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Intraoperative phlebotomies and bleeding in liver transplantation: a historical cohort study and causal analysis

Phlébotomies et saignements peropératoires dans les cas de transplantation hépatique : une étude de cohorte historique et une analyse causale

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Abstract

Background *Liver transplantation is associated with major bleeding and red blood cell (RBC) transfusions. No well-designed causal analysis on interventions used to reduce transfusions, such as an intraoperative phlebotomy, has been conducted in this population.*

Methods *We conducted a historical cohort study among liver transplantations performed from July 2008 to January 2021 in a Canadian centre. The exposure was intraoperative phlebotomy. The outcomes were blood loss, perioperative RBC transfusions (intraoperative and up to 48 hr after surgery), intraoperative RBC transfusions, and one-year survival. We estimated marginal multiplicative factors (MFs), risk differences (RDs), and hazard ratios by inverse probability of treatment weighting both among treated patients and the whole population. Estimates are reported with 95% confidence intervals (CIs).*

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Results *We included 679 patients undergoing liver transplantations of which 365 (54%) received an intraoperative phlebotomy. A phlebotomy did not reduce bleeding, transfusion risks, or mortality when estimated among the treated but reduced bleeding and transfusion risks when estimated among the whole population (MF, 0.85; 95% CI, 0.72 to 0.99; perioperative RD, −15.2%; 95% CI, −26.1 to −0.8; intraoperative RD, −14.7%; 95% CI, −23.2 to −2.8). In a subgroup analysis on 584 patients with end-stage liver disease, slightly larger effects were observed on both transfusion risks when estimated among the whole population while beneficial effects were observed on the intraoperative transfusion risk when estimated among the treated population.*

Conclusion *The use of intraoperative phlebotomy was not consistently associated with better outcomes in all targets of inference but may improve outcomes among the whole population.*

Study registration *www.ClinicalTrials.gov (NCT04826666); registered 1 April 2021.*

Résumé

Contexte *La transplantation hépatique est associée à des saignements importants et à de multiples transfusions de globules rouges (GR). Aucune analyse causale bien conçue sur l'effet d'interventions servant à réduire les transfusions, comme une phlébotomie peropératoire, n'a été menée dans cette population.*

Méthode *Nous avons mené une étude de cohorte historique incluant toutes les transplantations hépatiques réalisées dans un centre canadien de juillet 2008 à janvier 2021. L'exposition d'intérêt était une phlébotomie peropératoire. Les critères d'évaluation étaient le saignement peropératoire, les transfusions de GR périopératoires (peropératoires et jusqu'à 48 heures après la chirurgie), les transfusions de globules rouges peropératoires et la survie à un an. Des facteurs multiplicatifs (FM), des différences de risque (DR) et des rapports de risques instantanés marginaux ont été estimés en utilisant une pondération par l'inverse de la probabilité de traitement parmi les patients traités et parmi l'ensemble de la population. Les effets estimés ont été rapportés avec des intervalles de confiance (IC) à 95 %.*

Résultats *Nous avons inclus 679 transplantations hépatiques dont 365 (54 %) ont bénéficié d'une phlébotomie peropératoire. La phlébotomie n'a pas réduit les saignements, le risque de transfusion ou la mortalité lorsque ses effets ont été estimés parmi les patients traités, mais a réduit les risques de saignement et de transfusion lorsque ses effets ont été estimés parmi l'ensemble de la population (FM = 0,85 (IC 95 %, 0,72 à 0,99); DR périopératoire = −15,2 % (IC 95 %, −26,1 % à −0,8 %); DR peropératoire = −14,7 % (IC 95 %, −23,2 % à −2,8 %)). Dans une analyse de sous-groupe portant sur 584 patients atteints d'une hépatopathie terminale, des effets légèrement plus importants ont été observés sur les deux risques transfusionnels lorsqu'estimés dans l'ensemble de la population, tandis que des effets bénéfiques ont été observés sur le risque transfusionnel peropératoire lorsqu'estimés parmi les patients traités.*

Conclusion *L'utilisation de la phlébotomie peropératoire n'a pas été systématiquement associée à de meilleurs résultats dans toutes les populations cibles, mais semble améliorer les résultats lorsque les effets sont estimés dans l'ensemble de la population.*

Enregistrement de l'étude *www.ClinicalTrials.gov (NCT04826666); enregistrée le 1er avril 2021.*

Keywords liver transplantation · bleeding · red blood cell transfusion · phlebotomy · causal inference

Liver transplantation is associated with significant bleeding and often requires perioperative red blood cell (RBC) transfusions.[1,2] Overall, between 20 and 85% of liver transplant recipients receive at least one RBC transfusion during their surgery.[3] Perioperative transfusions have been consistently associated with a higher morbidity and mortality, although a causal relationship is still under debate in many surgical populations.[4–12] Despite this, minimizing bleeding and transfusions is a goal for improving postoperative outcomes in liver transplant recipients.[13,14]

Few perioperative interventions have been shown to reduce bleeding and transfusion requirements in liver transplant recipients.[15,16] Among them, the use of an intraoperative phlebotomy has been promising.[16–20] An intraoperative phlebotomy consists of removing some blood in a blood donation bag at the beginning of surgery and transfusing it at the end of surgery to reduce portal hypertension and splanchnic congestion observed in end-stage liver disease (ELD) during liver dissection, thus potentially reducing blood loss and subsequent RBC transfusions.[17,18,21] In fact, this intervention has been associated with less bleeding, fewer RBC transfusions, and lower mortality in different multivariable analyses conducted in liver transplant recipients.[10,16,22–24] Similar associations have also been recently observed in patients undergoing a liver resection.[19,20] In a recent systematic review, the use of intraoperative phlebotomy was also the only fluid management strategy associated with a lower mortality in observational studies conducted in liver transplantation.[25]

The potential effects of phlebotomy in liver transplant recipients have always been assessed using conditional

multivariable models not targeted specifically on the association between phlebotomies and outcomes, but rather using analyses in which phlebotomy was a variable within models that included many potential outcome determinants. Such models might have been biased because of misspecifications of the relations between independent variables and outcomes or by insufficient adjustment.[26,27] Since phlebotomies are mostly used in liver transplant recipients with near normal renal function and without severe anemia, some of our previous results may have been extrapolated in covariable subgroups without any observed phlebotomized patient.[10,22,24,28–30]

The objective of this study was to estimate the causal effects of intraoperative phlebotomy on hemorrhagic outcomes in liver transplantation using a well-defined causal analytical framework.

Methods

Study design and participants

We conducted a historical cohort study at the Centre hospitalier de l'Université de Montréal (CHUM). We included all adult patients who underwent a liver transplantation between July 2008 and January 2021. Patients who received renal replacement therapy prior to surgery and those who had a glomerular filtration rate below 30 $mL{\cdot}hr^{-1}$ (based on the Modification of Diet in Renal Disease study equation) were excluded to include only patients who could be at risk of receiving the intervention of interest, since phlebotomies are mostly made in patients with a near normal renal function.[16,17,31] The study was registered at ClinicalTrials.gov (NCT04826666; 1 April 2021) and is reported according to STROBE guidelines.[32] The study was approved by the Research Ethic Board (REB) of the CHUM. The need for consent was waived by the REB.

Exposure

The exposure of interest was the use of intraoperative phlebotomy at the beginning of surgery compared with not using it.[33,34] A phlebotomy consists of withdrawing 7–10 $mL{\cdot}kg^{-1}$ of blood from the patient before the dissection phase.[17,18] When a phlebotomy is performed, hypotension is managed using vasopressors rather than fluid administration and it is interrupted if the hypotension is severe. Phlebotomized blood is transfused back to patients in the reperfusion phase or before in case of major bleeding.

Outcomes

The primary outcome was intraoperative bleeding measured through a cell saver device.[35,36] The secondary outcomes were any intraoperative RBC transfusions, any perioperative RBC transfusions (intraoperative and postoperative transfusions up to 48 hr) and survival rate up to one year after surgery. Clinicians deciding to use an intraoperative phlebotomy were also the ones who made decisions regarding intraoperative transfusions of RBC. Since such transfusion decisions might be biased by knowing an intraoperative phlebotomy was used, the need for perioperative RBC transfusions was considered as a less biased outcome that better reflects the need for RBC transfusions associated with intraoperative blood loss.[29] The 48-hr time point was selected since changes in hemoglobin concentration within the first 48 hr after surgery have been shown to be a good surrogate of intraoperative bleeding.[35] Survival time was computed from liver transplantation to death or censoring. Patients who needed a retransplantation were censored at retransplantation. All patients were censored at one year after surgery or when the data set was last updated (15 April 2021).

Covariables

Many perioperative variables have been associated with higher blood loss and need for perioperative transfusions in liver transplant recipients, including liver disease severity, preoperative anemia and coagulopathy, higher cardiac filling pressures, and higher fluid administration.[2,10,16,17,22,25,37] Many of these variables are also associated with the use of an intraoperative phlebotomy and are thus known or potential confounders. In our centre, phlebotomies are more often carried out in non-anemic cirrhotic patients with high portal and central venous pressure (CVP), and less often in patients with severe acute disease with end-organ damage such as renal failure.[17,18] An observational study from our centre also suggested that intraoperative bleeding and transfusions have increased since recipients are prioritized by the model for end-sage liver disease (MELD) score.[10,38] Because of all these confounders, patients who received a phlebotomy might have different baseline prognostic characteristics than those who did not receive a phlebotomy. A sufficient set of important covariables was thus selected based on previously exposed published data and knowledge of the local clinical practice to control for confounding (see the Directed Acyclic Graph on eFig. 1 and eTable 5 in the Electronic Supplementary Material [ESM]). The following variables measured at the time of surgery were thus included in the propensity score model: age, sex, MELD,

hemoglobin ($g \cdot L^{-1}$), creatinine ($\mu mol \cdot L^{-1}$), international normalized ratio, platelets ($\times 10^3 \cdot \mu L^{-1}$), fibrinogen ($g \cdot L^{-1}$), CVP (mm Hg), acute liver failure status, retransplantation status, and year of transplantation.

Institutional intraoperative practice

Graft procurement occurred almost exclusively in neurologically deceased donors (98%). Grafts were mostly transplanted using a total cross-clamping technique with vena cava replacement, although the use of a piggyback technique was introduced in 2019 and is now used in around 50% of patients. A cell saver device was used in every case and a leukocyte filter added before transfusion in patients with hepatocellular carcinoma. Anesthesiologists used an intraoperative restrictive low-CVP fluid management strategy.[39,40] Blood products were transfused based on the presence of clinical bleeding and abnormal coagulation tests from the central laboratory. Coagulation disturbances were not corrected preemptively. Tranexamic acid was used prophylactically for almost every case unless active thrombosis was suspected.

Data sources and management

For all patients, intraoperative data had been collected prospectively by anesthesiologists using a standardized reporting form. Data for patients who received their transplantation between 2008 and 2017 were already available in a data set used for previous analyses.[24,29,30] We added transplantations conducted between January 2018 and January 2021 to the previous data set, inflating the available number of observations for analyses by more than 30% compared with previous studies. Data were merged by one author (F.M.C.) and the merging was double checked by a second author (E.A.). Supplemental data were extracted from patients' charts.

Statistical analyses

Study size

No previous study size calculation was performed as we used a convenience sample of all transplanted patients that met the inclusion criteria during the period of interest.

Main analyses

Baseline characteristics and crude outcome incidences were reported for the full sample as well as for each exposure category. Frequencies and proportions for categorical variables and means with standard deviations for continuous variables (or medians with interquartile ranges [IQRs] for skewed distributions) were used. Crude survival was estimated by the Kaplan–Meier formula. Intraoperative bleeding was analyzed as a continuous variable and RBC transfusions were analyzed as a dichotomous variable ("no transfusion" and "any transfusion") because most patients did not receive any intraoperative or perioperative RBC transfusions (ESM eFig. 2). Every liver transplantation was analyzed as a unit of observation.

Causal effects for all outcomes were estimated both among the treated (causal effects among the treated) and among the whole population (marginal causal effects).[41,42] Using inverse probability of treatment weights, we created pseudo-populations in which treatment indication was independent of the covariables distribution at cohort entry (i.e., the potential confounders). Our analyses were based on the comparison of treated and untreated patients in these pseudo-populations. The weights we used either created a pseudo-population of untreated patients having the same covariables distribution as the treated patients (causal effects among the treated) or created a pseudo-population with the covariables distribution corresponding to the whole sample (marginal causal effects) (see section A of the ESM for further details on weights and causal effects estimation). Selected estimands were analogous to the average treatment effect in the treated and the average treatment effect if the causal parameters were mean differences. Their interpretation corresponds to causal effects that could be estimated if all treated patients were randomized to the intervention (causal effects among the treated) or if all patients were randomized to the intervention (marginal causal effects among the whole population). Since a phlebotomy is a well-defined intervention that yields the same effects, notwithstanding who is the clinician performing it (stable unit of treatment value assumption), that all known potential confounders were included in our propensity score model (conditional exchangeability), and that we observed a good overlap of the propensity score distribution (positivity, see ESM eFig. 3), we considered that necessary causal assumptions were met.[34]

Since the distribution of blood loss was highly asymmetrical, we fitted a weighted log-transformed linear regression and reported a multiplicative factor (MF) as the causal parameter of interest for the effect of intraoperative phlebotomy on bleeding.[43] This factor represents the multiplicative effect of a phlebotomy on the mean blood loss observed in the control group: a value below 1 means that a phlebotomy reduces blood loss by 100*(1 – MF)%, while a value above 1 means that a phlebotomy increases blood loss by 100*(MF – 1)%. For the effect of phlebotomy on transfusions, causal risk differences (RDs) were calculated by weighted exact computation. Marginal

structural models were also fitted using a weighted proportional hazards Cox model to estimate the effect of phlebotomy on survival.[34] The risk proportionality assumption was tested using the score test and hazard ratios (HR) were reported. All effect estimates (MF, RD, and HR) are reported for both target populations of inference (treated patients or whole population).[42] Since some patients had more than one transplantation, 95% percentile confidence intervals (CI) were estimated by nonparametric bootstrap using 500 iterations of clustered resampling with replacement (both patients and transplantations were resampled with replacement) to handle intracluster correlation.[44] We used R software version 4.0.3 (R Foundation, Vienna, Austria) to conduct the analyses.

Subgroup and sensitivity analyses

We conducted a subgroup analysis restricted to liver transplantations performed for ELD. The effect of phlebotomy is considered to be mechanistically mediated by a reduction in portal pressure and splanchnic congestion, which may have a greater impact in patients with portal hypertension such as patients with ELD.[17] We restricted the analyses to ELD patients by excluding retransplantations and transplantations for acute liver failure, primary liver cancer without cirrhosis, or amyloidosis. We also conducted three sensitivity analyses to better explore our modelling assumptions (see section A of the ESM for further details).

Results

We included 679 liver transplantations performed in 631 different patients who met the inclusion criteria (Fig. 1). A phlebotomy was used in 365/679 (54%) liver transplantations. Patients' baseline characteristics were very unbalanced between the groups (Table 1 and ESM eTable 1 and eFig. 5). The median [IQR] bleeding for the full sample was 1 [0.6–1.8] L and 312/679 (46%) transplant patients received at least one RBC transfusion from the start of surgery up to 48 postoperative hours. The overall one-year survival was 93.8% (95% CI, 92.0 to 95.7) (Table 2 and ESM eTable 1). Based on crude data, patients who received a phlebotomy bled less, had a lower risk of receiving at least one RBC transfusion either intraoperatively or perioperatively, and had a better crude one-year survival (Table 2 and ESM eTable 1 and eFig. 9).

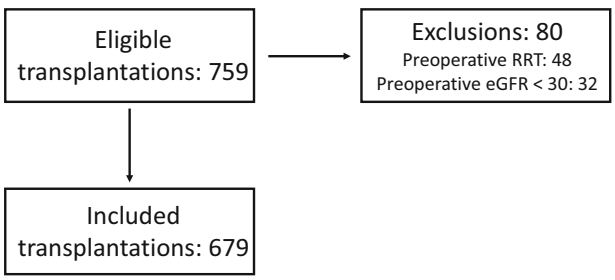

Fig. 1 Flowchart

Our weighting strategy well balanced the distribution of covariables between groups for either target population (ESM eFig. 5). Using a phlebotomy did not significantly reduce bleeding (MF, 0.90; 95% CI, 0.75 to 1.13), perioperative transfusions (RD, −9.6%; 95% CI, −20.3 to 3.3), intraoperative transfusions (RD, −6.6%; 95% CI, −15.6 to 0.7), and survival (HR, 0.46; 95% CI, 0.16 to 1.86) among treated patients since all 95% CIs covered the null value, precluding rejection of a potential null or harmful effect (Tables 3 and 4). Nevertheless, when estimating marginal effects among the whole population rather than the treated population, point estimates for bleeding and transfusion risks were larger and the upper confidence limit did not cover the null value (bleeding MF, 0.85; 95% CI, 0.72 to 0.99; perioperative RD, −15.2%; 95% CI, −26.1 to −0.8; intraoperative RD, −14.7%; 95% CI, −23.3 to −2.8) (Table 3), suggesting a possible beneficial causal effect of phlebotomy among the whole population. Survival was similar in both target populations with an upper confidence limit covering the null value (Table 4 and Fig. 2).

In the subgroup analysis conducted in 584 patients with ELD, observations were similar to those in the full sample, although point estimates suggested slightly larger effects on transfusion risks estimated among the whole population (ESM eTable 2).

Results for bleeding and transfusion risks among the treated were similar when using a propensity score-based matching technique rather than an inverse probability of treatment weighting (IPTW) technique (ESM eTable 3). Nevertheless, when using a matching technique to estimate marginal effects among the whole population, effect estimates were smaller, although the null value was still not covered by the upper confidence limit for most outcomes (ESM eTable 3). When removing time as a confounder, the point estimates were similar to those from main analyses, but the CIs were slightly narrower (ESM eTable 4 and eFig. 10). Finally, changing the categorization threshold of our transfusion outcomes to two or more RBC transfusions reduced the estimated effect of a phlebotomy on the intraoperative transfusion risk among both target populations, with CIs always covering the null value (ESM eTable 5).

Table 1 Cohort baseline characteristics

Variables	No phlebotomy $N = 314$	Phlebotomy $N = 365$
Preoperative characteristics and potential confounders		
Age (yr), mean (SD)	53.1 (11.7)	51.8 (11.6)
Sex (male), *n*/total *N* (%)	198/314 (63%)	260/365 (71%)
MELD, mean (SD)	22.2 (8.0)	17.2 (7.7)
Hemoglobin ($g \cdot L^{-1}$), mean (SD)	92 (19)	119 (21)
Creatinine (μmol/L), median [IQR]	86 [64–112]	69 [58–90]
INR, median [IQR]	1.7 [1.4–2.2]	1.4 [1.2–1.7]
Platelets ($10^{-3} \cdot \mu L^{-1}$), median [IQR]	74 [49–115]	86 [55–128]
Fibrinogen ($g \cdot L^{-1}$),[a] median [IQR]	1.8 [1.1–2.6]	2.3 [1.5–3.1]
Liver disease etiology, *n*/total *N* (%)		
Alcoholic cirrhosis	66/314 (21%)	71/365 (20%)
Viral cirrhosis	51/314 (16%)	80/365 (22%)
NASH cirrhosis	50/314 (16%)	33/365 (9%)
Mixed cirrhosis	13/314 (4%)	23/365 (6%)
Other cirrhosis	5/314 (2%)	7/365 (2%)
Chronic autoimmune disease	57/314 (18%)	84/365 (23%)
Primary liver cancer[b]	1/314 (0.3%)	4/365 (1%)
Other	20/314 (6%)	25/365 (7%)
Acute liver failure[c]	14/314 (5%)	14/365 (4%)
Retransplantation[c]	42/314 (13%)	27/365 (7%)
Baseline CVP (mm Hg), mean (SD)	13.2/314 (5.4)	13.3/365 (4.9)
Intraoperative fluid administration		
Crystalloids (L), median [IQR]	3.5 [2.5–5.0]	3.5 [2.8–4.3]
Colloids (L), median [IQR]	0 [0–0.5]	0 [0–0.5]
Albumin 5% (L), median [IQR]	0 [0–1.0]	0 [0–0.5]

[a] Four missing values

[b] Without confirmed cirrhosis prior to transplantation

[c] These categories both include primary non-function (retransplantation and acute liver failure are thus not mutual exclusive categories)

CVP = central venous pressure; INR = international normalized ratio; IQR = interquartile range; MELD = model for end-stage liver disease; SD = standard deviation

Table 2 Crude outcomes

Outcomes	No phlebotomy $N = 314$	Phlebotomy $N = 365$
Bleeding (L), median [IQR]	1.2 [0.7–2.5]	0.8 [0.5–1.4]
Any intraoperative RBC transfusions, *n*/total *N* (%)	151/314 (48%)	38/365 (10%)
Any postoperative RBC transfusions, *n*/total *N* (%)	158/314 (51%)	79/365 (22%)
Any perioperative RBC transfusions,[a] *n*/total *N* (%)	215/314 (69%)	97/365 (27%)
One-year survival[b]	91.4% (88.3 to 94.6)	95.9% (93.8 to 98.0)

Survival is reported as Kaplan–Meier estimates with 95% confidence intervals.

[a] Includes any intraoperative and postoperative RBC transfusions up to 48 hours after surgery

[b] Kaplan-Meier estimates

IQR = interquartile range; RBC = red blood cells

Table 3 Estimated effects on bleeding and transfusions

Target population	Bleeding (multiplicative factors)	Perioperative transfusions (risk differences)	Intraoperative transfusions (risk differences)
Treated population[a]	0.90 (0.75 to 1.13)	−9.6% (−20.3 to 3.3)	−6.6% (−15.6 to 0.7)
Whole population[b]	0.85 (0.72 to 0.99)	−15.2% (−26.1 to −0.8)	−14.7% (−23.3 to −2.8)

Estimates are presented with 95% clustered bootstrap confidence intervals

[a] Estimated causal effects among the treated population

[b] Estimated marginal effects among the whole population

Table 4 Estimated effects on survival

Target population	Hazard ratio
Treated population[a]	0.46 (0.16 to 1.86)
Whole population[b]	0.44 (0.18 to 1.15)

Estimates are presented with 95% clustered bootstrap confidence intervals

[a] Estimated causal effects among the treated population

[b] Estimated marginal effects among the whole population

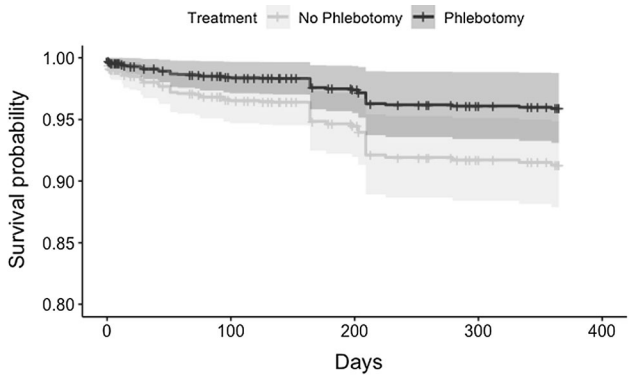

Fig. 2 Weighted survival analysis

Discussion

In this cohort of liver transplant recipients without severe preoperative renal failure, the use of a phlebotomy during liver transplantation was not associated with less bleeding, a lower perioperative RBC transfusion risk, or better survival when effects were estimated among the treated but may reduce bleeding and RBC transfusion risks (either intraoperatively or perioperatively) when estimated among the whole population. The directionality of the estimated effects was consistent across target populations of inference, although they were larger and statistically significant only when estimated among the whole population. In the subgroup of patients with ELD, estimates were similar to the main analyses. Overall, our results were consistent across sensitivity analyses.

Previous studies suggested that phlebotomy was associated with lower blood loss and a lower transfusion risk in liver transplantation.[17,22,23] These previous results came from multivariable models that did not incorporate covariables carefully selected for causal inference on the effects of phlebotomy. Also, it is possible that previous results were extrapolated within some covariables strata. When analyzing treated patients, we observed clinically meaningful associations that, most of the time, could not exclude a null effect. Nevertheless, when analyzing the sample using the full covariable distribution (marginal effects among the whole population) rather than the covariable distribution of the treated patients (effects among the treated), associations were greater with CIs always excluding the null value for hemorrhagic outcomes. The observed differential effect across target populations may be explained by the fact that treated patients selected by clinicians were, on average, at a lower risk of bleeding and transfusions based on their baseline covariables distributions. Moreover, marginal effects in the whole population are probably more relevant in a clinical setting. These marginal effects represent potential effects that would be observed if all patients would have received the intervention, effects similar to those that would be observed in a randomized controlled trial including all eligible patients. If a phlebotomy is used within or outside a clinical trial, all patients should be eligible to it notwithstanding their baseline characteristics. Finally, a phlebotomy did not improve one-year survival in any target population, but 95% CIs did not exclude beneficial effects and point estimates did not suggest harm. Nonetheless,

such harm could not be excluded. Also, many other postoperative complications were not measured, limiting conclusions on safety of a phlebotomy on many postoperative outcomes.

The use of a phlebotomy has been extensively studied for liver resection surgeries.[19,20] Such a technique seems to be a promising for reducing bleeding and blood transfusion in liver surgery. A multicentre randomized controlled trial is under way in this population to confirm such benefits (NCT03651154). Patients with ELD often have portal hypertension, fluid retention, and systematic volume overload.[21] Liver transplant recipients also lose more blood, receive more transfusions, and have more postoperative complications than patients undergoing a liver resection do.[9,19,20,45] Finding strategies to reduce transfusion exposure and improve outcomes in this population is paramount and strategies to reduce portal pressure and fluid overload seem to be an interesting path.[18,25] When we only looked at the subgroup of patients that may have more beneficial effects, i.e., ELD patients with a potential high portal pressure, we observed associations similar to those observed in the whole sample.[18] Nevertheless, compared with the whole sample, CIs for the estimates of the effects among the whole population were narrower and the lower confidence limit was further away from the null value for both transfusion outcomes in this subgroup of patients. Also, CIs excluded the null value for the intraoperative transfusion risk when estimated among the treated ELD patients. These observations were probably due to a lower variability of effect among ELD patients. Phlebotomy could thus be part of a blood-sparing strategy, especially in ELD patients, although more studies are needed to better define its effect in this subgroup of patients.

Strengths

To conduct this study, we used balancing score-based techniques, which allowed us to assess positivity among our sample and estimate marginal effects. We chose the IPTW analytical technique as our primary analysis because of its convenience assessing balancing properties of the propensity score while using as many observations as possible and because it is the preferred technique for estimating RDs.[46] Despite this, we explored its potential pitfalls by sensitivity analyses using a matching technique. We also explored effects in a potential subgroup that may benefit more from the intervention. We also estimated causal parameters among the treated population and the whole population, since both could be reasonably estimated based on propensity score overlapping and clinical assumptions, although marginal effects among the whole population are probably more clinically relevant.[41] We used clustered bootstrap percentile CIs to account for correlation between transplantations performed on the same individuals. Finally, overall, our study population is representative of liver transplant recipients, enhancing generalizability.

Limitations

Our study has several limitations. Our proposed causal framework is theoretical and may not have included all the intangibles used in clinical practice to perform a certain procedure.[47] Thus, we may still observe some potential beneficial associations due to uncontrolled or residual confounding, such as preoperative disease severity not captured by our baseline variables or other unmeasured preoperative organ dysfunctions (such as hemodynamic instability and hepatic encephalopathy). Some significant results were observed in our analyses with the intraoperative transfusion risk, an outcome based on a decision made by the same clinicians who carried on the exposure and were thus potentially biased by "non-indication". Also, blood loss measurement is highly variable across and within centres and at risk of non-differential measurement errors, inducing potential biases toward the null value and limiting interpretability.[35] In fact, we observed possible benefits for the perioperative transfusion risk, which is the most objective outcome in this study. Nonetheless, we did not collect extensive data on other postoperative complications, limiting our interpretation to transfusion risks and survival. We obtained CIs that were large in all analyses and crossed the null value when estimated among the treated population, potentially because we had an overall moderate sample size and relatively limited power. Indeed, we conducted several sensitivity analyses, increasing the risk of finding a significant effect by chance alone (please see section D of the ESM for further details on limitations of modelling strategies). Finally, our centre may have specific practices and perioperative management procedures associated with benefits from a phlebotomy (effect modification). Such characteristics may limit the external validity of our findings.

Conclusion

In this historical cohort study, the use of intraoperative phlebotomy was not consistently associated with less bleeding, lower perioperative transfusion risk, or better one-year survival in liver transplant recipients when effects were estimated in different target populations. According to our estimated marginal effects among the whole

population, an intraoperative phlebotomy may reduce blood loss and intraoperative and perioperative transfusion risks without improving one-year survival. Clinical trials are needed to better define the effects of phlebotomy on bleeding, transfusions, and other postoperative outcomes in liver transplant recipients.

Author contributions *François Martin Carrier* participated in research design, research performance, data acquisition, data analysis, and writing the manuscript. *Steve Ferreira Guerra* participated in research design, data analysis, and writing the manuscript. *Janie Coulombe* participated in research design, data analysis, and writing the manuscript. *Éva Amzallag* and *Luc Massicotte* participated in data acquisition and writing the manuscript. *Michaël Chassé* and *Helen Trottier* participated in data analysis and writing the manuscript.

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Data availability statement Due to national regulations in the Province of Quebec (Canada), health medical data cannot be made available publicly. Nevertheless, complete access to the research data set is possible for research purposes after appropriate privacy agreements between research parties have been made. Data access requests may be sent to the corresponding author (francois.martin.carrier@umontreal.ca), or directly to the CHUM REB (ethique.recherche.chum@ssss.gouv.qc.ca). The R code will be available upon request to the corresponding author.

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