Background and objectives: Homozygous sickle cell disease (SCD) patients frequently are alloimmunized resulting in restricted compatible blood supply. Methods to reduce exposure to allogeneic blood are not used frequently. Autologous blood from HbSS patients cannot be used because there is not a safe way to determine if it is suitable for re-transfusion. This feasibility study aims to establish the sickle cell index (SCI) as a monitor for the safe use of HbSS blood from autologous predonation (PAD), storage, and oxygenated cell salvage.

Materials and methods: HbSS blood units were stored as whole blood (WB) in CPDA-1 (n=4) or as packed red blood cells (PRBC) in SAG-M (n=4) for 42 days. Following storage at day 42, 3 stored WB and PRBC units were processed by oxygenated cell salvage in buffered washing solution. Quality of HbSS blood was assessed weekly by SCI during storage. Results were compared to HbA data obtained from healthy donor units with respective storage solutions.

Results: SCI correlated well with hemoglobin-S-fraction fraction (r=0.9625). During storage in SAG-M, sickling indicated by SCI increased continuously from day 21 on. In contrast, SCI in CPDA-1 units increased from day 1 on. In stored blood units with high SCI levels (>10‰), cell salvage reduced cell sickling and hemolysis.

Conclusion: SCI is feasible and useful for monitoring sickling in HbSS blood during exchange transfusion, storage, and cell salvage. As indicated by SCI, sickling of SCD blood in-vitro increased during storage but occurred later in SAG-M as opposed to CPDA-1. Monitoring of SCI revealed that oxygenated and buffered cell salvage improves SCD blood quality. Autologous methods in combination with SCI monitoring therefore may be appropriate to reduce allogeneic blood transfusion in SCD.

ABBREVIATIONS

2,3 DPG: 2,3 Diphosphoglycerate; ASAT: Aspartate Aminotransferase; ATP: Adenosine-5′-Triphosphate; CPDA-1: Storage Solution of Red Cells Containing Citrate, Phosphate, Dextrose-Adenine; CS: Oxygenated Cell Salvage; Hb: Hemoglobin; Hct: Hematocrit; HbS: Sickle Cell Hemoglobin; HbSS: Homozygous Patients Producing Red Cells Containing High Hbs Contents; FC: Fold Change; HU: Hydroxyurea; K+: Potassium; LDH: Lactate Dehydrogenase; NaCl: Sodium Chloride; OR: Osmotic Resistance; PRBC: Packed Red Blood Cells; SAG-M: Storage Solution of Red Cells Containing Saline, Adenine, Glucose, Mannitol; SCD: Sickle Cell Disease; SCI: Sickle Cell Index; WB: Whole Blood

INTRODUCTION

Despite an improvement in health care for patients with sickle cell disease (SCD) or HbSC, allogeneic blood transfusions still might be necessary to immediately treat severe complications or to avoid their recurrence. Due to altered T-cell responses and innate immunity abnormalities, frequent transfusions may induce further formation of allo-antibodies [1]. In special populations, a fourth of all SCD patients already are alloimmunized against a high-prevalence Rh antigen [2]. This reduces the compatibility of available allogeneic blood units dramatically [3]. In addition, an atypical form of life threatening hemolytic transfusion reaction may aggravate the hemolysis in SCD patients due to multispecific HLA antibodies [4].

Although not frequently used, autologous blood transfusions might be safe in SCD. Of concern is the retransfusion of sickle cells and thereby the induction of thromboses, emboli, infarctions, and sickle crises. Thus, physiological sickling triggers are responsible for the underuse of autologous HbS blood, i.e. processing conditions such as low temperature, acidity of washing and storage solutions, blood stasis or mechanical stress and others. There are only very few publications reporting the experimental use of autologous techniques to avoid these problems. Mainly
in sickle cell trait (with a modest HbS content), autologous techniques have been used safely [5,6]. With heterozygous SCD and HbSC blood of low HbS content, hemolysis of HbSS red cells was neither observed during storage [7,8] nor following intra operative blood salvage [9-11]. Testing the extreme, for homologous HbSS blood with high HbS contents, even frozen storage is reported without considerable damage [12,13]. However, to date, there is no monitor or indicator so far, which could translate these experimental results into clinical practice. In theory, if the HbS content is comparable to HbS levels in sickle cell trait, it is unlikely that autologous techniques are harmful. For example, during surgery with significant expected blood loss, the use of autotransfusion unlikely is harmful to the honorary SCD (HbSS>70%) recipient if before limited allogeneic HbA cell transfusion had reduced the HbS content below 50% [6,14-16].

Therefore, a combination of autologous and autologous techniques requires some guidance, i.e. by monitoring of actual HbS contents. Since hemoglobin electrophoresis is a time consuming method, more practical alternatives are modified automated high performance liquid chromatography (HPLC) [17,18] or the method we suggest in this article: The sickle cell count in the microscopic blood smear [19], termed by us “sickle cell index” (SCI). Since sickle cell prevalence is highest in underequipped health care systems of third world countries, a simple, cost-effective and common method is warranted to monitor autologous blood management in SCD.

This feasibility study was undertaken to demonstrate that SCI can assist clinicians to implement a combined program of autologous and limited allogeneic blood conservation techniques for SCD. Therefore, in only a few pilot patients with SCD, we used the SCI for both, the monitoring of blood storage in various additive solutions and blood processing during exchange transfusion and cell salvage for blood of various HbS contents. Microscopic monitoring of HbSS blood by SCI was compared to the electrophoretic pattern of hemoglobin fractions, particularly to the HbS-content. We hypothesized that SCI is a reliable monitor which would enable modified autologous techniques such as storage of predonations and cell salvage in SCD. Primary aim of the study was feasibility and accuracy in comparison to hemoglobin electrophoresis. Secondary aim was to investigate the quality of sickle cell containing blood products by a set of established quality and viability laboratory variables.

MATERIALS AND METHODS

After approval by the local ethics committee (2015-654N-MA) and informed consent of all subjects, exchange transfusion was performed in 4 sickle cell patients and 8 units of Hb SS blood units were stored. The primary aim of our study was to examine the feasibility of the combination of autologous and allogeneic blood transfusion techniques and the use of SCI for the guidance of this combination. For the latter aim, we investigated if monitoring the SCI could be used to target safe HbS contents in blood exchange by indicating the limit of allogeneic blood transfusion aiming at SCI of 5 o/oo. In patients and produced blood products, SCI results were compared to HbS contents in the hemoglobin electrophoresis. Secondary, a further study aim was to exclude a minor viability and quality of produced and stored HbS blood in comparison to HbA products; we applied established standard lab tests and compared them with HbA blood products.

Patients were Hb SS subjects, suffering from SCD osteonecrosis scheduled for primary or hip replacement surgery. All had at least one history of previous complicated surgery by a sickling event. Withdrawn HbSS blood was replaced by compatible allogeneic HbA units. HbSS units of each patient were stored in random fashion either as whole blood (WB) or packed red cells (PRBC) in case a double or quadriple unit donation clinically was not indicated; otherwise units were stored in pairs in both WB and PRBC. WB was stored in CPDA-1 (n=4; citrate, phosphate, dextrose-adenine) and packed red cells were stored in SAG-M (n=4; saline, adenine, glucose, mannitol). Data of those 8 units were compared to HbA units in identical storage solutions (own unpublished data from our quality assessment program with healthy donor blood synchronously before and during the study period).

Exchange transfusion

Prior to hospital admission, two of our patients received a long-term treatment with hydroxyurea (HU), but had not developed a protective Hb F-level over 20% [20,21] due to an assumed low medication intake compliance (for workflow, see Figure 1). Exchange transfusion was scheduled 2 to three days prior to surgery. Monitoring comprised pulse oximetry, continuous electrocardiography and invasive blood pressure measurement. Blood exchange was accomplished by large venous access in the femoral vein and a radial arterial line. The amount of withdrawn volume was not calculated in an iterative fashion since withdrawal and replacement was performed synchronized. Replacement of each withdrawn unit of 450 +/- 25 ml.

Figure 1: Workflow of patients and study procedures: predonation, exchange transfusion, storage, oxygenated cell salvage

Abbreviations: WB- Whole Blood; CPDA-1- Storage Solution of WB Containing Citrate Phosphate Dextrose Adenine; PRBC- Packed Red Blood Cell; SAGM- Storage Solution Of PRBC Containing Saline Adenine, Glucose, Mannitol; SCI- Sickle Cell Index, O2- Oxygen
ml was performed by transfusion of 300 +/- 25 ml allogeneic HbA blood and the volume substitution with 150 +/- 25 ml gelatine 6% solution (Haemaccel, TheraSelect, and Marburg, Germany). A colloid solution was preferred over a crystalloid volume substitute due to comparable volumes in the replacement calculation as well as for rheological advantages.

Oxygenated cell salvage (CS)

Modified cell salvage (Cell Saver® 5, Hemonetics, Munich, Germany) was performed on day 42 after storage. HbSS units were infused into the standard 5L reservoir at room temperature with a flow rate of 80-100 mL h⁻¹. In parallel, oxygen was insufflated via an extra hose with 10 L min⁻¹. The resulting oxygen fraction of 90% was measured online by a gas analyzer (O₂ Sensor S, Dräger Medical Germany, Lübeck, Germany). A washing volume of 1500 mL during 2 cycle’s phosphate buffered washing solution (PBS according to Dulbecco, pH 7.4) di-sodium-hydrogen-phosphate-dihydrate 7.12 g, sodium hydrogen-phosphate-monohydrate 1.38 g, sodium chloride 40.0 g, aqua dest, ad 2000 mL) was used (Figure 1). In our institution, data and experience for washing and processing red cells in the cell saver previously were derived by processing of outdated autologous blood (unpublished) using osmotic resistance and established viability variables of blood quality. Since the buffered washing solution provided better results with respect to acidity as compared to the routine washing (saline 0.9%) solution, we used the identical washing solution for this study. The same autologous transfusion device and identical storage solutions were used for both Hb SS and HbA units.

Laboratory tests / monitoring

During blood storage, quality of blood units was assessed by determination of the viability variables adenosine-5’-triphosphate (ATP) and 2,3 diphosphoglycerate (2,3 DPG) at 7, 14, 21, 28 and 35 days. Furthermore, the osmotic resistance was monitored at the respective days. Measurements of hemolysis indicators potassium (K⁺), lactate dehydrogenase (LDH), aspartate amino transferase (ASAT) and free hemoglobin content were performed during blood storage and CS procedure at 7, 14, 21, 35 and 42 days.

Reticulocyte count (RC): Reticulocyte count was performed by staining with thiazol orange using a benchtop flow cytometer equipped with a 488 nm argon laser and an 635 nm red diode laser (FACS Calibur™, Becton Dickinson Immunocytometry Systems, San Jose, CA). To ensure day-to-day reproducibility of flow cytometry data, commercial calibration beads were used (Calibrite™-Beads, Becton Dickinson). Reproducibility of the staining procedures was controlled daily by stabilized reference control cells (Reti-Chex™, Streck Laboratories INC., Omaha, USA).

Lysis resistance: The known lysis resistance of red cells in patients with sickle cell disease (SCD) [22,23] was measured on the Cell Dyn 3500™ haematology analyzer (Abbott Diagnostics, Wiesbaden, Germany). It requires the procedure performance in the “normal lye” mode (7s) and subsequently, if a RRBC (resistant red blood cells)-alert occurred, in an “extended lye” mode (28s) for accurate measurement of leucocytes differentials and red blood counts. Daily, reproducibility was evaluated by stabilized reference control cells (Cell Dyn Hematology Control 26™, Abbott Diagnostics, and Wiesbaden, Germany).

Sickle cell index (SCI): SCI is measured by microscopic examination of blood smears. Morphological examination and SCI-counting was performed following Pappenheim-stain. The index is calculated as the relation of sickle cells with banana and sickle shape with and without elongated edges as well as boxed shaped cells with sharp protusions to regular red cells. Irreversible sickle cells (ISCs) are unable to change their shape even during full oxygenation. RSCs are morphologically not distinguishable from irreversible sickle cells (ISCs), so the SCI includes RSCs and ISCs.

Microscopy and index counts were performed by experienced staff of a specialized laboratory. However, the method is easy to establish once video-microscopy with blood smears of sickle cells and other hemoglobinopathies and thalassemia can be trained regularly.

In each blood unit, predefined sickle cells were counted in triplicates in 20 visual fields (500 erythrocytes each) by experienced investigators. SCI results were reported in ‰. Microphotographs were taken with a KY F1030 Digital camera (JVC Deutschland GmbH, Friedberg, Germany) mounted on a Leitz Diaplan microscope.

Hemoglobin electrophoresis: The hemoglobin fractions were separated on agarose gels at pH8.6 (Hydragel 7 hemoglobin™) with a semi-automated Hydrazis LC™ electrophoresis system (both Sebia, Pulda, Germany). The contents of HbA, HbS, HbF and HbA₂ were measured densitometrically and the results were given in % of total hemoglobin. Hemoglobin AF, ASC and AA, hemolysate controls run on each gel (Hemoglobin Electrophoresis Control™, Beckman Coulter, Krefeld, Germany and β-thalassemic patient-in-house control). At primary diagnosis, patient’s HbS was additionally verified by a sickle cell test using the method of Schneider et al, [24].

Viability and hemolysis indicators (ATP, 2,3 DPG, K⁺, LDH, ASAT and free hemoglobin): Since intracellular loss of 2,3 DPG and ATP is well documented in both SAG-M and CPDA-1 (day 35 in CPDA vs. SAG-M: 2,3 DPG 0.6 to 1.3 vs. 0.0 to 1.5 µmol·g Hb⁻¹; ATP 2.4 to 3.3 vs. 0.7 to 3.0 µmol·g Hb⁻¹) [25,26], we did not expect major differences between sickle and Hb A red cells.

In contrast, these molecules served as additional indicators for hemolysis in the plasma. Both ATP and 2,3 DPG were determined by coupled enzymatic tests (both obtained from Sigma Diagnostics, Taufkirchen, Germany) based on the methods by Lowry et al., and Adams [27,28] and measured on a Hitachi U2000™ clinical chemical analyzer (Boehringer Mannheim, Germany). For quality control of ATP and 2,3 DPG, two concentration levels were prepared and measured in each experiment (A 2383, Sigma, Taufkirchen, Germany; 43380, Fluka, Seelze, Germany).

Plasma levels of K⁺, LDH and ASAT were measured using a Dimension™ clinical chemistry system (Dade Behring, Marburg, Germany). K⁺ was determined by the Electrolytes Quiklyte™ indirect integrated multisensor (IMT), LDH by the lactate dehydrogenase method and ASAT were measured according to the recommendations of the International Federation of Clinical Chemistry (IFCC) [29,30]. Quality controls were performed according to the RILI-BÄK national guidelines (Richtlinien der
Bundesärztekammer) with Precinorm UPX™ (Roche Diagnostics, Mannheim, Germany), Biochem II™ (Biorad, München, Germany) and the Monitril control (Dade Behring Diagnostics, Marburg, Germany). Values of free hemoglobin were obtained photometrically with a Dr. Lange spectrophotometer LSS00™ by the 3-wavelength method of Harboe [31]. The quality control was performed with Accumark Hemoglobin control™ (Sigma Diagnostics, Taufkirchen, Germany).

Osmotic resistance: Osmotic resistances of the red cells were evaluated by incubation in a dilution sequence of sodium chloride (NaCl) ranging from 0.1% to 0.85% [32-35]. After incubation for 20 minutes and subsequent centrifugation, the grade of hemolysis was determined spectrophotometrically from the supernatant. The values indicating the start of hemolysis and total hemolysis were reported in relation to the NaCl concentration (%). Quality assessment of the procedure was performed by examination of peripheral blood of a preoperative HbAA-donor in each experiment.

Statistical analyses: Feasibility was assumed if SCI monitoring of blood exchange achieved the targeted HbS level at the patient (primary aim of the study). Correlation of SCI to hemoglobin electrophoresis was examined with the correlation coefficient according to Spearman (r).

For the secondary aim of the study, guidance by SCI to identify minor quality of blood products, group comparisons for laboratory tests and SCI by Mann-Whitney U test with a non-parametric approach (employing the SAS® software Version 8.2 (SAS Institute, Cary, NC, USA)) was used. P-values below 0.05 were considered significant.

RESULTS

Exchange transfusion guidance by SCI
Orientation of removal and replacement of blood successfully achieved the fast and exact performance of the procedure to the target HbS content of below 30% (Figure 2). SCI correlated well with HbS contents during the procedure and remained stable until the rerise of HbS following 20 hours (Figure 2B). For all 4 subjects, the SCI correlation with HbS was r = 0.9625 see also Table (1). Remarkably, lysis resistance disappeared in conditions with HbS-levels below 60% (Figure 2A).

SCI during blood storage under various storage conditions: Hb-fractions at various hematocrit levels of the stored blood units are shown in Table (1). The SCI increased significantly during storage from day 1 today 42 in all blood units (Figure 3), in both WB-CPDA-1 (P = 0.033) and PRBC-SAG-M storage (P=0.034). Sickling was more prominent during storage in WB-CPDA-1 units compared to storage in PRBC-SAG-M units at day 1 (P = 0.049) and day 42 (P = 0,021). During storage, there was no positive correlation between Hb S contents and SCI counts.

Storage of HbSS blood: PRBC/SAG-M units with higher Hct-values (61.5, 65.2 and 35.1%) showed lower SCI than WB/CPDA-1 units with lower Hct-value (35.5, 38.0 and 26.6%) (Table 1).

Figure (4) contains microphotographs of Pappenheim-stained slides of the stored blood units at day 42. Figure 4B shows a slide of a PRBC/SAG-M unit of a preoperative donor (Hb: 25.6, Hct: 77%, HbA: 97.3%, HbF: <2.00%, HbA2: 2.70%). Erythrocyte ghosts (occasionally) and akanthocytes are found. This slide indicates a slight dehydration of erythrocytes during storing. In Figure (4C) and E, the patient’s blood units before oxygenated cell

**Figure 2** 2A: Course of Hb fractions and SCI during the clinical observation. Hemoglobin fractions in % (HbA, HbF, HbA2, HbS) are shown in comparison to the SCI (%) for different time points during the clinical course of the patient S.M. The SCI were counted in triplicates and the mean values and standard deviations are shown. The observation began 27 days before implantation of a total endoprosthesis (TEP). At that time point, the patient shows an HbSS phenotype with no HbA detectable and 79.5% HbS and 17.7% HbF. The follow up was done until day 72 after TEP. Additionally, SCI were counted intra-operatively. Lysis resistance was monitored: -: no lyse resistant cells were clustered by the hematology analyzer after 7 s of lysis; +: lyse resistant cells were clustered after 7 s of lysis (RRBC-alert); ++: lyse resistant cells were clustered after additional 15 s of lysis.

2B: Preoperative exchange transfusion. Hemoglobin fractions in % (HbA, HbF, HbA2, HbS) are shown in comparison to the (%) at the beginning (hour 0) and hour 1, 2, 3, 4 and 5 during exchange transfusion. 20 hours depicts a time point after exchange transfusion. The SCI were counted in triplicates and the mean values and standard deviations are shown.
Frietsch et al. (2016)

Email: thomas.frietsch@urz.uni-heidelberg.de

Table 1: Hemoglobin fractions and SCI in the perioperative period.

<table>
<thead>
<tr>
<th></th>
<th>3 month prior to admission</th>
<th>prior to blood exchange</th>
<th>following exchange</th>
<th>prior to surgery</th>
<th>following surgery</th>
<th>prior to discharge</th>
<th>3 month following surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA (mean, %)</td>
<td>0.0</td>
<td>10.2</td>
<td>72.4</td>
<td>70.4</td>
<td>64.5</td>
<td>57.6</td>
<td>11.3</td>
</tr>
<tr>
<td>Hbf (mean, %)</td>
<td>19.6</td>
<td>15.0</td>
<td>0.9</td>
<td>1.2</td>
<td>2.2</td>
<td>3.1</td>
<td>14.5</td>
</tr>
<tr>
<td>Hbs (mean, %)</td>
<td>78.1</td>
<td>73.0</td>
<td>24.9</td>
<td>26.9</td>
<td>31.4</td>
<td>37.0</td>
<td>72.0</td>
</tr>
<tr>
<td>Hba2 (mean, %)</td>
<td>2.3</td>
<td>1.8</td>
<td>1.8</td>
<td>1.6</td>
<td>1.9</td>
<td>2.3</td>
<td>2.2</td>
</tr>
<tr>
<td>SCI (mean, 0/00)</td>
<td>9.00</td>
<td>9.12</td>
<td>2.84</td>
<td>3.13</td>
<td>3.37</td>
<td>3.34</td>
<td>9.73</td>
</tr>
<tr>
<td>SD</td>
<td>0.09</td>
<td>0.08</td>
<td>0.22</td>
<td>0.18</td>
<td>0.21</td>
<td>0.28</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Abbreviations: SCI- Sickle Cell Index; SD – Standard Deviation, Hb- Hemoglobin

Figure 3 Sickle cell index during blood storage in different storage additives
Eight phlebotomized HBSS blood units were stored as PRBC/SAG-M (n=4, white bars) and WB/CPDA-1 (n=4, grey bars) over a storage period of 42 days. The SCI (‰) were counted in triplicates and the mean values and standard deviations are shown. P ≤ 0.05 for each storage solution from day 1 to 42 (group comparison by Mann-Whitney U test).

Figure 4 Morphological examination of HbSS peripheral blood and the stored and processed blood units at day 42
Shown are microphotographs of Pappenheim-stained slides of the peripheral blood (A) and the stored blood units of patient S.M. (C-F, day 42) and a preoperative donor (B, day 42). A: peripheral blood of patient S.M. 27 days before hip surgery (HbSS phenotype); B: WB/CPDA-1 blood unit of a preoperative donor; C and D: PRBC/SAG-M blood unit of patient S.M. before (C) and after (D) oxygenated cell salvage; E and F: WB/CPDA-1 blood unit of patient S.M. before (E) and after (F) oxygenated cell salvage. Black arrows: Sickle cells with banana and sickle shape with and without elongated edges and boxed shaped cells with sharp protrusions in patients' blood. Magnification: 630x.

In all SCD units, K+, LDH, ASAT and free hemoglobin increased during storage (Figure 5C-H). Absolute values of K+ and free hemoglobin were higher in PRBC/SAG-M units, whereas absolute values of LDH- and ASAT-activities were greater in WB/CPDA-1 units. In WB/CPDA-1 and PRBC/SAG-M units of healthy HbA subjects, all variables considerably increased during storage, except ASAT and free hemoglobin in PRBC/SAG-M. At day 42, comparable potassium values were seen in the WB/CPDA-1 and PRBC/SAG-M units of SCD compared to the HbA units. In contrast, considerably higher values of ASAT (median values: 347 U/L and 62.9 U/L) and LDH (median values: 3998 U/L and 792 U/L) were measured in WB/CPDA-1 and PRBC/SAG-M units of SCD blood compared to HbA units.

Osmotic resistance of stored blood units: Osmotic resistance measures the lysis of red cells by photometry at increasing concentration of saline solution. During storage from day 7 to day 35, osmotic resistance did not change in PRBC/SAG-M units of SCD blood (Figure 5G) at the first signs of hemolysis. In WB-CPDA-1 units, osmotic resistance decreased. For achievement of complete hemolysis, osmotic resistance decreased during storage in WB/CPDA-1 (median values: 0, 15 to 0, 39% NaCl) as well as in PRBC/SAG-M units (median values:
Comparing to stored HbA units, no remarkable differences at day 42 were seen for the onset of hemolysis in WB/CPDA-1 and PRBC/SAG-M units. Hemolysis after 24 hours of autoincubation revealed a higher osmotic resistance in SCD blood units compared to the HbA units.

SCI before and after oxygenated cell salvage: Cell salvage and oxygenation were performed in six HBSS-blood units at day 42 (PRBC/SAG-M and WB/CPDA-1, n=3 each). Additionally, blood units of three HbA donors (n=3) underwent the same procedure. The SCI were determined before and after cell salvage and oxygenation from the SCD blood.
The difference of the SCI values indicates the amount of reversible sickle cells (RSCs).

Hemoglobin fractions and Hct levels were measured before the stored blood units were processed. After cell salvage and oxygenation, a significant reduction of the SCI was observed in the WB/CPDA-1 HBSS-units (P = 0.0433). No significant reduction of SCI by cell salvage and oxygenation, however, resulted for RBC/SAG-M units (group comparison by Mann Whitney U-test, P = 0.1913, Figure 6). Less sickled red blood cells were present in one visual field in a Pappenheim stained slide from WB/CPDA-1 after cell processing (Figures 4F) compared to a slide taken before cell processing (Figure 4E).

Viability variables of stored blood units before and after cell salvage and oxygenation: In Table (3), the viability variables (K+, LDH, ASAT, and free Hb) for HBSS- and HBAA-blood units measured before and after cell salvage and oxygenation at day 42 are shown. Overall, a marked reduction of almost all viability variables by cell salvage could be demonstrated.

**DISCUSSION**

A case series on HbSS high risk patients for surgical complications and preoperative high Hbs content was used for this feasibility study. It results in the demonstration of SCI as an easy and safe monitoring method for exchange transfusion in SCD and autologous HbSS blood predonation prior to potential major blood loss surgery.

Using SCI, a reduced Hbs content similar to those in a heterozygous state can be produced and quantified by a mixture of allogeneic and autologous blood management- potentially resulting in a reduced risk for sickling complications in homozygous SCD and reduced exposure to allogeneic blood and alloimmunization. SCI correlates well to ensure the Hbs

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**Table 2: Hematological variables of the stored SCD blood units.**

<table>
<thead>
<tr>
<th>Hemoglobin fraction</th>
<th>PRBC/SAGGM</th>
<th>WB/CPDA-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=4</td>
<td>n=4</td>
</tr>
<tr>
<td>HbA (%)</td>
<td>44.6</td>
<td>31.7</td>
</tr>
<tr>
<td>HbF (%)</td>
<td>8.20</td>
<td>13.8</td>
</tr>
<tr>
<td>HbS (%)</td>
<td>44.4</td>
<td>52.2</td>
</tr>
<tr>
<td>HbA2 (%)</td>
<td>2.80</td>
<td>2.30</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>61.5</td>
<td>54.9</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>4.00</td>
<td>3.55</td>
</tr>
</tbody>
</table>

The variables were determined at the beginning of the storage (day 1).

**Abbreviations:** PRBC: Packed Red Blood Cells; SAG-M: Storage Solution of Red Cells Containing Saline, Adenine, Glucose, Mannitol; WB- Whole Blood; CPDA-1: Storage Solution of Red Cells Containing Citrate, Phosphate, Dextrose-Adenine

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**Table 3: Viability and hemolysis indicators of stored blood units before and after oxygenated cell salvage at day 42.**

<table>
<thead>
<tr>
<th>Additive solution</th>
<th>K+ (mmol/l)</th>
<th>LDH (U/l)</th>
<th>ASAT (U/l)</th>
<th>free Hb (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before CS</td>
<td>after CS</td>
<td>before CS</td>
<td>after CS</td>
</tr>
<tr>
<td><strong>SCD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WB/CPDA-1 (n=3)</td>
<td>28.3 (± 2.75)</td>
<td>1.60 (± 0.00)</td>
<td>3998 (2345)</td>
<td>614 (± 70.5)</td>
</tr>
<tr>
<td>PRBC/SAG-M (n=3)</td>
<td>49.3 (± 2.00)</td>
<td>0.50 (± 0.10)</td>
<td>792 (66.5)</td>
<td>172 (± 19.5)</td>
</tr>
<tr>
<td><strong>HBA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WB/CPDA-1 (n=2)</td>
<td>33.9 (± 5.75)</td>
<td>1.25 (± 0.55)</td>
<td>1101 (397)</td>
<td>178 (± 23.0)</td>
</tr>
</tbody>
</table>

Shown are the mean values and the standard deviations.

**Abbreviations:** CS: Oxygenated Cell Salvage; PRBC: Packed Red Blood Cells; SAG-M: Storage Solution of Red Cells Containing Saline, Adenine, Glucose, Mannitol; WB- Whole Blood; CPDA-1: Storage Solution of Red Cells Containing Citrate, Phosphate, Dextrose-Adenine
level prior to retransfusion and during the surgical procedure. As demonstrated in this study, SCI monitoring has the capacity to indicate the safety of blood products by a threshold of less than 5 sickled cells per 1000 red cells, correlating to a safe HbS content of 40 +/- 5%. Microscopic monitoring of the HbS content by SCI requires prepared and available laboratory personnel to perform the test within the respective time frame. SCI can be produced within 60 minutes. Benefits of this easy cell count under the microscope such as short time to the test's result, low cost and simple equipment offer an alternative to hemoglobin electrophoresis or automated HPLC based perioperative determination of HbS contents [37,38]. SCI successfully was used in this study (i) for monitoring of the exchange transfusion prior to surgery for blood donation and reduction of hemoglobin S content by a limited allogeneic blood transfusion, (ii) guidance of exchange and perioperative transfusion therapy by the sickle cell index, (iii) storage of autologous HbSS blood in regular SAG-M solution, and (iv) retransfusion of stored HbSS blood via modified oxygenated cell saving. In comparison to lysis resistance, SCI better is suited to monitor changes in Hb S contents, since it indicates also lower HbS contents than 60%. Lysis resistance is lost in the HbS range of 50-60%. Using SCI, exchange transfusion could be guided to a target HbS content around 30%, since this is the level of heterozygous sickle trait patients in which sickle cell related complications are extremely rare. Thus, over-transfusion and more than necessary exposure to allogeneic blood potentially can be avoided by this method - even in small health centers with little laboratory capacity.

The secondary aim of the study was to demonstrate the quality of HbS autologous products following storage and autotransfusion in comparison to HbA blood. Since both methods expose HbS red cells to sickle triggers such as low temperature, shear stress, acidity, deoxygenation, hemolysis and sickling reliable is indicated by SCI. SCI indicated that storage of HbSS packed red cell SAG-M solution is preferable over CPD and a reliable and safe method even for higher HbS contents. In congruence to previous results [8], sickling in SAG-M does not occur prior to day 42. Retransfusion of stored sickle cell blood with minor HbS contents clinically should be effective. Castro and co-worker demonstrated sufficient recovery and survival of stored SS erythrocytes in autologous recipients. The storage solution of HbS containing blood should be considered: Although, SCI cannot discriminate reversibly from irreversibly sickled red cells (this would add further time and material requirements to an easy test [39]) in this study, sickling occurred predominantly in WB/CPDA-1. Probably due to a higher ATP content, component production and storage of sickle cell blood in SAG-M is favourable over storage of whole blood in CPDA-1. In comparison to normal HbA blood however, storability of HbS blood is minor due to decreased 2,3-DPG levels and increased indicators of cell lysis (free hemoglobin, LDH, ASAT).

The quality of stored units additionally can be improved by oxygenated cell salvage. Especially units with high SCI levels (>10%) benefit from cell salvage and oxygenation by reduction of SCI. SCI indicated a significant reduction of sickling in previously stored HBSS-units by washing and oxygenation. All cell saver units had tolerable low sickle cell content. Whether the storage solution makes a difference of the sickle cell content or if SCI also can be used for cell salvage of fresh HbS wound blood remains unclear.

Since this is a feasibility study using little numbers, efficacy of this concept could not be demonstrated. However, based on this study, improved perioperative outcome of high risk and alloimmunized SCD patients such as reduced allogeneic transfusion rate without increased sickle cell complication rate can be expected.

In conclusion, SCI monitoring correlates well with the HbS content during various autologous blood conservation techniques for SCD patients. A tight control of HbS content of autologous blood harvested during exchange transfusion, storage or cell salvage in Hb SS patients is feasible by this easy and simple method. Thus, SCI might enable the reduction to alloimmunization of high risk SCD patients by monitoring autologous blood conservation techniques.

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REFERENCES


