Blood Markers of Portal Hypertension Are Associated with Blood Loss and Transfusion Requirements during Orthotopic Liver Transplantation

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Blood Markers of Portal Hypertension Are Associated with Blood Loss and Transfusion Requirements during Orthotopic Liver Transplantation

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Abstract

There is increasing evidence that portal hypertension plays a major role in bleeding risk during orthotopic liver transplantation (OLT). We investigated the association between preoperative blood levels of von Willebrand factor (VWF) and soluble CD163 (sCD163), which are established markers of portal hypertension, and blood loss and transfusion requirements during OLT. We measured levels of VWF and sCD163 in preoperative serum samples of 168 adult patients undergoing a primary OLT between 1998 and 2012. Preoperative levels of VWF and sCD163 correlated with the model of end-stage liver disease (MELD) score ($r = 0.414$, $p < 0.001$ and $r = 0.382$, $p < 0.001$, respectively). Patients with high VWF or sCD163 levels (VWF and sCD163 levels above the median) had a substantially increased risk of needing red blood cell transfusion compared with patients with low VWF or sCD163 levels (VWF and sCD163 levels below the median) (odds ratio 3.5 [95% confidence interval, CI 1.7–7.0] and 2.3 [95% CI 1.1–4.5], respectively). Blood loss was highest in patients with both high VWF or sCD163 levels and a high preoperative international normalized ratio. Elevated blood levels of markers of portal hypertension are associated with increased blood loss and transfusion requirements during OLT and support the notion that portal hypertension is an important contributor to perioperative blood loss.

Keywords
- cirrhosis
- red blood cell
- von Willebrand factor
- CD163
- bleeding

Orthotopic liver transplantation (OLT) can be complicated by substantial perioperative blood loss which frequently necessitates transfusion of blood products. As a result of medical and technical advances, perioperative transfusion requirements have declined substantially over time.[1,2] Nevertheless, a substantial proportion of patients still require blood product transfusions, which are associated with significant side effects.[3–6] We have previously demonstrated a dose-dependent increase in postoperative mortality as a consequence of blood product transfusion during OLT,[3] which is in line with studies on other surgical procedures, notably cardiac surgery.[5]

The perioperative transfusion requirements in patients with cirrhosis undergoing OLT have historically been attributed to preoperative changes in the hemostatic system, which further aggravate intraoperatively.[7–10] Clinical and laboratory studies from the past 15 years, however, have demonstrated that the hemostatic balance in patients with cirrhosis and in patients during OLT is relatively well preserved, although routine indices of hemostasis (platelet count, prothrombin time) may suggest otherwise.[11–13] In addition, it has been demonstrated that such routine diagnostic tests fail to predict perioperative blood loss.[14]

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Apart from the preexisting coagulopathy, portal hypertension has been implicated as a contributor to perioperative blood loss during OLT.[2,15] Multiple centers have reported reductions in blood transfusion requirements when enforcing a restricted fluid infusion policy, which is aimed at preventing venous congestion and aggravation of portal hypertension.[16,17] One center has taken this concept a step further and performs preoperative phlebotomy in a proportion of patients to lower central venous pressure to prevent excessive blood loss.[18] Despite indirect clinical evidence, no studies providing direct evidence for a role of portal hypertension in bleeding during OLT have to our knowledge been performed. The difficulty in quantifying portal hypertension (e.g., by hepatic-venous pressure gradient [HVPG] measurements) is likely responsible for this lack of data.

Recently, it was shown that plasma levels of von Willebrand factor (VWF) and soluble CD163 (sCD163) in patients with cirrhosis correlate with HVPG and thus may be useful minimally invasive markers for portal hypertension.[19–21] VWF is released from endothelial cells, whereas sCD163 is the extracellular part of a transmembrane receptor specific for macrophages (including Kupffer cells). It thus appears that VWF and sCD163 relate to portal hypertension via distinct mechanisms. In this study we investigated the hypothesis that high preoperative blood levels of VWF and sCD163 are associated with increased blood loss and transfusion requirements during OLT.

Methods

Patients

Since 1996, we prospectively collect serum samples from patients who are candidates for OLT in the University Medical Center Groningen. Between 1996 and 2012, 833 patients underwent OLT in our center. We excluded patients undergoing a retransplantation ($n = 145$), pediatric patients (defined as age < 18 years at the time of OLT, $n = 301$), and patients undergoing OLT for acute liver failure ($n = 63$). Of the remaining 325 patients, serum samples taken within 30 days prior to OLT were available for 168 patients.

Ethics Statement

This study was approved by the Institutional Review Board of the University Medical Center Groningen.

VWF and sCD163 Analyses

We determined VWF antigen and sCD163 antigen levels with enzyme-linked immunosorbent assays using polyclonal antibodies to VWF (Dako) and monoclonal antibodies to sCD163 (R&D System).

Demographic Data

Patient charts were used to collect demographic data, type of liver disease, preoperative international normalized ratio (INR), model of end-stage liver disease (MELD) score, and estimated blood loss (referred to as blood loss) and red blood cell (RBC) transfusion requirements during OLT.

Statistical Analyses

Data are presented as medians with interquartile range (IQR) or as numbers with percentages. The Pearson's correlation coefficient was used to determine correlations between VWF, sCD163, the INR, blood loss, and transfusion requirements. We additionally analyzed the relation between VWF and sCD163 levels and the INR and blood loss/transfusion requirements by defining high and low levels. For this, data were divided into categories of patients with levels below or at and above the median. The Mann Whitney-U and the Kruskal–Wallis test were used to determine differences in blood loss and transfusion requirements between the different categories of VWF-, sCD163-, and INR levels, as appropriate. The risk for RBC transfusion for the different categories was determined using the Fisher's exact test and odds ratios (ORs) were calculated with 95% confidence intervals (CIs) according to the approximation of Woolf. Analyses were performed using the statistical software package SPSS/PC 22.0 (SPSS Inc.) and GraphPad Prism.

Results

Patient Characteristics

Patient characteristics and data on surgical and transfusion variables are summarized in ►**Table 1**.

VWF and sCD163 Levels

We measured levels of VWF and sCD163 in serum samples in 168 patients for whom a serum sample was available. The median VWF level was 37.5 µg/mL (IQR 23.6–58.9) and the median sCD163 level was 1,330 ng/mL (IQR 761–2189). There was a significant linear correlation between VWF- and sCD163-levels, and both markers also correlated with the MELD score (►**Table 2**).

Increased Blood Loss and RBC Transfusion in Patients with High Levels of VWF and sCD163

We analyzed blood loss and RBC transfusion requirements in patients with high or low levels of VWF and sCD163 as defined in earlier sections. Patients with high serum levels of VWF or sCD163 had significantly increased blood loss and RBC transfusion requirements compared with patients with low levels of these markers. In addition, patients with a high preoperative INR had increased blood loss and RBC transfusion requirements (►**Figs. 1** and **2**).

When patients had a combination of a high INR and high VWF or sCD163 levels, blood loss and RBC transfusion requirements were higher as compared with those patients with a low INR and high VWF or sCD163 levels and vice versa (►**Table 3**). Also, a combination of high VWF and high sCD163 levels was associated with increased blood loss and RBC transfusion requirements as compared with low levels of either or both VWF and sCD163. Finally, a combination of high MELD score and high VWF or sCD163 levels was associated with higher blood loss and RBC transfusion requirements compared with patients with low MELD and high VWF or sCD163 levels and vice versa.

Table 1 Patient characteristics

Patient population ($n = 168$)		
Age (y)		52 (46–58)
Gender	Female	74 (44%)
	Male	95 (56%)
Liver disease	Biliary	40 (24%)
	Postnecrotic	65 (38%)
	Metabolic	20 (12%)
	Cryptogenic	17 (10%)
	Miscellaneous	27 (16%)
MELD-score		13 (9–20)
INR		1.3 (1.1–1.6)
Duration of surgery (min)		609 (515–700)
Cold ischemia time (min)		464 (412–574)
Warm ischemia time (min)		46 (40–57)
Implantation	Piggyback	144 (85%)
	Conventional	24 (14%)
Donor type	Donation after brain death	125 (74%)
	Donation after circulatory death	31 (18%)
	Domino	1 (0.6%)
Blood loss (mL)		3,440 (1,800–6,725)
RBC transfusion (mL)		750 (0–2,100)
FFP transfusion (mL)		0 (0–900)
Platelet transfusion (mL)		0
Transfusion-free OLT		51 (30%)

Abbreviations: FFP, fresh frozen plasma; IQR, interquartile range; MELD, model of end-stage liver disease; OLT, orthotopic liver transplantation; RBC, red blood cell.
Note: Data are presented as medians with IQR or number with percentages. Total numbers may be less than 168, representing missing data.

Table 2 Correlations between the various markers, EBL, and RBC transfusion

	INR	VWF	sCD163
INR			
VWF	0.281***		
sCD163	0.282***	0.470***	
Adjusted MELD score	0.686***	0.414***	0.382***

Abbreviations: EBL, estimated blood loss; INR, international normalized ratio; MELD, model of end-stage liver disease; RBC, red blood cell; sCD163, soluble CD163; VWF, von Willebrand factor.
Note: Numbers represent Pearson's correlation-coefficient.
*indicate statistical significance with $p < 0.05$
**indicate $p \leq 0.01$
***indicate $p \leq 0.001$.

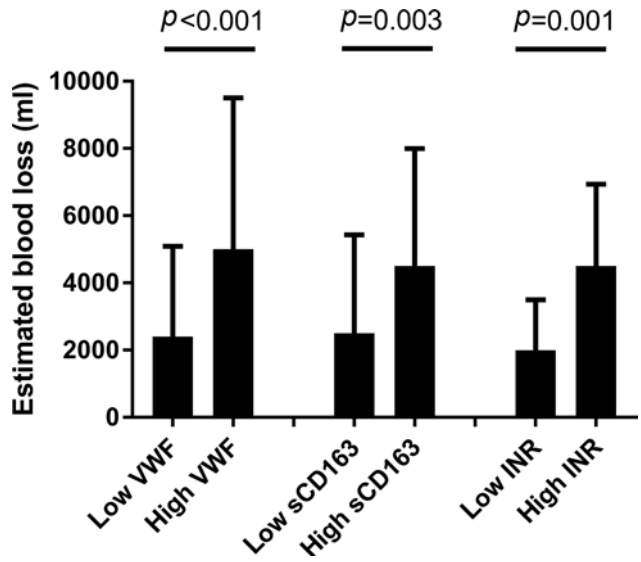

Fig. 1 Blood loss in patients stratified according to serum levels of VWF or sCD163, or according to INR. High levels represent values above, low values below the median. Bars indicate medians, error bars indicate interquartile range. INR, international normalized ratio; sCD163, soluble CD163; VWF, von Willebrand factor.

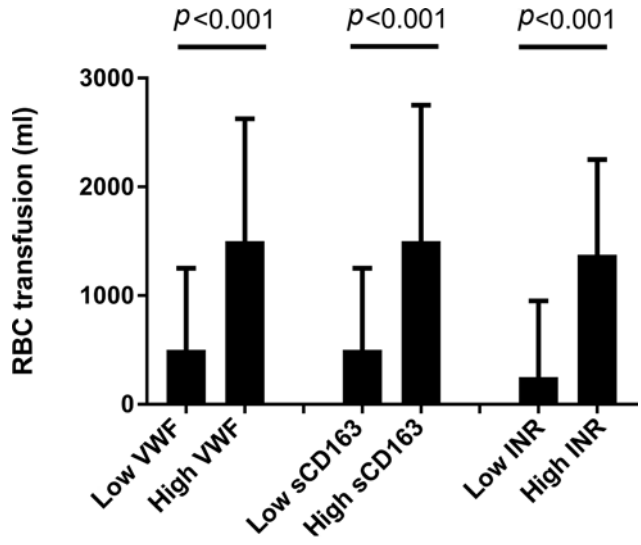

Fig. 2 RBC transfusion requirements in patients stratified according to serum levels of VWF or sCD163, or according to INR. High levels represent values above, low values below the median. Bars indicate medians, error bars indicate interquartile range. INR, international normalized ratio; RBC, red blood cell; sCD163, soluble CD163; VWF, von Willebrand factor.

More Transfusion Free-OLT in Patients with Low Levels of VWF and sCD163

The proportion of patients undergoing OLT without the requirement for RBC transfusion was 43% with low levels of VWF compared with 18% in the group of patients with high levels of VWF. This translates to a more than threefold increased risk of receiving RBCs in patients with high preoperative VWF levels (OR 3.4 [95% CI 1.7–7.0]). The proportion of patients that did not require RBC transfusion was 39% with low preoperative levels of sCD163 versus 22% in those with high sCD163 levels (OR 2.3 [95% CI 1.1–4.5]). Patients

Table 3 Blood loss and RBC transfusion requirements in different categories of INR, VWF, and sCD163

A					
	Low INR and low VWF ($n = 44$)	**Low INR and high VWF ($n = 22$)**	**High INR and low VWF ($n = 32$)**	**High INR and high VWF ($n = 49$)**	***p*-Value**
Blood loss (mL)	2,000 (1,050–3,420)	3,750 (2,000–6,000)	3,500 (1,525–5,950)	5,000 (2,500–8,615)	≤ 0.001
RBC transfusion (mL)	0 (0–750)	1,000 (63–2,437)	750 (0–2,000)	1,750 (500–2,500)	≤ 0.001
B					
	Low INR and low sCD163 ($n = 49$)	**Low INR and high sCD163 ($n = 17$)**	**High INR and low sCD163 ($n = 30$)**	**High INR and high sCD163 ($n = 51$)**	***p*-Value**
Blood loss (mL)	2,000 (1,200–3,500)	3,250 (1,525–6,137)	4,500 (1,500–10,480)	4,500 (2,350–7,300)	0.001
RBC transfusion (mL)	0 (0–750)	1,000 (0–2,250)	1,250 (500–2,375)	1,500 (500–2,500)	≤ 0.001
C					
	Low VWF and low sCD163 ($n = 49$)	**Low VWF and high sCD163 ($n = 17$)**	**High VWF and low sCD163 ($n = 30$)**	**High VWF and high sCD163 ($n = 51$)**	***p*-Value**
Blood loss (mL)	2,000(1,200–4,000)	3,500 (1,650–6,500)	4,500 (1,850–10,480)	5,000 (2,500–8,750)	≤ 0.001
RBC transfusion (mL)	250 (0–775)	1,250 (0–2,250)	1,250 (500–2,757)	1,625 (500–2,688)	≤ 0.001
D					
	Low MELD and low VWF ($n = 40$)	**Low MELD and high VWF ($n = 23$)**	**High MELD and low VWF ($n = 27$)**	**High MELD and high VWF ($n = 51$)**	***p*-Value**
Blood loss (mL)	1,950 (1,025–2,925)	3,000 (1,500–6,100)	2,150 (1,000–5,950)	5,000 (2,650–7,700)	< 0.001
RBC transfusion (mL)	0 (0–500)	750 (0–1,500)	750 (0–2,000)	1,750 (750–2,625)	≤ 0.001
E					
	Low MELD and low sCD163 ($n = 45$)	**Low MELD and high sCD163 ($n = 17$)**	**High MELD and low sCD163 ($n = 27$)**	**High MELD and high sCD163 ($n = 51$)**	***p*-Value**
Blood loss (mL)	2,000 (1,050–3,220)	3,000 (1,850–5,800)	4,500 (1,500–7,400)	4,000 (2,350–6,750)	0.001
RBC transfusion (mL)	0 (0–775)	500 (0–2,000)	750 (0–220)	1,500 (500–2,250)	≤ 0.001

Abbreviations: INR, international normalized ratio; MELD, model of end-stage liver disease; RBC, red blood cell; sCD163, soluble CD163; VWF, von Willebrand factor.
Note: Data are presented as medians with IQR. "High" represents values above the median; "low" represents values below the median. Total number (*n*) is less than 168, as a result of missing data. *p*-Values were calculated using the Kruskal–Wallis test.

with a low preoperative INR were also more likely not to require RBC transfusion compared with those with high preoperative INR levels (44 vs. 22%, OR 2.8 [95% CI 1.4–5.8]).

More Massive Transfusions in Patients with High Levels of VWF and sCD163

The proportion of patients undergoing OLT with the requirement for 6 or more units of RBC (i.e., a massive transfusion) was 13% in the low VWF group compared with 36% in the high VWF group. This translates to an almost fourfold increased risk of a massive transfusion in patients with high VWF levels (OR 3.9 [95% CI 1.8–8.3]). Similarly, 15% of the patients in the low sCD163 group required massive transfusion, compared with 34% in the high sCD163 group, OR 2.9 (95% CI 1.4–5.8).

Discussion

Our data show that patients with high preoperative levels of VWF and sCD163, which are established makers for portal hypertension, have a significantly higher risk for RBC transfusion, experience more blood loss, and require more RBC transfusions compared with patients with low values of these markers. High preoperative INR values and a combination of a high INR and high levels of VWF or sCD163 also constitute an increased risk for bleeding and RBC transfusion. Thus, perioperative blood loss appears to be dependent on a combination of severity of liver dysfunction or derangement in hemostasis (as indicated by the INR), and portal hypertension (assessed by serum levels of VWF and sCD163). Although portal hypertension is increasingly considered a prime determinant of blood loss during OLT, this study is to our knowledge the first to link established markers for portal hypertension with intraoperative blood loss. In addition, the results of our study suggest an interaction between portal hypertension and the extent of liver synthetic dysfunction in determining bleeding risk.

Although serum levels of VWF and sCD163 correlated moderately well, blood loss was clearly higher in those patients with elevated levels of both markers compared with patients with elevated levels of only one of the two. As VWF and sCD163 assess distinct consequences of portal hypertension (i.e., activation of endothelial cells and Kupffer cells, respectively), it may be that the combination of these two markers is a better predictor of the extent of portal hypertension than the levels of either one.

Whether the association between INR and blood loss in our study indicates a role for preexisting coagulopathy in bleeding during OLT is uncertain. The INR represents the deficiency in procoagulant proteins in cirrhosis and does not reflect the net hemostatic balance in cirrhosis.[22] We have previously demonstrated intact hemostatic capacity in samples taken at various time points during OLT despite profound prolongations in the INR, which we explained by a concomitant decline in pro- and anticoagulants, whereas the INR is only sensitive for alterations in procoagulant proteins.[11] It thus appears more plausible that the relation between preoperative INR and blood loss reflects severity of disease. Nevertheless, we cannot exclude that, although the patients with elevated INR remain in hemostatic balance, those patients are more susceptible to bleeding as the hemostatic balance is less stable in those patients with severe deficiencies in both pro- and anticoagulant proteins. It may be that improvement of the hemostatic status by infusion of low-volume prohemostatics, such as prothrombin complex concentrates, helps in further reducing perioperative blood loss. Such low-volume products lack the increase in portal hypertension associated with infusion of fresh frozen plasma. Anecdotal clinical experience supports the use of low-volume products over blood components.[23]

A limitation of our study is that the serum samples used were collected without a strict timing protocol. We studied samples that were taken anywhere from 0 up to 30 days prior to OLT, and assumed levels of VWF and sCD163 in these samples to reflect the extent of portal hypertension at the time of surgery. VWF antigen levels are generally assessed in (citrated) plasma, but we only had stored serum samples available for analyses, which is another limitation of our analyses. It has been established that VWF antigen levels are somewhat lower in serum compared with plasma,[24] and that in serum particularly the high molecular weight multimers are lower,[25] presumably due to entrapment in the clot. Additionally, we did not directly relate our serum markers of portal hypertension to clinical markers of portal hypertension or to direct measures such as HVPGs as these data were not available. Future studies in this setting should therefore assess VWF levels in plasma, preferably by automated methods available in diagnostic laboratories, and relate these to both direct measures of portal hypertension and intraoperative transfusion requirements.

Conclusion

We have shown increased blood loss and transfusion requirements during OLT, in patients with elevated levels of established markers of portal hypertension. These results provide evidence for the contribution of portal hypertension in the increased bleeding risk in cirrhotic patients undergoing OLT and support a restrictive transfusion policy and perioperative interventions that decrease or prevent further aggravation of portal hypertension.

Conflict of Interest
None.

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