Normothermic Machine Perfusion Enhances Intraoperative Hepatocellular Synthetic Capacity: A Propensity Score-matched Analysis

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Background. Normothermic machine perfusion (NMP) of liver grafts is increasingly being incorporated in clinical practice. Current evidence has shown NMP plays a role in reconditioning the synthetic and energy capabilities of grafts. Intraoperative coagulation profile is a surrogate of graft quality and preservation status; however, to date this aspect has not been documented. **Methods.** The liver transplantation recipients who received NMP liver grafts in the QEHB between 2013 and 2016 were compared in terms of intraoperative thromboelastography characteristics (R time, K time, α-angle, maximum amplitude, G value, and LY30) to a propensity score-matched control group, where the grafts were preserved by traditional static cold storage (SCS). **Results.** After propensity matching, none of the thromboelastography characteristics were found to differ significantly between the 72 pairs of SCS and NMP organs when measured preimplantation. However, postimplantation, NMP organs had significantly shorter K time (median: 2.8 vs 3.6 min, $P = 0.010$) and R + K time (11.4 vs 13.7 min, $P = 0.016$), as well as significantly larger α-angle (55.9° vs 44.8°, $P = 0.002$), maximum amplitude (53.5 vs 49.6 mm, $P = 0.044$), and G values (5.8 vs 4.9k dynes/cm^2, $P = 0.043$) than SCS organs. Hyperfibrinolysis after implantation was also mitigated by NMP, with fewer patients requiring aggressive factor correction during surgery (LY30 = 0, NMP vs SCS: 83% vs 60%, $P = 0.004$). Consequently, NMP organs required significantly fewer platelet units to be transfused during the transplant procedure (median: 0 vs 5, $P = 0.001$). **Conclusions.** In this study, we have shown that NMP liver grafts return better coagulation profiles intraoperatively, which could be attributed to the preservation of liver grafts under physiological conditions.

(*Transplantation* 2019;103:e198–e207)

INTRODUCTION

The increased interest in normothermic machine perfusion (NMP) in the clinical transplant setting is based on the hypothesis that organ preservation under physiological conditions, as opposed to traditional cold storage, would best preserve the deceased organs.[1] Considering that the availability of good quality organs is on the decline, the perceived benefits of NMP would enable expansion of the available donor pool by allowing for increased utilizations of extended criteria organs.[2] NMP has also been proven to be an effective method of performing viability assessment and reconditioning of liver grafts.[3,4] Although

the mechanisms through which NMP improves graft function are still unknown, it is hypothesized that they mainly involve maintaining the integrity of the endothelium and restoring the ATP levels.[4]

Ischemia-reperfusion injury (IRI) is one of the major concerns in orthotopic liver transplantation (LT) and is considered the fundamental mechanism causing graft damage following static cold storage (SCS).[5,6] It consists of hepatocellular injury, caused by an inflammatory immune response mechanism. The latter is, in turn, triggered by the efflux of toxic cellular compounds caused by IRI, which are released to the systemic circulation of the recipient at the time of reperfusion. As SCS is not capable of complete abolition of metabolism, cellular metabolism continues at a slower pace in the milieu of oxygen deprivation; the resultant anaerobic metabolism and synthesis of reactive oxygen species brings about much of the reperfusion syndrome.[7] The graft inflammation and injury caused by IRI generally reverts to normal graft function eventually, depending on the overall graft quality and preservation status of the graft. Hepatic synthetic recovery determines the coagulation status in the immediate postreperfusion phase of a liver graft, and this is one way of assessing graft function.

Among the intrahepatic metabolic pathways, the production of coagulation factors is a well-established marker of liver synthetic function,[8] especially if it is gauged in real-time with appropriate means, ie, thromboelastography (TEG). Based on observations made on the lactate clearance and bile production, both of which experience a steep increase once the machine perfusate temperature reaches 35°C, it has been hypothesized that the coagulopathic syndrome occurring after implantation is related to the deleterious effect of SCS.[9] Imber et al[10] published the first report that documented the positive effect of NMP on the synthetic function of the perfused livers, reflected in higher levels of measured factor V Leiden compared with SCS. At present, clinical studies ascertaining whether NMP of donor livers before implantation mitigates hyperfibrinolysis in recipients after LT are still lacking. TEG has been used perioperatively during LT to provide a real-time global hemostasis assessment for targeted blood product replacement.[11] Attributable to its real-time application, TEG has become a vital component for detecting and managing coagulopathies in high bleeding–risk scenarios, such as LT. Additionally, preoperative TEG may also identify LT recipients at greater risk of developing serious complications (eg, HAT).[12]

The aim of this study is to characterize the coagulation profile, as reflected in TEG measurements, in a cohort of transplant recipients of NMP liver grafts, in comparison to a control group of patients receiving grafts preserved via SCS. To the best of our knowledge, this is the first study published in the literature to date which aims to ascertain the synthetic capacity of hepatocytes in implanted livers previously perfused normothermically.

MATERIALS AND METHODS

Study Population

This retrospective study was approved by the Clinical Audit and Research Management System (CARMS) of the University Hospitals Birmingham NHS Foundation Trust (registration No. CARMS-14049). A retrospective analysis of a prospectively collected database of all liver transplants between January 2013 and December 2016 in the Liver Surgery Department of the Queen Elizabeth Hospital Birmingham was performed. Exclusion criteria consisted of live donors, split/partial grafts, and pediatric recipients. In the first analysis, those receiving NMP organs from this cohort were matched 1:1 with patients undergoing transplantation of conventional SCS livers, on the basis of a propensity score. The variables included in the propensity score consisted of graft and recipient factors that could potentially influence the immediate reperfusion outcomes. Recipient factors included recipient's age, gender, ethnicity, diagnosis, and UK End-stage Liver Disease (UKELD) score as a marker of disease severity, which in turn encompasses INR, serum sodium, serum creatinine, and serum bilirubin. Donor characteristics consisted of age, type of organ (donation after brain death [DBD] vs donation after circulatory death [DCD]), cause of death, donor warm ischemia time in DCD patients, and extended cold ischemia time (CIT) (>8 h). A secondary subgroup analysis was performed, comparing the NMP grafts to the SCS grafts of the Consortium for Organ Preservation in Europe (COPE) trial contributed by Queen Elizabeth Hospital Birmingham.[13]

Normothermic Machine Perfusion

Within the COPE trial, NMP was performed with the OrganOx *metra* device (OrganOx Ltd., Oxford, United Kingdom) at the donor hospitals once the liver grafts were procured after a period of short cold ischemia, during which bench preparation was completed. NMP of the liver consists of circulating a red cell-based perfusate through both the portal vein and hepatic artery at physiological pressures. The perfusion is performed at physiological temperatures, and the arterial flow is continuous. After exiting the organ through the inferior vena cava, the perfusate is recirculated in a close circuit back to the inflow vessels. The machine is completely automated in terms of adjusting vascular pressures, flow rates, and blood gas regulation. The technique of NMP using the OrganOx *metra* device is as previously published.[13]

Surgical Technique

All patients undergoing transplantation had a uniform surgical approach in major steps, barring few variations, for example avoidance of temporary portocaval shunt and caval anastomosis technique (classical and modified piggyback technique). No patients had venovenous bypass. All transplants were supported intraoperatively by autologous cell salvage and autotransfusion with a cell saver device (Cell Saver 5; Haemonetics Corp., Braintree, MA), unless the transplant recipient had previously diagnosed malignant hepatic lesions. Transfusion of the autosalvaged blood was at the discretion of the anesthetist.

TEG Data

Before the start of the liver implantation procedure, baseline (preimplantation) TEG samples were drawn in the theatre in the period after intubation but before skin incision. Postimplantation TEG samples were drawn within 30 minutes of reperfusion and subsequent samples postreperfusion
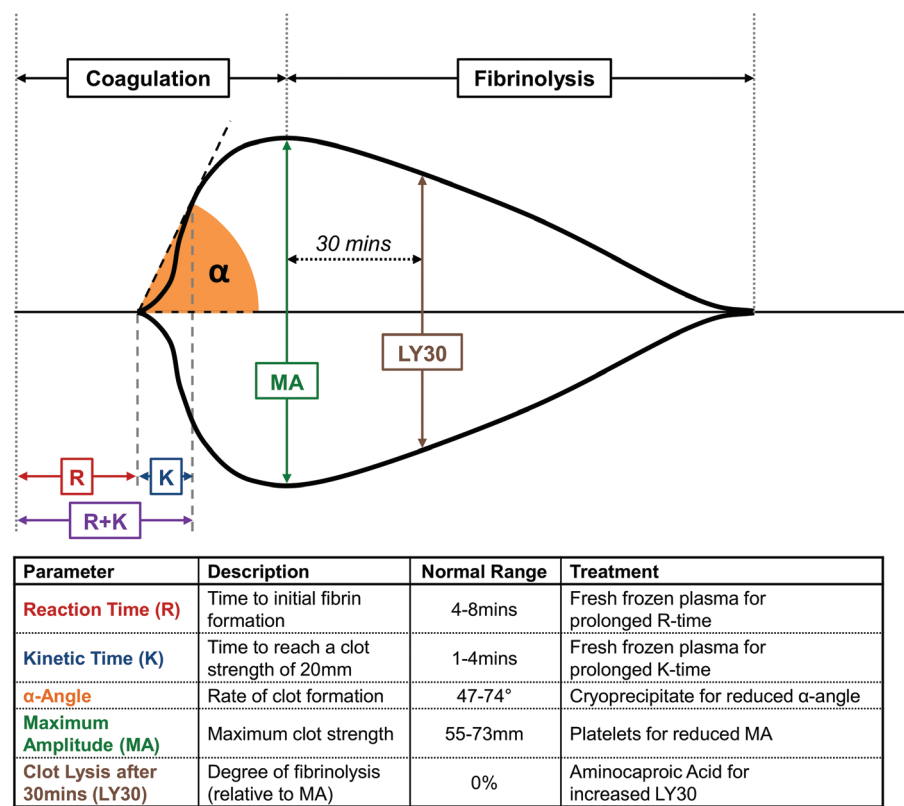
Parameter	Description	Normal Range	Treatment
Reaction Time (R)	Time to initial fibrin formation	4-8mins	Fresh frozen plasma for prolonged R-time
Kinetic Time (K)	Time to reach a clot strength of 20mm	1-4mins	Fresh frozen plasma for prolonged K-time
α-Angle	Rate of clot formation	47-74°	Cryoprecipitate for reduced α-angle
Maximum Amplitude (MA)	Maximum clot strength	55-73mm	Platelets for reduced MA
Clot Lysis after 30mins (LY30)	Degree of fibrinolysis (relative to MA)	0%	Aminocaproic Acid for increased LY30

FIGURE 1. Schema of thromboelastography assay and blood product guidance. K, kinetic; R, reaction.

were determined by the length and coagulation status of the recipient. Where multiple postimplantation samples were available, the first postreperfusion sample was selected for inclusion in the study, as subsequent measurements would bear a higher risk of being influenced by confounding factors, such as transfusions. The citrated samples were assayed using the TEG 5000 Thrombelastograph Hemostasis Analyzer (Haemoscope Corp., Niles, IL).

Clot formation and strength, as well as fibrinolysis, were measured using reaction time (R time), kinetic time (K time), α-angle, maximum amplitude (MA), G value, and clot lysis 30 minutes (LY30) after MA. In TEG analysis, the R time is defined as the latency time of fibrin formation until the clot reaches 2 mm in amplitude, while the K time represents the duration of time from the end of R time until the clot is 20 mm in diameter (Figure 1).[14,15] When prolonged, both markers prompt administering fresh frozen plasma (FFP). As a result, in clinical practice, the sum of R and K times is widely used to exclude potential redundancy. α-Angle (in degrees) represents the kinetics of clot formation and is a measure of the rate of fibrin formation, as well as of the cross-linking of platelets.[16] MA is a surrogate marker for clot strength and represents the maximum dynamic properties of fibrin and platelet bonding, with values being lower in hypocoagulable states or consumption of platelets.[11] The G value measured in K dynes/cm² is calculated from platelet and fibrin performance and is a measure of clot strength or stability. G value may be considered the most important marker in TEG, because it illustrates the overall function or effectiveness of the clot. G values below 5k dynes/cm² are associated with increased risk of hemorrhage, while values above 10k dynes/cm² are associated with increased risk of thrombosis. Hyperfibrinolysis is an indicator of clot lysis at 30 minutes (LY30; in percentage), which measures clot dissolution 30 minutes after MA is reached. All TEG parameter data of patients undergoing NMP and SCS were collated for the study purpose.

Statistical Analysis

The statistical review of the study was performed by a biomedical statistician (J.H.). Initially, graft and recipient factors were compared between the NMP and SCS groups. Continuous data were tested for normality before analysis, with normally distributed variables reported as mean ± SD and compared using independent samples t tests. Variables that were not normally distributed were reported as medians and interquartile ranges (IQRs) and analyzed using Mann-Whitney tests. Fisher exact or Chi-square tests were used for nominal variables. A propensity score was then produced, which was generated using a binary logistic regression model, including all of the demographic factors described previously for which data were available. For continuous variables, the goodness of fit was assessed using Hosmer and Lemeshow tests. Cases from the NMP cohort were then matched to control patients on the basis of this propensity score in a 1:1 ratio without replacement. Exact matches were prioritized, with the case order randomized for fuzzy matches. The comparisons between cohorts were then repeated for the matched cohort to assess the quality of the matching.

The TEG measurements were then compared between the 2 matched groups at both pre- and postimplantation. In addition, the changes from pre- to postimplantation were calculated for each case, and the resulting values were also compared between the groups to adjust for preimplantation variability. Since LY30 was highly skewed, values

were dichotomized into hyperfibrinolysis 'PRESENT' versus hyperfibrinolysis 'ABSENT'. The "change" in LY30, for both NMP and SCS groups, was analyzed by comparing the pre- and postimplantation values. All analyses were performed using IBM SPSS 22 (IBM Corp., Armonk, NY), with $P < 0.05$ deemed to be indicative of statistical significance throughout.

RESULTS

Between January 2013 and December 2016, a total of 699 transplants met the inclusion criteria of the study outlined under the Methods section, with NMP used in 76 (11%) cases and the remainder being SCS. The NMP cohort had significantly lower UKELD scores than SCS group (mean 52.6 vs 54.9, $P = 0.002$), likely as a result of the selection of patients for NMP trials in the early safety studies. The recipient diagnoses also differed between the groups ($P < 0.001$), with fewer cases of ALF (1% vs 10%) but more PSC/PBC (33% vs 22%) in the NMP cohort. CIT was found to be considerably shorter for organs receiving NMP, at a median of 162 minutes, compared with 461 minutes in the SCS cohort ($P < 0.001$). This reflected the practice of NMP, in which the majority of organs were

TABLE 1.
Patient demographics in the SCS and NMP groups for the cohort as a whole and after matching

Factor	Unmatched			Propensity matched		
	SCS (N = 623)	NMP (N = 76)	P	SCS (N = 72)	NMP (N = 72)	P
Recipient age, y	54 (44–62)	55 (45–62)	0.463	57 (45–61)	55 (45–62)	0.930
Recipient sex			0.704			0.467
Female	222 (36%)	25 (33%)		19 (26%)	24 (33%)	
Male	401 (64%)	51 (67%)		53 (74%)	48 (67%)	
Recipient ethnic origin			0.165			0.369
Black	15 (2%)	2 (3%)		3 (4%)	2 (3%)	
Euro-Caucasian	540 (87%)	71 (93%)		62 (86%)	67 (93%)	
Other	68 (11%)	3 (4%)		7 (10%)	3 (4%)	
Indication for LT			**<0.001**			0.869
Alcoholic liver disease	165 (27%)	16 (21%)		15 (21%)	16 (22%)	
Acute liver failure	65 (10%)	1 (1%)		2 (3%)	1 (1%)	
Budd-Chiari	6 (1%)	0 (0%)		0 (0%)	0 (0%)	
Hemochromatosis	5 (1%)	3 (4%)		1 (1%)	1 (1%)	
HBV	19 (3%)	2 (3%)		4 (6%)	2 (3%)	
Hepatocarcinoma	4 (1%)	0 (0%)		0 (0%)	0 (0%)	
HCV	91 (15%)	9 (12%)		15 (21%)	9 (13%)	
NASH	51 (8%)	13 (17%)		12 (17%)	12 (17%)	
Noncirrhotic portal HTN	6 (1%)	3 (4%)		2 (3%)	3 (4%)	
Polycystic liver	14 (2%)	1 (1%)		0 (0%)	0 (0%)	
PSC/PBC	137 (22%)	25 (33%)		18 (25%)	25 (35%)	
Other	59 (9%)	3 (4%)		3 (4%)	3 (4%)	
UKELD	54.9 ± 6.1	52.6 ± 5.0	**0.002**	53.1 ± 5.1	52.9 ± 4.8	0.886
Preoperative hemoglobin, g/L	NA[a]	NA[a]		120 (105–136)	120 (100–128)	0.248
Preoperative platelets, ×10⁹/L	NA[a]	NA[a]		85 (52–129)	104 (64–139)	**0.028**
Preoperative INR	NA[a]	NA[a]		1.3 (1.2–1.5)	1.2 (1.1–1.4)	0.085
Donor age, y	53 (40–64)	52 (42–63)	0.945	50 (37–65)	53 (43–63)	0.727
Type of donor			0.105			0.862
DBD	452 (73%)	48 (63%)		47 (65%)	45 (63%)	
DCD	171 (27%)	28 (37%)		25 (35%)	27 (38%)	
Donor cause of death			0.067			0.530
Anoxia	97 (16%)	18 (24%)		19 (26%)	17 (24%)	
Cerebrovascular accident	358 (57%)	37 (49%)		32 (44%)	36 (50%)	
Other	84 (13%)	15 (20%)		10 (14%)	13 (18%)	
Trauma	84 (13%)	6 (8%)		11 (15%)	6 (8%)	
Cold ischemia time, min						
Median (IQR)	461 (384–547)	162 (112–536)	**<0.001**	425 (359–480)	161 (111–524)	**<0.001**
Cases >8 h	263 (42%)	22 (29%)	**0.035**	18 (25%)	21 (29%)	0.708

Continuous data are reported as mean ± SD with P value from t tests or as median (IQR) with P value from Mann-Whitney tests, as applicable. P value from ordinal factors are from Fisher exact groups for dichotomous factors and Chi-square tests otherwise. Bold P values are significant at <0.05.
[a]Data were only available for the propensity-matched cohort.
DBD, donation after brain death; DCD, donation after circulatory death; HTN, hypertension; INR, international normalized ratio; IQR, interquartile range; LT, liver transplantation; NA, not available; NASH, nonalcoholic steatohepatitis; NMP, normothermic machine perfusion; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; SCS, static cold storage; SD, standard deviation; UKELD, UK End-stage Liver Disease.

perfused soon after organ procurement. Comparisons between the SCS and NMP cohorts are reported in Table 1.

Propensity Score Matching

To account for the differences in demographics between the 2 cohorts, transplants from the NMP group were matched to controls using a propensity score. CIT values showed little overlap between the groups, since reduced CIT is an intrinsic result of NMP, as mentioned above. As such, attempting to match the groups on CIT would result in large numbers of NMP cases being excluded, due to insufficient matches in the SCS group with sufficiently short CIT. However, to provide some degree of adjustment of CIT, a dichotomous variable identifying cases of extended (>8h) periods of CIT was included in the propensity score.

Of the 76 cases in the NMP cohort, suitably matched controls were identified for 72 patients. Comparisons between the 2 groups after matching found that the previously observed difference in UKELD scores was negated, with means of 52.9 versus 53.1 ($P = 0.886$) in the propensity-matched NMP versus SCS groups. The difference in the distribution of diagnoses also became nonsignificant after propensity matching ($P = 0.869$). While the average duration of CIT remained significantly longer in the matched SCS group, compared with the NMP group (median: 425 vs 161 min, $P < 0.001$), the number of cases with extended (>8h) periods of CIT were similar in the 2 groups after matching (25% vs 29%; $P = 0.708$).

Additional variables that could potentially influence the graft behavior were then compared between the propensity-matched NMP and SCS groups, namely presence of portal hypertension (HTN) and donor warm ischemic time. Neither of these factors were found to differ significantly between the propensity-matched groups, with presence of portal HTN rates of 42% versus 43% ($P = 1.000$) and donor warm ischemic time of 26 minutes (IQR: 17–31) versus 20 minutes (14–24, $P = 0.100$) in NMP versus SCS, respectively (Table 1).

TEG Values

None of the TEG parameters were found to differ significantly between the NMP and propensity-matched SCS groups when measured preimplantation (Table 2). However, in the postreperfusion period, the NMP group was found to have significantly shorter K times (median: 2.8 vs 3.6 min, $P = 0.010$) and R + K times (11.4 vs 13.7 min, $P = 0.016$) and significantly greater α-angles (55.9° vs 44.8°, $P = 0.002$), MA (53.5 vs 49.6 mm, $P = 0.044$), and G values (5.8k vs 4.9k dynes/cm², $P = 0.043$) than the matched SCS group. In addition, the LY30 was also found to differ significantly between the groups in the postimplantation period ($P = 0.004$), with 83% of the NMP group having absent hyperfibrinolysis (LY30 of 0) compared to 60% of the SCS group.

The analysis was also repeated to assess the changes in TEG values from pre- to postimplantation. The prolongation of clotting times in the NMP group

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TABLE 2.

Differences in TEG measurements between propensity-matched groups

	SCS		NMP		
	N	Statistic	N	Statistic	P
Preimplantation					
R time, min	64	6.9 (5.9–8.1)	64	6.8 (5.8–7.8)	0.556
K time, min	64	1.9 (1.5–2.7)	64	1.7 (1.5–2.3)	0.253
R + K time, min	64	8.9 (7.6–10.9)	64	8.5 (7.7–10.0)	0.460
α-Angle, °	64	63.8 (57.0–68.6)	64	66.4 (60.1–69.3)	0.163
MA, mm	64	61.1 (51.1–67.7)	64	63.0 (55.9–69.2)	0.127
G value, dynes/cm² [k]	64	7.8 (5.2–10.5)	64	8.5 (6.4–11.2)	0.123
LY30 = 0[a]	64	34/64 (53%)	65	32/65 (49%)	0.726
Postimplantation					
R time, min	65	8.9 (6.7–11.4)	67	7.9 (6.4–10.2)	0.115
K time, min	62	3.6 (2.4–6.4)	66	2.8 (2.0–4.2)	**0.010**
R + K time, min	62	13.7 (9.9–18.0)	66	11.4 (8.7–13.3)	**0.016**
α-Angle, °	65	44.8 (33.3–58.3)	67	55.9 (43.2–62.7)	**0.002**
MA, mm	65	49.6 (38.2–57.1)	67	53.5 (46.3–59.9)	**0.044**
G value, dynes/cm² [k]	65	4.9 (3.1–6.7)	67	5.8 (4.3–7.5)	**0.043**
LY30 = 0[a]	65	39/65 (60%)	66	55/66 (83%)	**0.004**
Change from pre- to postimplantation					
R time, min	63	2.7 ± 5.5	64	1.4 ± 3.2	0.089
K time, min	60	2.8 ± 3.6	63	1.5 ± 2.2	**0.014**
R + K time, min	60	5.3 ± 7.4	63	2.9 ± 4.7	**0.031**
α-Angle, °	63	−17.3 ± 14.0	64	−10.9 ± 13.0	**0.009**
MA, mm	63	−11.5 ± 11.7	64	−9.6 ± 10.1	0.332
G value, dynes/cm² [k]	63	−2.9 ± 2.6	64	−3.1 ± 3.3	0.613

Data are reported as mean ± SD with P value from t tests or median (IQR) with P value from Mann-Whitney tests, as applicable. Bold P values are significant at <0.05.
[a]The n/N (%) of patients with an LY30 of 0, with P value from Fisher exact test.
IQR, interquartile range; K, kinetic; LY30, clot lysis after 30 min; MA, maximum amplitude; NMP, normothermic machine perfusion; R, reaction; SCS, static cold storage; SD, standard deviation; TEG, thrombelastography.

TABLE 3.

Blood product use in propensity-matched groups

Type, units	SCS		NMP		
	N	Median (IQR)	N	Median (IQR)	P
FFP	72	4 (0–8)	72	2 (0–5)	0.070
Platelets	72	5 (0–15)	72	0 (0–15)	**0.001**
Red packed cells	72	2 (0–6)	72	2 (0–5)	0.665

Data are reported as median (IQR) with P values from Mann-Whitney tests. Bold P values are significant at <0.05.
FFP, fresh frozen plasma; IQR, interquartile range; NMP, normothermic machine perfusion; SCS, static cold storage.

had significantly smaller increases in K (mean: 1.5 vs 2.8 min, $P = 0.014$) and R + K times (2.9 vs 5.3 min, $P = 0.031$) and a significantly smaller reduction in α-angle (−10.9° vs −17.3°, $P = 0.009$) than propensity-matched SCS controls. For LY30, the patients were divided into subgroups with values of 0 (absence of hyperfibrinolysis) or >0 (presence of hyperfibrinolysis) in the preimplantation period. In both subgroups, the proportion of patients with absence of hyperfibrinolysis in the postimplantation period were significantly greater in the NMP versus SCS group, with rates of 97% versus 73% ($P = 0.013$) and 73% versus 43% ($P = 0.023$), respectively.

Blood Product Use

The group of patients who received NMP before graft implantation had significantly lower platelet use with a median of 0 units compared to 5 units in matched SCS group ($P = 0.001$). In total, only 34% (25/72 patients) of the NMP group required platelet transfusion compared to 64% (46/72) in the SCS group. No statistically significant differences in the numbers of units of FFP ($P = 0.070$), red packed cells ($P = 0.665$), or cryoprecipitate ($P = 1.000$) were detected between the matched NMP and SCS groups (Table 3). We have also analyzed Cell Saver use across the study groups. Across the propensity-matched cohort and NMP patients as a whole (N = 144), cell saver was used in 75% (n = 108) of cases with similar usage rates in the NMP and SCS groups (71% vs 79%, $P = 0.336$). In those that used cell saver, the median volumes were similar in the 2 groups, at 775 cc (IQR: 496–1677) and 737 cc (480–1781) for NMP and SCS, respectively ($P = 0.614$).

Across the COPE trial subgroup as a whole (N = 118), cell saver was used in 75% (n = 89) of cases. Usage rates did not differ significantly between the NMP and SCS groups (70% vs 86%, $P = 0.074$). Where the cell saver was used, the median volumes were similar in the 2 groups at 764 cc (IQR: 496–1588) and 772 cc (504–1789) for NMP and SCS, respectively ($P = 0.982$).

TABLE 4.

Differences in TEG measurements between DBD and DCD organs in the propensity-matched groups

	SCS			NMP		
	DBD (n = 47)	DCD (n = 25)	P	DBD (n = 45)	DCD (n = 27)	P
Preimplantation						
R time, min	7.1 (6.1–8.4)	6.7 (5.3–8.1)	0.259	6.9 (6.0–7.7)	6.7 (5.7–8.4)	0.997
K time, min	1.9 (1.5–2.8)	2.0 (1.7–2.3)	0.760	1.7 (1.4–2.2)	2.1 (1.7–2.5)	0.069
R + K time, min	9.0 (7.8–11.1)	8.5 (7.1–10.4)	0.281	8.5 (7.6–9.6)	8.7 (7.8–10.6)	0.528
α-Angle, °	65.0 (56.7–69.5)	62.9 (59.7–67.1)	0.777	67.5 (60.8–70.2)	62.1 (58.6–66.8)	**0.043**
MA, mm	58.7 (49.5–67.7)	63.6 (56.5–68.0)	0.241	65.0 (59.8–71.6)	58.5 (54.6–63.9)	**0.015**
G value, dynes/cm² [k]	7.1 (4.9–10.5)	8.7 (6.5–10.6)	0.262	9.3 (7.4–12.6)	7.1 (6.0–8.8)	**0.015**
LY30 = 0[a]	24/44 (55%)	10/20 (50%)	0.791	22/41 (54%)	10/24 (42%)	0.443
Postimplantation						
R time, min	3.7 (2.4–6.2)	3.2 (2.6–6.4)	0.638	7.9 (6.6–9.9)	8.1 (6.0–10.4)	0.910
K time, min	14.5 (10.4–18.2)	12.5 (9.2–15.0)	0.240	2.9 (2.0–4.2)	2.5 (2.1–4.2)	0.883
R + K time, min	43.8 (31.8–58.3)	47.2 (33.4–58.8)	0.493	11.6 (9.2–12.9)	11.0 (8.3–16.5)	0.903
α-Angle, °	46.7 (34.6–57.1)	52.9 (44.7–60.0)	0.271	54.5 (44.8–62.7)	56.9 (43.0–62.1)	0.745
MA, mm	4.4 (2.6–6.7)	5.6 (4.0–7.5)	0.277	52.8 (45.4–60.4)	53.6 (48.7–59.7)	0.631
G value, dynes/cm² [k]	3.7 (2.4–6.2)	3.2 (2.6–6.4)	0.638	5.6 (4.2–7.6)	5.8 (4.8–7.4)	0.627
LY30 = 0[a]	25/43 (58%)	14/22 (64%)	0.791	35/42 (83%)	20/24 (83%)	1.000
Change from pre- to postimplantation						
R time, min	2.4 ± 5.0	3.6 ± 6.4	0.421	1.5 ± 2.7	1.2 ± 4.0	0.734
K time, min	2.6 ± 2.9	3.2 ± 4.7	0.523	1.5 ± 2.2	1.4 ± 2.3	0.801
R + K time, min	4.6 ± 5.3	6.8 ± 10.5	0.388	3.1 ± 3.9	2.5 ± 5.8	0.662
α-Angle, °	−17.7 ± 13.5	−16.4 ± 15.4	0.725	−11.5 ± 11.5	−10.0 ± 15.4	0.658
MA, mm	−11.6 ± 12.6	−11.1 ± 10.0	0.861	−11.7 ± 10.4	−6.0 ± 8.6	**0.027**
G value, dynes/cm² [k]	−2.8 ± 2.8	−2.9 ± 2.2	0.899	−4.0 ± 3.4	−1.8 ± 2.7	**0.009**

Data are reported as mean ± SD, with P value from t tests, or as median (IQR), with P value from Mann-Whitney tests, unless stated otherwise. Bold P values are significant at <0.05.
[a]The n/N (%) of patients with an LY30 of 0, with P value from Fisher exact test.
DBD, donation after brain death; DCD, donation after circulatory death; IQR, interquartile range; K, kinetic; LY30, clot lysis after 30 min; MA, maximum amplitude; NMP, normothermic machine perfusion; R, reaction; SCS, static cold storage; SD, standard deviation; TEG, thromboelastography.

Patient demographics in the SCS and NMP groups for the subgroup of organs from the COPE trial

| Factor | COPE trial arm | | P |
	SCS (N = 42)	NMP (N = 76)	
Recipient age, y	54 (48–63)	55 (45–62)	0.931
Recipient sex			0.683
Female	12 (29%)	25 (33%)	
Male	30 (71%)	51 (67%)	
Recipient ethnic origin			0.975
Black	1 (2%)	2 (3%)	
Euro-Caucasian	39 (93%)	71 (93%)	
Other	2 (5%)	3 (4%)	
Final diagnosis			0.382
ALD	14 (33%)	16 (21%)	
ALF	2 (5%)	1 (1%)	
Hemochromatosis	0 (0%)	3 (4%)	
HBV	2 (5%)	2 (3%)	
HCV	6 (14%)	9 (12%)	
NASH	7 (17%)	13 (17%)	
Noncirrhotic portal HTN	0 (0%)	3 (4%)	
Polycystic liver	0 (0%)	1 (1%)	
PSC/PBC	8 (19%)	25 (33%)	
Other	3 (7%)	3 (4%)	
UKELD	52.4 ± 6.1	52.6 ± 5.0	0.857
Donor age, y	56 (45–64)	52 (42–63)	0.486
Donor height, cm	172.3 ± 9.2	170.4 ± 9.7	0.311
Type of donor			0.308
DBD	31 (74%)	48 (63%)	
DCD	11 (26%)	28 (37%)	
Donor cause of death			0.075
Anoxia	7 (17%)	18 (24%)	
CVA	28 (67%)	37 (49%)	
Other	2 (5%)	15 (20%)	
Trauma	5 (12%)	6 (8%)	
Cold ischemia time, min			
Median (IQR)	443 (382–504)	162 (112–536)	**<0.001**
Cases >8 h	13 (31%)	22 (29%)	1.000

Continuous data are reported as mean ± SD with P value from t tests or median (IQR) with P value from Mann-Whitney tests, as applicable. P value from categorical factors are from Fisher exact tests for dichotomous factors and Chi-square tests otherwise. Bold P value is significant at <0.05.
COPE, Consortium for Organ Preservation in Europe; DBD, donation after brain death; DCD, donation after circulatory death; HTN, hypertension; IQR, interquartile range; NASH, nonalcoholic steatohepatitis; NMP, normothermic machine perfusion; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; SCS, static cold storage; SD, standard deviation; UKELD, UK End-stage Liver Disease.

DBD versus DCD Organs

Subgroup analyses were then performed within propensity-matched NMP and SCS groups, to compare TEG parameters between DBD and DCD organs (Table 4). For the SCS group, no significant differences in any of the TEG parameters were detected, either for pre- or postimplantation. In contrast, in the NMP group, those receiving DCD organs were found to have significantly lower α-angles (median: 62.1° vs 67.5°, P = 0.043), MA (58.5 vs 65.0 mm, P = 0.015), and G values (7.1 vs 9.3k dynes/cm², P = 0.015) than DBD organs preimplantation and all these 3 parameters then improved in the DCD group, relative to DBD, such that no significant differences between DCD and DBD organs were detected in any TEG parameter postimplantation.

Analysis of the COPE Trial Subgroup; NMP versus SCS

Since the NMP organs had been part of the COPE trial, a subgroup analysis was performed in which these were compared with the SCS organs from the trial (N = 42). Comparisons of graft and recipient factors between these 2 groups found no significant differences, as would be expected, since the group allocation had been randomized (Table 5). Moreover, the duration of NMP was not found to be significantly correlated with any of the TEG parameter, as measured either pre- or postimplantation (NMP duration mean: 10.0 ± 3.9 h across the 2 groups). The only significant difference between the groups was in the CIT, which was significantly longer in the SCS group (median: 443 vs 162 min, P < 0.001), as expected. Comparisons of TEG parameters between these groups (Table 6) returned results that were consistent with the propensity-matched analysis. No significant differences between groups were detected preimplantation, but postimplantation R, K, and R + K times were significantly lower in NMP (with P values of 0.014, 0.029, and 0.039, respectively), while α-angle, MA, and G values were significantly higher in NMP relative to SCS (with P values of 0.004, 0.044, and 0.042, respectively).

DISCUSSION

NMP has undergone the process from feasibility assessment to multicenter randomized control trials and is in the transition phase, where various groups are considering incorporating it in their routine clinical practice. While the initial studies have proven the clinical safety of the procedure,[17,18] the focus of the large trials has been on mitigating preservation reperfusion injury.[13] Consequently, other aspects of the perireperfusion period including technical aspects, implantation times, coagulation management, and reperfusion syndrome have not yet been studied in detail. Previously our group published data on postreperfusion syndrome in a limited cohort of patients.[19] Given our expanded experience with NMP, including significant contributions to the recently published randomized controlled trial, our aim here was to address some of the aforementioned aspects for which the literature is presently deficient.

While the main advantage of NMP is proven to be reduced IRI, thereby better preserving allografts, we postulated that NMP would also preserve the synthetic function of liver grafts as a corollary. Furthermore, we envisaged that this improved synthetic ability of a graft would lead to immediate functional return upon reperfusion, thereby producing clotting factors, leading to better hemostasis as reflected in TEG characteristics. The results have proved our hypothesis. Since the cohorts were propensity-matched to minimize the impact of confounders, our results suggest that NMP independently improved the synthetic capabilities of the hepatocytes, as compared to SCS. One of the fundamental roles of the hepatic parenchyma is the synthesis of anti- and procoagulant proteins alongside components of the fibrinolytic system.[20] Karangwa et al[21] described the secretion patterns of prothrombin and plasminogen as potential surrogates for the activation of coagulation and hyperfibrinolysis in discarded liver grafts that received NMP without being implanted. The majority of transplant recipients with end-stage liver disease present with reduced

TABLE 6.

Differences in TEG measurements between SCS and NMP organs from the COPE trial

| | COPE trial arm | | | | |
| | SCS | | NMP | | |
	N	Statistic	N	Statistic	*P*
Preimplantation					
R time, min	35	6.6 (5.6–7.9)	68	6.8 (5.8–7.8)	0.837
K time, min	35	1.6 (1.3–2.0)	68	1.7 (1.4–2.2)	0.312
R + K time, min	35	8.7 (7.3–10.0)	68	8.5 (7.6–9.9)	0.974
α-Angle, °	35	67.1 (60.5–70.5)	68	66.4 (60.3–69.5)	0.656
MA, mm	35	66.8 (59.4–69.4)	68	63.5 (56.2–69.8)	0.410
G value, dynes/cm^2 [k]	35	10.1 (7.3–11.4)	68	8.8 (6.4–11.6)	0.402
LY30 = 0[a]	35	20/35 (57%)	69	35/69 (51%)	0.678
Postimplantation					
R time, min	38	9.3 (7.5–14.0)	71	7.9 (6.4–10.1)	**0.014**
K time, min	32	3.7 (2.7–6.8)	70	2.7 (2.0–4.2)	**0.029**
R + K time, min	32	13.8 (9.5–19.2)	70	11.1 (8.4–13.0)	**0.039**
α-Angle, °	38	44.9 (24.8–55.7)	71	56.4 (43.3–62.6)	**0.004**
MA, mm	38	49.9 (29.8–60.0)	71	53.6 (46.5–60.4)	**0.044**
G value, dynes/cm^2 [k]	38	5.0 (2.1–7.5)	71	5.8 (4.4–7.6)	**0.042**
LY30 = 0[a]	38	22/38 (58%)	70	59/70 (84%)	**0.005**
Change from pre- to postimplantation					
R time, min	34	5.9 ± 9.4	68	1.3 ± 3.1	**<0.001**
K time, min	28	3.3 ± 4.0	67	1.4 ± 2.1	**0.025**
R + K time, min	28	6.0 ± 8.4	67	2.8 ± 4.6	**<0.001**
α-Angle, °	34	−24.2 ± 21.0	68	−10.9 ± 12.6	**<0.001**
MA, mm	34	−19.6 ± 18.1	68	−9.4 ± 9.9	**0.004**
G value, dynes/cm^2 [k]	34	−4.5 ± 2.9	68	−3.1 ± 3.2	**0.045**

Data are reported as mean ± SD, with *P* value from *t* tests, or as median (IQR), with *P* value from Mann-Whitney tests, unless stated otherwise. Bold *P* values are significant at <0.05.
[a]The *n*/*N* (%) of patients with an LY30 of 0, with *P* value from Fisher exact test.
COPE, Consortium for Organ Preservation in Europe; IQR, interquartile range; K, kinetic; LY30, clot lysis 30 min; MA, maximum amplitude; NMP, normothermic machine perfusion; R, reaction; SCS, static cold storage; SD, standard deviation; TEG, thromboelastography.

synthetic capacity of coagulation factors and a rebalanced rather than decreased hemostasis, albeit with a decreased functional reserve.[14] Considering that the rebalancing of the coagulation cascade is caused by a reduction in both anti- and procoagulants, TEG seems to be the best means of assessing the ability to synthetize coagulation and anticoagulation factors by the liver parenchyma. Liver graft quality at the time of LT, as reflected in its synthetic capability, is a major determinant of early graft performance and, therefore, also influences graft survival and morbidity in the longer term.[22]

The first TEG-based intraoperative transfusion algorithms for LT recipients were reported by Kang et al in 1985.[23] Bed-side hemostasis monitoring devices, for example thrombelastography (TEG; Haemonetics, Niles, IL), allow discrimination between different phases of the coagulation system and consequently have a statistically proven advantage over conventional tests (INR, aPTT, fibrinogen values, and platelet count) in regard to predicting massive transfusion and fine-tuning the coagulation features to avoid catastrophic intraoperative bleeding.[24] This is probably because whole blood viscoelastic assays (TEG and thromboelastometry) containing both coagulation factors and platelets possess a greater ability to predict in vivo hemostasis alterations in comparison to classical artificial laboratory assays, which assess platelet-poor plasma.[25] The present study represents a report of the use of NMP in adult LT, aiming to evaluate its impact on the intraoperative synthetic capability of the graft reflected in TEG parameters, and these data look at the advantages of NMP from a different angle to commonly reported outcomes. TEG was chosen as an outcome over conventional coagulation tests, since PT and aPTT testing do not include thrombomodulin. For this reason, they are not suitable for assessing acquired deficiency of both pro- and anticoagulant factors, which occur in end-stage liver disease.[26] The choice of intraoperative TEG parameters as surrogate markers of graft synthetic capacity is, in our opinion, especially relevant considering the results of a recent study published by Trautman et al.[11] This analysis of a large cohort of 441 LT recipients found lengthened R, K, and R + K times to be independently associated with increased length of hospital stay. This may suggest that coagulopathy per se at the time of transplant exerts a deleterious effect on how patients fare postoperatively. The fact that our data show improved α-angle and MA values in recipients of NMP liver grafts is especially relevant for the postoperative management of the recipient, considering the recent study of Lawson et al.[27] This found that decreased α-angle and MA values predict massive transfusion requirements in liver transplant recipients. Therefore, by increasing these parameters, NMP might be able to indirectly decrease the

need for massive requirements of blood products. This was actually observed in our study, with the recipients of NMP organs having significantly lower platelet requirements. While the difference in platelet transfusion between the 2 groups in terms of platelet transfusion requirement can be attributed to NMP, this potentially could also result from baseline differences between groups in platelet count. Furthermore, TEG-guided transfusion decreases the necessary of FFP in patients undergoing LT,[28] which means that NMP graft enhancement, as reflected in TEG parameters, might also improve the intraoperative management of the recipient.

Ex situ NMP of livers is progressing at an advanced pace, and the benefits of assessing for viability as well as reconditioning the grafts are emerging from multiple teams all over the world. A milestone study published by He et al[29] reporting the first case ischemia-free liver transplant with the total elimination of both warm ischemia time and CIT made possible by uninterrupted NMP has interestingly revealed that no plasma, fibrinogen, or any other coagulation factor was used after reperfusion. This validates the results of our study, which suggest that coagulopathy is mitigated in recipients of NMP livers, on one hand, but also seems to suggest that in the future, ischemia-free organ transplantation with continuous NMP might become standard of care.

A major limitation of the study consists of assessing the outcomes by kaolin-TEG, which has a reduced capacity to differentiate between fibrinogen and platelet contributions to clot formation.[30] New viscoelastic assay devices, such as Functional Fibrinogen (FF-TEG), have been introduced in recent years with promising results,[31] including at our center. However, at the time of study conclusion, the investigation did not have this capability. The data were collated retrospectively; however, the informatics system has recorded all TEG data as real-time data. Therefore, we do not believe this in any way impacted the analysis and results. We believe the patient population and size are adequate and study and control groups are well matched to draw these conclusions, especially considering that a randomized study especially targeted at analyzing these parameters is virtually impossible. On the other hand, the major advantage of the retrospective collation of the data is the elimination of subjective bias of setting transfusion thresholds, avoiding under/over correction, whereas in all the cases in the study considered here transfusion has been based on the clinical need only.

We conclude NMP is capable of improving the synthetic function of liver grafts as reflected in TEG measurements of coagulation in the recipient. It is one of the aspects which herald a paradigmatic shift in organ preservation before transplantation, from metabolic suppression during SCS to metabolic support in NMP. Taking into consideration that the optimal range of hemostatic balance is significantly narrower in liver transplant recipients compared with the healthy population, the fact that NMP can enhance the coagulation characteristics of liver grafts is especially relevant in the clinical setting.

ACKNOWLEDGMENTS

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