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Introduction of the Concept of Diagnostic Sensitivity and Specificity of Normothermic Perfusion Protocols to Assess High-Risk Donor Livers

Hynek Mergental ,[1,2,3] Richard W. Laing,[1,2,3] James Hodson,[4] Yuri L. Boteon ,[2,3] Joseph A. Attard,[2,3] Laine L. Walace,[2,3] Desley A. H. Neil ,[5] Darren Barton,[6] Andrea Schlegel,[1,3] Paolo Muiesan,[1] Manuel Abradelo,[1] John R. Isaac,[1] Keith Roberts,[1] M. Thamara P. R. Perera,[1] Simon C. Afford,[2,3] and Darius F. Mirza[1,2,3]

[1]Liver Unit, Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom; [2]National Institute for Health Research, Birmingham Biomedical Research Centre, University of Birmingham and University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom; [3]Centre for Liver and Gastrointestinal Research, Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, United Kingdom; [4]Department of Statistics, Institute for Translational Medicine, Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham; [5]Department of Cellular Pathology, Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom; and [6]D[3]B Team, Cancer Research UK Clinical Trials Unit, University of Birmingham, Birmingham, United Kingdom

Normothermic machine perfusion (NMP) allows objective assessment of donor liver transplantability. Several viability evaluation protocols have been established, consisting of parameters such as perfusate lactate clearance, pH, transaminase levels, and the production and composition of bile. The aims of this study were to assess 3 such protocols, namely, those introduced by the teams from Birmingham (BP), Cambridge (CP), and Groningen (GP), using a cohort of high-risk marginal livers that had initially been deemed unsuitable for transplantation and to introduce the concept of the viability assessment sensitivity and specificity. To demonstrate and quantify the diagnostic accuracy of these protocols, we used a composite outcome of organ use and 24-month graft survival as a surrogate endpoint. The effects of assessment modifications, including the removal of the most stringent components of the protocols, were also assessed. Of the 31 organs, 22 were transplanted after a period of NMP, of which 18 achieved the outcome of 24-month graft survival. The BP yielded 94% sensitivity and 50% specificity when predicting this outcome. The GP and CP both seemed overly conservative, with 1 and 0 organs, respectively, meeting these protocols. Modification of the GP and CP to exclude their most stringent components increased this to 11 and 8 organs, respectively, and resulted in moderate sensitivity (56% and 44%) but high specificity (92% and 100%, respectively) with respect to the composite outcome. This study shows that the normothermic assessment protocols can be useful in identifying potentially viable organs but that the balance of risk of underuse and overuse varies by protocol.

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The demand for liver transplantation is being met by the progressive use of extended criteria donors.[1,2] Consideration of liver transplantability depends on multiple factors, including donor characteristics and the sickness of the recipient, in the context of mortality on the waiting list. Although organ transplantation is 1 of the most advanced medical specialties, the rates of graft acceptance may vary and are dependent on largely subjective interpretations of the relevant risk factors.[3,4] The standard transplantability criteria are well defined and accepted, but high-risk features are less distinct, without described firm limits.[5-7]

Abbreviations: ALT, alanine aminotransferase; BP, Birmingham protocol; CIT, cold ischemia time; CP, Cambridge protocol; CT, computed tomography; DBD, donation after brain death; DCD, donation after circulatory death; DRI, donor risk index; ET-DRI, Eurotransplant

Efficient use of donor livers is becoming increasingly challenging because of the ongoing decline in the quality of donor organs and the increasing levels of sickness and complexity of recipients. For example, in the United Kingdom, almost 40% of livers transplanted in 2018 were recovered from donors older than 60 years, one-third were from donors with body mass indexes greater than 30 kg/m^2, and only 3% of donors had died as a consequence of trauma.[2] To avoid suboptimal transplant outcomes with marginal livers in sick recipients, 15% of retrieved livers were discarded. Salvaging these organs could result in up to 142 additional transplants per year in the United Kingdom alone.

Organ preservation and reconditioning by normothermic machine perfusion (NMP) provides livers with oxygen and nutrients to prevent inevitable quality decline during static cold storage.[8] In addition, NMP allows functional assessment and provides objective information that could potentially be used to guide decision making regarding organ transplantability. This can lead to increased use of marginal grafts and enable access to transplantation for more patients. These benefits of NMP were observed in a recent European randomized trial comparing NMP with static cold storage, which reported 50% fewer discards in the NMP arm, resulting in 20% more transplants compared with static cold storage.[9]

Several teams, including our own, have pushed liver use boundaries and investigated the potential for functional parameters recorded during NMP to predict the viability of organs initially deemed not suitable for transplantation. We reported a composite measure based on lactate clearance, bile production, pH maintenance, vascular flows, and liver appearance.[10] Similarly, Watson and colleagues successfully transplanted livers with low perfusate transaminase release and bile pH >7.50.[11] The emerging opportunity to objectively assess organ transplantability brings a ground-breaking advancement but also adds another layer of complexity.[12] The currently used protocols were developed on small discarded liver series, without clearly defined inclusion and exclusion criteria. With the cumulatively growing experience with NMP viability testing, the initially proposed benchmarks are now being updated and extended.[13,14] Pushing the boundaries of use of the highest-risk livers increases access to transplantation; however, extending the limits too far might negatively impact outcomes.

The aim of this study was to introduce the concept of the NMP protocols' diagnostic accuracy to predict the most serious graft-related posttransplant complications, specifically primary nonfunction (PNF) and nonanastomotic biliary strictures, assessed by graft survival at 90 days and 24 months, respectively. The real-world dilemma of selecting the appropriate assessment protocol was illustrated by the application of 3 different viability protocols to a rigorously

donor risk index; GP, Groningen protocol; IQR, interquartile range; mCP, modified Cambridge protocol; mGP, modified Groningen protocol; NA, not available; NMP, normothermic machine perfusion; NPV, negative predictive value; PNF, primary nonfunction; PPV, positive predictive value; SD, standard deviation; UK-DCD, UK donor after circulatory death score; UK-DLI, UK donor liver index; VITTAL, Viability Testing and Transplantation of Marginal Livers.

Address reprint requests to Hynek Mergental, M.D., F.E.B.S., Liver Unit, Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, B15 2TH, United Kingdom. Telephone: 0044 121 371 4638; E-mail: hynek.mergental@uhb.nhs.uk

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Hynek Mergental conceived the study. Hynek Mergental, Richard W. Laing, James Hodson, Simon C. Afford, and Darius F. Mirza contributed equally to the study delivery. Hynek Mergental and Richard W. Laing collected the data. James Hodson devised and performed the statistical analyses. M. Thamara P. R. Perera, Andrea Schlegel, Paolo Muiesan, Keith Roberts, Manuel Abradelo, John R. Isaac, Hynek Mergental, Richard W. Laing, Yuri L. Boteon, Joseph A. Attard, and Darius F. Mirza were involved in the transplantations, machine perfusions, and posttransplant patients' management. Hynek Mergental and James Hodson prepared the article draft. Hynek Mergental, Richard W. Laing, James Hodson, Yuri L. Boteon, Joseph A. Attard, Laine L. Walace, Desley A. H. Neil, Darren Barton, Andrea Schlegel, Paolo Muiesan, Manuel Abradelo, John R. Isaac, Keith Roberts, M. Thamara P. R. Perera, Simon C. Afford, and Darius F. Mirza conducted the study and reviewed the final version of the article.

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characterized cohort of discarded livers that underwent testing within the framework of the Viability Testing and Transplantation of Marginal Livers (VITTAL) clinical trial.[14]

Materials and Methods

STUDY DESIGN, ENDPOINTS, AND DATA COLLECTION

This study was a secondary analysis of data from the VITTAL clinical trial, which was a prospective, open-label, phase 2 adaptive single-arm trial assessing whether NMP viability assessment of currently discarded, high-risk marginal livers could allow transplantation of these organs. The trial protocol and outcomes were reported previously.[14,15]

The primary aim of the study was to demonstrate the diagnostic accuracy of 3 currently available viability assessment protocols for the prediction of long-term graft survival. Because not all livers were transplanted, we created a composite outcome, which treated livers that were transplanted and remained functional at 24 months as having a positive outcome, whereas organs that were either not transplanted or were transplanted and failed within 24 months were treated as having a negative outcome. An organ that failed as a result of technical issues (hepatic artery thrombosis) was excluded from the diagnostic accuracy analysis because this outcome was likely to be a result of external factors that could not be predicted from the viability testing.

The secondary objectives were to assess short-term (90 day) graft survival, quantify the number of livers classified as viable by each protocol, and assess how their use could impact organ utilization rates.

The outcomes at 90 days and 24 months were assessed through the patients' clinical follow-ups at 1 to 3 monthly intervals, with clinical reviews and blood analyses, including liver function tests. In addition, each patient underwent a protocol magnetic resonance cholangiopancreatography at 6 months (unless indicated earlier).

DISCARDED LIVER INCLUSION CRITERIA

The VITTAL trial aimed to include only the highest risk marginal organs, which was achieved by a 2-tier inclusion process embedded within its design. Tier 1 consisted of the liver being discarded by all UK centers following a fast-track national offering. All included organs were therefore allocated within the nonclinical offering scheme. The second tier for inclusion consisted of meeting at least 1 of the following high-risk criteria: donor risk index (DRI) >2.0,[5] balance of risk score >9,[16] liver steatosis >30%, donor warm ischemia time (defined as the period between the systolic blood pressure <50 mm Hg to the time of commencing donor aortic perfusion) in donation after circulatory death (DCD) >30 minutes, peak donor aspartate or alanine transaminase level >1000 IU/mL, anticipated cold ischemia time (CIT) >12 hours for donation after brain death (DBD) livers or >8 hours for DCD livers, or suboptimal liver graft perfusion as assessed by a consultant transplant surgeon and documented by photography (details shown in Supporting Table 1). The transplantation procedures were performed at the Liver Unit, Queen Elizabeth Hospital, Birmingham, UK.

NMP PROCEDURE

All livers were initially preserved by static cold storage, with assessment by NMP commencing upon arrival at our unit. NMP was performed using the OrganOx *metra* (OrganOx Ltd, Oxford, UK) device with red blood cells and Gelofusine (B.Braun Medical Ltd, Sheffield, UK) base perfusate and added infusions of heparin, prostacyclin, bile salts, insulin, glucose, and amino acids.[8] The protocol stipulated the NMP duration to be between 4 and 24 hours, and livers that met the predefined viability parameters (referred to as the Birmingham protocol [BP]) were transplanted into low-risk to moderate-risk recipients who consented to the trial.

THE REGULATORY APPROVAL

Each participant was fully informed in advance of being offered a very high-risk graft and gave written consent for the study. The VITTAL trial was registered at ClinicalTrials.gov (number NCT02740608) and approved by both the National Research Ethics Service in London-Dulwich (Research Ethics Committee reference 16/LO/1056, protocol number RG 15–240) and the Medicines and Healthcare Products Regulatory Agency. The project was endorsed by the Research, Innovation and Novel Technologies Advisory Group committee of the National Health Service Blood and Transplant.

LIVER VIABILITY ASSESSMENT AND COMPARED PROTOCOLS

Blood and bile were analyzed from serial measurements made with regularly calibrated devices used for clinical transplantation, which were available in the liver operating room. The blood gas analyses were obtained using the Cobas b 221 blood gas analyser (Roche Diagnostics, Indianapolis, IN). Except for a single (10-20 mL) sodium bicarbonate bolus within the initial 30 minutes of perfusion, the perfusate pH was not further corrected. Regarding the presence of bile production, volumes ≥10 mL were considered positive. The presence of glucose metabolism was considered positive if the values started to decrease and maintained a consistent downward trend. The perfusate transaminase levels were obtained from the hospital biochemistry labs at 2-hour and 4-hour time points.

To introduce the concept of the viability assessment diagnostic value, this study compared the latest versions of clinically used protocols introduced by the Birmingham, Cambridge, and Groningen teams[15,17,18] (published until December 2020; listed in alphabetical order). The measured variables included in each of those protocols are summarized next, with details provided in Table 1.

The BP was based principally on the perfusate lactate clearance to levels ≤2.5 mmol/L, in combination with minor criteria, including the presence of bile production, perfusate pH >7.30, glucose metabolism, vascular perfusion or homogeneous liver perfusion, and soft parenchyma consistency.[15] To be considered viable, a liver had to meet the lactate clearance component in combination with at least 2 of the other minor components of the protocol within 4 hours (240 minutes).

The Cambridge protocol (CP) consisted of maintenance of perfusate pH >7.20, falling perfusate glucose beyond 120 minutes, perfusate alanine aminotransferase (ALT) <6000 IU, peak lactate fall ≥4.4 mmol/L/kg/hour, maximum bile pH >7.50, and bile glucose of either ≤3 mmol/L or ≥10 mmol/L less than the perfusate glucose.[17] It must be noted that the authors described these as a set of favorable features rather than cutoff thresholds. They did not explicitly define an assessment time point, but instead stated that livers expected to have a good outcome would meet the assessed components within 120 to 240 minutes; hence, assessments at 4 hours were used in the analysis.

TABLE 1. Details of Viability Protocols and Proportion of Livers Meeting Each Component

Component	n/N (%) of Livers
BP at 4 hours	24/31 (77)
Lactate concentration ≤2.5 mmol/L	24/31 (77)
Bile production	22/31 (71)
Perfusate pH >7.30	8/31 (26)
Glucose metabolism*	23/31 (74)
Vascular perfusion	30/31 (97)
Homogeneous perfusion present	29/31 (94)
CP at 4 hours	0/31 (0)
Maximum bile pH >7.50†	17/31 (55)
Bile glucose ≤3 mmol/L or ≥10 mmol/L less than perfusate†	3/31 (10)
Maintenance of perfusate pH >7.20	26/31 (84)
Falling glucose*	23/31 (74)
Peak lactate fall ≥4.4 mmol/L/kg/hour	18/31 (58)
ALT level <6000 IU	19/26 (73)‡
mCP without bile glucose	8/31 (26)
GP at 2.5 hours	1/31 (3)
Cumulative bile production ≥10 mL and increasing in the last hour	22/31 (71)
Lactate concentration <1.7 mmol/L	19/31 (61)
Perfusate pH 7.35-7.45	3/31 (10)
Bile pH >7.45†	13/31 (42)
mGP without perfusate pH	11/31 (35)

NOTE: The BP requires lactate ≤2.5 and at least 2/5 of the remaining components to be met for a liver to be classified as viable. CP and GP require all of the components to be met for a liver to be classified as viable; it should be noted that the Cambridge measures are favorable features rather than a formal protocol.

*"Glucose metabolism" and "Falling glucose" were alternative names for the same variable.

†Organs that did not produce bile were treated as not having met the bile pH requirements.

‡Measures of ALT were unavailable in n = 5 organs at 4 hours; for these, the ALT component was not considered when assessing whether the protocol was met.

The Groningen protocol (GP) consisted of cumulative bile production ≥10 mL and increased production within the last hour, bile pH >7.45, perfusate pH within the range 7.35 to 7.45, and lactate concentration <1.7 mmol/L.[18] To be considered transplantable, a liver had to meet all of these criteria within 2.5 hours (150 minutes).

STATISTICAL ANALYSIS

Continuous variables were reported as mean ± standard deviation (SD) if normally distributed or as median and interquartile range (IQR) otherwise, with comparisons

between groups using independent-samples *t* tests and Mann-Whitney U tests, respectively. Nominal data were reported as numbers and percentages and compared between groups using Fisher's exact tests.

The diagnostic accuracy of the protocols was assessed against the composite outcome (transplanted with 24-month graft survival) and quantified using sensitivity and specificity as well as the positive predictive value (PPV; the proportion of livers with positive test results that were correctly diagnosed) and negative predictive value (NPV; the proportion of livers with negative test results that were correctly diagnosed). The Youden Index was additionally calculated as a summary of overall predictive accuracy. All analyses were performed using IBM SPSS 22 (IBM Corp., Armonk, NY), with $P < 0.05$ deemed to be indicative of statistical significance throughout.

Results

DISCARDED LIVER CHARACTERISTICS

The study enrolled 31 discarded livers consisting of 17 (55%) DBD and 14 (45%) DCD organs. Donors had a median age of 57 years (IQR, 45-63 years), body mass index of 29 kg/m^2 (IQR, 25-32 kg/m^2), and DRI of 2.2 (IQR, 1.9-2.9). The median liver weight was 1.8 kg (IQR, 1.4-2.0 kg). Further details of the cohort are provided in Table 2 and Supporting Table 2.

BILE PRODUCTION DURING NORMOTHERMIC PERFUSION

A total of 9 (29%) livers did not produce bile during the course of the NMP perfusion. For these organs, checks for potential technical problems relating to the bile duct cannulation were performed, including tube kinks, obstruction from the fixing tie, and misplacement of the tip of the cannula, with removal and reinsertion of the tube also being performed. In all cases, the lack of bile production persisted after these checks; hence this was contributed to the intrinsic liver function. The organs without bile production were found to have significantly longer CITs (median 11.9 versus 7.1 hours; $P = 0.03$) and also had a tendency to be heavier and lower risk on the UK donor liver index (UK-DLI) score, although neither of these comparisons reached statistical significance (Table 3).

VIABILITY PROTOCOL PREDICTIVE VALUES AND THE MEASURED COMPONENTS ASSESSMENT

Of the 31 enrolled livers, 24 (77%) met the BP, whereas no livers (0%) met the CP and only a single liver (3%) met the GP. A further breakdown of the individual components of the 3 protocols is reported in Tables 1 and 2 and detailed next.

For BP, the viable organs had to clear the perfusate lactate condition and meet at least 2 of the remaining components. Of these, the vascular flows and homogenous perfusion were achieved by almost all organs (97% and 94%, respectively). As a result, all of the organs that met the lactate condition were deemed viable, with none of the other components causing organs to be excluded.

CP included 2 components related to measures of the bile, namely, the pH and glucose concentrations. All 9 (29%) organs that did not produce bile were treated as having failed to meet these components. Data relating to the ALT were unavailable in 5 organs, 1 of which met the remaining components of the protocol, with the remaining 4 organs failing to meet at least 1 component. For these 5 organs, the ALT component of the protocol was not considered when assessing the CP in subsequent analysis. No organs were found to meet all components of the score, most commonly failing in the measure of bile glucose, which was only achieved by 3 (10%) livers. Removing the bile glucose component from the protocol to form the modified CP (mCP) identified 8 (26%) organs that would be classified as viable.

GP also included 2 components related to measures of the bile, and organs that did not produce bile were treated as failing to meet these components. The perfusate pH was the component most commonly resulting in organs failing to meet the protocol, with only 3 (10%) having a pH of 7.35 to 7.45. Only 1 organ (3%) met the GP; removing the perfusate pH component to form the modified GP (mGP) increased the number of organs meeting the protocol to 11 (35%).

To summarize, 77%, 0%, and 3% of the livers met the BP, CP, and GP, respectively. By removing the most stringent measures (bile glucose and perfusate pH, respectively) the mCP and mGP would achieve

TABLE 2. Details of Liver Perfusion Parameters and Viability Protocols

Organ Number	Donor Type	Liver Weight (kg)	CIT (Hours)	Lactate (mmol/L)		Perfusate pH		Bile Produced		Bile pH		ALT (iU)		Protocol Met			Trans-planted	Graft Survival	
				2 Hours	4 Hours	2 Hours	4 Hours	≥10 mL	mL	2 Hours	4 Hours	2 Hours	4 Hours	BP at 4 Hours	mCP at 4 Hours	mGP at 2.5 Hours		90 Days	24 Months
1	DCD	1.6	9.2	2.3	1.0	7.02	7.13	Yes	18	—	7.89	NA	NA	Yes	No	No	No	—	—
2	DBD	2.4	7.3	0.3	0.6	7.08	7.16	No	—	—	—	NA	NA	Yes	No	No	Yes	Yes	Yes
3	DBD	1.2	6.0	1.1	0.7	7.23	7.30	Yes	46	7.86	7.80	NA	NA	Yes	No	Yes	Yes	Yes	Yes
4	DBD	1.2	13.2	1.4	1.2	7.21	7.24	Yes	64	7.80	7.65	NA	NA	Yes	Yes	Yes	Yes	Yes	Yes
5	DBD	1.2	10.4	3.2	2.5	7.35	7.38	Yes	>99	7.70	7.79	572	658	Yes	No	No	Yes	Yes	Technical problems†
6	DBD	1.3	14.8	1.8	1.5	7.27	7.26	Yes	60	7.93	7.93	2027	2198	Yes	Yes	Yes	Yes	Yes	Yes
7	DBD	1.8	6.5	1.1	1.3	7.20	7.24	Yes	>99		7.75	1561	1585	Yes	No	Yes	Yes	Yes	Yes
8	DCD	2.0	9.6	2.6	2.5	7.32	7.35	Yes	90	7.58	7.91	3822	4328	Yes	No	No	Yes	Yes	Yes
9	DCD	1.5	6.0	0.7	1.0	7.21	7.24	Yes	>99	7.43	7.58	7995	8625	Yes	No	No	No	—	—
10	DCD	2.0	7.3	7.4	6.7	7.33	7.36	Yes	11	—	7.40	6063	6511	No	No	No	No	—	—
11	DBD	1.8	5.4	2.1	2.1	7.44	7.45	Yes	60	—	7.93	2379	2681	Yes	Yes	No	Yes	Yes	Yes
12	DCD	1.9	10.0	6.7	5.2	7.16	7.26	No	—	—	—	7044	8021	No	No	No	No	—	—
13	DCD	2.0	6.5	1.5	1.9	7.28	7.26	No	—	—	—	2080	2335	Yes	No	No	Yes	Yes	Yes
14	DCD	1.7	7.2	0.7	0.7	7.32	7.30	No	—	—	—	958	1034	Yes	No	No	Yes	Yes	No
15	DCD	2.0	5.5	0.6	0.8	7.30	7.31	Yes	>99	7.85	7.80	2770	3158	Yes	Yes	Yes	Yes	Yes	Yes
16	DBD	1.2	11.9	0.2	0.6	7.24	7.23	No	—	—	—	NA	NA	Yes	No	No	Yes	Yes	Yes
17	DBD	1.7	12.0	0.4	0.4	7.32	7.29	No	—	—	—	931	1068	Yes	No	No	Yes	Yes	Yes
18	DCD	1.6	7.7	0.3	0.5	7.41	7.37	Yes	75	7.49	7.65	3165	4168	Yes	Yes	Yes*	Yes	Yes	Yes
19	DCD	1.8	7.0	0.5	0.9	7.33	7.31	Yes	>99	7.68	7.65	948	1076	Yes	Yes	Yes	Yes	Yes	Yes
20	DCD	1.4	5.9	0.9	1.3	7.29	7.24	Yes	48	7.45	7.44	856	921	Yes	No	No	Yes	Yes	No
21	DCD	1.9	6.9	0.9	1.1	7.19	7.22	Yes	15	7.57	7.73	2153	2219	Yes	Yes	Yes	Yes	Yes	Yes
22	DBD	2.0	8.6	1.1	1.3	7.16	7.23	Yes	18	7.93	7.51	3046	3390	Yes	No	Yes	No	—	—
23	DCD	1.3	10.0	0.6	1.2	7.20	7.26	Yes	>99	7.25	7.26	2205	2535	Yes	No	No	Yes	Yes	Yes
24	DBD	2.4	12.0	17.7	>2.5	7.13	7.20	No	—	—	—	5785	<6000	No	No	No	No	—	—
25	DBD	1.8	13.4	7.9	6.3	7.13	7.16	No	—	—	—	7189	7692	No	No	No	No	—	—
26	DBD	2.4	6.0	1.6	2.8	7.21	7.24	Yes	30	7.41	7.51	10,648	7841	No	No	No	No	—	—
27	DBD	2.6	11.9	10.4	5.1	7.04	7.18	No	—	—	—	20,325	20,750	No	No	No	No	—	—
28	DCD	1.4	5.6	2.6	2.0	7.29	7.32	Yes	56	7.37	7.63	2660	3187	Yes	No	No	Yes	Yes	No
29	DBD	2.0	6.3	2.3	2.6	7.13	7.14	Yes	15	—	7.75	6246	6940	No	No	No	Yes	Yes	Yes
30	DBD	1.7	7.8	0.7	1.0	7.32	7.21	Yes	>99	7.82	7.84	1954	1931	Yes	Yes	Yes	Yes	Yes	Yes
31	DBD	1.6	7.6	1.3	1.6	7.24	7.25	Yes	63	7.93	7.93	4475	4700	Yes	No	Yes	Yes	Yes	Yes

*Organ also met the unmodified GP.
†Graft failed as a result of technical problems with the transplant.

TABLE 3. Associations With Bile Production

Variable	n	Bile Produced: No	Bile Produced: Yes	*P* Value
Donor type, % DBD	31	6/9 (67)	11/22 (50)	0.46
Liver weight, kg	31	1.9 (1.7-2.4)	1.7 (1.4-2.0)	0.15
CIT, hours	31	11.9 (7.3-12.0)	7.1 (6.0-9.2)	**0.03**
UK-DLI	31	1.4 ± 0.3	1.7 ± 0.6	0.15
ET-DRI	31	2.9 ± 0.5	3.1 ± 0.7	0.46
UK-DCD*	14	6.3 ± 3.2	6.7 ± 1.8	0.78
ALT at 4 hours, iU	26	6000 (1068-8021)	3158 (1931-4700)	0.46
Transplanted	31	5/9 (56)	17/22 (77)	0.39
Functional at 24 months†	21	4/5 (80)	14/16 (88)	>0.99
Composite outcome‡	30	4/9 (44)	14/21 (67)	0.42

NOTE: Data are reported as mean ± SD with *P* values from independent-sample *t* tests, median (interquartile range) with *P* values from Mann-Whitney U tests, or as n/N (%) with *P* values from Fisher's exact tests, as applicable. Bold *P* values are significant at $P < 0.05$.
*For DCD organs only.
†Transplanted organs only; 1 organ had technical failure.
‡A composite of transplanted and functional at 24 months and excludes 1 organ that had technical failure.

26% and 35% transplantability, respectively. The numbers of organs needed to be perfused before 1 would be classed as viable were 1.3 for the BP, 3.9 for the mCP, and 2.8 for the mGP.

TRANSPLANT RATES AND POSTTRANSPLANT OUTCOMES

The decision to transplant organs was made based on the BP, which was met by 24 (77%) organs. Of these, 2 livers were not transplanted for reasons unrelated to perfusion, namely, a complex hepatic arterial anatomy and an extrahepatic donor malignancy. A further organ met the BP at 4 hours, but the lactate levels subsequently started to rise, and the liver was discarded. Of the organs not meeting the BP at 4 hours, 2 did initially meet the protocol after 2 hours of NMP perfusion and were classified as viable. However, lactate levels rose again, meaning that the organs were classified as nonviable by the BP at 4 hours. Of these livers, 1 was discarded. For the other organ, the team had already commenced the transplantation, hence the graft was transplanted despite not meeting the BP at 4 hours; this graft remained functional at 24 months.

TABLE 4. Outcomes by Viability Testing Protocol

Testing Protocol	Transplanted, n/N (%)	Functional at 24 Months, n/N (%)*,†
BP at 4 hours		
No	1/7 (14)	1/1 (100)
Yes	21/24 (88)	17/20 (85)
mCP at 4 hours		
No	14/23 (61)	10/13 (77)
Yes	8/8 (100)	8/8 (100)
mGP at 2.5 hours		
No	12/20 (60)	8/11 (73)
Yes	10/11 (91)	10/10 (100)

*In transplanted organs.
†Excludes the organ with technical failure.

In total, 22 (71%) livers were transplanted; the detailed characteristics of the transplanted cohort are shown in Supporting Table 3 and early posttransplant outcomes in Supporting Table 4. All 22 (100%) transplanted grafts were functional at 90 days. Of the patients, 1 developed hepatic artery thrombosis within several hours after transplantation. Although the arterial flow was urgently restored, the organ subsequently developed ischemic cholangiopathy. Because this was the result of a technical failure that could not had been predicted during the viability assessment, this liver was excluded from the analysis of long-term outcomes. Of the remainder, 18/21 (86%) were functional at 24 months; all of these patients had magnetic resonance cholangiopancreatography without biliary strictures at the 6-month scans, were free of any biliary complication clinical symptoms, and had normal liver function tests. Of the patients, 1 died 23 months after the transplantation as a result of a metastatic spread of hepatocellular cancer with normal liver function, hence this organ was considered to be functional at 24 months for analysis. All 3 graft failures were caused by nonanastomotic biliary strictures in DCD livers, requiring retransplantation at 120, 225, and 375 days.

Comparisons of transplant rates and long-term graft function across the viability protocols are reported in Table 4. As described previously, 21/24 (88%) of the organs meeting the BP were transplanted. Of these, 1 was lost as a result of technical failure, with 85% of the remainder being functional at 24 months.

For the other 2 viability protocols, all 8 (100%) livers meeting the mCP and 10/11 (91%) organs meeting the mGP were transplanted, all of which remained functional at 24 months. However, 61% and 60% of livers

that did not meet mCP and mGP, respectively, were transplanted, and 77% and 73% of those, respectively, remained functional at 24 months.

PROTOCOL SENSITIVITY AND SPECIFICITY TO PREDICT POSITIVE 24-MONTH COMPOSITE TRANSPLANT OUTCOME

To better quantify the predictive accuracy of the protocols, a composite outcome was generated in which a transplant with 24-month graft survival was treated as a positive outcome and organs being discarded or failing within 24 months of transplant were treated as negative outcomes. The distribution of organs and outcomes by protocol are visualized in Fig. 1, with the diagnostic accuracy quantified in Table 5.

After excluding the organ that was lost as a result of technical failure, the composite endpoint was achieved in 18/30 (60%) livers. Of organs meeting the BP, 74% had a positive outcome compared with 14% that did not meet the protocol, yielding high sensitivity (94%) but poor specificity (50%). All 8 of the organs meeting the mCP had positive outcomes, giving 100% specificity; however, 45% of those that failed to meet the protocol had a positive outcome, giving moderate sensitivity (44%). Similarly, a total of 91% (10/11) meeting the mGP had a positive outcome, giving high specificity (92%) with a moderate sensitivity (56%). The test predictive accuracy was also summarized using the Youden index, which was found to be similar across the 3 protocols, ranging from 0.44 to 0.47. Although the overall diagnostic accuracy was similar for the 3 protocols, the BP has a tendency to be too lenient and overuse organs, whereas the mCP and mGP tended to be too strict and underuse organs.

In addition to analyzing the cohort as a whole, subgroup analyses were also performed, which considered the DCD and DBD organs separately. Of the 6 organs that did not achieve the composite outcome after being classified as viable by the BP, 5 were DCD organs. After excluding these organs, the diagnostic accuracy of the BP improved considerably, with a sensitivity of 91% and a specificity of 80% when applied to DBD organs, which was superior to both the mCP and mGP. However, performance of the BP was poor for DCD organs, with only 58% of the organs classified as viable achieving the composite outcome, yielding 100% sensitivity and 29% specificity.

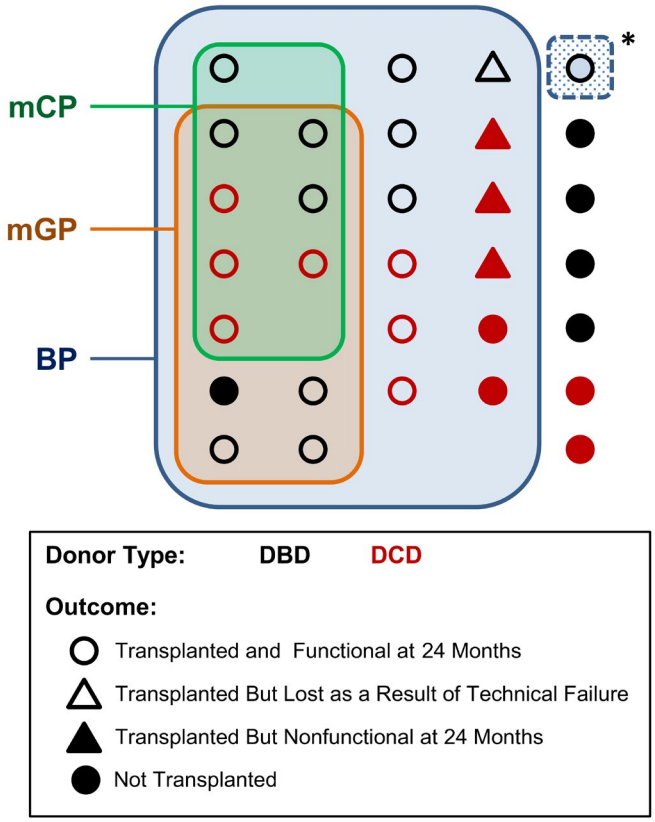

FIG. 1. Visualization of the livers meeting each criterion and subsequent outcomes. The figure shows 31 points, each representing a single organ, with the symbol corresponding with the transplant outcome. Black points represent DBD organs, and red points represent DCD organs. Points within the colored areas represent organs classified as viable by the stated criteria, with the overlapping areas identifying organs classified as viable by multiple criteria. *The decision to transplant the organ was made at 2 hours, as the BP was met—however, lactate levels subsequently rose to the point that the organ no longer met the BP at 4 hours; hence this organ was treated as not meeting the BP in the analysis.

It must be noted that the purpose of this study was to introduce the concept of the diagnostic value of the viability protocols. The resulting accuracy statistics will be highly dependent on the characteristics of the organs being assessed that, in the case of this study, were very high risk. As such, application of the protocol to organs of more typical risk profiles may give different results.

ANALYSIS OF THE VIABILITY ASSESSMENT PERIOD

The assessment period differed between the protocols, with BP and mCP based on assessments at 4

TABLE 5. Predictive Accuracy of Viability Protocols According to the Donor Cohort Type

Testing Protocol	Composite Outcome, n/N (%)*	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Youden Index
All organs, n = 31						
BP at 4 hours		94	50	74	86	0.44
No	1/7 (14)					
Yes	17/23 (74)†					
mCP at 4 hours		44	100	100	54	0.44
No	10/22 (45)†					
Yes	8/8 (100)					
mGP at 2.5 hours		56	92	91	58	0.47
No	8/19 (42)†					
Yes	10/11 (91)					
DCD, n = 14						
BP at 4 hours		100	29	58	100	0.29
No	0/2 (0)					
Yes	7/12 (58)					
mCP at 4 hours		57	100	100	70	0.57
No	3/10 (30)					
Yes	4/4 (100)					
mGP at 2.5 hours		57	100	100	70	0.57
No	3/10 (30)					
Yes	4/4 (100)					
DBD, n = 17						
BP at 4 hours		91	80	91	80	0.71
No	1/5 (20)					
Yes	10/11 (91)†					
mCP at 4 hours		36	100	100	42	0.36
No	7/12 (58)†					
Yes	4/4 (100)					
mGP at 2.5 hours		55	80	86	44	0.35
No	5/9 (56)†					
Yes	6/7 (86)					

*A composite of transplanted and functional at 24 months.
†Excludes the organ with technical failure.

hours, whereas the mGP was assessed at 2.5 hours. The effect of reducing the assessment period to 2 hours for the BP and mCP or increasing this to 4 hours for the mGP was analyzed (Table 6). For BP, shortening the assessment period from 4 to 2 hours led to 2 additional organs being classified as viable and 3 organs to be reclassified as nonviable, all of which resulted from differences in lactate clearance between 2 and 4 hours.

Performing the assessment for the mCP at 2 hours led to 4 organs becoming nonviable, all attributed to failing to meet the bile or perfusate pH components. All of these organs achieved the composite outcome, leading to a reduction in the diagnostic accuracy of the protocol. Increasing the assessment period for mGP from 2.5 to 4 hours would cause an additional 2 organs to become viable, again attributed to improvements in bile pH, although neither of these achieved the composite outcome because they were not transplanted, again reducing the diagnostic accuracy.

Discussion

This is the first study that investigates the differences between liver viability protocols and also introduces the concept of NMP assessment as a predictive tool, similar to other clinical tests. We took 3 different protocols to demonstrate that these vary in their specificity and sensitivity with respect to long-term

TABLE 6. Effects of Changing the Organ Assessment Period

Testing Protocol	Number of Organs Viable, n/N (%) or n	Composite Outcome Rate, n/N (%)*
BP		
Viable at 4 hours	24/31 (77)	17/23 (74)†
Viable at 2 hours	23/31 (74)	17/23 (74)
Difference		
Becomes viable	2	1/2 (50)
Becomes nonviable	3	1/2 (50)†
mCP		
Viable at 4 hours	8/31 (26)	8/8 (100)
Viable at 2 hours	4/31 (13)	4/4 (100)
Difference		
Becomes viable	0	—
Becomes nonviable	4	4/4 (100)
mGP		
Viable at 2.5 hours	11/31 (35)	10/11 (91)
Viable at 4 hours	13/31 (42)	10/13 (77)
Difference		
Becomes viable	2	0/2 (0)
Becomes nonviable	0	—

*A composite of transplanted and functional at 24 months.
†Excludes 1 organ that had technical failure.

graft outcomes. Such a concept and the real-world dilemmas of choosing the most appropriate viability evaluation protocols in specific circumstances are novel within the transplant field. If we use an analogy with another clinical diagnostic method, for example, computed tomography (CT), the presented study looks at NMP as if it were a scanner and the assessed criteria as different scanning protocols. As with CT imaging, there are different scans required to look for different conditions, but when an adequate technique is used, the scans can provide physicians with information needed to establish the likely diagnosis, regardless of the moderate differences in the scan timing, the scanner manufacturer, or contrast dye used.

Similarly, our article focused on the assessment of diagnostic values to predict the most significant posttransplant complications, namely, PNF and nonanastomotic biliary strictures. The use of high-risk livers has to be interpreted within the context of the allocation system, recipient sickness, and the waitlist mortality. The relevance of diagnostic information obtained may vary between regions and centers, and a direct comparison regarding organ use might be misleading. Viability testing, however, produces objective measures that are applicable globally, and the important findings from this comparison are provided next.

First, all 3 of the assessed protocols resulted in 100% 90-day graft function of the livers transplanted. In addition, 24-month graft survival was 85% in those meeting the BP, and 100% in those meeting the mCP and mGP. Therefore, it is clear that carefully selected high-risk livers can be successfully transplanted and that viability assessment helps to achieve excellent long-term outcomes.

Second, the NMP allowed total preservation times ranging from 11.4 to 25.5 hours, even in very marginal livers, without an obvious detrimental impact on the early transplant outcomes. This finding alone may provide an opportunity to transform algorithms for allocation of high-risk livers to improve their use. The extension of the assessment period is now possible because of advancements in the perfusion devices, allowing preservation times to 24 hours and beyond,[9,19,20] and highlights the opportunity to revise future criteria as the field progresses. Our data also suggest that the NMP diagnostic value to predict PNF remains very high, regardless of the duration of the total preservation time. We believe this finding is of key importance for the assessment of steatotic and other high-risk DBD livers.

In terms of the DCD livers, where biliary complications are of utmost concern, the bile production and its constitution provide additional important information regarding the integrity of the biliary tree.[17,21-25] The evaluation of DCD livers may therefore benefit from an extended assessment period. Regarding the optimal evaluation period for the lactate clearance, however, our experience shows conflicting results. Following the encouraging NMP experiences from our initial viability testing series,[26] we explored strategies to further increase the organ rescue rates by relaxing the criteria.[15] After 1 PNF in a liver where the trough lactate level dropped to only 4.5 mmol/L,[9] we extended the assessment period to 4 hours rather than relaxing the 2.5 mmol/L lactate cutoff value. Unexpectedly, this change brought up a phenomenon of rising lactate in a liver that initially achieved its clearance threshold, and the mechanism behind this observation remains unclear. A possible explanation may be that this is a consequence of the liver's ongoing exposure to substances and toxic products washed out and accumulated within the

circulating perfusate following the static cold storage. Testing this hypothesis is 1 of the areas of our ongoing research interest.

When comparing the proportions of livers deemed transplantable across the 3 viability protocols, the differences were larger than we had expected. It must be noted that if the assessments were performed in real time by the authors' teams, the outcomes might have been different. The presented comparisons are meant to illustrate the real-world trade-offs rather than benchmarking the criteria. For example, removing the perfusate pH component from the GP considerably increased the number of organs classified as viable (from 1 to 11) without losing the specificity to predict nonanastomotic biliary strictures. The actual difference in predicted transplantability is likely to be related to the high proportion of DCD livers included in the CP and GP protocol development sets (79% and 83%, respectively) and the teams' research focus on preventing biliary complications specific to DCD organs.[25,27] This would also explain why the mCP and mGP performed best in the subgroup of DCD livers, whereas the BP had superior performance in DBD organs. More research is needed to determine whether optimal diagnostic accuracy can be achieved using 1 set of comprehensive measures or whether separate criteria are required for DBD and DCD livers. It is important to realize that application of a stringent diagnostic criterion to organs with a low incidence of a specific complication (eg, bile pH to bile measures to prevent nonanastomotic biliary strictures in DBD livers) might prevent the use of transplantable grafts. For example, this was observed in the PROCEED II trial using heart machine preservation.[28]

Regarding the reported clinical outcomes, the presented 24-month outcome analysis excluded 1 liver that failed as a result of technical reasons. If this study's aim was to assess overall transplant outcomes, then such exclusion would not be appropriate. However, our study was focused on the diagnostic accuracy of the NMP and therefore we believe that retaining that particular perfusion data would introduce bias to the results. Although there is currently no evidence that machine perfusion increases incidences of vascular complications, we acknowledge that there might be a certain risk of hepatic artery damage from the vessel cannulation and handling related to the NMP procedure.

Lastly, our study demonstrated that some measures might not add to the decision making (eg, vascular flows and homogeneous perfusion) and that minor amendments might significantly improve transplantability rates without noticeable impact on the long-term outcomes (eg, perfusate pH). These particular observations might help teams starting NMP programs to choose and tailor viability criteria according to their needs.

The findings of this study should be interpreted in the context of its several limitations, 1 of which might be a bias toward favoring BP because this study was performed under similar conditions to the protocol development set. The VITTAL trial was designed to explore the usage boundaries of the highest risk organs with the perceived risks of PNF, accessing the benefits of rigorous peer review and continual safety appraisal within the framework of a clinical trial. The attitude to use only high-risk organs was reflected by the 2-tier liver inclusion process embedded within its design and the fact that many discarded livers were not included because they were not considered to be sufficiently marginal.[14] This might explain its higher rate of late graft failures compared with other series that applied NMP to livers deemed suitable for transplantation in an attempt to improve outcomes.[29] Although the average DRI and UK-DLI of 2.4 (DBD 2.0/DCD.2.8) and 1.6 (DBD 1.2/DCD 2.0), respectively, were comparable with other series, the CITs were 2-fold to 3-fold longer.[30-32] A second limitation might be the strict application of the protocols' cutoff readings that perhaps did not accurately reflect the authors' practice. Furthermore, the perfusion and testing protocols varied widely. For example, the GP was proposed based on blood-based perfusate experiments, but the clinical validation was performed in a rewarming clinical trial with an artificial oxygen carrier-based fluid.[30,33] We assumed that the viability criteria for NMP could be applied universally, as those were proposed based on the similarities with clinical physiological conditions and routine blood gas analyser or liver function readings. We acknowledge that the decision to proceed with transplantation is multifactorial and that NMP readings might be evaluated in the context of the overall benefit for its intended recipient. Regarding the bile volume and composition assessment, the findings might be affected by the addition of bile acids included in our protocol.[23] Of the transplanted organs, 1 failed as a result of hepatic artery thrombosis and was excluded from the analysis of 24-month graft survival. We acknowledge that this

outcome may have, in part, been related to the cannulation and instrumentation required to perform the NMP—if this were the case, then the exclusion may have resulted in a small underestimate or overestimate of the predictive accuracy of the protocols.

In conclusion, this study aimed to introduce the new concept of diagnostic accuracy in liver viability assessment and demonstrated that this should be interpreted in the context of the desired outcome measure and the liver type. Our findings suggest that the currently used protocols differ in their predictive value for different clinical endpoints. Application of lactate-based viability criteria might yield higher use rates in DBD organs, whereas inclusion of bile composition assessment is likely to prevent late graft loss for biliary strictures in DCD grafts. Some of the cutoff values of the present protocols seem to be restrictive, and minor adjustments might improve the liver use rates while achieving excellent long-term outcomes. Regardless of the protocol used, the viability assessment provides objective data about high-risk liver metabolic function and allows safe transplantation of selected organs. This key finding should provide clinicians with access to the technology with the confidence to increase use of marginal livers for patients with urgent needs for transplantation.

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