Chapter 3

Blood loss in orthotopic liver transplantation: a retrospective analysis of transfusion requirements and the effects of autotransfusion of cell saver blood in 164 consecutive patients

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Abstract
Liver transplantation is associated with excessive blood loss. In order to identify factors influencing blood loss and to provide a basis for a pilot study to evaluate recombinant factor VIIa as a hemostatic agent, a retrospective study was performed in 164 consecutive patients with cholestatic or noncholestatic liver disease, who underwent orthotopic liver transplantation at a single centre between 1989 and 1996. Transfusion of allogeneic and autologous (cell saver) blood was used as a measurement of blood loss. Transfusion requirements were associated with age, gender, primary disease, Child-Pugh classification, serum levels of activated partial thromboplastin time, antithrombin III, urea and creatinine, platelet number, year of transplantation, length of cold ischaemia time and autologous blood transfusion. Of these variables, Child-Pugh classification \((P = 0.001)\), urea \((P = 0.0007)\), year of transplantation \((P = 0.002)\), cold ischaemia time \((P = 0.01)\) and autologous blood transfusion \((P < 0.0001)\) were identified as independent predictors of transfusions requirements by multivariate analysis. Thus, blood loss and transfusion requirements, depend primarily on the severity of liver disease, quality of the donor liver, experience of the transplantation team and use of autologous (cell saver) blood transfusion. These findings emphasise the need for appropriate drug therapy and a critical reappraisal of current transfusion policy.
**Introduction**

The first human liver transplantation was performed in 1963 by Starzl (1). In the early years of transplantation success failed because of frequent perioperative exsanguination (2). Although advances in surgical and anaesthesiological techniques and in graft preservation have reduced blood loss (3), uncontrollable bleeding still occurs, necessitating the use of large amounts of blood products (4,5). Primary causes of massive blood loss are surgical bleeding and impaired haemostasis (6). Surgical blood loss is aggravated by portal hypertension associated with the formation of numerous large collaterals in end stage liver disease (3,7,8). Impaired haemostasis in liver disease arises for a number of reasons, including preoperative and perioperative disturbances in the platelet-vessel interaction, and changes to the coagulation system and/or the fibrinolytic system (5,6).

Excessive blood loss during liver transplantation is related to increased risk of postoperative morbidity and mortality (2,6,9). Blood transfusion in these immuno-compromised patients has been correlated with an increased risk of infections (10,11). Autologous transfusion of blood salvaged during surgery is advocated to reduce morbidity caused by exposure to allogeneic blood and to reduce demand on blood bank supplies.

The present study sought to identify predictors of blood loss during liver transplantation and to evaluate the putative benefits of cell saving. Study results will provide reference data for a pilot study evaluating the use of recombinant activated factor VII (rFVIIa; NovoSeven®, Novo Nordisk A/S, Bagsvaerd, Denmark) in the management of bleeding during orthotopic liver transplantation.

**Materials and methods**

*Patients*

Blood loss and transfusion requirements were studied retrospectively in 198 consecutive adult patients who underwent primary orthotopic liver transplantation in our hospital between January 1989 and December 1996. To obtain a homogeneous group, patients with fulminant liver failure (n = 23), familial amyloid polyneuropathy (n = 3), haemangioma (n = 2), Budd Chiari’s syndrome (n = 5) and angiosarcoma (n = 1) were excluded from the study. The final analysis contained the remaining 164 patients with cholestatic (n = 60) or noncholestatic liver disease (n = 104).
Anaesthesia

Total intravenous anaesthesia was performed using sufentanil, midazolam and vecuronium. Patients were ventilated in a volume-controlled mode with an oxygen/air mixture (fractional inspired oxygen = 40%). Volatile anaesthetics were not used. Arterial and central venous catheters were inserted for haemodynamic monitoring and blood sampling. Two intravenous cannulas (14 G) were secured for transfusion purposes. Glucose, calcium chloride and potassium chloride were administered intravenously to maintain plasma concentrations within normal ranges. Dopamine (5 μg/kg/min) was continuously infused. Of 83 patients transplanted from March 1991 to March 1994, 68 patients received aprotinin. An initial intravenous dose of 2 million kallikrein inactivator units (KIU) was administered, followed by continuous intravenous infusion of 0.5 million KIU/h until the end of the operation.

Operative techniques

Donor livers were harvested from haemodynamically stable brain-dead donors with normal or near normal liver function tests. Organs were harvested using standard procedures (12). All donor livers were perfused and stored at 4°C until transplantation in University of Wisconsin solution. ABO-incompatible donors were not accepted. Orthotopic liver transplantation was performed using standard surgical techniques as described by Starzl (3). In 136 patients (83%) a veno-venous bypass was used. The splanchnic system was drained by insertion of a cannula in the portal vein (n = 70,52%) (13) or in the inferior mesenteric vein (n = 55, 40%) (14). In the remaining 11 patients (8%), the femoral vein was drained with a portosystemic shunt. In all cases, drained blood was returned by a biopump to the axillary vein. The ‘Piggyback’ procedure was applied in seven of the remaining 28 transplantations that were performed without a veno-venous bypass (15).

Transfusion protocol.

The transfusion regimen was standardised. Blood loss was counteracted by transfusion of allogeneic packed red blood cells. Ninety-nine patients also received autologous blood collected with a cell saver (Haemonetics, MA, USA). The aim was to maintain a haematocrit level of 0.30. Plasma was administered to maintain the prothrombin time (PT) at < 1.5 fold the upper limit of the normal range, i.e. < 24 s, and cryoprecipitate was administered to
maintain fibrinogen levels above 1.0 g/l. Six (donor) units of platelet concentrates were given at platelet counts below 50 x 10⁹/l. Saline (NaCl 0.9%) was continuously supplied at a rate of 5 to 15 ml/kg/h to compensate for fluid loss by evaporation. A plasma expander (Gelofusine) was administered if central venous pressure dropped below 6-8 mmHg.

Laboratory
Haematological (haematocrit, number of platelets) and coagulation [PT, activated partial thromboplastin time (APTT), fibrinogen and antithrombin III (ATIII)] parameters, as well as blood chemistry (sodium, potassium, urea, creatinine and urate serum levels) were measured preoperatively. Serial arterial blood samples were taken every 15 minutes in the anhepatic stage and every 30 minutes in the pre- and postanhepatic stage to monitor haematocrit, platelet numbers, sodium, potassium, calcium, glucose, PT, APTT, fibrinogen, ATIII, and for blood gas analyses.

Statistical methods
The total amount of allogeneic and autologous red blood cell concentrates (RBC) administered, was used as a measure of blood loss. A unit of allogeneic (bank) blood or autologous (cell saver) blood had a volume of 250 ml and a haematocrit of 0.70. Univariate analysis was used to assess a relationship between transfused RBC and patient characteristics. These included gender, age, primary disease, Child-Pugh classification, previous right upper abdominal operations, preoperative coagulation parameters, platelet numbers and serum levels of urea and creatinine. Child-Pugh classification A, B or C expressed the severity of liver disease (16). Previous right upper abdominal surgery included major operations of the liver and biliary tract and portal decompression procedures, such as portal caval shunts. The relationship between transfused RBC and cold ischaemia time, year of transplantation, type of veno-venous bypass (if applicable) and individual surgeons and anaesthetists were also analysed. Patients were arbitrarily assigned to three groups based on the cold ischaemia time: group A (4-10h), group B (10-15h) and group C (15-24h). Finally, the relationship between transfused RBC and transfusion of autologous (cell saver) blood was assessed. The X² test or Fisher’s exact test were used for qualitative variables and Student’s T test or the Mann Whitney U test were used for quantitative variables. Multiple logistic regression analysis was
performed to identify predictors of RBC transfusion requirements. All characteristics that showed a $P$-value of $<0.15$ in the univariate logistic analysis were included in the multiple regression model. Using backward selection with relevant first order interactions the final multiple regression model for RBC transfusion requirements was constructed with only significant variables. $P$-values $<0.05$ were considered statistically significant; all tests were two sided. The SAS version 6.12 (Cary, North Carolina, USA) was used for all analyses.

Results
Of 164 adult liver transplant patients with cholestatic ($n = 60$) or noncholestatic ($n = 104$) liver disease, 87 were female and 77 male. Their age ranged from 17 to 66 years (mean ± SD, 43 ± 13). The category cholestatic liver disease included primary biliary cirrhosis ($n = 26$), primary sclerosing cholangitis ($n = 32$) and secondary biliary cirrhosis ($n = 2$). The noncholestatic liver disease category included all other diagnoses. Liver disease was classified as Child A in 12 patients (7%), Child B in 45 patients (27%) and Child C in 107 patients (65%). Fifty patients (30%) had a previous right upper abdominal operation. Mean preoperative values (± SD) of coagulation parameters were: PT 21 ± 8 s, aPTT 44 ± 18 s, fibrinogen 2.6 ± 1.4 g/l, and AT III 51 ± 26 % (normal ranges of PT = 11-16 s, aPTT = 26-36 s, fibrinogen = 1.7-3.5 g/l and AT III = 80-120%). Variables selected by univariate analysis as possible determinants of the need for RBC transfusions are summarised in Table 1 (categorical variables) and Figure 1 (continuous variables). Men required more RBC than women, and patients with Child B or C liver disease required more than those with Child A. There was a trend towards increased transfusions in patients with noncholestatic liver disease, compared to those with cholestatic liver disease, and in patients who had previous right upper abdominal operations. Transfusion requirements increased with length of cold ischaemia time and use of cell saver blood. Transfusion requirements also increased with age, baseline values of aPTT and serum levels of urea (Figure 1) and creatinine (not shown) ($r = 0.1$, $p = 0.06$), but were inversely related to AT III levels and platelet numbers at baseline. Patients received a mean (± SD) of 40 (± 37) units (allogeneic and autologous) blood in 1989, compared to 22 (± 22) units in 1996, suggesting a reduction in transfusion requirements with time. RBC transfusions needs in the 68 patients who received aprotinin were similar to the 96 patients who did not (24.3 U versus 22.0 U).
With regard to portal or inferior mesenterical veno-venous bypasses, no significant difference in RBC transfusions was demonstrated (22.4 U versus 22.4 U).

**Table 1** Transfusion requirements in liver transplantation - univariate analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>category</th>
<th>N</th>
<th>RBC (U) Transfusion&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>male</td>
<td>87</td>
<td>27 ± 21</td>
<td>P = 0.002</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>77</td>
<td>19 ± 15</td>
<td></td>
</tr>
<tr>
<td>Primary disease</td>
<td>cholestatic</td>
<td>60</td>
<td>20 ± 17</td>
<td>P = 0.051</td>
</tr>
<tr>
<td></td>
<td>non-cholestatic</td>
<td>104</td>
<td>25 ± 19</td>
<td></td>
</tr>
<tr>
<td>Severity of liver disease</td>
<td>Child-Pugh A</td>
<td>12</td>
<td>10 ± 5</td>
<td>A vs B: P = 0.02</td>
</tr>
<tr>
<td></td>
<td>Child-Pugh B</td>
<td>45</td>
<td>21 ± 15</td>
<td>A vs C: P = 0.005</td>
</tr>
<tr>
<td></td>
<td>Child-Pugh C</td>
<td>107</td>
<td>26 ± 19</td>
<td></td>
</tr>
<tr>
<td>Previous operation</td>
<td>yes</td>
<td>50</td>
<td>27 ± 19</td>
<td>P = 0.1</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>114</td>
<td>22 ± 18</td>
<td></td>
</tr>
<tr>
<td>CIT*</td>
<td>A</td>
<td>4 – 10 h</td>
<td>39</td>
<td>18 ± 14</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>10 – 15h</td>
<td>72</td>
<td>22 ± 14</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>15 – 24h</td>
<td>34</td>
<td>32 ± 27</td>
</tr>
<tr>
<td>Use of cell saaver blood</td>
<td>yes</td>
<td>99</td>
<td>27 ± 20</td>
<td>P = 0.0003</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>65</td>
<td>18 ± 12</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>mean ± SD of blood (RBC) transfusions  *retrospective data not traced in all patients

*RBC, red blood cell concentrates; CIT, cold ischaemia time*
Figure 1 Prognostic factors of transfusion requirements in liver transplantation, univariate analysis. RBC, red blood cell concentrates

Multivariate analysis identified gender, Child-Pugh classification, serum urea level, year of transplantation, cold ischaemia time and use of autologous (cell saver) blood as independent predictors of RBC transfusion requirements (Table 2).

Table 2 Prognostic factors of transfusion requirements in liver transplantation, multivariate analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>Standard error</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, male vs female</td>
<td>0.44</td>
<td>0.09</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Child-Pugh</td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>class A vs C</td>
<td>-0.60</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>class B vs C</td>
<td>0.007</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>0.021</td>
<td>0.006</td>
<td>0.0007</td>
</tr>
<tr>
<td>Year of transplantation</td>
<td>-0.081</td>
<td>0.025</td>
<td>0.002</td>
</tr>
<tr>
<td>Cold ischaemia time</td>
<td>0.005</td>
<td>0.0002</td>
<td>0.01</td>
</tr>
<tr>
<td>Use of autologous blood</td>
<td>0.17</td>
<td>0.03</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Patients receiving cell saver blood had greater overall blood and plasma requirements than patients in whom this procedure was not used (Figure 2, Table 3).

Figure 2 Relation of autotransfusion of cell saver blood and total amount of allogeneic and autologous blood transfused in liver transplantation patients.

RBC, red blood cell concentrates

![Graph showing the relationship between units of RBC (allogeneic + autologous) and units of autologous blood. The graph includes a line with a positive slope, indicating a positive correlation. The text states that the correlation is statistically significant with a p-value of less than 0.01 and a correlation coefficient r of 0.83.

Table 3 Blood product consumption in patients receiving autologous (cell saver) blood in addition to allogeneic blood versus patients only receiving allogeneic blood

<table>
<thead>
<tr>
<th>Transfusion</th>
<th>Without cell saver N=65</th>
<th>With cell saver N=99</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total blood (U)</td>
<td>17 ± 12</td>
<td>27 ± 20</td>
<td>0.0004</td>
</tr>
<tr>
<td>Allogeneic blood (U)</td>
<td>17 ± 12</td>
<td>17 ± 10</td>
<td></td>
</tr>
<tr>
<td>Autologous blood (U)</td>
<td>0</td>
<td>10 ± 12</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>32 ± 14</td>
<td>39 ± 16</td>
<td>0.005</td>
</tr>
<tr>
<td>Cryoprecipitate (ml)</td>
<td>219 ± 150</td>
<td>256 ± 158</td>
<td>0.1</td>
</tr>
<tr>
<td>Platelets (ml)</td>
<td>207 ± 117</td>
<td>214 ± 78</td>
<td>0.6</td>
</tr>
</tbody>
</table>
Discussion

The present study showed that gender, Child-Pugh classification, serum urea level, year of transplantation, length of cold ischaemia time and use of autologous (cell saver) blood were the primary determinants of RBC transfusions requirements during liver transplantation surgery.

RBC transfusion requirements provide an easily quantifiable measure of blood loss, since the accuracy of direct measurement can be hampered both by ascites and ongoing intraperitoneal hypersecretion. Since RBC transfusions are commonly given according to haematocrit values, lower haematocrit levels at baseline are more likely to result in a need for transfusions, although actual blood loss may remain limited. In this study, baseline haematocrit values were normally distributed and are unlikely to have influenced the results of our analysis. Analysis of blood loss (data not shown) revealed similar results to our analysis of RBC transfusions. Women received a lower volume of RBC than men, a difference attributed to their smaller body size. When adjusted for body surface, RBC requirements are comparable in woman and men (P = 0.2). Transfusions requirements are dependent on severity of liver disease (Child-Pugh classification), surgical procedure (year of transplantation) and condition of the graft (cold ischaemia time). In addition, RBC transfusions are related to the use of autologous blood. The Child-Pugh classification assesses reduction in preoperative albumin, increases in creatinine serum levels, prolongation of PT, presence of ascites and grade of encephalopathy. With the exception of encephalopathy, each of these characteristics has been associated with increased intraoperative blood loss in previous studies (17-20). Univariate analysis revealed the existence of a relationship between RBC transfusions and preoperative values of aPTT, ATIII, platelet number and serum levels of urea and creatinine. However, with the exception of serum urea levels, multivariate analysis did not identify these parameters as independent predictors of transfusion requirements. This is probably because they all express severity of liver disease that was already represented by the Child-Pugh classification as a composite variable in the multiple logistic regression analysis. The year of transplantation as a determinant of required transfusion requirements suggests a positive effect of increasing experience within the transplantation team, as has been demonstrated in other studies (20,21). It should be noted, however, that the administration of aprotinin to the majority of patients transplanted between March 1991 and March 1994 may have influenced transfusion requirements. The
value of aprotinin in liver transplantation in terms of a reduction of blood loss and the need for transfusion has been recently established (22). However, no difference in transfusion requirements between those patients administered aprotinin and those who were not was observed in the present study. This may be a result of the selective administration of aprotinin to patients who were at high risk of bleeding. The observation that RBC transfusion requirements increased with the length of cold ischaemia time has not been consistently reported (18,19,23-25). A longer cold ischaemia time may result in more blood loss, due to poor initially function of the graft – a reflection of preservation damage. Improved long-term clinical outcome and higher survival rate has been reported for cold ischaemia times of < 12 hours (26), and may be related to reductions in blood loss. This is supported by our finding that mortality within three months of transplantation is markedly related to transfusion requirements (P < 0.0001).

Remarkably, transfusion requirements were significantly increased in patients to whom cell saver blood was returned (P < 0.0001). The cell saver was not routinely used during the study and use of this procedure was dependent on the availability of the cell saver rather than on the amount of blood loss. It is therefore, likely that excessive blood loss was a consequence rather than a cause of transfusion of cell saver blood. This finding is in agreement with some previous studies, in which autotransfused patients received more RBC, fresh frozen plasma, cryoprecipitate and/or platelets than patients not undergoing this procedure (27,28).

Differences were not statistically significant, while in earlier studies patient number was too small to demonstrate statistical significance. A possible explanation for the increased blood loss in patients receiving cell saver blood is the release of fibrinolytic compounds from blood cells in the collected blood and/or from the transplanted liver, that are not washed out by the cell saver.

Although we recognise the limitations of a retrospective study, it should be noticed that our study contained a large, homogeneous group of consecutive patients. Attempts to reduce blood loss and transfusion requirements should focus on repair of haemostasis, further improvement of surgical and anaesthetic techniques and preservation of the donor liver. In particular, drug therapy should be considered, as the current use of supplementary coagulation factors and platelets is obviously insufficient to correct the multiplicity of hemostatic disturbances. The antifibrinolytic potency of aprotinin (22) and the coagulation-enhancing
action of rFVIIa (29) make them suitable candidates.

In conclusion, this retrospective study shows that transfusion requirements, and hence blood loss, during orthotopic liver transplantation depend on severity of liver disease, quality of the donor liver and experience of the transplantation team. Autologous blood transfusion by a cell saver unexpectedly increased transfusion requirements. These findings emphasise the need for an evaluation of prohaemostatic drugs and a critical reappraisal of current transfusion policy to reduce blood loss. The data obtained from this retrospective analysis will provide a reference for a pilot study to evaluate rFVIIa, a prohaemostatic drug, for the management of excessive blood loss during orthotopic liver transplantation.
References

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