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ORIGINAL ARTICLE

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Discarded livers tested by normothermic machine perfusion in the VITTAL trial: Secondary end points and 5-year outcomes

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Abstract

Normothermic machine perfusion (NMP) enables pretransplant assessment of high-risk donor livers. The VITTAL trial demonstrated that 71% of the currently discarded organs could be transplanted with 100% 90-day patient and graft survivals. Here, we report secondary end points and 5-year outcomes of this prospective, open-label, phase 2 adaptive single-arm study. The patient and graft survivals at 60 months were 82% and 72%, respectively. Four patients lost their graft due to nonanastomotic biliary

Abbreviations: AEs, adverse events; BDI, bile duct injury; DBD, donation after brain death; DCD, donated after circulatory death; IQR, interquartile range; MRC, magnetic resonance cholangiography; NAS, nonanastomotic biliary stricture; NMP, normothermic machine perfusion; QoL, quality of life; SCS, static cold storage; VITTAL, viability testing and transplantation of marginal livers.

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strictures, one caused by hepatic artery thrombosis in a liver donated following brain death, and 3 in elderly livers donated after circulatory death (DCD), which all clinically manifested within 6 months after transplantation. There were no late graft losses for other reasons. All the 4 patients who died during the study follow-up had functioning grafts. Nonanastomotic biliary strictures developed in donated after circulatory death livers that failed to produce bile with pH >7.65 and bicarbonate levels >25 mmol/L. Histological assessment in these livers revealed high bile duct injury scores characterized by arterial medial necrosis. The quality of life at 6 months significantly improved in all but 4 patients suffering from nonanastomotic biliary strictures. This first report of long-term outcomes of high-risk livers assessed by normothermic machine perfusion demonstrated excellent 5-year survival without adverse effects in all organs functioning beyond 1 year (ClinicalTrials.gov number NCT02740608).

INTRODUCTION

The demand for liver transplantation surpasses donor supply and the waiting list mortality, exceeding 10% in many countries, and has become a worldwide problem. A rising proportion of organs are procured from high-risk donors. In the context of closely scrutinized post-transplant outcomes (expecting 1- and 5-year survival rates around 95% and 84%, respectively), the declining donor quality means many procured livers remain unused.[1] For example, in the United Kingdom, organ donation increased by 50% between 2010 and 2020, yet the number of liver transplants performed each year has been falling since 2017, and the liver discard rate nearly tripled during the same period.[2–4]

Aiming to answer the question if viability testing by normothermic machine perfusion (NMP) can allow safe transplantation of high-risk livers, we conducted the VITTAL clinical trial.[5] Applying lactate-based criteria during NMP commenced after a period of static cold storage (SCS), we demonstrated that 71% of organs deemed unsuitable for transplantation by all the UK centers could be transplanted with 100% 90-day patient and graft survivals.[6] Others confirmed similarly high rescue rates of declined high-risk livers in clinical trials performed in different regions, donor allocation policies, and health care systems, making it evident that implementation of objective assessment can lead to a significantly increased utilization of organs from sub-optimal donors.[7–10]

While the short-term VITTAL trial outcomes were excellent, there were several graft losses in livers donated after circulatory death (DCD) due to the development of nonanastomotic biliary strictures (NAS). The development of NAS was not anticipated as the importance of bile composition in predicting biliary strictures following end-ischemic NMP was only reported at the end of the patient enrollment phase.[11] Others reported alternative perfusion approaches such as hypothermic oxygenated perfusion, normothermic regional perfusion, normothermic preservation, and their combinations with favorable outcomes.[12–16] The results of the VITTAL trial should not be directly compared with other normothermic perfusion series, however, as its design allowed only enrollment of the highest-risk livers considered for donation in the UK, and it excluded many discarded organs for being “too good,” which has been unprecedented in any other perfusion study protocol reported to date.[7,9,17]

With the growing experience of machine perfusion technologies, it seems that a tailored approach based on the type of donation might further improve post-transplant outcomes.[18] Yet, there is still a large majority of livers that are procured without consideration given to using any machine perfusion, and an increasing proportion of these are not used for transplants.[1] In this context, applying machine perfusion *ad hoc* on liver inspection in the transplant center remains the only opportunity for assessing their quality and salvaging some of those organs for patients in need of lifesaving transplantation. For instance, our team has established a program targeting fast-track donation after brain death (DBD) livers (ie, livers already retrieved but declined following the donor or accepting team’s inspection) and successfully applies the lactate-based viability criteria to use them for patients requiring liver retransplantation.[19]

The lack of data about the long-term post-transplant outcomes of perfused livers, in particular those from DCD donors, is often a concern for physicians and patients who are offered these organs. To address the

question of longevity of NMP-perfused livers, we report here the 5-year VITTAL trial results, including a detailed analysis of biliary features, recipient quality of life (QoL), and adverse events (AEs).

METHODS

The trial design, study intervention, and primary outcomes

The study design, methodology, and primary outcomes have been reported.[5,6] Briefly, VITTAL was a prospective, open-label, phase 2 adaptive single-arm trial comprising livers rejected as unsuitable for transplant by all UK transplant centers that used end-ischemic NMP to select transplantable organs. The study was registered at ClinicalTrials.gov (reference number NCT02740608), funded by the Wellcome Trust, conducted in accordance with the Declarations of Helsinki and Istanbul, and approved by the National Research Ethics Service in Dulwich, London (REC reference 16/LO/1056), and the Ethical Committee at the University Hospitals Birmingham (CARMS-17170). The livers were used for primary liver transplant in recipients deemed suitable to receive a high-risk graft. Each participant was fully informed in advance about the nature of these livers and gave written consent. The NMP using the OrganOx *metra* device was commenced following a period of cold storage upon the liver's arrival at our center. The principal criterion for a liver to be considered viable was to metabolize perfusate lactate levels to $\leq$2.5 mmol/L within 4 hours. More details are provided in the Supplemental Material online, http://links.lww.com/LVT/A502.

Patient and graft survivals and comparison with matched controls

The post-transplant immunosuppression consisted of tacrolimus in combination with either azathioprine or mycophenolate mofetil, and a 3-month course of steroids. The standard clinical follow-up consisted of routine blood analyses including liver and renal function tests, full blood count, and tacrolimus levels, performed weekly within the first month, fortnightly for up to 3 months, monthly for up to 1 year, and every 3–6 months afterward. Each participant underwent magnetic resonance cholangiography (MRC) at 6 months (or earlier if clinically indicated). Some patients with normal blood tests and performance status were followed by local hepatologists with ongoing annual reviews by the VITTAL team.

The patient and graft survival data were compared with contemporary controls (1:2), matched in order of the priority for the donor graft type, UKELD Score, donor age, and donor sex. These patients underwent the same follow-up except for the routine MRC. In both groups, patients with abnormal liver function tests underwent a Doppler ultrasound, with the investigation algorithm consisting of an MRC, a contrast CT scan of the abdomen and pelvis, a liver biopsy, or a combination of these investigations until the cause of the abnormal liver function was identified. Patients diagnosed with biliary problems typically underwent multiple MRC investigations in combination with biliary stenting using endoscopic retrograde cholangiography or percutaneous transhepatic cholangiography approaches. The survival analyses include graft survival censored for death, transplant-specific survival (graft survival noncensored for death), and overall patient survival.

The preplanned comparisons with the control group were not powered to demonstrate any differences and were included to put the study results within the context of the unit's contemporary outcomes.

Biliary features

The bile duct biopsies were taken before commencing the perfusion and after the graft implantation prior to performing the biliary anastomosis. The tissues were processed routinely to paraffin blocks and sections stained with hematoxylin and eosin and assessed for bile duct injury (BDI) scores based on semiquantitative grading of the deep peribiliary glands, stromal necrosis, medial necrosis of arteries, thrombi, and interstitial hemorrhage features.[20,21] If the liver produced bile during the NMP, the volume was measured in hourly intervals and its biochemistry was analyzed from the vial collected at 4 hours. Histological BDI was correlated with the development of clinically manifest NAS. To assess biliary complications, all patients underwent per-protocol MRC at 6 months, or earlier if clinically indicated.

Post-transplant complications, QoL, AEs reporting, and analysis

The EuroQol EQ-5D-5L questionnaire was used before transplant (at time of consent) and at day-7, day-30, and month-6 follow-up visits. The collection and reporting of AEs were performed in accordance with the Research Governance Framework for Health and Social Care and the requirements of the National Research Ethics Service. Definitions of different types of AEs were as per the study protocol.[5] The reporting period for AEs commenced at visit 1 and ended at the 24-month follow-up, and the seriousness, causality, and Clavien-Dindo grade were assessed by the study investigator team.[22] The patient outcomes collected beyond 24 months included only graft performance (normal or abnormal liver function tests) and patient status (alive or dead).

Statistical analysis

Descriptive analyses include a number of observations, with data presented as frequency with percentage for categorical data, and median and interquartile range (IQR) for continuous and ordinal measures. The data in Table 1 were tested for differences in proportions using a 2-sample proportion test. The analyses were two-sided at the 5% significance level, but none of those were powered nor adjusted for multiple testing. Patient and graft survivals were analyzed using the Kaplan-Meier method with 95% CIs and compared with the log-rank test. Repeated-measures mixed-effects model fixed estimates were used to produce mean trend and 95% confidence bands for blood tests and bile assessment results, and the blood test graphical data have been transformed to aid visualization. Repeated-measures mixed-effects models were fitted to QoL data with and without a step-change point at visit 2, with models selected using likelihood ratio tests and Akaike statistical criteria. Continuous perfusate biochemical analysis data were investigated using restricted cubic spline mixed-effects regression and plotted with 95% CIs. Results were rounded to a relevant precision, percentages in the text to whole numbers, and *p*-values to 3 decimals. Missing data variables were omitted from the analyses. The analyses were performed using STATA software package version 17.0 for Windows (StataCorp LLC, TX).

RESULTS

Discarded liver characteristics and patient and graft survival rates

The study's primary outcomes and 24-month follow-up were reported.[6] Briefly, the 31 enrolled livers consisted of 17 (55%) DBD and 14 (45%) DCD organs. The median SCS period was 464 minutes (IQR 389–625). Twenty-two (71%) livers were transplanted with 100% 90-day patient and graft survivals.

The graft and patient survivals at 1, 3, and 5 years were 91%, 82%, and 82%, and 100%, 91%, and 82%, respectively (Table 1 and Figure 1). Four patients lost their graft due to the development of NAS, one caused by hepatic artery thrombosis in DBD liver, and 3 in elderly DCD livers, which all became symptomatic within 6 months (Table 2). There were no late graft losses for other reasons. Four patients died, all with functioning grafts (3 from cancer recurrence and 1 from chronic rejection based on poor compliance with treatment), 2 of them being previously retransplanted for NAS. When excluding such causes, the transplant-specific patient survival was 100% at 5 years.

The contemporary matched control graft and patient survivals at 1, 3, and 5 years were 98%, 98%, and 95%, and 95%, 91%, and 86%, respectively, and transplant-specific patient survival 93% at 5 years. The

TABLE 1 VITTAL study and controls patient and graft survival rates (frequency/%)

	VITTAL patients (n = 22)	Control patients (n = 44)	*p*[a]
Graft survival[b] (y), N/n (%)			
≤1	20/22 (90.9)	43/44 (97.7)	0.486
≤2	18/22 (81.8)	43/44 (97.7)	0.119
≤3	18/22 (81.8)	43/44 (97.7)	0.054
≤5	18/22 (81.8)	42/44 (95.4)	0.127
Transplant-specific survival[c] (y), N/n (%)			
≤1	22/22 (100)	43/44 (97.7)	No test performed as low number of events
≤2	22/22 (100)	42/44 (95.4)	0.280
≤3	22/22 (100)	41/44 (93.2)	0.257
≤5	22/22 (100)	41/44 (93.2)	0.176
Patient survival[d] (y), N/n (%)			
≤1	22/22 (100)	42/44 (95.4)	No test performed as low number of events
≤2	21/22 (95.4)	41/44 (93.2)	0.242
≤3	20/22 (90.9)	40/44 (90.9)	0.848
≤5	18/22 (81.8)	38/44 (86.4)	0.960
HR test results			
Graft survival	HR = 3.992	95% CI: 0.715–22.287	0.115
Transplant-specific survival	HR = $45.37e^{-17}$	95% CI: $1.50e^{-17}$ to $1.28e^{-16}$	< 0.001
Patient survival	HR = 1.087	95% CI: 0.330–3.586	0.891

[a]The statistical comparison tests were not powered, and these results should be interpreted with caution.
[b]Graft survival was defined as the period from transplantation to retransplantation.
[c]Transplant-specific survival was defined as the period from transplantation to death related to graft failure.
[d]Patient death was defined as the period from transplantation to death from any cause.

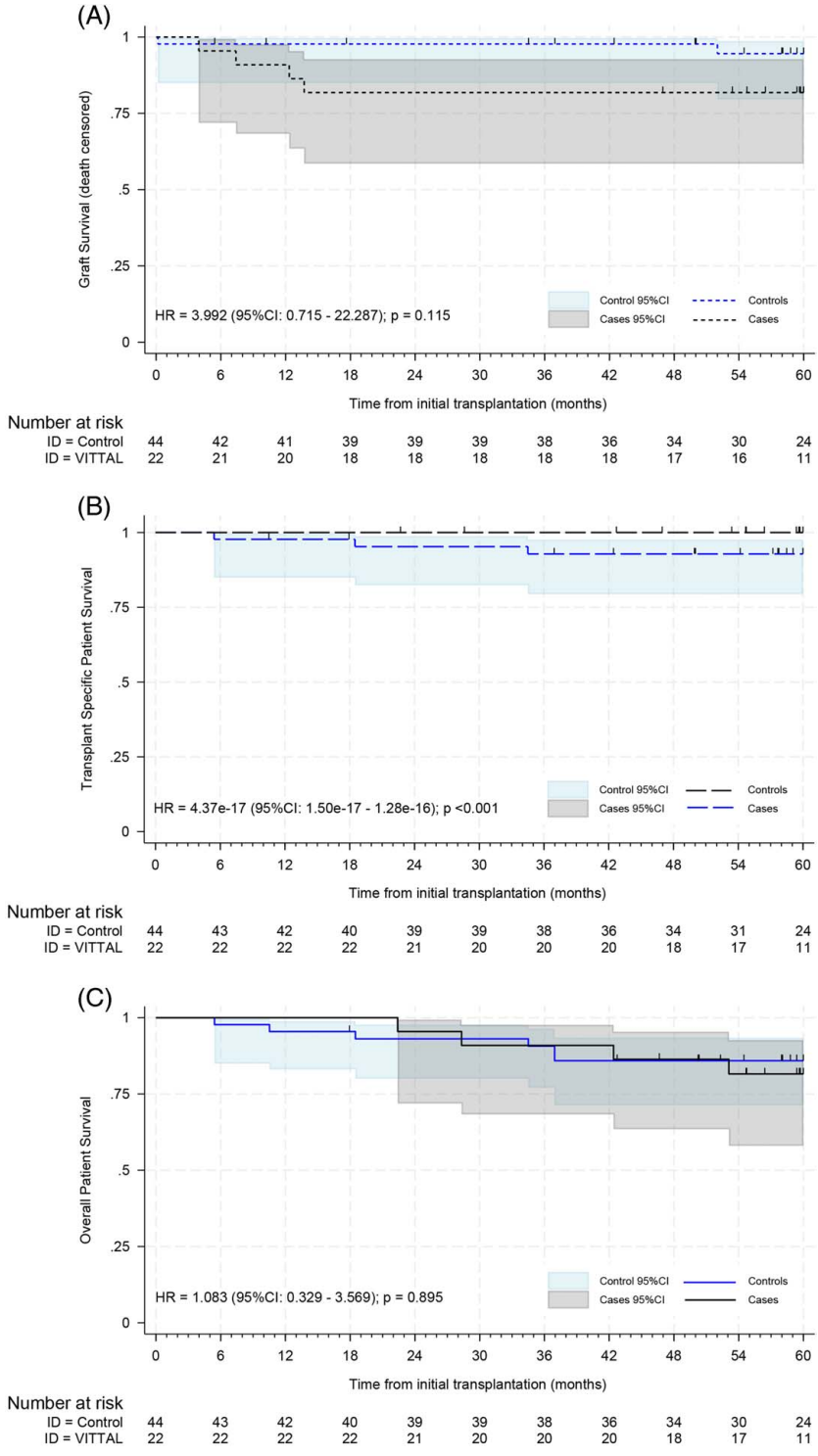

FIGURE 1 Kaplan-Meier plots of 5-year survival estimates. Five-year graft survival estimates censored for patient death (A), transplant-specific survival censored for graft unrelated deaths such as cancer (B), and 5-year overall patient survival (C), respectively. While graft and patient survivals were similar (p-value 0.115 and 0.891, respectively), the transplant-specific survival was improved in the VITTAL group ($p < 0.001$). The VITTAL patients are presented in black, and controls by blue lines, with 95% CIs shaded in the respective colors. Abbreviation: VITTAL, viability testing and transplantation of marginal livers.

HR for graft survival for the VITTAL group was 3.992 (95% CI: 0.715–22.287; $p = 0.115$), and patient survival was also lower than in controls (HR = 1.087, 95% CI: 0.330–3.586; $p = 0.891$), though these differences were not significant at the 5% level. The transplant-specific survival, however, was significantly better compared to controls (HR = $45.37e^{-17}$, 95% CI: $1.50e^{-17}$ to $1.28e^{-16}$; $p < 0.001$). As these comparisons were not powered, the results should be interpreted with caution.

TABLE 2 Details of livers with nonanastomotic biliary strictures requiring retransplantation

	DCD 1	DCD 2	DCD 3	DBD
Liver characteristics				
Donor age (y)	54	69	69	84
Liver weight (kg)	1.7	1.4	1.4	1.3
Steatosis > 30%	No	No	No	No
Donor warm ischemia (min)[a]	40	19	20	NA
Cold ischemic time (min)	429	354	334	625
Total preservation time (min)	1049	681	874	1205
Donor risk index[b]	2.9	3.1	3.8	2.5
UK DCD score	10	4	9	NA
UK Donor liver index	1.70	2.65	2.77	1.23
Bile duct histology[c]				
Bile duct injury score pre-NMP (points)	Severe (6)	Minimal (1)	Mild/moderate (4)	Mild/moderate (5)
Deep peribiliary glands	2	0	2	2
Stromal nuclear loss	2	0	1	2
Medial nuclear loss	2	1	1	1
Thrombosis	0	0	0	0
Hemorrhage	0	0	0	0
Bile duct injury score postimplant (points)	Severe (10)	Mild/moderate (5)	Severe (11)	Severe (6)
Deep peribiliary glands	3	1	3	2
Stromal nuclear loss	3	3	3	3
Medial nuclear loss	3	1	3	0
Thrombosis	0	0	1	1
Hemorrhage	1	0	1	0
Biliary NMP criteria				
Presence of bile	No	Yes	Yes	Yes
Bile volume (mL)	0	48	56	> 99
Bile pH at 4 h	NA	7.44	7.63	7.79
Bile HCO3 at 4 hrs	NA	22.8	11.5	24.9
Bile O_2 at 4 h (kPa)	NA	20.8	19.8	30.6
Perfusion criteria				
Lactate level at 2 h (mmol/L)	0.7	0.9	2.6	3.2
Lactate level at 4 h (mmol/L)	0.8	1.3	2.0	2.5
Arterial flow at 4 h (mL/min/kg)	177	296	292	229
Portal flow at 4 h/weight (mL/min/kg)	531	741	875	700
Alanine aminotransferase at 0 h (IU/mL)	644	693	2181	NA
Alanine aminotransferase at 2 h (IU/mL)	958	856	2660	NA
Alanine aminotransferase at 4 h (IU/mL)	1034	921	3187	NA
Clinical course				
Abnormal liver function tests (mo)	11	2	From the LT	From the LT
NAS confirmed by MRC (days)	11 (323 d)	2 (58 d)	2 (39 d)	2 (47 d)
Patient listed for retransplantation (days)	11 (345)	2 (64)	9 (284)	7 (205)
Graft survival (mo)	12	4	14	7 (225 d)

[a]Donor warm ischemic time is defined as the period from the systolic blood pressure decrease below 50 mm Hg to commencing the aortic cold flush.
[b]Donor risk index as described by Feng et al.[23]
[c]Injury to extrahepatic bile ducts was determined by grading injury to deep peribiliary glands (0–3), stromal nuclear loss (0–3), loss of nuclei in the media of arteries/arterioles (0–3), the extent of hemorrhage (0–4), and the presence of thrombi (Y/N)[20,21]; an overall bile duct injury grade was assigned based on the severity/presence of each, for example, minimal = mild of 1 or 2 features, severe = moderate to severe in all.
Abbreviations: DBD, donation after brain death; DCD, donation after circulatory death; MRC, magnetic resonance cholangiography; NAS, nonanastomotic strictures; NMP, normothermic machine perfusion.

Analyses of biliary features

Bile volume and composition during NMP

During the study follow-up, all the graft losses were attributed to NAS, 3 of those occurring in DCD grafts, and 1 in DBD liver as a consequence of perioperative hepatic artery thrombosis and biliary ischemia. As the thrombosis was considered to be technical and not predictable from the perfusion parameters, this liver was excluded from the statistical analyses looking for correlation with NAS development (note this liver remained included in all the other outcomes analyses).

Overall, 22 (71%) livers produced bile during the 4-hour NMP assessment period, and the absence of bile production seemed to be unrelated to technical problems with the bile duct cannulation or drain occlusion. Bile composition data were not available for all patients, and the assessed parameters included only bile volume and its pH, bicarbonate, and oxygen concentrations. Four livers (2 DBDs and 2 DCDs) without bile production were transplanted, and one of those (DCD liver) developed NAS. The detailed characteristics of grafts that developed NAS are provided in Table 2. The recipients developed a progressive confluence-dominant type of NAS and were retransplanted after 4, 13, and 14 months, respectively. Two of these recipients were transplanted initially for primary sclerosing cholangitis. Two other patients developed anastomotic biliary strictures requiring endoscopic retrograde cholangiopancreatography. Three patients had mild biliary irregularities on MRC, but remained asymptomatic with normal liver function tests throughout the 5-year follow-up.

Post-transplant patient blood biochemistry

Sequential post-transplant liver and renal biochemical blood tests show a similar pattern for all livers within the initial 30 days. The transaminase curves peaked on the first day and were normalized between days 7 and 30. Parameters related to biliary functions (bilirubin, alkaline phosphatase, and gamma-glutamyl transferase) peaked between days 3 and 7, and normalized beyond day 30. The patient with hepatic artery thrombosis diverged from day 7, while patients developing NAS in DCD grafts split from the general pattern between days 30 and 180 (Figure 2).

Bile duct histology

There were no differences in preperfusion BDI grades between the group transplanted and those deemed nontransplantable by perfusion criteria, nor between DBD and DCD livers. In the transplanted livers, the median BDI increased from 2 preperfusion to 6 postimplantation biopsies and the progression seemed amplified in the DCD grafts. This was most prominent in the DCD grafts that required retransplantation, reaching mostly the highest grade 7 of severity (Table 3, Figures 3 and 4). Of the individual BDI features assessed, only the injury to the media of arteries seemed to correlate with the development of NAS. There was limited injury in DBD livers (absent to moderate), with more injury to the arteries in the DCDs, in particular those requiring retransplantation (median of severe, details in Table 3).

Post-transplant complications, QoL, and AEs assessment

Seven (32%) patients developed Clavien-Dindo complication grade ≥ 3. Eight (36%) suffered from acute kidney injury, including 4 (18%) of the patients who required renal replacement therapy. The median intensive care and in-hospital stays were 3.5 days (IQR 3–4) and 10 days (IQR 8–17), respectively.

The patients' median score of QoL assessment, using the EQ-5D-5L questionnaire "Health today" scores, before the transplantation was 69 points (IQR 50–80). This high score reflects that the study included only stable, low-risk recipients; however, the per-protocol QoL collection timepoints did not enable the capturing of any changes in QoL during the waiting list period. The QoL scores remained similarly high at 70 (IQR 50–83) at the day-30 post-transplant visit. In the further follow-up, it increased to 85 (IQR 65–90) at 6 months. The detailed assessment showed reduced scores in patients who developed NAS but suggested that even very high-risk DBD livers deemed not suitable for transplantation achieved good patient QoL within 30 days of the procedure.

A total of 450 AEs were reported during the trial, of which 31 were classified as being serious. More than half (55%) of the AEs reported were abnormal blood test findings, and 76% were grade I Clavien-Dindo complications. None of the AEs were assessed as being NMP device related. The details are shown in Table 4 and Figure 5.

DISCUSSION

We report here for the first time long-term transplant outcomes (5+ years) of high-risk livers assessed by NMP applied following a period of SCS. Our initial VITTAL trial publication reported the lactate-based NMP viability criteria that had high diagnostic accuracy (100%) to prevent primary nonfunction or severe dysfunction, and this finding was subsequently confirmed by others.[6–8,10,24] That was a fundamental discovery for patients in need of timely access to lifesaving transplantation. In this subsequent report, we

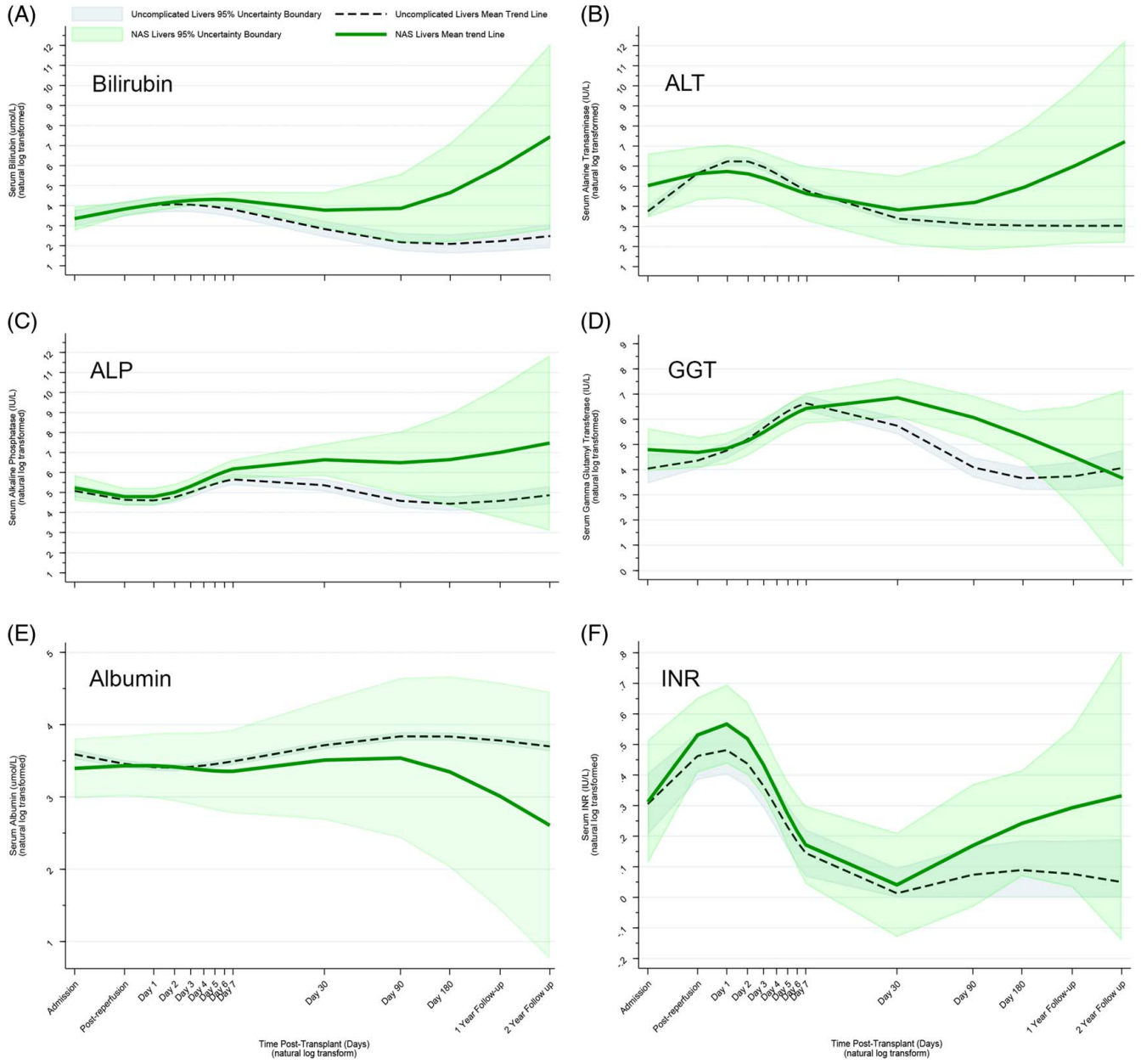

FIGURE 2 Mean trend and 95% confidence bands of post-transplant blood results. (A–D) Patients' post-transplant liver function tests, consisting of bilirubin, ALT, ALP, and GGT, respectively. The ALT peaked at the first day and normalized between day 7 and day 30. Parameters related to biliary functions (bilirubin, ALP, and GGT) peaked between day 3 and day 7, and normalized beyond day 30. The deteriorating overall condition in patients suffering from NAS is demonstrated by declining albumin and INRs of prothrombin times beyond 3 months. Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; GGT, gamma-glutamyltransferase; INR, international normalized ratio; NAS, non-anastomotic biliary strictures.

now provide clinicians and recipients, offered high-risk livers that might be salvaged by NMP assessment, with reassurance that such intervention does not impose any risk for the longevity of the assessed grafts.

All graft losses in the VITTAL trial were attributed to NASs. In DBD livers, where the risks of developing NAS are, in our experience, very low (< 1%), we believe lactate clearance is sufficient to guide the principal decision regarding liver transplantability.[24,25] The benefits of early access to transplantation, enabled by an objective assessment of suboptimal livers, clearly outweigh the risks that were demonstrated by improvements in the recipients' QoL at 6 months and subsequent excellent long-term outcomes.

Although we assume that bile production is not necessary to proceed with transplantation in DBD livers, its presence is a reassuring feature of good organ function and quality.[9,24,26] In organs without bile secretion in the VITTAL series, we could not identify any technical problems related to bile duct cannulation, although a misplaced, kinked, or occluded drain could often be a reason causing such a problem. The

TABLE 3 Biliary features in livers donated after circulatory death (median, interquartile range)

	Overall (n = 10)	Good long-term function (n = 7)	Nonanastomotic strictures (n = 3)
Liver characteristics			
Donor age (y)	57 (45–69)	52 (37–65)	69 (54–69)
Donor BMI (kg/m^2)	30 (24–33)	30 (21–33)	29 (27–34)
Liver weight (kg)	1.8 (1.4–1.9)	1.9 (1.6–2.0)	1.4 (1.3–1.7)
Donor warm ischemia (min)[a]	23 (19–35)	23 (18–35)	20 (19–40)
Cold ischemic time (min)	416 (354–464)	420 (389–573)	354 (334–429)
Total preservation time (min)	1000 (874–1097)	1023 (878–1440)	874 (681–1049)
Donor risk index[b] (points)	3.1 (2.5–3.2)	3.0 (2.2–3.2)	3.1 (2.9–3.8)
UK DCD score (points)	7.5 (6.3–8.8)	7 (6.5–8)	9 (6.5–9.5)
UK Donor liver index (points)	1.97 (1.75–2.47)	1.95 (1.73–2.16)	2.65 (2.18–2.71)
Bile duct histology[c]			
Bile duct injury score pre-NMP	2 (1–3)	2 (1–3)	4 (1–5)
Deep peribiliary glands	1 (1–2)	1 (1–1.5)	2 (1–2)
Stromal nuclear loss	3 (2–3)	3 (0–3)	3 (3–3)
Medial nuclear loss	2.5 (1–3)	2 (0–3)	3 (1–3)
Thrombosis	0	0	0.5 (0–1)
Hemorrhage	1 (1–1)	1 (1–2)	1 (0–1)
Bile duct injury score postimplant	6 (4–7)	5 (2–7)	7 (4–7)
Deep peribiliary glands	2 (1–3)	2 (1–2.5)	3 (2–3)
Stromal nuclear loss	3 (2–3)	3 (0–3)	3 (3–3)
Medial nuclear loss	2.5 (1–3)	2 (0–3)	3 (1–3)
Thrombosis	0	0	0.5 (0–1)
Hemorrhage	1 (1–1)	1 (1–2)	1 (0–1)
Biliary NMP criteria			
Presence of bile, n (%)	8 (80)	6 (86)	2 (67)
Bile volume (mL)	66 (15–>99)	90 (15–>99)	48 (0–56)
Bile pH	7.65 (7.53–7.76)	7.69 (7.65–7.80)	7.53 (7.44–7.62)
Bile HCO3	23.1 (20.8–28.6)	27.3 (23.1–28.6)	17.2 (11.5–22.8)
Bile O_2 (kPa)			
Perfusion criteria			
Lactate clearance <2.5 mmol/L within 4 h	10 (100)	7 (100)	3 (100)
Arterial flow at 2 h/weight (mL/min/kg)	275 (236–292)	257 (203–273)	292 (236–296)
Arterial flow at 4 h/weight (mL/min/kg)	257 (236–292)	257 (203–273)	292 (236–296)
Portal flow at 2 h/weight (mL/min/kg)	661 (559–784)	655 (559–705)	815 (531–875)
Portal flow at 4 h/weight (mL/min/kg)	608 (559–769)	600 (559–769)	741 (531–875)
Alanine transferase at 0 h (IU/mL)	1666 (1413–2125)	(1666 (1445)	1437 (693–2181)
Alanine transferase at 2 h (IU/mL)	2205 (2080–2770)	2488 (2153–3165)	958 (856–2660)
Alanine transferase at 4 h (IU/mL)	2535 (2219–3187)	2847 (2335–4168)	1034 (921–3187)
Glucose metabolism (%)	10 (100)	7 (100)	3 (100)

Note: Body mass index is the weight in kilograms divided by the square of the height in meters.

[a]Donor warm ischemic time is defined as the period from the systolic blood pressure decrease below 50 mm Hg to commencing the aortic cold flush; this variable is applicable only for donors after circulatory death.

[b]Donor risk index as described by Feng et al.[23]

[c]Injury to extrahepatic bile ducts was determined by grading injury to deep peribiliary glands (0–3), stromal nuclear loss (0–3), loss of nuclei in the media of arteries/arterioles (0–3), the extent of hemorrhage (0–4), and the presence of thrombi (Y/N)[20,21]; an overall bile duct injury grade was assigned based on the severity/presence of each, for example, minimal = mild of 1 or 2 features, severe = moderate to severe in all.

Abbreviations: BMI, body mass index; DCD, donation after circulatory death; NMP, normothermic machine perfusion.

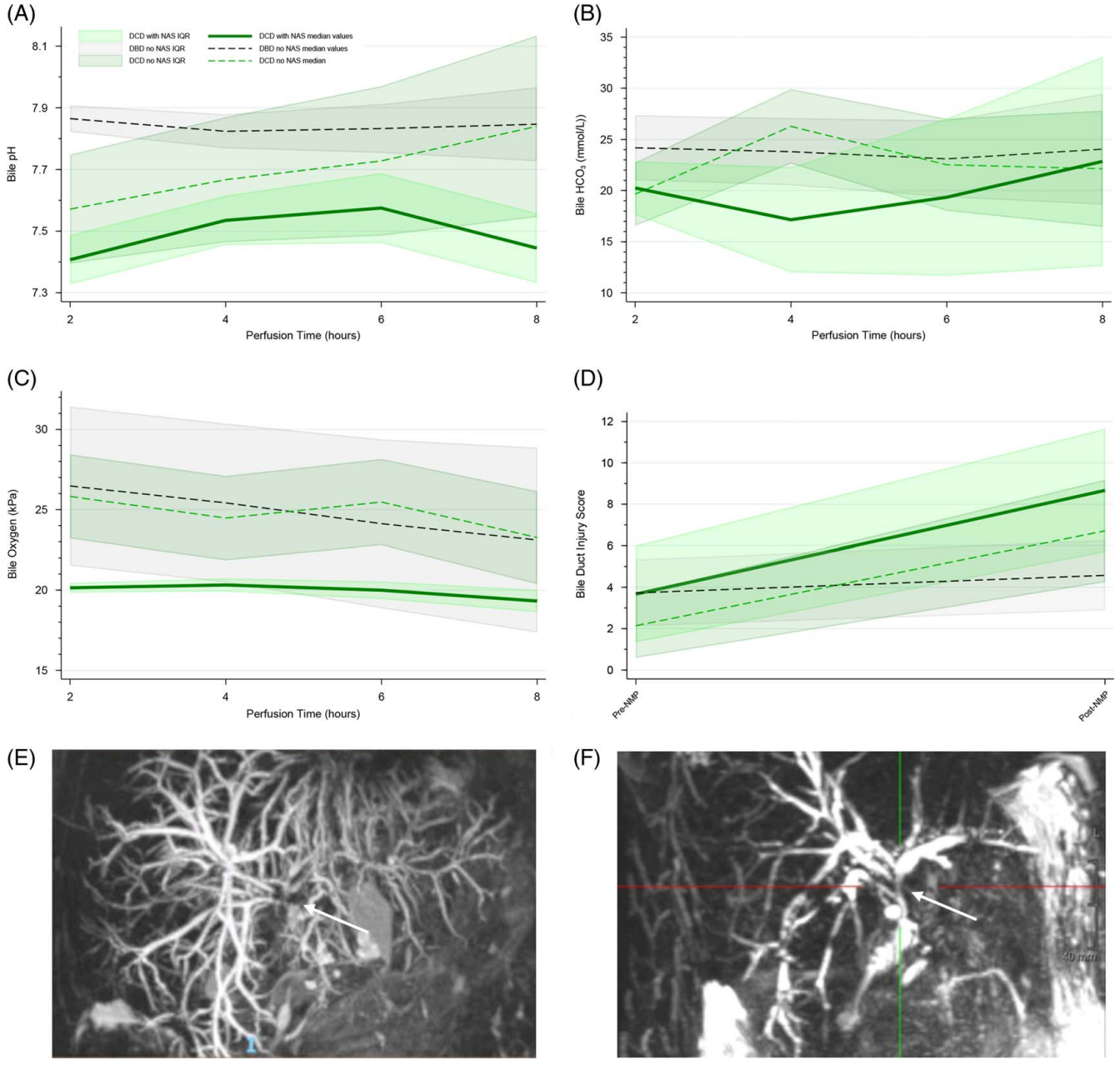

FIGURE 3 Biliary features and risks for development of nonanastomotic biliary strictures. (A–C) Bile composition analyses, consisting of pH, HCO3, and partial oxygen pressures. (D) Captures the evolution of the bile duct injury scores from bile duct samples taken before commencing the perfusion and after the liver implantation. Repeated-measures mixed-effects model fixed estimates were used to plot the mean trend and 95% confidence bands. The dotted black and green lines demonstrate DBD and DCD livers with good long-term outcomes, respectively. While those lines are to some extent overlapping, the solid green line representing DCD livers that failed due to the development of nonanastomotic biliary strictures is set apart. (E, F) typical MRC images of failed DCD livers, capturing separation of main bile ducts in the liver hilum in patients who became symptomatic at 4 and 10 months, respectively. Abbreviations: DBD, donation after brain death; DCD, donated after circulatory death; IQR, interquartile range; NAS, nonanastomotic biliary strictures; NMP, normothermic machine perfusion.

absence of bile production might be explained by impaired hepatocyte function related to exposure of the organ to prolonged ischemic periods, as one of these DCD organs had a donor warm ischemic time (blood pressure below 50 mm Hg to aortic flush) of 40 minutes, and in both DBD livers, the SCS time was approaching 12 hours. The 4-hour viability assessment period was long enough only to recover the metabolic function to clear lactate, but insufficient to restore bile secretion, and 3 out of 4 of those livers developed early allograft dysfunction.[27]

The implementation of objective evaluation of livers from donors currently deemed too high risk can unlock a pool of organs that until now have not been considered of sufficient quality for transplantation, including steatotic organs.[8] The unique feature of the VITTAL

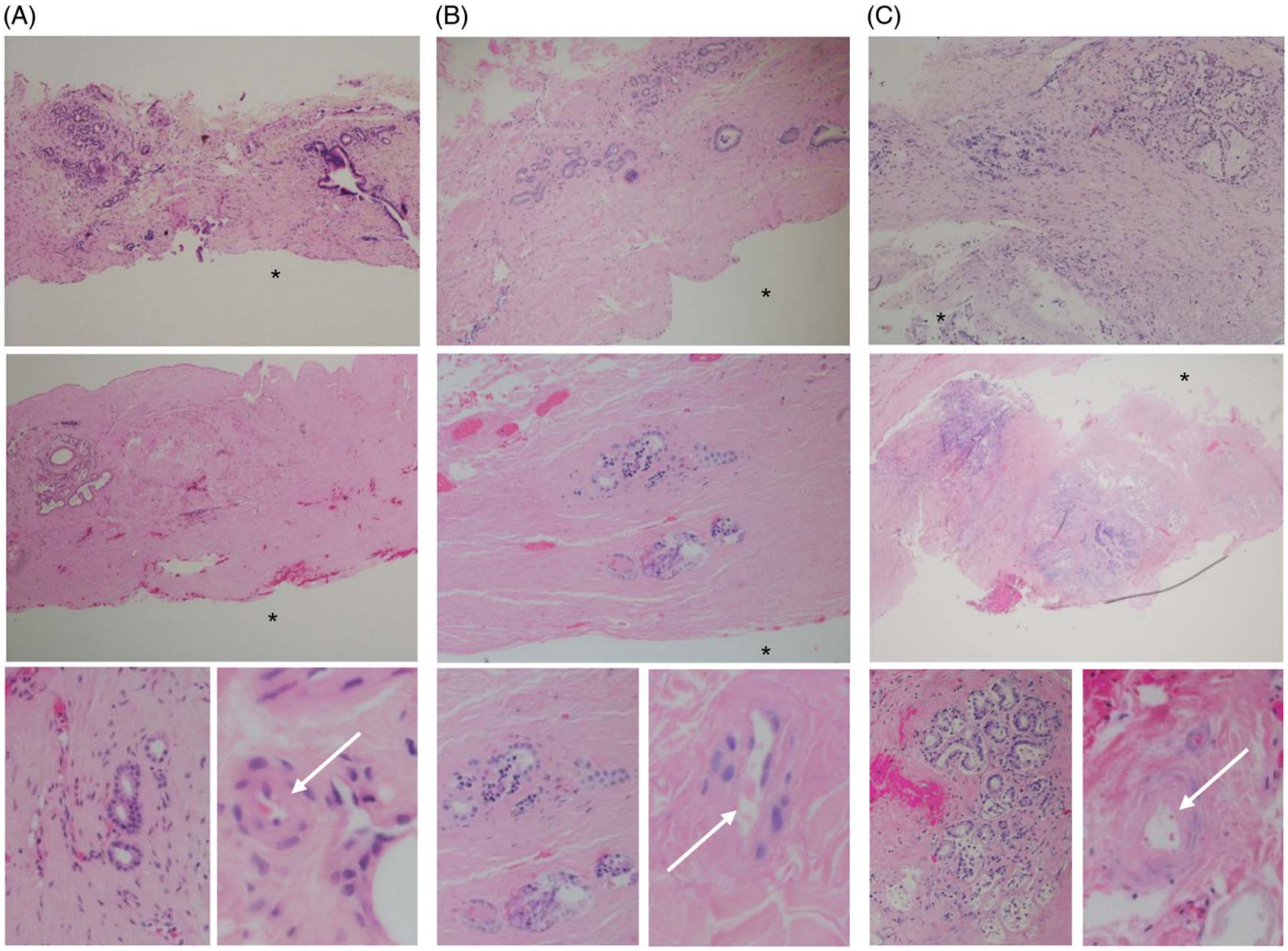

FIGURE 4 Bile duct histology assessment. (A–C) grouped bile duct histology sections, capturing typical findings in donation after brain death (DBD) liver, DCD with good long-term outcomes, and DCD that developed NAS, respectively. The top figures capture preperfusion and the middle postimplantation biopsies. The bottom images show high-power slides of deep peribiliary glands left, and artery right, respectively. The degree of bile duct injury was similar in all livers before commencing the NMP. The postimplantation biopsies revealed the injury degree progressed in all livers, but the features were significantly more prominent in DCD livers, reaching high scores in livers that failed as a consequence of NAS. This was demonstrated by the bile duct injury score, with the difference between DBD and DCD livers being most prominent in the medial necrosis assessment. There was no difference in the presence of periglandular vascular thrombi and the hemorrhage features were more prominent in livers without biliary problems. * indicates the lumen side of the bile duct. The arrows point at arteries. DBD liver (A) shows normal medial nuclei, suggesting no medial necrosis. DCD with the good long-term outcome (B) demonstrates some loss of medial nuclei (blue elongated dots), and DCD developing NAS (C) captures the complete loss of medial nuclei from the wall of the artery. Normal deep peribiliary glands DBD, moderate to severe injury DCD no clinical biliary problems, and DCD NAS, respectively.

trial design was its aim to enroll livers likely to fail the viability criteria. An extreme example of a fatty liver NMP characteristic was perfusion number 24, with histologically proven 80% steatosis, whose lactate levels became unmeasurably high and pH <7.00 after 2 hours' perfusion time. This observation supports the use of NMP to assess fatty livers, as it evidently outperforms the histological assessment of the suitability for transplantation.[8] Liver macrosteatosis, in particular large droplet fat, is a known risk for early post-transplant outcome. In this study, it was not a factor due to the limited number of these livers being transplanted.[28,29] Clinicians' concerns about liver steatosis are often aggravated by logistical difficulties in achieving a short cold ischemic time. In this context, the adoption of objective NMP assessment combined with removing the timing constraints would improve patient access to lifesaving transplantation.[30] Of note, other teams in North America and Europe have also achieved 70% transplantability rates when applying lactate-based viability criteria to very high-risk declined livers, corroborating the universal potential to increase donor liver use by embracing NMP technology.[7,8,10,31]

Regarding the assessment of high-risk DCD livers, and specifically those from elderly donors or exposed to warm ischemia beyond 30 minutes, our results confirm the observation that end-ischemic NMP does not prevent the development of NAS.[18] The post hoc analysis suggests that DCD livers with bile pH ≤ 7.64 and bicarbonate concentrations ≤25 mmol/L are at risk of developing

TABLE 4 Study adverse events by Clavien-Dindo grading

	Overall (n = 22)	DBD (n = 12)	DCD (n = 10)
No. adverse events per patient—median, (IQR)	21 (13–26)	21 (13–30)	25 (23–30)
The highest grade of complication per patient—median, (IQR)	2 (2–3)	2 (2–2)	2.5 (2–5)
Clavien-Dindo grade	No. adverse events		
Grade I (%)	347 (76.4)		
Grade II (%)	78 (17.2)		
Grade III (%)	14 (3.1)		
Grade IIIa	5 (1.1)		
Grade IIIb	9 (1.9)		
Grade IV (%)	15 (3.3)		
Grade IVa	14 (3.1)		
Grade IVb	1 (0.2)		
Grade V (%)	0 (0)		
Total	**454 (100)**		
Type of event			
Abnormal blood test	250 (55.1)		
Other	139 (30.6)		
Recipient infection	21 (4.6)		
Hospital admission	14 (3.1)		
Biliary stricture (nonanastomotic)	8 (1.8)		
Biopsy proven acute rejection	4 (0.9)		
Medication-related	4 (0.9)		
Bleeding	4 (0.9)		
Biliary stricture (anastomotic)	3 (0.7)		
Reoperation	3 (0.7)		
Bile leak	2 (0.4)		
Hepatic artery thrombosis	2 (0.4)		
Total	**454 (100)**		

Abbreviations: DBD, donation after brain death; DCD, donation after circulatory death; IQR, interquartile range.

NAS. We therefore concur with the opinion of other teams that the inclusion of bile composition improves the diagnostic accuracy of viability assessment in DCD organs, possibly preventing the later development of NAS.[17,24,32,33] This is in keeping with bile duct histological findings, revealing higher BDI scores from postimplantation biopsies in such organs. Although it does not necessarily mean these changes would be visible if the biopsies were taken during the course of NMP, injury to the media of arteries seemed to be a discriminatory factor also from preperfusion assessment.[20] The low partial oxygen levels in bile in livers developing NAS are in keeping with impaired blood circulation in peribiliary arterioles. The published evidence and the presented histology suggest that the bile duct damage evolves during SCS and the end-ischemic NMP may not sufficiently mitigate the onset of ischemia-reperfusion injury.[34–37] The preretrieval damage and overall poor quality of the included DCD livers may explain why NAS manifested in these grafts rather early.[10,18] To conclude, end-ischemic NMP does not seem to reduce risks of NAS development in DCDs, but as a diagnostic tool it may allow the selection of DCD livers with limited biliary damage based on favorable bile composition or provide an opportunity to evaluate bile duct histology.[17,38] Our data acknowledge that if machine perfusion were used as a premeditated intervention, the DCD organs would benefit from alternative strategies, such as commencing NMP in the donor hospital, normothermic regional perfusion, or hypothermic oxygenated perfusion.[14–16,36,39]

The VITTAL trial was designed in line with the prevalent experts' and ethicists' view that declined high-risk livers are not suitable for enrollment into a randomized controlled trial against cold storage.[40] The protocol therefore included a comparison with the unit's contemporary matched controls, revealing that NMP prevented early patient mortality related to severe graft dysfunction or nonfunction seen in literally all historical deceased liver transplant series. Interestingly, in the VITTAL DCD series, we observed earlier manifestations of severe NAS that became symptomatic in weeks, and required retransplantation in 6–12 months. That was rarely seen in our historical or contemporary controls who were typically diagnosed

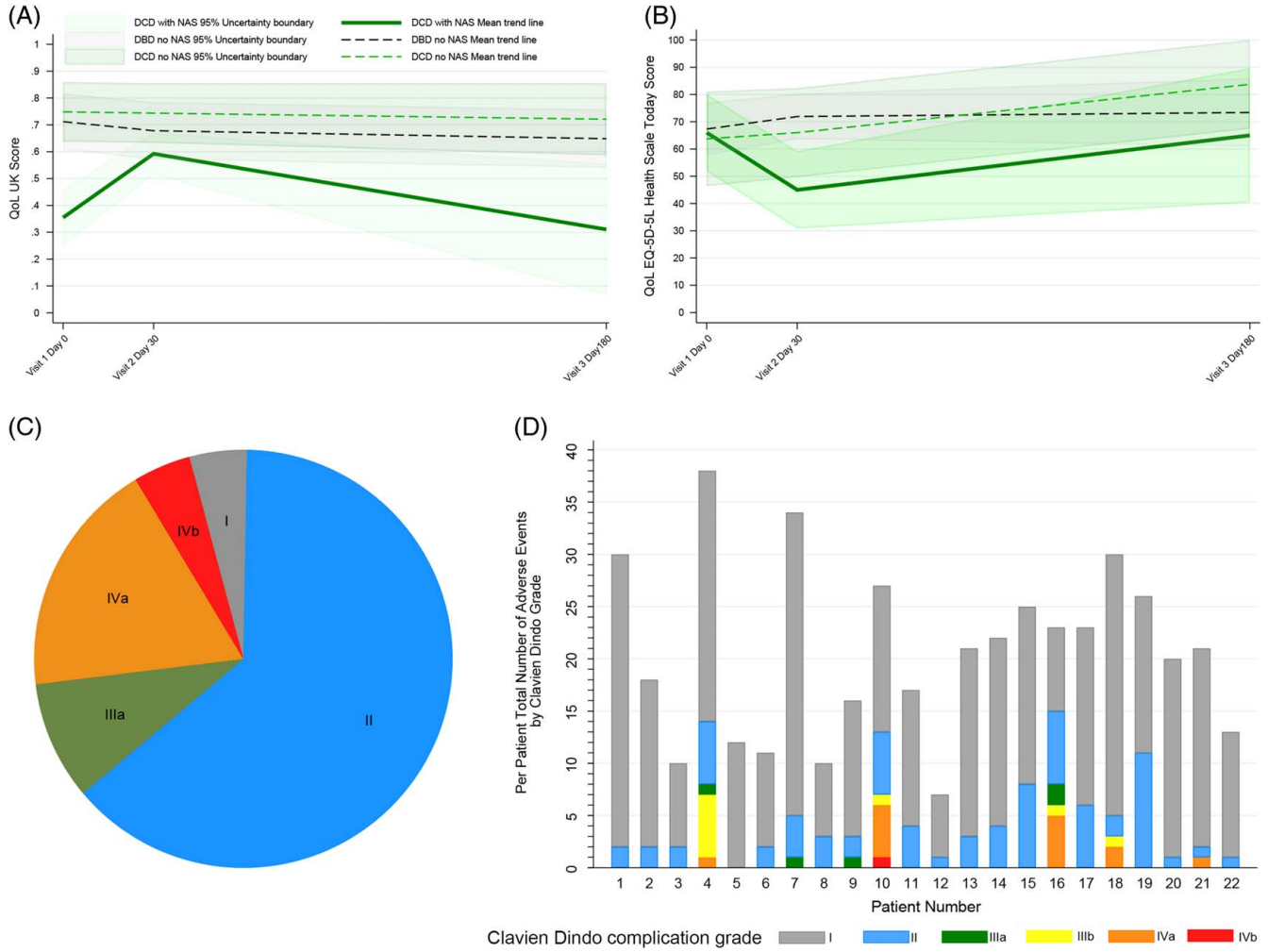

FIGURE 5 Post-transplant quality of life, complications grade, and serious adverse events. (A and B) repeated-measures mixed-effects model fixed estimate plots of mean trend and 95% confidence bands for quality of life scores based on the DBD, DCD with good long-term results (dotted black and green line, respectively), and DCD that failed due to NAS patient (solid green line), demonstrating inferior scores in DCD NAS patients. Panel C illustrates the highest Clavien-Dindo complication grade experienced by each patient, demonstrating that the large majority (69%) experienced minor complications. (D) Actual number of adverse events in each patient, with grade 1 (elevated liver and renal blood tests) and grade 2 (predominantly infection requiring antibiotics, or rejection episodes requiring high-dose steroids) constituting the majority of complications. The high grade of complications (grade 4) includes 3 patients who suffered from acute renal injury requiring renal replacement therapy, and partly overlaps with 4 patients who needed retransplantation. Abbreviations: DBD, donation after brain death; DCD, donated after circulatory death; NAS, nonanastomotic biliary strictures; QoL, quality of life.

with NAS beyond 12–18 months after transplantation, presenting with slowly deteriorating liver biochemistry, progressive itch, and eventually suffering from recurrent episodes of cholangitis.[25,41] Such scenarios often lead to a more profound decline in patients' performance status, which combined with ubiquitously present colonization by multiresistant bacteria frequently means those patients die before being saved by retransplantation. This might explain the better transplant-specific survival in the VITTAL cohort compared to controls. As for the overall long-term patient survival, 3 study patients died from liver cancer recurrence and 1 from chronic rejection caused by noncompliance, while in the control group 6 patients died: 2 from graft failure related to biliary strictures, 2 from cancer recurrence, and 2 from noncompliance caused by alcohol recidivism.

The VITTAL study has some special design features and limitations that need to be kept in mind when interpreting its results. First, it was designed to explore the boundaries of high-risk liver utilization. To achieve this aim, it required enrollment of livers likely to fail the assessment. This was achieved by rigorous two-tier inclusion criteria to objectively ensure the organs' marginality status. For this reason, the trial did not include 25 discarded livers that were deemed too good to be tested. We consider this to be a major strength of the VITTAL trial, which allowed validation of the tested criteria to prevent primary nonfunction. Second, the median 7.5 hours of SCS is nearly twice as long as in other end-ischemic NMP series, which most likely contributed to poorer survival of DCD livers.[8–10,42] However, while the DCD outcomes seem to be inferior

to other machine perfusion series, the actual number of symptomatic NAS is too low to draw firm conclusions from the presented cutoffs for the bile composition analyses. Corroborated by per-protocol MRC imaging at 6 months and 5-year median follow-up, however, the trial allowed robust analysis of the biliary complications, in particular in DBD livers including those failing to produce bile. Our confirmation of the likelihood of developing NAS in DCD livers failing to produce alkalotic bile with high concentrations of bicarbonate is also an important observation of benefit to the transplant community. A relative flaw of this current report is its limited novelty. Indeed, the graft loss data are identical to those reported with the primary VITTAL outcomes. However, we believe that our confirmation of NMP relevance to early transplant outcomes, while demonstrating the absence of negative impact on graft longevity (through rigorous prospective 5+ years' follow-up within a clinical trial framework), is a great strength of this publication.[43] Finally, our exclusion of high-risk recipients might be perceived as a shortcoming for informing adoption into real-world practice, given the increasing complexity and sickness of patients on transplant waiting lists. Back in 2016, when VITTAL was designed, the ethical committee requested the inclusion of this safety feature when considering the trial application. Reflecting on the VITTAL trial outcomes, our team commenced the NAPLES project, applying NMP viability assessment to fast-track livers, and allocating those to patients requiring retransplantation or having complex PVT. These are universally considered to be the highest-risk recipients requiring good-quality grafts to achieve acceptable outcomes.[44] Using the end-ischemic NMP assessment, we transplanted 26 livers with 96% and 92% 3-month patient and graft survivals, respectively.[19] Reflecting on the VITTAL trial outcomes, however, our subsequent series considered only DBD livers. Taken together, the VITTAL and normothermic machine perfusion of the liver to enable transplantation in difficult recipients (NAPLES) projects provide compelling evidence that DBD livers that meet NMP viability criteria can be safely used in any recipients and achieve excellent outcomes that are well within established liver transplant benchmarks.[44]

In summary, the VITTAL trial explored the boundaries of high-risk liver utilization. Our three key findings are that (1) the lactate-based viability criteria are suitable for selecting DBD livers with excellent long-term outcomes regardless of their bile production, (2) we confirm that the production of alkalotic bile may be relevant for predicting longevity in DCD livers, and (3) objective liver assessment by NMP does not impose any risks, and these organs achieve excellent long-term outcomes. The VITTAL trial findings have been confirmed by several other teams, clearly demonstrating that NMP viability assessment allows expansion of the donor pool and improves access to lifesaving transplantation.

DATA AVAILABILITY STATEMENT

The source data underlying figures and tables included in the paper would be provided upon request according to the process available on the Cancer Research UK Clinical Trials Unit website (https://www.birmingham.ac.uk/research/crctu/data-sharing-policy).

AUTHOR CONTRIBUTIONS

Hynek Mergental, Simon C. Afford, and Darius F. Mirza conceived the study; Christina Yap, Darius F. Mirza, Hynek Mergental, Amanda J. Kirkham, and Darren Barton designed the trial; M. Thamara P.R. Perera, John R. Isaac, Keith J. Roberts, Manuel Abradelo, Hynek Mergental, Andrea Schlegel, James W. Ferguson, Hentie Cilliers, Bobby V.M Dasari, Richard W. Laing, Yuri L. Boteon, and Darius F. Mirza were involved in the transplantations, machine perfusions and post-transplant patient management; Richard W. Laing, Yuri L. Boteon and George Clarke were responsible for samples and data collection. Desley A.H. Neil was responsible for histological assessment; Amanda J. Kirkham performed the statistical analyses; Hynek Mergental wrote the first draft of the paper with input from Desley A.H. Neil and Amanda J. Kirkham; all authors contributed to the study conduct and reviewed the final manuscript version.

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CONFLICTS OF INTEREST

James Ferguson advises and is on the speakers' bureau for Albireo. Chris Morris is employed by OrganOx Ltd. Peter J Friend is a co-founder, chief medical officer of OrganOx Ltd., has stock in, is employed by consults, and has received grants from OrganOx Ltd. Thamara Perera is on the speakers' bureau for OrganOx Ltd. Darius Mirza has stock in OrganOx Ltd. The remaining authors have no conflicts to report.

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