Hypothermic oxygenated perfusion(HOPE) improves ECD liver graft function and reduces duration of hospitalisation without extra cost: The PERPHO Study

Michel Rayar, Jean-Marie Beaurepaire, Emma Bajeux, Stephanie Hamonic, Thomas Renard, Clara Locher, Véronique Desfourneaux, Aude Merdrignac, Damien Bergeat, Mohamed Lakehal, et al.

DR. AUDE MERDRIGNAC (Orcid ID : 0000-0002-0284-4755)

Article type : Original Articles

Hypothermic oxygenated perfusion(HOPE) improves ECD liver graft function and reduces duration of hospitalisation without extra cost: The PERPHO Study

Michel Rayar MD[1,2,3], Jean-Marie Beaurepaire MD[1], Emma Bajeux MD[4], Stéphanie Hamonic Eng[4], Thomas Renard MD[1], Clara Locher PharmD[2,5], Véronique Desfourneaux MD[1], Aude Merdrignac MD PhD[1,2] , Damien Bergeat MD[1], Mohamed lakehal MD[1], Laurent Sulpice MD PhD[1,2] , Pauline Houssel-Debry MD[6], Caroline Jezequel MD[6], Christophe Camus MD PhD[7], Edouard Bardou-Jacquet MD PhD[2,3,6], Bernard Meunier MD PhD[1,2]

[1] CHU Rennes, Service de Chirurgie Hépatobiliaire et Digestive, F-35033 Rennes, France
[2] Université Rennes1, Faculté de médecine, F-35043 Rennes, France
[3] INSERM, CIC 1414, F-35033 Rennes, France
[4] CHU Rennes, Service d'Epidémiologie et de santé publique, F-35033 Rennes, France
[5] Service de Pharmacologie clinique, F-35033 Rennes, France
[6] CHU Rennes, Service des maladies du foie, F-35033 Rennes, France
[7] CHU Rennes, Service de Maladies Infectieuses et Réanimation Médicale, F-35033 Rennes, France

Corresponding author:
Michel Rayar michel.rayar@chu-rennes.fr
Service de Chirurgie Hépato-Biliaire et Digestif.
CHU Pontchaillou – Rennes (France) +33 2 99 28 91 89

Clinical trial number : NCT03376074
Key Words: liver transplantation, machine perfusion, ECD graft, HOPE
Word count: 4228

Number of figures and tables: 6

Short Title: HOPE for ECD grafts

Communication: This work was presented as an oral presentation in plenary session at the Congress of the French Association of Hepato-Biliary and Transplantation Surgery (ACHBT) in Marne-la-Vallée, France in 2018

Author contributions:

MR, JMB, BM wrote and reviewed the manuscript.

MR, JMB, TR gathered the data.

MR, CJ, CL performed the statistical analysis.

EB, SH performed the medico-economic analysis.

All authors reviewed and approved the manuscript.

Disclosures: The authors declare no conflict of interest regarding this study.

Funding: This study received financial support from the Rennes University Hospital.

ABSTRACT

Few studies have evaluated the efficacy or the cost of Hypothermic Oxygenated PErfusion(HOPE) in the conservation of extended criteria donor (ECD) grafts from brain-dead donors (DBD) during liver transplantation(LT). The PERPHO Study (NCT03376074) is a prospective, monocentric, study designed to evaluate the interest of HOPE for ECD-DBD grafts. For comparison, a control group was selected after propensity score matching among patients transplanted between 2010 and 2017. Between February and November2018, the HOPE procedure was used in 25 LT. Immediately after LT, the median AST level was significantly lower in the HOPE-group (724vs 1284, p=0.046), as was ALT (392vs 720, p=0.01), lactate (2.2vs 2.7, p=0.01) and creatinine levels (73vs 89, p=0.01). There was a significant reduction in ICU stay (3vs 5days, p=0.01) and hospitalization (15vs 20days, p=0.01) in the HOPE-group. There was no significant difference regarding the incidence of early allograft dysfunction (EAD) (28% vs 42%, p=0.22) or PNF (8% vs 3%, p=0.29) between the HOPE and control-groups. A level of AST or ALT in perfusate> 800 was found to be highly predictive of EAD occurrence (AUC 0.92 and 0.91, respectively). The 12-month graft (88% vs 89.5%, p=1) and patient survival (91% vs 91.3%, p=1) were similar. The additional cost of HOPE was estimated at €5298 per patient. The difference between costs and revenues, from the hospital's perspective, was not different between the HOPE and control groups (respectively +€3023 vs +€4059, IC[-€5470; +€8652]). In conclusion, HOPE may improveECD graft function and reduced hospitalization stay without extra cost. These results must be confirmed in a randomized trial.

Introduction

Liver transplantation (LT) is the best treatment for end-stage liver disease and primary liver malignancies. Owing to the growing gap between the number of candidates and transplanted patients, transplantation teams have been forced to increase the donor pool by using more and more grafts from so-called "extended criteria donors" (ECD).

Although they may currently represent more than 50% of the grafts used [1], there is still no consensual definition of an ECD graft. However, they are mostly represented by elderly donors, circulatory death donors (DCD) or fatty liver grafts and are well known to be more vulnerable to ischemia-reperfusion injuries (IRI) [2] created during conventional static cold storage. These grafts are therefore associated with higher rates of graft dysfunction [3–6].

Thus, there has been increasing interest in perfusion machines in order to improve the quality of conservation and reduce the consequences of IRI. Among the different techniques of perfusion, hypothermic oxygenated perfusion (HOPE) is simple and has already proved its efficacy in preserving DCD grafts by reducing biliary complications and improving graft and patient survival [7]. However, few studies have evaluated the interest of HOPE in preserving ECD grafts procured from brain-dead donors (DBD) nor its cost from a hospital's perspective since the procedure is not covered by health insurance in most countries.

We hypothesized that HOPE could benefit liver transplantation performed with ECD grafts and implemented a prospective trial: the PERPHO Study in order to confirm it.

Patients and Methods

Study design and purpose

The PERPHO Study (the interest of hypothermic oxygenated perfusion in the preservation of extended criteria donor; NCT03376074) was a prospective, monocentric, single arm, pilot study designed to evaluate the interest of HOPE in the preservation and functional recovery of ECD grafts during liver transplantation.

For comparison, a control group was selected after propensity score matching among patients transplanted with ECD grafts in our institution between 2010 and 2017.

Ethic statement

Informed and written consent was obtained from all included patients in the HOPE group and absence of opposition was obtained from patients in the control group.

The study was approved by the National Ethics Committee and received authorization from the French National Drug Safety Agency (ANSM).

Inclusion criteria

HOPE group

All adult patients with cirrhosis (whatever the cause), candidates for a first LT between February and November 2018, without the need for combined organ transplantation were eligible to participate in the study and were finally included if they received ECD grafts from DBD donors.

Patients who required emergency transplantation for acute liver failure or retransplantation without cirrhosis, or who received split grafts, were not eligible.

Patients who received grafts from DCD donors were also not eligible since in France, those grafts systematically receive in situ normothermic regional perfusion.

An ECD graft was defined by the presence of at least one of the following criteria as previously reported [8]: age >65 years; BMI >30; ICU stay prior to procurement >7 days; natremia >155 mmol/L; liver enzymes 3 times higher than the normal value (i.e. aspartate aminotransferase (AST) blood level >150 IU/mL, alanine aminotransferase (ALT) blood level >170 IU/mL); occurrence of cardiac arrest before procurement, biopsy-proven macrovesicular steatosis >30%.

Patients were recruited during pre-LT consultations or just before the procedure.

Control group

The control group was selected after propensity score matching (1:3 ratio) among liver transplantations with ECD grafts performed in our institution between 2010 and 2017.

The variables used in the calculation of propensity score were:

- Recipients: age, gender, BMI, MELD score, CHILD PUGH grade, indication for LT, location at time of LT (i.e. home or hospital or ICU), intubation;
- Donors: age, gender, BMI, ICU stay prior to procurement >7 days, natremia >155 mmol/L, AST blood level >150 IU/mL, ALT blood level >170 IU/mL, occurrence of cardiac arrest before procurement;
- duration of cold ischemia.

All variables were attributed the same weight in the propensity score calculation.

Study protocol

Procurement and machine perfusion settings

After graft acceptance and standard procurement, the graft was initially preserved in a static cold ischemia phase using CUSTODIOL®. After arrival at our center, the liver graft was prepared during the back-table procedure and then flushed with 1 liter of machine perfusion solution (UWMP®) before connection to the machine perfusion device (Liver Assist®).

According to the Karangwa et al. classification [9], perfusion was performed through the portal vein at a target pressure of 3 to 5 mmHg with 2 liters of machine perfusion solution (UWMP®) oxygenated with 1 liter/min at a temperature of 11°C. The duration of perfusion was ideally between 2 and 4 hours with a minimum of 1 hour and a maximum of 6 hours.

Our aim was to achieve the shortest ischemia time (ideally below 8 hours) and not to test HOPE to increase ischemia time. Perfusion was therefore started immediately after the back-table procedure and during native liver hepatectomy and then stopped just before graft implantation.

Liver transplantation and postoperative care

All patients had orthotopic liver transplantation with inferior vena cava preservation. Briefly, after standard wound incision and exposition, the liver pedicle was first dissected. The native liver was removed and careful hemostasis was performed. The graft was removed from the perfusion machine, flushed with 500ml of 5% albumin, and its implantation started with side-to-side caval anastomosis followed by end-to-end portal vein anastomosis. The graft was then vascularized prior to artery and biliary anastomosis.

After the procedure, standardized immunosuppression (associating calcineurin inhibitor [usually tacrolimus], mycophenolate mofetil and a short course of corticosteroids) was systematically administered. Systematic Doppler ultrasonography was performed on postoperative days (POD) 1 and 7 and if hepatic dysfunction or vascular complication was suspected. When suspected, CT was

systematically performed to confirm vascular complications and treatment (medical, radiological or surgical) was systematically discussed in multidisciplinary meetings.

Patients were discharged from hospital only when they presented normal liver graft function, had immunosuppressive treatment within the therapeutic range and demonstrated sufficient autonomy (eating, physical movement, able to correctly take their medications).

Study endpoint

Primary endpoints

The primary endpoint was the incidence of early allograft dysfunction (EAD) as described by Olthoff et al. [10] (i.e. the presence of one or more of the following criteria: bilirubin ≥171 µmol/L on day 7 after LT, or international normalized ratio (INR) ≥1.6 on day 7 after LT, or peak alanine-aminotransferase (ALT) >2,000 U/L within the first 7 days after LT) and/or occurrence of primary non-function defined as liver failure requiring retransplantation or leading to death within 7 days after transplantation.

Secondary endpoints

The secondary endpoints were:

- Intraoperative parameters: number of intraoperative transfusions, incidence of reperfusion syndrome (defined as a decrease of more than 30% of the mean arterial pressure value for at least 1 minute and occurring within 5 minutes after revascularization, [11] duration of procedure.
- Biological parameters:
    - Graft function: prothrombin level, serum bilirubin, serum AST and ALT, Factor V, INR, during the 7th day after LT and arterial lactate (dosed until normalization).
    - Renal function: serum creatinine, clearance estimated by the CKD-EPI formula [12] and acute kidney injuries (with KDIGO classification) observed at 48 hours after LT [13].
- Postoperative parameters: incidence of severe postoperative complications assessed by Clavien-Dindo classification ≥3 during initial hospitalization, duration of hospital and intensive care unit stays, biliary complications at 12 months and patient and graft survival at 12 months.
- Economic impact from the hospital's perspective: evaluation of the additional cost of the machine perfusion procedure, estimation of the costs and incomes of the hospital stay.

Cost analysis

In France, the hospital income is paid by French National Health Insurance and is mainly determined by the characteristics of each inpatients stay (taking in consideration patient's age and comorbidities, diagnosis, duration of stay, medical and surgical procedures, intensive care unit stays, treatments …). These inpatients stays are then classified in a medically and economically homogenous group (Groupe Homogène de Malade ; GHM). A standard national tariff is applied to each group. For each French hospital, all the hospital stays, their characteristics, the GHM and associated tariff are recorded in a Database called *Programme de Médicalisation des Systèmes d'Information* database; PMSI. Data on hospital revenues for hospital stays of the patients included in the study were identified from the Rennes CHU PMSI database.

The tariffs of each GHM are defined by the national authorities. They correspond to an estimate of the average costs of hospital stays for this GHM (e.g. for an average length of stay). Data on hospital costs for hospital stays of the patients included in the study were retrieved from the French National Cost Database called ENC (Echelle Nationale des Coûts) which collects average real-world costs for each GHM from a French hospital representative sample. We adjusted the costs for each GHM with the actual length of stay of patients included in the study.

Additionally, for each hospital stay in the HOPE group, cost data included amortization of the machine perfusion device, perfusion kits, machine perfusion solution and maintenance of the machine.

Cost and revenue were expressed as mean value ± standard deviation and compared using bootstrap confidence intervals of the difference of mean cost between the two groups. Owing to sampling fluctuations, the distribution of DRG was not necessarily the same in the two groups. We therefore estimated the average costs of hospital stays in the HOPE group by performing standardization, i.e. by applying the distribution of DRG of the control group to the HOPE group.

Statistical analysis

Comparative cohort matching

After calculation of propensity scores, matching was performed with a 1:3 ratio between patients in the HOPE and control groups. Exact match was prioritized followed by selection of the closest control and the maximum distance allowed between two matched patients was set at 0.2 (i.e. caliper restriction).

Quantitative variables were expressed as mean value ±standard deviation or by median with extreme values and compared using Student's *t*-test or Wilcoxon test as appropriate. Qualitative variables were expressed as number and percentage and compared using Chi-squared or Fisher's exact test, as appropriate.

All statistical analyses were made with R software version 3.4.3 while the medico-economic analysis was performed with SAS software version 9.4. The propensity score analysis was performed using the "Matchit" R-package version 3.0.2. and ROC curve analysis was made using the "ROCR" R-package version 1.0-7. A $p<0.05$ value was considered as statistically significant.

Results

Recipient and graft characteristics (Table 1)

Between February and November 2018, 97 LT were performed in our institution. An ECD grafts was used in 54 (56%) cases. After taking into consideration exclusion criteria, 43 LT could have been potentially included in the PERPHO study, among them 25 patients were finally included and constituted the HOPE group. The remaining 18 LT were already included in another study [14] or were not included owing to operator availability for the machine perfusion (Figure 1).

Patients were men in 80% (n=20) of the cases with a median age of 63 [43-69] years and a median MELD before LT of 18 [7-37]. Seventy-two percent (n=18) of the patients were at home prior to LT while 28% (n=7) were hospitalized including 16% (n=4) in the ICU with intubation.

The donor was a man in 54% (n=14) of the cases with a median age of 70 [45-87] years and in 19 (76%) cases, donor age was >65 years.

Procedure and perfusion characteristics

Median ischemia time was 525 [379-824] min with a median perfusion time of 117 [75-252] min. Median perfusion pressure was 5 [2-6] mmHg and the median flow rate was 448 [205-624] ml/min. No adverse event due to machine perfusion was observed.

Perfusion time was greater than 3 hours in 5 cases. In 2 cases (cases 11 and 13), surgery had to be delayed owing to another liver transplantation at the same time (case 11) or to another emergency surgical procedure (case 13). In the 3 other cases (cases 14, 15, 18), prolonged perfusion time was related to prolonged hepatectomy due to extended portal thrombosis (cases 14 and 18) requiring extensive portal thrombectomy or recipient obesity (case 15).

Comparison after propensity score matching (Table 2)

Propensity score matching

Between 2010 and 2017, 932 LT were performed in our institution. Among them, 534 LT were performed with an ECD graft and constituted the "pool" of potential controls. After propensity score matching, only 69 patients were included in the control group instead of 75 owing to caliper restriction (Figure 1). In particular, there were no controls found for case 13 (probably due to prolonged ischemia time).

In order to avoid giving an advantage to one group or the other, we decided to keep the unmatched patients in the HOPE group in the comparative analysis since the 2 groups were still comparable.

Endpoints and outcomes

As for the primary outcome, EAD was present in 28% (n=7) of patients in the HOPE group and 42% (n=29) in the control group without significant difference (p=0.22). Retransplantation within the 7th day was observed in 8% (n=2) in the HOPE group (patient 4 on POD 4 for hepatic vein thrombosis with liver failure and patient 15 on POD 7 for hepatic artery thrombosis with normal liver function, discovered on systematic Doppler ultrasound) and 2.9% (n=2) in the control group without significant difference (p=0.29). Primary outcome was therefore present in 32% (n=8) of the patients in the HOPE group and 42% (n=29) in control group without significant difference (p=0.38).

Predictive value of liver enzyme levels measured on perfusate at the end of perfusion

Liver enzyme dosage on the perfusion solution was analyzed for 20 patients in the HOPE group (dosage not performed or failed in 3 cases and excluded in 2 cases (cases 4 and 15) due to the presence of vascular complications which could impact postoperative biological parameters).

Median AST level in the perfusion solution was 248.5 [48-2,076] UI/L and median ALT level was 285.5 [38-1,397] UI/L.

Among the 20 patients, 4 patients presented EAD and had a significantly higher AST level (980.5 UI/L vs 170 UI/L, p=0.01) and ALT level (1073.5 UI/L vs 143.5 UI/L, p=0.01) in the perfusion solution compared with the patients who did not present EAD.

ROC curve analysis (Figure 2) revealed that AST and ALT levels in perfusate were excellent predictors of EAD occurrence with AUC respectively of 0.92 and 0.91. The best predictive cut-off was 800UI/L for AST and ALT.

As a consequence, postoperative blood liver enzymes were significantly increased in the early postoperative days when liver enzyme levels were increased in the perfusate (Figure 3).

Intraoperative parameters

Surgical time was significantly reduced in the HOPE group compared with the control group (300 vs 395 min, p<0.001). There was no difference regarding the median number of packed red blood cells transfused (5 vs 5, p=0.29) while the difference was close to being significant for the median number of fresh frozen plasma transfused (4 vs 5, p=0.07) or the median number of platelet count (0 vs 1, p=0.06). Post reperfusion syndrome was present in 52% (n=13) patients in the HOPE group and could not be evaluated retrospectively in the control group.

Postoperative parameters (Figure 4)

The median AST level was significantly lower in the HOPE group on POD 0 (724 U/L vs 1,284 U/L, p=0.046) as was the ALT level (392 U/L vs 720 U/L, p=0.01), the lactate level (2.2 vs 2.7, p=0.01) and the creatinine level (73 µmol/L vs 89 µmol/L, p=0.01).

On POD 1, the lactate level was still lower in the HOPE group (1.5 vs 2, p=0.03) while the difference was no more significant regarding the AST level (599 U/L vs 870 U/L, p=0.09) and ALT (454 U/L vs 668 U/L, p=0.06).

The acute kidney injuries incidence (calculated at 48 hours after LT) was no different between groups (44% (n=11) vs 28.9%(n=20), p=0.17). As for cholestasis enzymes, the GGT level was significantly increased in the HOPE group from POD 5 (253 vs 191, p=0.04) as in the ALP level on POD 7 (186 vs 129, p=0.024) whereas bilirubin levels were not different.

Median ICU stay was significantly reduced in the HOPE group (3 vs 5 days, p=0.01) as was hospital stay (15 vs 20 days, p=0.01). Six patients (24%) had severe complications (i.e. Clavien-Dindo score ≥ 3) in the HOPE group with 31 (44.9%) patients in the control group, without significant difference (p=0.07).

Occurrence of biliary complications during the first 12 months was similar between groups (8% (n=2) vs 11.6% (n=8), p=1). In the HOPE group, 2 patients presented anastomotic complications, requiring surgical revision for bile leakage in one case and endoscopic treatment for anastomotic stricture in the other. In the control group, 7 patients presented anastomotic complications, represented by 4 bile leakage (requiring surgery in 2 cases and medical treatment only in 2 cases) and 3 anastomotic strictures treated by surgery in 1 case and endoscopic treatment in 2 cases. One patient in the control group presented biliary necrosis requiring retransplantation. No patients presented non-anastomotic stricture.

At 12 months, graft survival was similar between the 2 groups (88% in the HOPE group vs 89.5% in the control group, p=1) as was patient survival (91% in the HOPE group vs 91.3% in the control group, p=1).

Cost analysis

In the HOPE group, machine perfusion device cost was estimated at €429 per patient according to an amortization time of 7 years and an annual number of LT procedures of 25 patients. The costs of perfusion kits and machine perfusion solution were estimated at €4,195 and €338 per patient respectively. Maintenance cost was estimated at €336 per patient. Finally, total additional cost for the procedure was estimated at €5,298 per patient.

Consequently, the average cost of hospital stay was €46,136 ± €39,994 in the HOPE group and €42,756 ± €27,943 in the control group.

Average hospital income was estimated at €49,159 ± €24,740 in the HOPE group and €46,815 ± €17,886 in the control group.

The average difference between cost and revenue for an hospital stay from an hospital perspective was not statistically significant between the HOPE and the control groups (respectively +€3,023 ±€16,537 and +€4,059 ±€16,266, IC [-€5,470-€8,652]).

DISCUSSION

Liver transplantation is the best treatment for end-stage liver disease but it is limited by the scarcity of grafts. Expanding the donor pool with DCD or ECD grafts appears to be the most effective solution despite their higher risk of dysfunction. In order to decrease this risk and improve outcome, machine perfusion has been evaluated and shown promising results [15,16].

The PERPHO Study is the first prospective trial evaluating the interest of HOPE in the preservation of ECD grafts procured from DBD donors. The study was not designed to test an increase of the total conservation time or to increase the number of LT performed by reconditioning discarded grafts.

We found a significant reduction in AST and ALT levels in the early postoperative period attesting to a reduction in hepatocyte damage as well as a significant decrease in lactate levels reflecting faster graft function recovery. However, we did not note a significant decrease in EAD incidence (28% in the HOPE group vs 42% in the control group) which was probably due to a lack of power. Interestingly, liver enzyme levels in the perfusion liquid, measured at the end of the HOPE procedure, were highly predictive of EAD incidence and correlated to postoperative serum liver enzymes.

Interestingly, we observed that the serum level of ALP and GGT started to increase from POD2 (as well as bilirubin level) in both groups. However, this increase was higher in the HOPE group and became significant at POD5 for GGT and POD7 for ALP before spontaneously resolving. We believe this phenomenon may represent the liver regeneration which was described as positively correlated with serum level of biliary marker [17] instead of a marker of biliary damage.

We also observed a significant reduction in ICU (3 vs 5 days, p=0.01) and total hospital stays (15 vs 20 days, p=0.01) which could be explained by better graft function recovery as well as the reduced incidence of severe complications which was close to being significant (p=0.07). Consequently, from a hospital's perspective, the additional cost of the machine perfusion procedure (estimated at €5,298 per patient) was compensated by a reduction of hospitalization length resulting in a non-significant difference between cost and revenue between the 2 groups. This analysis was made from the hospital’s perspective (and not from the healthcare system’s) since the decision to invest or not into machine perfusion program, with charge of the extra-costs which is the main obstacle to its implementation, is up to the hospitals with no participation of the national healthcare system in most countries.

Moreover, even if the aim of the study was not to increase the number of LT performed, we believe that one procedure was only allowed by the HOPE procedure. Indeed, in the case of patient 13, an unpredictable weather event led to the graft arriving with more than 10 hours of cold ischemia and without immediate access to an operating room (due to another urgent procedure). In this particular case, the graft would have probably considered as unfit for transplantation without the potential benefit of HOPE since we would not have taken the risk of using an ECD graft with such prolonged ischemia. Instead, we decided to put the liver on machine perfusion and wait until another operating room was available. As a consequence, we did not find a suitable control for this case – highlighting its exceptional nature.

In the same way, when the hepatectomy is complicated, we believe that HOPE makes it possible to partially mitigate the stress and the pressure of "cold ischemia".

Another change of paradigm was represented by recipient-graft matching. Indeed, ECD grafts are usually allocated to "good" recipients (mostly HCC patients with compensated cirrhosis) while most "high-MELD" recipients receive non-ECD grafts. However, in our study, we transplanted 3 patients with MELD >30 with grafts from donors >75 years. Since the postoperative outcomes were uneventful, we believe that HOPE makes it possible to safely allocate ECD grafts to "high-MELD" recipients.

Similar to kidney transplantation, liver perfusion started in the beginning of this decade with the first clinical series in 2010 reported by Guarrera et al. [15]. In their pilot study, the authors reported the use of hypothermic machine perfusion without oxygenation for conservation of DBD "standard" donors and found an improvement in biological markers and shorter hospital stays in the machine group. Thereafter, the same team reported their experience with 31 liver grafts considered as "orphan" [18] and reported higher one-year patient survival and significantly shorter hospital stays, fewer biliary complications and acute kidney injuries. However, Schlegel et al. [19,20] showed that hypothermic oxygenated perfusion (HOPE) recharged depleted cellular energy stores (i.e. ATP level), restored the mitochondrial redox state by reversible suppression of oxidative metabolism and subsequently decreased the production of oxygen radical species, resulting in less IRI and improved liver function [21]. Thereafter, machine perfusion proved its utility in LT with DCD grafts by improving graft function as well as survival compared with conventional static conservation [7,22]. Recently, Nasrala et al. [23] reported in a prospective randomized trial using normothermic perfusion for preservation of all sorts of grafts, a significant decrease in postoperative liver enzyme levels as well as in the incidence of EAD and the rate of

discarded grafts. However, despite the fact that normothermic machine perfusion is supposed to be more efficient than HOPE (but also more complex), they failed to show any clinical improvement since the duration of hospital stays as well as the grafts and patient survival were no different. One could therefore think that machine perfusion is not required for all LT, especially those with "non-ECD grafts". Recently, Patrono et al. [24] reported their results of 25 patients transplanted with ECD grafts using D-HOPE. They noted a significant reduction in postoperative AST and ALT blood levels resulting in a decrease in EAD incidence and a significant reduction in stage 2-3 acute kidney injuries. However, they did not report a significant difference regarding hospital stay and survival which could be partly explained by the significantly higher duration of preservation in the perfusion group (499 min vs 371 min, $p<0.001$).

Our results are in line with previous studies relating the benefits of the HOPE procedure since we noted a reduction in liver enzyme levels [23] and better graft function recovery. However, we found that HOPE significantly reduced hospital stays resulting in equivalent hospitalization costs. Therefore, as with transplantations with DCD grafts [7,22], we believe that use of machine perfusion is justified and economically acceptable for LT with DBD-ECD grafts.

However, our results must be interpreted with caution. First, since this was a pilot study, we had a limited number of included patients (n=25). Moreover, we only found 69 patients for the control group (due to caliper restriction) instead of 75, which decreased the power of our study. However, since the unmatched patients of the HOPE group (which could be considered as having a poorer prognosis) were kept in the analysis, we believe that our results may underestimate the potential benefit of HOPE. Second, our control group was not prospective which could induce a methodological bias in both the clinical and cost analysis. However, we voluntarily decided to not make a prospective control group since we believe that a prospective randomized trial with only 25 patients in both the HOPE and control groups has a high risk of leading to unbalanced randomization and then to non-comparable groups. Third, our definition of ECD graft could be questionable and may not reflect the real quality of the graft. However, there is still no consensual definition of an ECD graft and we previously reported those criteria which are now used in prospective trials in our country [14,25].

In any case, our promising results must be confirmed in a prospective randomized trial which is currently ongoing [25]. Fourth, the wide range of the HOPE duration in our study may impact the liver enzymes level in the perfusate. However, since the plateau level is quickly reach and stay mostly stable until the end of perfusion[18], we believe that the HOPE procedure duration is not

represent a major bias. Finally, our cost analysis finding may not be directly applicable to other healthcare systems due to the specificities of the French hospital funding. However, since our estimation of the extra cost of the HOPE procedure will be the same whatever the country or the healthcare system, we believe that our results will provide informative data for further studies.

In conclusion, we believe that HOPE is a promising method to improve preservation of DBD-ECD grafts since it may provide better graft function. In our study, the additional cost of the procedure was compensated by better outcomes. These promising results must be confirmed in a prospective, multicentric, randomized trial.

REFERENCES

1. Rapport annuel médical et scientifique 2018, agence de la biomédecine.
2. Noack K, Bronk SF, Kato A, et al. The greater vulnerability of bile duct cells to reoxygenation injury than to anoxia. Implications for the pathogenesis of biliary strictures after liver transplantation. *Transplantation*. 1993;56:495–500.
3. Nemes B, Gámán G, Polak WG, et al. Extended-criteria donors in liver transplantation Part II: reviewing the impact of extended-criteria donors on the complications and outcomes of liver transplantation. *Expert Rev Gastroenterol Hepatol*. 2016;10:841–859.
4. Durand F, Renz JF, Alkofer B, et al. Report of the Paris consensus meeting on expanded criteria donors in liver transplantation. *Liver Transplantation*. 2008;14:1694–1707.
5. Eren EA, Latchana N, Beal E, et al. Donations After Circulatory Death in Liver Transplant. *Exp Clin Transplant*. 2016;14:463–470.
6. Foley DP, Fernandez LA, Leverson G, et al. Biliary Complications after Liver Transplantation from Donation after Cardiac Death Donors: An Analysis of Risk Factors and Long Term Outcomes from a Single Center. *Ann Surg*. 2011;253:817–825.
7. Dutkowski P, Polak WG, Muiesan P, et al. First Comparison of Hypothermic Oxygenated PErfusion Versus Static Cold Storage of Human Donation After Cardiac Death Liver Transplants: An International-matched Case Analysis. *Ann Surg*. 2015;262:764–770; discussion 770-771.
8. Rayar M, Levi Sandri GB, Cusumano C, et al. Benefits of temporary portocaval shunt during orthotopic liver transplantation with vena cava preservation: A propensity score analysis. *Liver Transpl*. 2017;23:174–183.
9. Karangwa SA, Dutkowski P, Fontes P, et al. Machine Perfusion of Donor Livers for Transplantation: A Proposal for Standardized Nomenclature and Reporting Guidelines. *Am J Transplant*. 2016;16:2932–2942.
10. Olthoff KM, Kulik L, Samstein B, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. *Liver Transplantation*. 2010;16:943–949.
11. Paugam-Burtz C, Kavafyan J, Merckx P, et al. Postreperfusion syndrome during liver transplantation for cirrhosis: outcome and predictors. *Liver Transpl*. 2009;15:522–529.
12. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604–612.
13. Khwaja A. KDIGO clinical practice guidelines for acute kidney injury. *Nephron Clin Pract*. 2012;120:c179-184.

14. APCext : Effect of Temporary Porto-caval Shunt During Liver Transplantation on Function of Liver Graft From Extended Criteria Donor - Full Text View - ClinicalTrials.gov Available from: https://clinicaltrials.gov/ct2/show/NCT02784119. Accessed January 15, 2020.

15. Guarrera JV, Henry SD, Samstein B, et al. Hypothermic Machine Preservation in Human Liver Transplantation: The First Clinical Series. *American Journal of Transplantation*. 2010;10:372–381.

16. Dutkowski P, Schlegel A, de Oliveira M, et al. HOPE for human liver grafts obtained from donors after cardiac death. *Journal of Hepatology*. 2014;60:765–772.

17. Otao R, Beppu T, Isiko T, et al. External biliary drainage and liver regeneration after major hepatectomy. *Br J Surg*. 2012;99:1569–1574.

18. Guarrera JV, Henry SD, Samstein B, et al. Hypothermic Machine Preservation Facilitates Successful Transplantation of "Orphan" Extended Criteria Donor Livers. *American Journal of Transplantation*. 2015;15:161–169.

19. Schlegel A, Rougemont O de, Graf R, et al. Protective mechanisms of end-ischemic cold machine perfusion in DCD liver grafts. *Journal of Hepatology*. 2013;58:278–286.

20. Schlegel A, Dutkowski P. Role of hypothermic machine perfusion in liver transplantation. *Transplant International*. 2015;28:677–689.

21. Lüer B, Koetting M, Efferz P, et al. Role of oxygen during hypothermic machine perfusion preservation of the liver. *Transplant International*. 2010;23:944–950.

22. van Rijn R, Karimian N, Matton APM, et al. Dual hypothermic oxygenated machine perfusion in liver transplants donated after circulatory death: Dual hypothermic oxygenated machine perfusion in liver transplantation. *Br J Surg*. 2017;104:907–917.

23. Nasralla D, Coussios CC, Mergental H, et al. A randomized trial of normothermic preservation in liver transplantation. *Nature*. 2018;557:50–56.

24. Patrono D, Surra A, Catalano G, et al. Hypothermic Oxygenated Machine Perfusion of Liver Grafts from Brain-Dead Donors. *Sci Rep*.;9 . Epub ahead of print June 27, 2019. DOI: 10.1038/s41598-019-45843-3.

25. Hypothermic Oxygenated Perfusion for Extended Criteria Donors in Liver Transplantation (HOPExt) - Full Text View - ClinicalTrials.gov Available from: https://clinicaltrials.gov/ct2/show/NCT03929523. Accessed January 15, 2020.

Table and figure legends

Table 1: Characteristics of recipients and donors in the HOPE group and postoperative outcome.

Table 2: Comparison of the demographic characteristics and outcomes between the 2 groups.

Figure 1 : Flow chart of the study

Figure 2: ROC curve analysis of predictive value of liver enzyme levels on perfusion liquid on occurrence of early allograft dysfunction.

Figure 3: Evolution of postoperative serum liver enzyme levels according to level in perfusat

Postoperative evolution of a) median AST level; b) median ALT level; *indicates a significant difference.

Figure 4: Biological parameter evolution during the first 7 days following liver transplantation.

Postoperative evolution of a) median AST level; b) median ALT level; c) median lactate level; d) median serum creatinine level; e) median bilirubin level; f) median ALP level; g) median GGT level; h: median Factor V level; *indicates a significant difference.

	Recipient characteristics					Donor characteristics				Intraoperative			Postoperative outcome						
	Age	Gender	CHILD	MELD at LT	Indication for LT	Age	BMI	Cardiac arrest	Steatosis	Perfusion Duration (min)	CIT (min)	PRS	EAD	PNF	Clavien-Dindo	Hospital stay (day)	3-month graft survival	3-month patient survival	Cause of graft loss or/and death
1	64	Male	5	7	HCC+ALC	80	23.8	No	5	93	642	Yes	Yes	No	1	8	Yes	Yes	
2	63	Female	9	15	ALC	68	43	No	30	109	536	No	No	No	3	24	Yes	Yes	
3	63	Male	7	9	HCC+ALC	83	28.2	No	0	75	425	No	No	No	2	12	Yes	Yes	
4	61	Male	6	10	HCC+ALC	69	25.2	No	15	125	491	No	Yes	Yes	4	97	No	Yes	HV thrombosis
5	62	Male	12	17	HCC+ALC	47	25	Yes	0	115	630	No	No	No	2	21	Yes	Yes	
6	64	Male	6	22	HCC+ALC	83	29	No	0	105	616	Yes	No	No	1	10	Yes	Yes	
7	58	Male	13	34	ALC	87	18	No	10	95	511	Yes	No	No	2	13	Yes	Yes	
8	66	Female	12	18	ALC	75	26.2	No	10	97	414	Yes	No	No	3	13	Yes	Yes	
9	49	Male	15	34	ALC	76	22.6	No	0	90	526	Yes	Yes	No	2	33	Yes	Yes	
10	43	Male	12	25	ALC	64	32.4	No	5	117	554	No	No	No	2	13	Yes	Yes	
11	50	Male	5	16	HCC+ALC	53	21.6	Yes	5	226	692	No	No	No	2	19	Yes	Yes	
12	63	Female	11	37	ALC	68	25.3	No	5	130	379	Yes	No	No	2	15	Yes	Yes	
13	63	Male	9	12	HCC+ALC	70	29.4	No	0	252	816	Yes	Yes	No	3	19	Yes	Yes	
14	57	Male	8	19	ALC+HCV	84	29	No	15	222	547	Yes	Yes	No	2	24	Yes	Yes	
15	62	Male	12	21	ALC	70	20.7	No	0	178	525	Yes	No	Yes	3	23	No	Yes	HA thrombosis
16	66	Male	5	12	HCC+ALC	70	27.3	No	0	130	615	Yes	No	No	2	13	Yes	Yes	
17	57	Male	12	22	HCC+ALC	68	23.4	Yes	0	116	512	No	No	No	2	15	Yes	Yes	
18	68	Male	9	17	HCC+ALC	82	23.7	No	5	195	530	No	No	No	2	12	Yes	Yes	
19	59	Male	10	22	OTHERS	71	22.1	No	0	124	444	No	No	No	2	10	No	No	Multivisceral failure
20	65	Male	5	18	HCC+ALC	51	25.1	Yes	NA	107	495	No	Yes	No	2	20	Yes	Yes	
21	62	Male	6	14	HCC+ALC	82	20.3	No	0	144	505	Yes	No	No	2	8	Yes	Yes	
22	56	Female	12	32	NASH	79	24.5	No	0	153	665	No	No	No	2	15	Yes	Yes	
23	66	Male	11	18	ALC	78	22.9	Yes	10	100	519	Yes	No	No	3	13	Yes	Yes	
24	69	Female	11	20	ALC	48	25.4	No	0	124	458	No	No	No	2	17	Yes	Yes	
25	66	Male	5	8	HCC+ALC	45	40.9	No	NA	90	445	Yes	Yes	No	2	11	Yes	Yes	

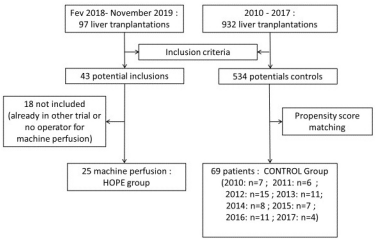
Table 1: Characteristics of recipients and donors in the HOPE group and postoperative outcomes

ALC: alcoholic disease; EAD: early allograft dysfunction; HA: hepatic artery; HCC: hepatocellular carcinoma; HV: hepatic vein; LT: Liver Transplantation; NA: not available; PRS: post-reperfusion syndrome; PNF: primary non function

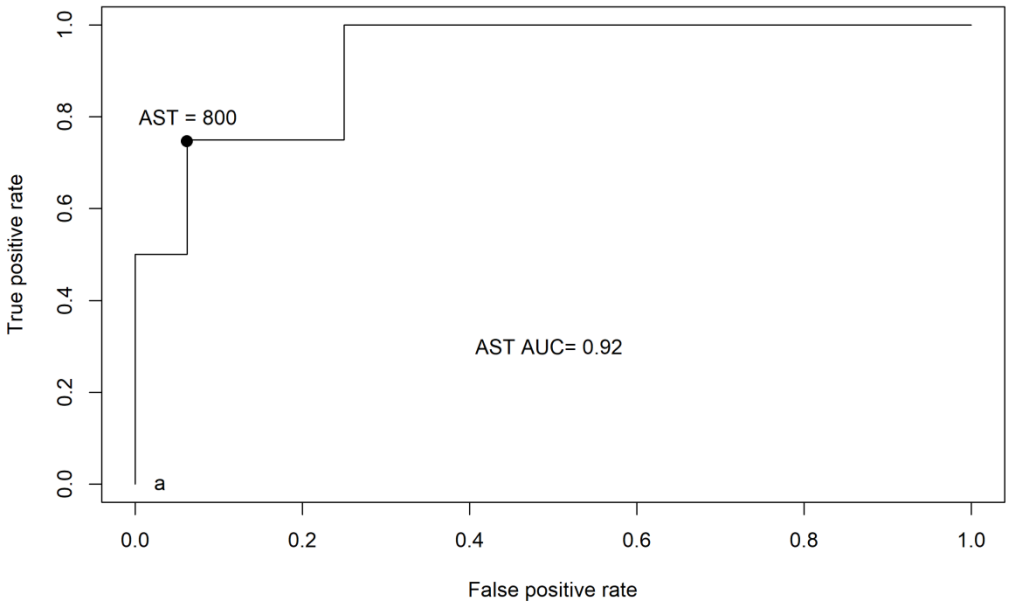
Table 2: Comparison of the demographic characteristics and outcomes between the 2 groups

	HOPE group (n=25)	Control group (n=69)	p-value
Recipient characteristic			
Gender (male)	20 (80%)	57 (82.6%)	0.77
Age (years)	63 [43-69]	62 [36-70]	0.32
BMI	26.7 [19.7-44.8]	27.4 [18.1-39.2]	0.88
MELD	18.3 [7-37]	18.3 [5-40]	0.63
CHILD	9 [5-15]	9 [5-15]	0.84
Creatinine level (day before LT)	75 [44-197]	76 [43-486]	0.96
Total Bilirubin level (day before LT)	35 [11 ; 625]	43.5 [5 ; 749]	0.83
Indication of LT			0.96
HCC	13 (52%)	33 (47.8%)	
Alcoholic cirrhosis	9 (36%)	24 (34.8%)	
HCV	1 (4%)	6 (8.7%)	
Others	2 (8%)	6 (8.6%)	
Donor characteristics			
Gender (male)	14 (56%)	43 (62.3%)	0.58
Age	70 [45-87]	72 [25-88]	0.88
BMI	25.1 [18-43]	24.7 [15.2-37.2]	0.61
ICU stay (days)	2 [0-13]	2 [1-10]	0.91
Cardiac arrest before procurement	5 (20%)	14 (20.3%)	1
Cold Ischemia Time (min)	525 [379-824]	555 [207-722]	0.55
Steatosis	0 [0 ; 30]	0 [0 ; 50]	0.45
Graft weight (g)	1340 [900 ; 2000]	1355 [650 ; 2270]	0.85
Primary outcomes			
Early allograft dysfunction	7 (28%)	29 (42%)	0.22
Primary non function	2 (8%)	2 (2.9%)	0.29

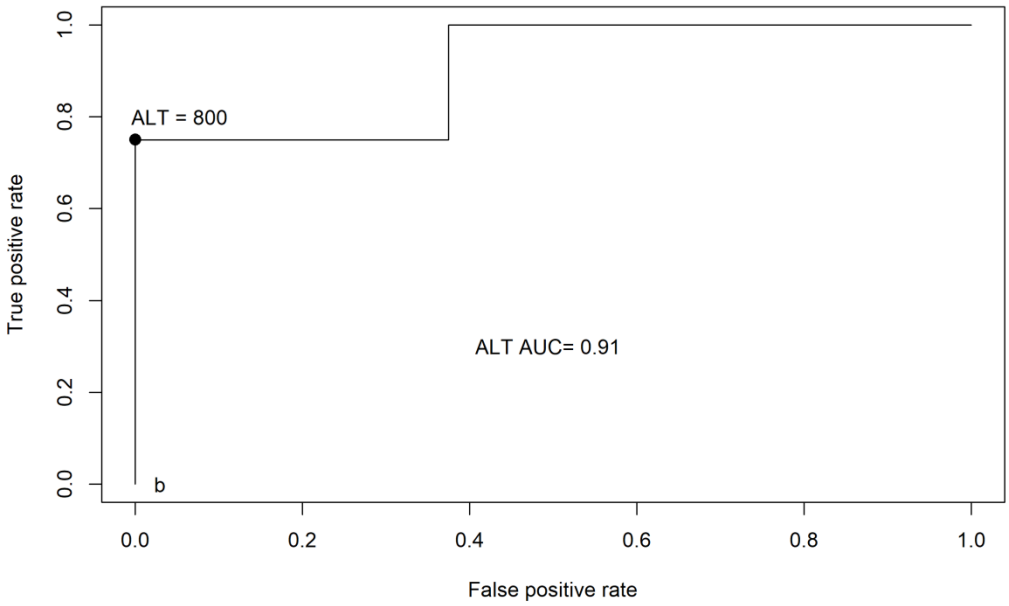
Secondary outcomes			
Intraoperative parameters			
Surgical time (min)	300 [206-387]	395 [169-582]	<0.001
Red blood cell transfusion	5 [0-10]	5 [0-23]	0.64
Fresh Frozen plasma transfusion	4 [0-14]	5 [0-20]	0.07
Platelet count transfusion	0 [0-2]	1 [0-3]	0.06
Post-reperfusion syndrome	13 (52%)	NA	
Postoperative parameters			
Acute Kidney Injury	11 (44%)	20 (28.9%)	0.17
KDIGO stage 1	7 (28%)	11 (15.9%)	0.86
KDIGO stage 2	3 (12%)	6 (8.7%)	
KDIGO stage 3	1 (4%)	3 (4.3%)	
Need for renal replacement	1 (4%)	2 (2.9%)	1
Peak ASAT level (within 24st hrs)	722 [184 ; 6673]	1301 [236 ; 10979]	0.07
Peak ALAT level (within 24st hrs)	493 [132 ; 4353]	722 [169 ; 5754]	0.02
Clavien-Dindo ≥3	6 (24%)	31 (44.9%)	0.07
ICU stay (days)	3 [1-72]	5 [1-43]	0.01
Hospital stay (days)	15 [8-92]	20 [9-92]	0.01
Biliary complication (at 12 months)	2 (8%)	8 (11.6%)	1
Anastomotic (leak or stenosis)	2 (8%)	7 (10.1%)	
Non anastomotic stricture	0	0	
Ischemic necrosis	0	1 (1.4%)	
12-month graft survival	22 (88%)	59 (89.5%)	1
12-month patient survival	23 (91%)	63 (91.3%)	1



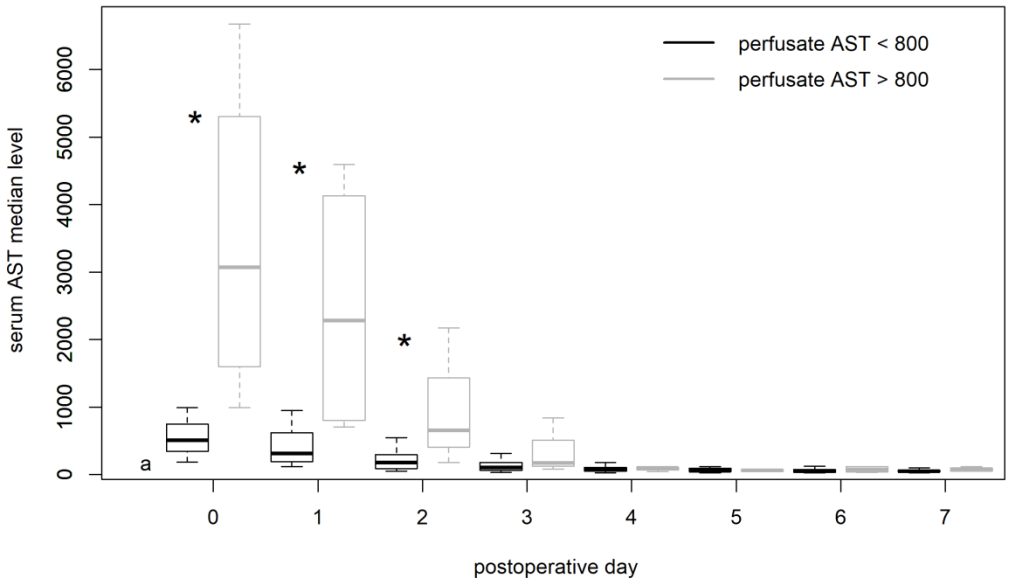
lt_25955_f1.tif



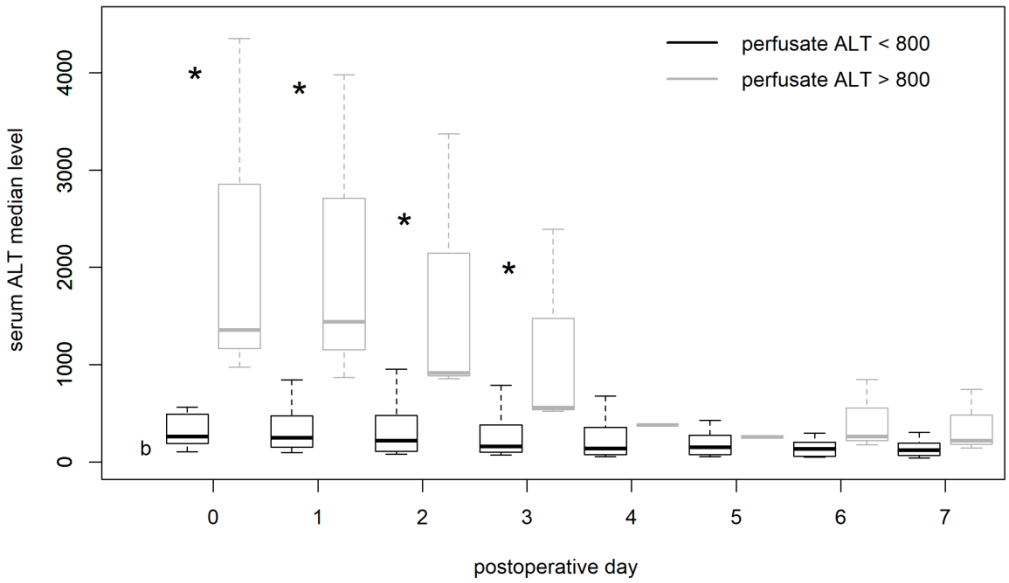
lt_25955_f2a.tiff



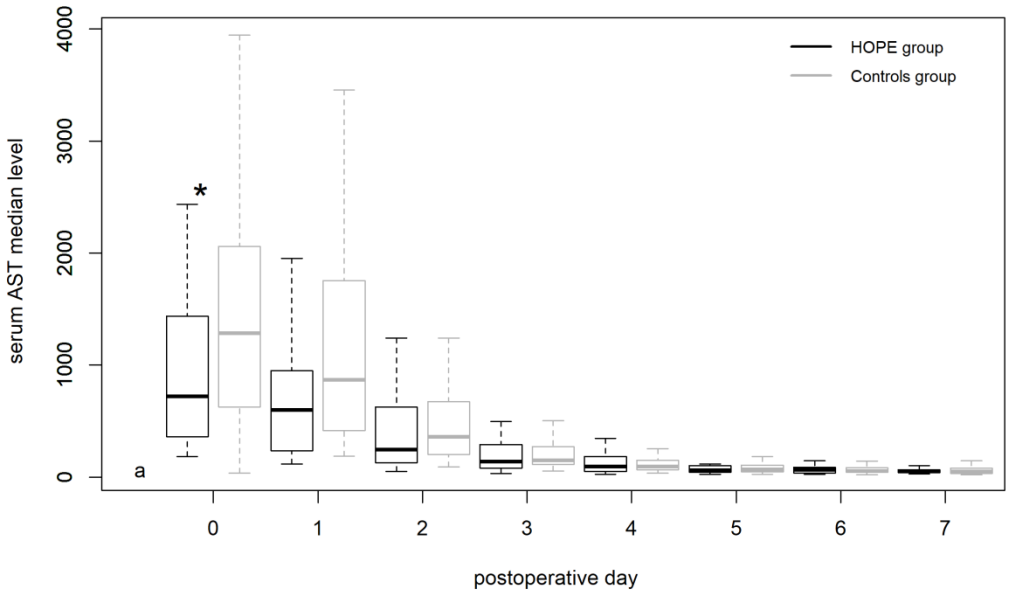
lt_25955_f2b.tiff



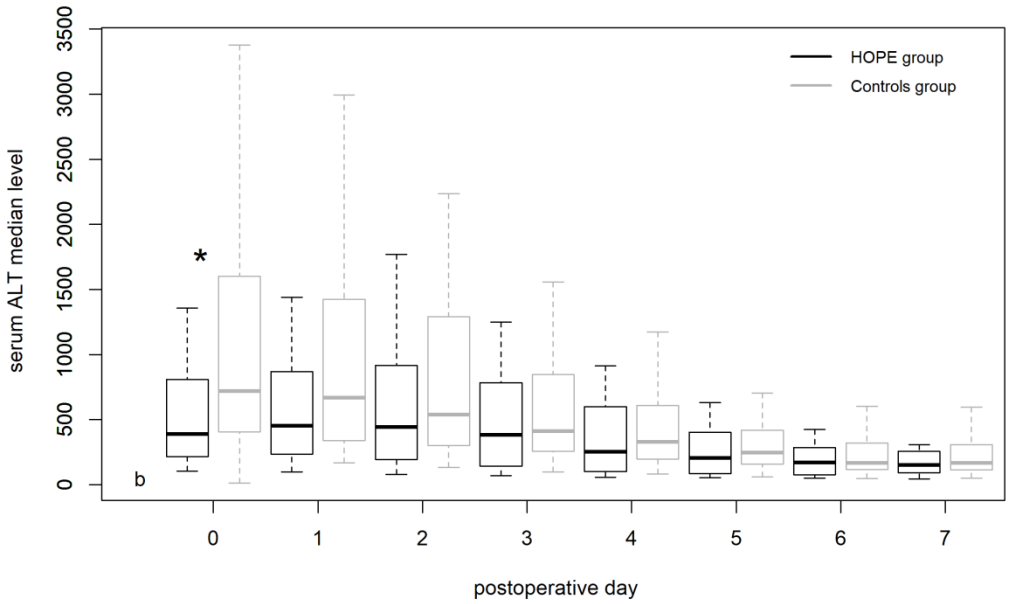
lt_25955_f3a.tif



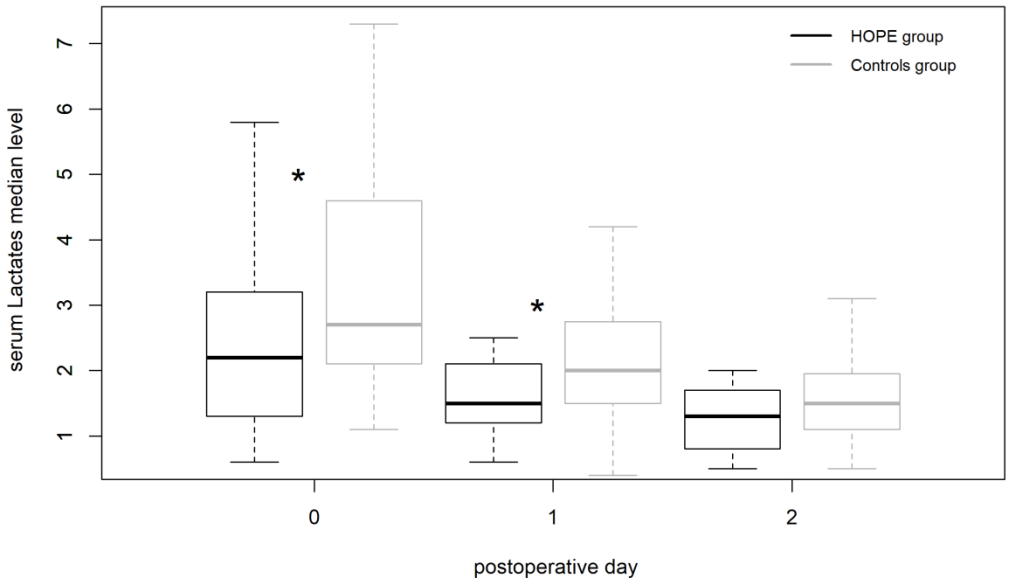
lt_25955_f3b.tif



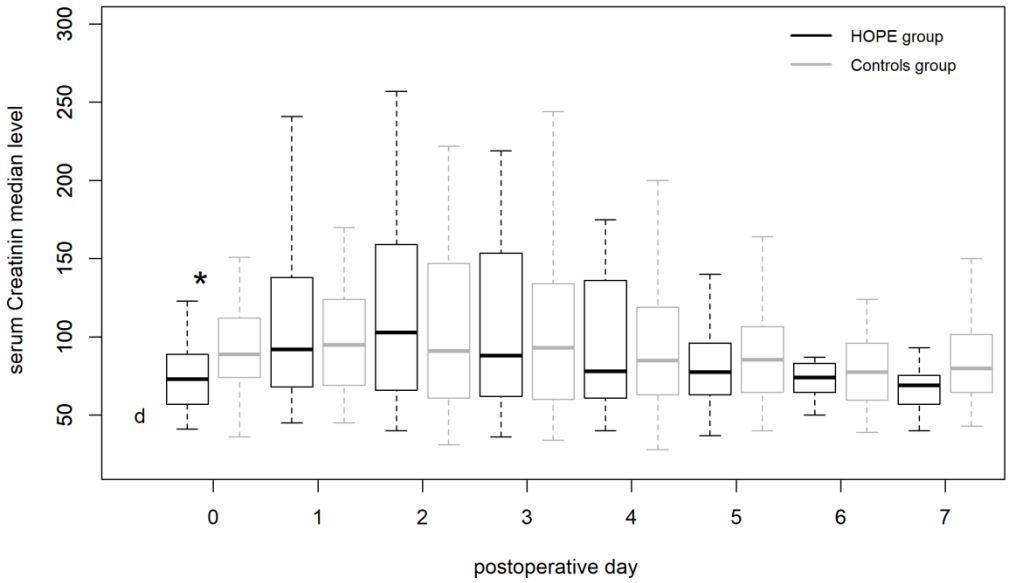
lt_25955_f4a.tif



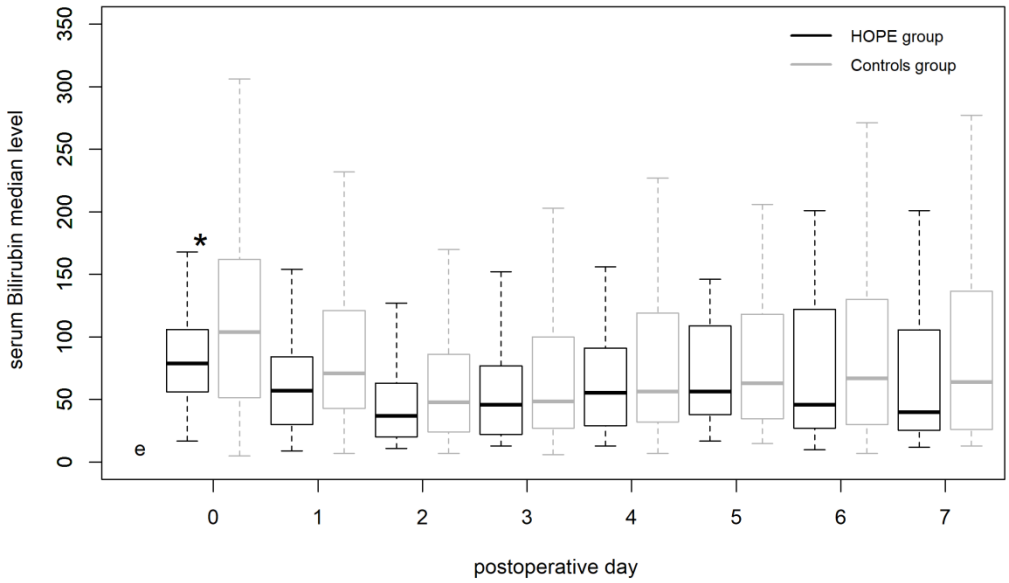
lt_25955_f4b.tif



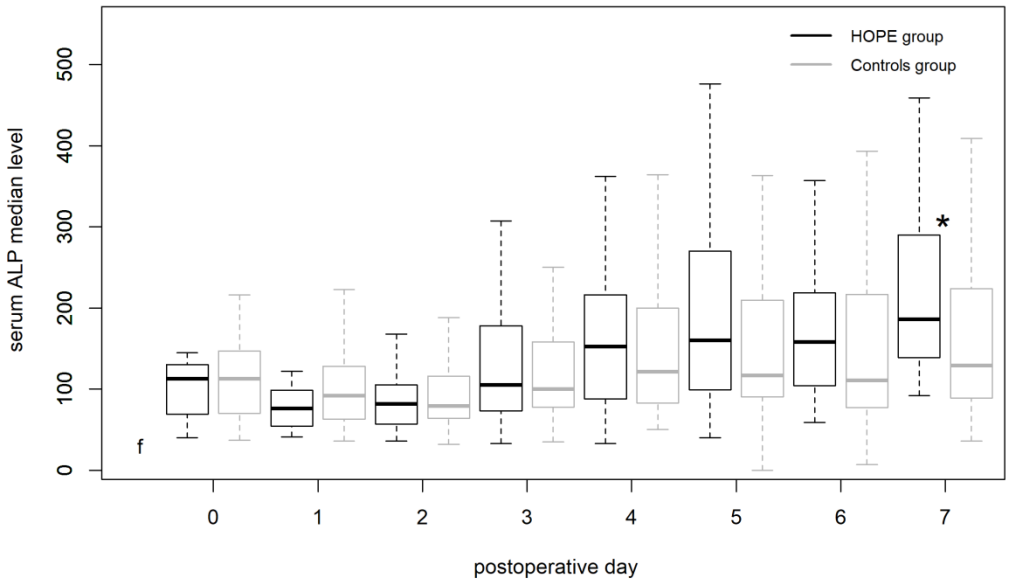
lt_25955_f4c.tif



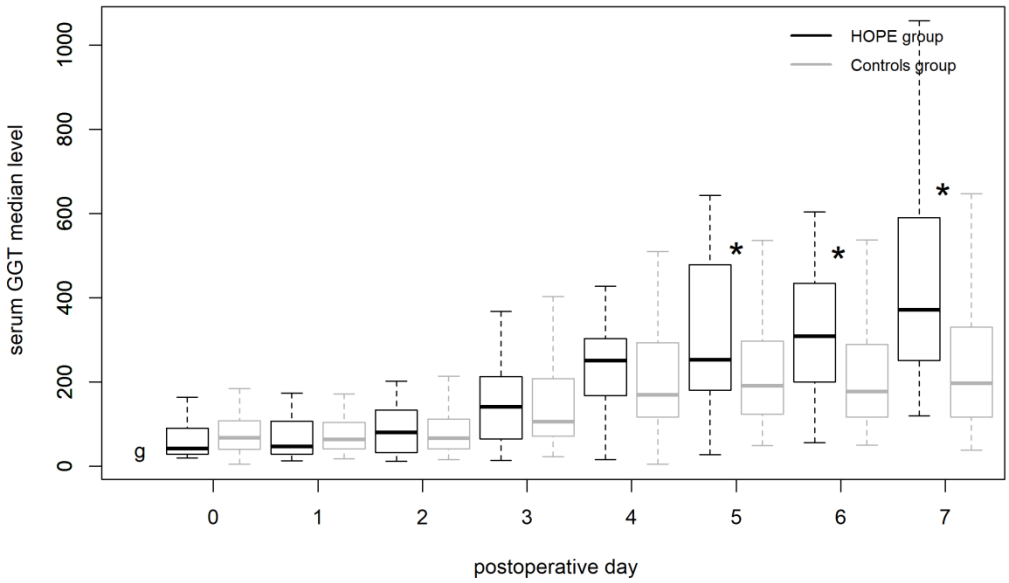
lt_25955_f4d.tif



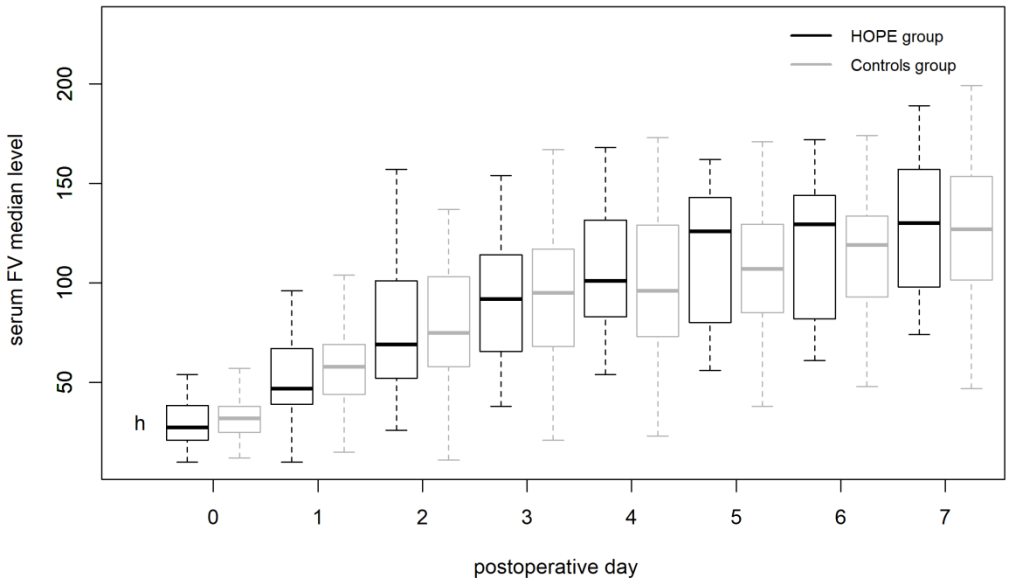
lt_25955_f4e.tif



lt_25955_f4f.tif



lt_25955_f4g.tif



lt_25955_f4h.tif