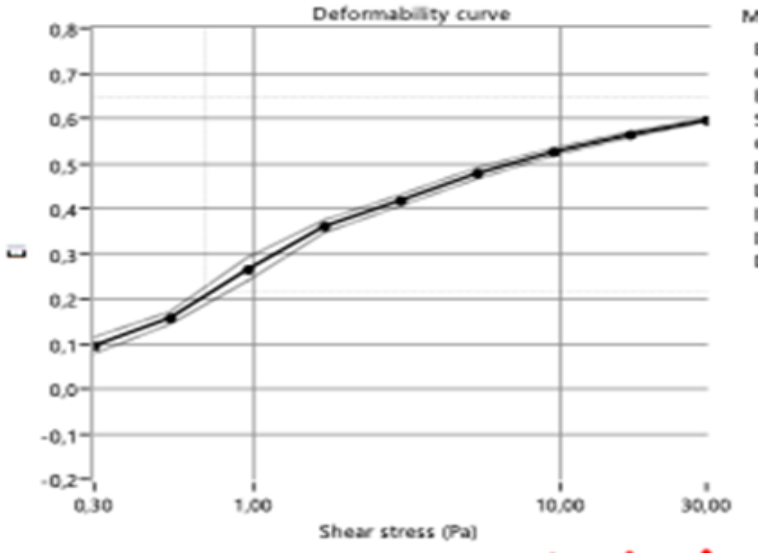
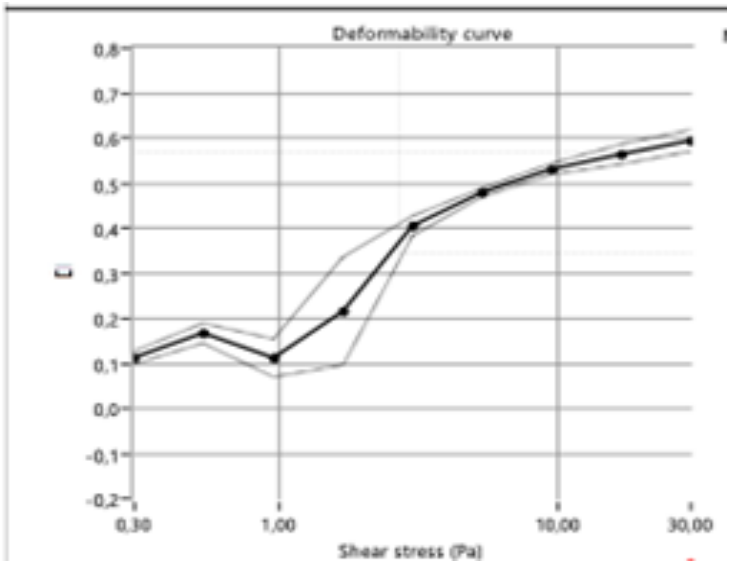


Figure 3: Deformability curve: deviation occurred at shear stresses below 3 Pa.



Conclusion

Our experiments indicate that gentle cell salvage preparation steps and short storage up to 6 hours until re-transfusion adds oxygen carrying capacity with deformable erythrocytes to the surgical patient.

Declaration of potential conflicts of interest

None.

Source of research support

This study was supported by the Department of Anesthesia and Intensive Care, Evangelical Hospital Vienna, Vienna, Austria.

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