Impact of postoperative shed blood transfusion, with or without leucocyte reduction, on acute-phase response to surgery for total knee replacement

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Background: In patients undergoing total knee replacement (TKR) most blood loss occurs postoperatively and return of unwashed filtered shed blood (USB) from postoperative drainage may represent an alternative to allogeneic blood transfusion (ABT). We evaluated the impact of USB return, with or without leucocytes, on the acute-phase response (APR) after TKR.

Patients and methods: Forty-eight TKR patients, intended to receive postoperative USB, entered the study. Blood samples were obtained before and 6, 24, 72 h and 7 days after surgery, and from the USB before and after it passed through a 40-μm filter (Group F40) or a leucocyte-reduction filter (Group LRF). Haematimetric parameters, APR proteins (albumin, prealbumin, ceruloplasmin, haptoglobin, C-reactive protein), complement C3 and C4, and cytokines (IL-1β, IL-6, IL-8, IL-10, and TNF-α) were measured in all samples.

Results: Twenty-eight patients (Group F40 = 14, Group LRF = 14) received a mean of 1.2 USB units, without any clinically relevant incident, and did not need additional ABT. Sixteen out of the 20 remaining patients who received neither USB nor ABT served as a control group for the postoperative APR study. All patients showed the typical postoperative APR profile and there were no significant differences between groups for APR parameters, postoperative complications, or hospital stay.

Conclusions: Postoperative blood salvage and return, with or without a LRF, after TKR does not present any clinically relevant side-effects and does not modify APR induced by surgery. These findings seem to confirm the clinical experience that postoperative USB return is safe and questions the beneficial effect of using LRF.

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Key words: Acute-phase reaction; allogeneic blood transfusion; leucocyte reduction filter; postoperative blood salvage; total knee replacement.

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tumour necrosis factor-α (TNF-α), but not IL1β or IL-6, in drain blood at the 5th postoperative hour, whereas at the same time triggered complement activation. However, they did not assess the impact of this procedure, which clearly involves manipulation of the blood salvage device on the patient’s inflammatory parameters. In the other hand, LRFs have been shown to decrease the number of leucocytes, as well as other debris such as fat particles, during USB transfusion (12), and we therefore evaluated whether a LRF placed in the USB infusion line may also influence the postoperative acute-phase response (APR) in TKR patients.

**Patients and methods**

**Patients and procedures**

After approval by the Ethics Committee of the University Hospital ‘Virgen de la Victoria’ (Málaga, Spain), 48 consecutive primary TKR patients, who were operated on between October 2003 and June 2004 and gave informed consent, entered the study. Patients with clinical signs of infectious disease, history of gastro-duodenal ulcer, bleeding disorder, abnormal hepatic or renal function on routine laboratory analyses, and those with known neoplasia, being treated with immunomodulatory agents (e.g. corticosteroids) within 2 weeks prior to the study, or having a blood transfusion prior to the operation were excluded.

All surgical procedures were performed under regional anaesthesia and involved application of a tourniquet which was deflated before knee closure. A total condylar knee (Duracon, Stryker, Kalamazoo, MI), the tibial component being cemented, was used in all patients. The anaesthesiologist, who was unaware of the study, estimated blood losses both at the operation theatre and at the anaesthesia recovery unit, and performed the return of USB. In the ward, measurement of postoperative blood loss and decisions on postoperative transfusions were made by the attending surgeon.

**Postoperative blood salvage and return**

In all patients, the collection blood canister (ConstaVac CBC II, Stryker, Kalamazoo, MI) was connected at the end of surgery to two drainage catheters through a Y-connector, and a USB was collected without anticoagulant at a negative pressure of 25 mmHg. The blood first passed a 260-μm filter before entering the container. The canister is connected to the return bag to which the USB is transferred, scraping the last 60–80 ml to minimize fat particles and other debris being transfused to the patient, and allowing for several returns if needed. If at least 400 ml of blood was collected within 6 h after surgery, the USB was returned to the patient. During return, the blood passed through an additional 40-μm screen filter (SQ40SJ4KL, Pall Biomedical, Portsmouth, UK) (Group F40) or a leucocyte-reducing filter (PureCell, Pall Biomedical, Portsmouth, UK) (Group LRF) intercalated in the patient’s line. The volume of recovered USB was converted into blood units according to the expression: \( U = \text{USB volume (ml)} \times \text{USB haematocrit (%)}/400 \text{ (ml)} \) (13). Those patients having less than 200 ml of USB collected within 6 h after surgery did not receive USB return and were assigned to the control group.

**Clinical data**

A set of demographic and clinical data was prospectively collected in all patients, including age, gender, height, weight, tourniquet time, operation length, 48-h postoperative blood loss, volume of USB returned, USB transfusion index (units per patient), ABT rate (n, %) and index, postoperative infection, and length of hospital stay (LOS).

**Blood samples**

Five blood samples were obtained from the patients at different perioperative stages: before anaesthesia (sample 1), on the sixth postoperative hour, before USB return (sample 2), and on the first, third and seventh postoperative days (samples 3, 4 and 5). Blood samples were obtained by means of venipuncture and collected in three tubes: one Venoject-EDTA-K₂ (3 ml) and two Venoject-II-AUTOSEP (4 ml) (Terumo, Leuven, Belgium). Additional samples were obtained from the USB, before (reinfusion bag) and after filtration. Serum samples were obtained by means of centrifugation (3000 r.p.m. per 5 min), and aliquot samples were stored at −70°C until assayed.

**Haematological parameters**

Red cell count (RBC), haematocrit, haemoglobin (Hb), total and differential white cell count (WBC) and platelet count were determined using a Pentra 120 Retic cell counter (ABX, Montpellier, France) in blood samples collected in EDTA. The cell counter was calibrated daily and is included in a quality-control programme by the Spanish Association for Haematology and Haemotherapy (AEHH), according to the International Committee for Standardization in Haematology (ICSH).
Measurement of serum proteins
Nephelometric measurements of serum levels of \( \alpha_1 \)-antitripsin (AAT), C-reactive protein (CRP), haptoglobin (HPT), albumin (ALB), prealbumin (PAL), and complement C3 and C4 fractions were performed (Immage II, Beckman-Coulter, Hialeah, FL). The nephelometer was calibrated daily and is included in a quality-control programme by the Spanish Society of Clinical Chemistry (SEQC), according to the National Committee for Clinical Laboratory Standards (NCLLS).

Measurement of cytokines
Plasma concentrations of pro-inflammatory (TNF-\( \alpha \), IL-1\( \beta \), IL-6, IL-8) and anti-inflammatory (IL-10) cytokines were assessed using a solid-phase enzyme-labelled chemiluminiscent immunometric assay (Immulite I, Diagnostics Products Corporation, Los Angeles, CA). Controls for the five cytokines studied provided by the manufacturer were measured routinely with each assay. Calculated intra-assay and interassay coefficients of variance were less than 10%. Assay detection limits (pg ml\(^{-1} \)) were 2 for IL-1\( \beta \) and IL-6, 4 for TNF-\( \alpha \), and 5 for IL-8 and IL-10.

Statistics
All results are presented as the mean ± SD (n) or incidence (n, %). Statistical analysis was carried out using a repeated-measures MANOVA test with a within-factor (up to five levels) and one between-factor (group). An unpaired Student’s \( t \)-test, Chi-squared test, or Fischer’s exact test was used for comparison of non-repeated measures between groups. \( P \)-values < 0.05 were considered statistically significant. All statistical analyses were performed with a SPSS 12.0 package (Licensed to the University of Málaga, Spain).

Results
Clinical outcomes
Although all patients received the postoperative blood collection device, USB return was possible in 28 patients (14 in Group F40 and 14 in Group LRF). In the remaining 20 patients there was low blood loss and less than 400 ml of USB was collected and not reinfused, and these patients served as a control group. There were no differences in age, height or weight between groups, although there were three anaemic patients in the control group, and more men in both USB groups (Table 1). Similarly, there were no differences in operation length, tourniquet time, postoperative infection, or length of hospital stay between groups (Table 1). Patients with USB return received a mean of 1.25 ± 0.70 U pte\(^{-1} \) and 1.22 ± 0.45 U pte\(^{-1} \), for groups F40 and LRF, respectively, without any clinically relevant incident, and no patient required additional ABT (Table 1). Despite a lower mean postoperative blood loss, the overall ABT index in the control group was 0.35 ± 0.75 U pte\(^{-1} \) (Table 1), and the four patients who received ABT were excluded for the APR study.

Laboratory characteristics of unwashed shed blood
As shown in Table 2, samples of USB contained less leucocytes and platelets counts and less haemoglobin than venous blood samples obtained from the patient before return; the values of some USB cellular components being further reduced after filtration through LFR, but not after filtration through F40 (Table 2). The USB also showed a reduction in the concentrations of some serum proteins, such as HPT (Table 2). However, when compared with the patient’s postoperative venous blood samples, there was a significant increase in the concentrations of IL-6, IL-8 and TNF-\( \alpha \) in the USB (Table 2), whereas those of IL-1\( \beta \) and IL-10 were below the limit of detection in most USB samples (data not shown). The values of these biochemical and immunologic parameters were not affected by filtration through either F40 or LRF (Table 2).

Impact of return of filtered drainage blood on patient’s blood parameters
Blood haematology. Despite ABT or USB transfusion, perioperative blood loss induced postoperative anaemia in all patients, with Hb values on the seventh postoperative day being significantly lower than in the preoperative period, without differences between groups (Fig. 1A). As expected, total WBC counts were raised at the end of surgery, but most of the WBC counts returned to preoperative values on the third postoperative day, and there were no differences between groups (Fig. 1B). Platelet counts decreased in the early postoperative period, but they were raised at postoperative day 7 (Fig. 1C), without differences between groups.

Serum cytokine levels. Preoperative IL-6, IL-8 and TNF-\( \alpha \) levels were within the normal range in all patients. Serum IL-8 levels increased at 24 h after
Table 1

Demographic and clinical data of patients undergoing total knee replacement. Control group: no return of postoperative unwashed shed blood (USB); Group F40; return of USB through a 40-μm filter (SQ-40, Pall Biomedical, Portsmouth, UK); Group LRF: return of USB through a leucocyte reduction filter (PureCell, Pall Biomedical).

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Group F40</th>
<th>Group LRF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>20</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>2/18</td>
<td>5/9</td>
<td>3/11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>73 ± 4</td>
<td>71 ± 6</td>
<td>71 ± 6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76 ± 10</td>
<td>75 ± 12</td>
<td>72 ± 12</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>154 ± 9</td>
<td>157 ± 9</td>
<td>156 ± 23</td>
</tr>
<tr>
<td>Surgery time (min)</td>
<td>107 ± 20</td>
<td>95 ± 27</td>
<td>90 ± 18</td>
</tr>
<tr>
<td>Ischaemia time (min)</td>
<td>58 ± 13</td>
<td>61 ± 22</td>
<td>61 ± 22</td>
</tr>
<tr>
<td>48 h blood loss (ml)</td>
<td>324 ± 175</td>
<td>857 ± 555</td>
<td>864 ± 301</td>
</tr>
<tr>
<td>USB reinfused (ml)</td>
<td>0</td>
<td>619 ± 251</td>
<td>577 ± 227</td>
</tr>
<tr>
<td>Patients with ABT (n,%)</td>
<td>4 (20)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Transfusion index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABT (U/pte)</td>
<td>0.35 ± 0.75</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>USB (U/pte)*</td>
<td>0</td>
<td>1.25 ± 0.70</td>
<td>1.22 ± 0.45</td>
</tr>
<tr>
<td>Total (U/pte)</td>
<td>0.35 ± 0.75</td>
<td>1.25 ± 0.70</td>
<td>1.22 ± 0.45</td>
</tr>
<tr>
<td>Postoperative infections (n,%)</td>
<td>1 (5)</td>
<td>1 (7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Hospital stay (days)</td>
<td>12.1 ± 3.1</td>
<td>12.1 ± 2.6</td>
<td>12.3 ± 2.9</td>
</tr>
</tbody>
</table>

ABT = allogenic blood transfusion; U/pte = units per patient.
*USB units = (volume USB × Hb USB)/(400 × preoperative Hb).
All data are the mean ± SD or incidence (%).

surgery and remained slightly elevated at postoperative day 7 (Fig. 2A), whereas serum levels of TNF-α did not change from baseline throughout the perioperative period (Fig. 2B). Serum IL-6 levels increased at 6 h after surgery and remained elevated after 24 and 72 h

Table 2

Haematimetric, biochemical and immunological parameters of postoperative unwashed shed blood (USB) taken from the reinfusion bag at the sixth postoperative hour, before (pre-F) and after (post-F) passing through a 40-μm filter (SQ-40, Pall Biomedical, Portsmouth, UK; Group F40) or a leucocyte reduce filter (PureCell, Pall Biomedical; Group LRF), compared with those in patient’s venous blood obtained simultaneously (postop 6 h).

<table>
<thead>
<tr>
<th></th>
<th>Group F40 (n = 14)</th>
<th>Group LRF (n = 14)</th>
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<tbody>
<tr>
<td></td>
<td>USB pre-F</td>
<td>USB post-F</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>10.8 ± 1.2</td>
<td>10.6 ± 1.7</td>
</tr>
<tr>
<td>Leukocytes (×10^3 μl⁻¹)</td>
<td>2.8 ± 0.8</td>
<td>2.7 ± 0.6</td>
</tr>
<tr>
<td>Platelets (×10^3 μl⁻¹)</td>
<td>28 ± 16</td>
<td>22 ± 15</td>
</tr>
<tr>
<td>Immunoglobulin G (mg dl⁻¹)</td>
<td>715 ± 101</td>
<td>676 ± 141</td>
</tr>
<tr>
<td>Immunoglobulin A (mg dl⁻¹)</td>
<td>178 ± 50</td>
<td>169 ± 49</td>
</tr>
<tr>
<td>Immunoglobulin M (mg dl⁻¹)</td>
<td>89 ± 34</td>
<td>89 ± 37</td>
</tr>
<tr>
<td>Complement C3 (mg dl⁻¹)</td>
<td>98 ± 19</td>
<td>90 ± 21</td>
</tr>
<tr>
<td>Complement C4 (mg dl⁻¹)</td>
<td>25 ± 6</td>
<td>24 ± 7</td>
</tr>
<tr>
<td>Albumin (mg dl⁻¹)</td>
<td>2982 ± 260</td>
<td>2959 ± 552</td>
</tr>
<tr>
<td>Prealbumin (mg dl⁻¹)</td>
<td>19 ± 5</td>
<td>19 ± 5</td>
</tr>
<tr>
<td>α₁-antitrypsin (mg dl⁻¹)</td>
<td>99 ± 30</td>
<td>91 ± 18</td>
</tr>
<tr>
<td>Haptoglobin (mg dl⁻¹)</td>
<td>80 ± 42</td>
<td>74 ± 40</td>
</tr>
<tr>
<td>Ceruloiplasmin (mg dl⁻¹)</td>
<td>26 ± 3</td>
<td>25 ± 5</td>
</tr>
<tr>
<td>C-reactive protein (mg dl⁻¹)</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>IL-6 (pg ml⁻¹)</td>
<td>515 ± 402</td>
<td>461 ± 407</td>
</tr>
<tr>
<td>IL-8 (pg ml⁻¹)</td>
<td>271 ± 196</td>
<td>246 ± 184</td>
</tr>
<tr>
<td>TNF-α (pg ml⁻¹)</td>
<td>22 ± 15</td>
<td>22 ± 16</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01 USB vs. postop. 6 h.
Furthermore, IL-6 serum levels were largely normalized on the seventh postoperative day in both groups (Fig. 3A). Serum levels of IL-1β and IL-10 were below the detection limit of the assay in most perioperative samples (data not shown).

**Serum proteins.** Preoperative serum levels of CRP were within normal range (0–0.8 mg dl$^{-1}$) in all patients. Compared with preoperative values, postoperative serum levels of CRP increased significantly after 24–72 h, and decreased thereafter although remaining above the normal range on the seventh postoperative day ($P < 0.001$) (Fig. 3B). The peak CRP values were observed at 72 h, without differences between groups. Serum levels of AAT and HPT showed a slight decrease at the end of surgery, which was followed by a continuous increase from postoperative day 1 until postoperative day 7, without differences between groups (Fig. 4). Serum levels of ALB and PAL remained below the preoperative values for the entire postoperative time-course, except for those of PAL in groups F40 and LRF which returned to preoperative values by postoperative day 7 (Fig. 4), whereas those of CER did not change significantly throughout the perioperative period in any group (data not shown). Finally, serum levels of C3 and C4 showed a similar postoperative time-course, with those of C3 being significantly increased by postoperative day 7, and there were no differences between groups (data not shown).

**Discussion**

Perioperative collection and reinfusion of autologous blood is beneficial for the patient, as it may decrease the allogenic blood requirements, thus reducing or avoiding the risk of transfusion-associated complications. Preoperative donations of several blood units is becoming standard practice before major elective orthopaedic procedures, and there is a consensus on its safety and efficacy, while intraoperative red cell salvage seems to be cost-effective if blood loss higher than 1500 ml is expected (14). Postoperative return of shed blood in another established method to reduce the need for ABT, with total knee arthroplasty being the operation where it has been used the most (15). There are a number of devices for collecting postoperative shed blood, with the principal differentiating characteristic being the existence or not of a washing process for the salvaged blood. However, in knee surgery this procedure is normally performed using devices that recuperate and re-transfuse USB to the patient. Therefore, this work aimed to assess the haematological, biochemical and
immunological characteristics of USB collected in the postoperative period in elective TKR, and its impact on patient’s inflammatory parameters, and to ascertain whether a LRF placed in the USB infusion line influences the postoperative acute-phase response (APR) in TKR patients

Clinical results
In these series, only 8% (4/48) of the patients needed postoperative ABT. These results agree with those recently published by Strümpel et al. (9) and seem to support the effectiveness of the return of USB as a tool towards the achievement of bloodless knee surgery. In addition, we did not observe any adverse effect of USB return during the study, nor significant differences in the rate of postoperative complications or LOS in patients receiving USB, when compared with the control group (Table 1). This is in agreement with previous reports showing the re-infusion of USB to present very few adverse effects (mostly chills, febricula, tachycardia, and hypotension), which can be prevented by limiting the volume to return, using a slow infusion rate, and discarding the last 50–100 ml of USB, as does the ConstaVac CBC II (13, 16, 17).

Characteristics of postoperative unwashed drainage blood
From a haematological point of view, the USB samples contained lower leucocytes and platelet counts, and lower concentrations of haemoglobin and some proteins than the blood samples drawn from the patients in the early postoperative period (Table 1). The observed reduction in the haematological and biochemical parameters could be due to haemodilution and haemolysis. In regard to haemolysis, high levels of plasma-free haemoglobin has been observed in USB (13), and this most probably accounts for the observed reduction in the HPT levels (Table 2). However, it has been previously reported that if postoperative USB is reinfused up to 15% of the total blood volume (18) or 1000 ml (19), there seems to be enough HPT (an acute phase reactant) in the general circulation to bind free haemoglobin, avoiding possible renal damage (13, 20, 21).

Impact of unwashed shed blood return on patient inflammatory parameters
The content of immunologically bioactive substances, such as cytokines or complement fractions, and activated leucocytes which may modify the immune response of the patient, is one concern reported about the reinfusion of USB (15). In our study, except for a remarkable increase in pro-inflammatory cytokines, IL-6 and IL-8, there were no other major changes in these mediators in USB (Table 2). In addition, most leucocytes were removed when a LRF was used (Table 2). Moreover, after the reinfusion of either whole or leukodepleted USB, serum levels of cytokines, negative acute-phase proteins (ALB, PAL), and positive acute-phase proteins (HPT, AAT, CER), were not significantly different from those measured in the control patients (Figs 2–4), indicating that surgical...
trauma induces an acute-phase response not modified by USB return. These results are in agreement with published data for different orthopaedic surgeries (15, 22, 23), although a transient increase of pro-inflammatory cytokines and their receptors after USB return has been also reported (24). Moreover, in recently published studies, return of whole USB activated systemic immunity after joint replacement as shown by increased frequencies of NK precursors and the concentration of interferon-\(\gamma\), thereby reversing the immunosuppression associated with surgical trauma and blood loss (25), and by increased production of reactive oxygen species by neutrophils, thereby enhancing their bactericidal effects (26). Thus, removal of leukocytes or fluid from USB might remove these benefits. In addition, the beneficial effect of the use of expensive LRF to reduce the cytokine content of USB (11) or to remove some constituents of USB thought to be harmful, such as fat particles (12) or leukocytes (27), has to be also weighed against the
risk of episodes of hypotension due to bradykinin production following platelet exposure to negatively charged leucocyte filters (28).

Conclusions

Postoperative blood salvage and return with the ConstaVac CBC II, either as whole USB or leucocyte-reduced USB, after TKR does not present any clinically relevant side-effects and does not modify APR induced by surgery. However, these conclusions might not be extrapolated to other devices. Moreover, although more research is needed in areas such as activation of blood coagulation (29), these findings seem to confirm the clinical experience that postoperative USB return is safe and effective (6–9, 19–21, 30), and question the need for washing or leucocyte-reduction of postoperative salvaged blood in TRK.

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