The challenge of quality assessment and regional perfusion to increase donor organ utilisation

Leemkolk, F.E.M. van de

Chapter 7

Donor eligibility criteria and liver graft acceptance criteria during normothermic regional perfusion: a systematic review

Ivo J. Schurink, Fenna E.M. van de Leemkolk, Constantino Fondevila, Riccardo De Carlis, Eric Savier, Gabriel C. Oniscu, Volkert A.L Huurman, Jeroen de Jonge

Liver Transplantation. 2022; doi: 10.1002/lt.26512

Abstract

Background

Acceptance of liver grafts from donations after circulatory death (DCD) largely remains a 'black box', particularly due to the unpredictability of the agonal phase. Abdominal normothermic regional perfusion (aNRP) can reverse ischaemic injury early during the procurement procedure, and it simultaneously enables graft viability testing to unravel this 'black box'. This review evaluates current protocols for liver viability assessment to decide upon acceptance or decline during aNRP.

Material & Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline was used, and relevant literature databases were searched. The primary outcome consisted of criteria for liver graft viability assessment. Secondary outcomes included survival, primary non-function (PNF), early dysfunction, and biliary complications.

Results

A total of 14 articles were included in the analysis. In all protocols, a combination of criteria was used to assess suitability of the liver for transplantation. As many as 12 studies (86%) used macroscopic assessment, 12 studies (86%) used alanine transaminase (ALT) levels in perfusate, 9 studies (64%) used microscopic assessment, and 7 studies (50%) used lactate levels as assessment criteria. The organ utilisation rate (OUR) was 16% for uncontrolled donation after circulatory death (uDCD) and 64% for controlled donation after circulatory death (cDCD). The most used acceptation criterion in uDCD is ALT level (31%), while in cDCD macroscopic aspect (48%) is most used. Regarding postoperative complications, PNF occurred in 13% (6% - 25%) of uDCD livers and 3% (2% - 4%) of cDCD livers. In uDCD, the 1-year graft and patient survival rates were 75% (66% - 82%) and 82% (75% - 88%). In cDCD, the 1-year graft and patient survival rates were 91% (89% - 93%) and 93% (91% - 94%), respectively.

Conclusions

In conclusion, the currently used assessment criteria consist of macroscopic aspect and transaminase levels. The acceptance criteria should be tailored according to donor type to prevent an unacceptable PNF rate in uDCD and to increase the relatively modest OUR in cDCD.

Introduction

As a result of persisting donor organ shortage, many countries are now accepting liver grafts from donation after circulatory death (DCD).[1,2] The drawback of DCD liver transplantation compared with donation after brain death (DBD) liver transplantation is that DCD grafts lead to more complications, such as ischaemic cholangiopathy (nonanastomotic strictures (NAS)), early allograft dysfunction (EAD), and acute kidney injury.[3,4] In particular, the unpredictability of the agonal phase, with additional donor liver injury from hypoxia and hypotension preceding circulatory arrest, turns acceptance of a DCD liver graft into a 'black box' that can only be justified after successful transplantation in the recipient.[5] Because of this uncertainty about both long-term quality and ability to provide immediate life-sustaining function in the recipient, for DCD liver grafts a more stringent donor selection is performed compared with DBD grafts, with lower limits on donor age and donor body mass index, and short functional warm ischaemia time (fWIT). Thus, DCD livers are more often declined and then discarded, resulting in only 35% of all potential DCD livers being transplanted in the UK, compared with 82% of all liver grafts originating from donors after brain death.[1]

Abdominal Normothermic Regional Perfusion (aNRP) enables liver graft viability assessment after the agonal phase to reduce the 'black box' uncertainty, by restoring the abdominal circulation.[6] With aNRP, it is able to utilise uncontrolled DCD (uDCD) liver grafts (Maastricht category II and IV) and to transplant more safely controlled DCD (cDCD) grafts (Maastricht category III).[6]

Currently, countries across Europe, Asia, the United States, and Canada are using heterogeneous populations of DCD donors (uDCD and cDCD), differ in their policies and practices, and thus have various implementation levels of aNRP, ranging from routine use of aNRP in Spain, France, Norway, and Italy to selective use in the UK, the Netherlands, Belgium the United States (University of Michigan), Russia (Pavlov University St. Petersburg) and Korea (Ajou University, Suwon).[6] These differences across countries and transplant centres result in heterogeneous aNRP protocols, for instance, the opportunity to cannulate or perform interventions before withdrawal of life support. This also applies for protocols to donor liver evaluation and criteria to determine donor liver viability. The question remains unanswered as to which of the criteria currently used are able to identify as many viable donor livers as possible, without compromising the outcomes after transplantation. Such evidence is needed to reduce underutilisation of DCD donor livers and to allow wider clinical implementation, without increasing the risks for the recipient.

In this systematic review, we aim to analyse all published aNRP protocols to investigate both similarities and differences across the inclusion and acceptance criteria, and to relate these criteria to clinical outcome.

Methods

Literature search strategy

A systematic literature review was performed according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guideline.[7] The review was registered on the International Prospective Register of Systematic Reviews (PROSPERO; CRD:229013). A search strategy was developed and the following databases were explored: Embase, Medline Ovid, COCHRANE Library, web of science and Google scholar. The final search was performed on January 2, 2022. For the complete search strategy, see *Appendix S1*.

Inclusion and exclusion criteria

Articles on aNRP of human donor livers, describing liver graft viability assessment or liver graft acceptance criteria during aNRP and describing outcome data after liver transplantation were included. Case reports, editorials, letters to the editors, meeting abstracts, and (systematic) reviews without original data were excluded. Furthermore, only articles written in English were considered.

Outcomes

The primary outcome of interest was to identify the criteria used to assess liver graft viability, and to determine upon graft acceptance during aNRP, and to relate these assessment criteria to graft and patient survival.

Secondary outcomes included percentage of grafts transplanted after the evaluation protocol, Organ Utilisation Rate (OUR), acceptance rate during aNRP, primary non-function (PNF), EAD according to the Olthoff-criteria [8] and NAS.

To calculate the OUR and the acceptance rate during aNRP, we followed these definitions:

- *aNRP initiation rate*: number of donors in whom aNRP took place divided by the total number of all potential donors.
- *aNRP acceptance rate*: number of transplanted livers divided by the number of donors in whom aNRP was initiated.

- *Organ Utilisation Rate (OUR)*: number of transplanted livers divided by the number of potential donors. The number of potential donors is the total number of donors without the cases in which the family or a judge did not provide consent for donation or in which there were absolute contraindications for donation, for instance, a malignancy or an active infection.

Risk of bias

Analysis of the risk of bias via the Cochrane tools did not apply, as this systematic review primarily compares aNRP protocols, without comparing an actual intervention. Therefore, selection, performance, attrition, and detection bias in the primary studies could not be assessed. To detect evidence selection bias, we checked clinical trial registries and conference abstracts to identify unpublished studies or any outcomes that may have been selectively omitted from a study publication. We further tried to minimise the risk of bias and promote transparency by registering and publishing the protocol on PROSPERO before starting the review and by adhering to the PRISMA statements. We did not encounter search protocol deviations, and the comprehensive search for published and unpublished studies was supervised by a librarian from the Erasmus MC. No financial or industrial sponsorship exists in this review.

Data extraction and analysis

Title and abstracts were screened by two independent reviewers (IS and JJ) to meet predefined inclusion criteria, followed by full-text review of eligible articles. Consensus regarding inclusion was obtained between reviewers. Data extraction on current criteria for consideration of aNRP and liver graft viability assessment criteria was performed using a predetermined Microsoft Excel template. When additional information was needed, the corresponding authors of the studies were contacted.

The post transplantation pooled proportions for complications and outcome with 95% confidence intervals (CIs) were calculated with a random-effect model as described by DerSimonian and Laird.[9] Other pooled proportions with range were calculated with a fixed-effect model. Statistical heterogeneity was visually assessed by judging overlap in the 95% CIs and with I^2.

Differences in outcome between uDCD and cDCD were analysed based on a subgroup analysis. The articles that used any other kind of machine perfusion after aNRP were excluded for the pooling of post transplantation complications and outcome. Statistical analysis was performed using RStudio (Version 1.4.1106 RStudio, PBC, Boston, MA).

Results

Of the 630 articles found through the literature search, 585 articles were excluded after abstract screening and 31 reports were excluded after full-text analysis. In total, 14 studies were included in the analysis (**Figure 1**).

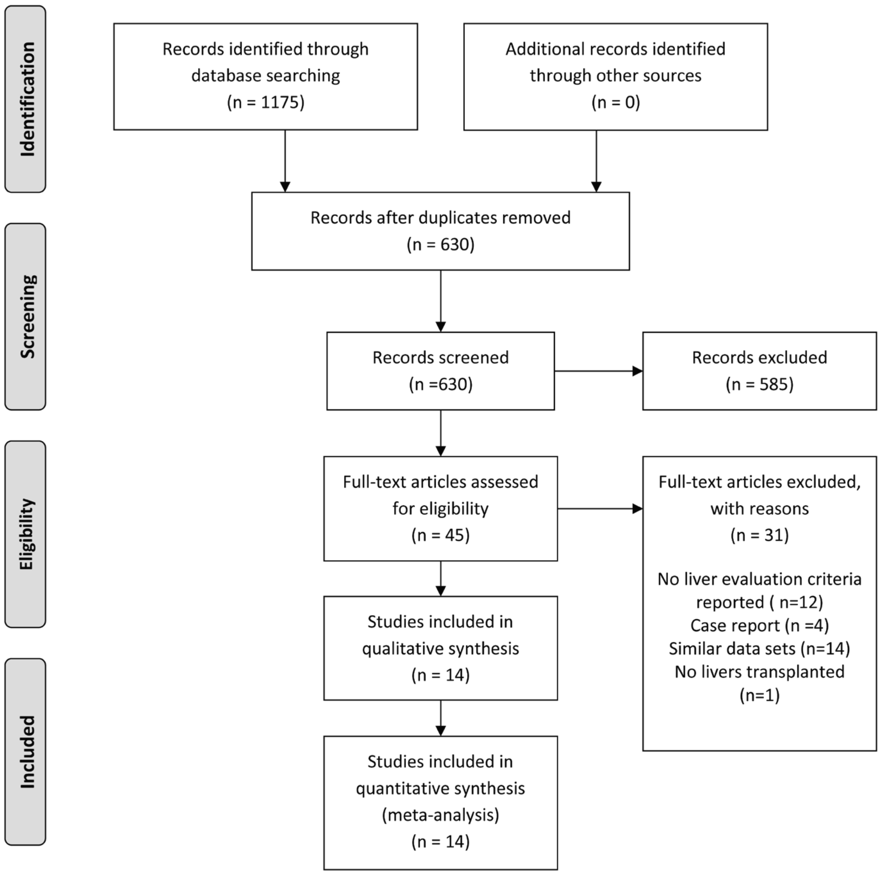

Figure 1. PRISMA flow diagram.

Description of included studies

Of the 14 studies published between 2003 and 2021, 12 studies (86%) were published after 2014 (**Table 1**).[10-23] All study designs were retrospective cohort (RC) studies. No randomised clinical trials were found. All studies, except one, were performed in Europe (n = 13; 93%)[10-22]; the other study was performed in the United States (**Table 1**).[23] Five reports (36%) described exclusive use of uDCDs.[19-23] In seven reports (50%), only cDCDs were included for aNRP.[10-16] Two reports (14%) described a combination of uDCDs and cDCDs for aNRP.[17,18]

Table 1: Baseline characteristics of studies included in the systematic review

Author	Year of publication	Period of inclusion	Country	Study design	DCD type (Maastricht category)	Eligible patients in whom aNRP is performed
De Carlis et al.[12]	2021	Sep 2015 – Apr 2019	Italy	RC	cDCD (III)	52
Hessheimer et al.[11]	2021	Jan 2012 – Dec 2019	Spain	RC	cDCD (III)	747
Muller et al.[10]	2021	Jan 2015 – Dec 2020	France	RC	cDCD (III)	88
Ghinolfi et al.[17]	2020	Jan 2018 – Apr 2019	Italy	RC	uDCD (II) & cDCD (III)	32
Justo et al.[20]	2020	Jan 2006 – Dec 2016	Spain	RC	uDCD (II)	75
Lazzeri et al.[19]	2020	Jun 2016 – Jun 2019	Italy	RC	uDCD (II)	30
Muller et al.[13]	2020	Jan 2015 – Dec 2019	France	RC	cDCD (III)	226
Watson et al.[14]	2019	Jan 2011 – Jun 2017	UK	RC	cDCD (III)	57
De Carlis et al.[18,A]	2018	Jan 2015 – Dec 2017	Italy	RC	uDCD (II) & cDCD(III)	25
Champigneulle et al.[21]	2015	Jan 2010 – Dec 2012	France	RC	uDCD (II)	76
Oniscu et al.[16]	2014	Jan 2010 – Jan 2014	UK	RC	cDCD (III)	20
Rojas-Peña et al.[15]	2014	Oct 2000 – Jul 2013	USA	RC	cDCD (III)	29
Fondevila et al.[22]	2012	Apr 2002 – Dec 2010	Spain	RC	uDCD (II)	201
Otero et al.[23]	2003	Dec 1995 – Mar 2000	Spain	RC	uDCD (II)	14

A = De Carlis et al[18] (2018) describes cDCD and uDCD liver grafts. Only the uDCD grafts are included in further analyses as De Carlis et al.[12] (2021) describes the extended cohort of the cDCD grafts from De Carlis et al.[18] (2018).

Current criteria for consideration of aNRP in uDCD

In total, seven articles that reported uDCD donation are included in this section.[17-23] In the series of uDCD, the duration of no-flow and low flow was mentioned in all articles. Cardiac arrest (CA) time is defined as the time between CA and basic cardiopulmonary resuscitation.[24] The advance ventilator support (AVS) phase is defined as the time between basic cardiopulmonary resuscitation and disconnecting the AVS resulting in the death of the donor.[24] In all reports the CA needed to be witnessed and in four reports (57%) cardiopulmonary resuscitation was to be started within 15 minutes [20-23] (**Table 2**). The AVS phase until the start of aNRP was set to a maximum of 120 minutes [21,23] or 150 minutes.[20,22] The AVS phase includes the legally mandatory no-touch time, which is different across countries (5 - 20 minutes). Additional consideration criteria were a donor age limitation in all studies, varying between 50 - 70 years (**Table 2**).[17-23] Furthermore, reports from the national Spanish protocol excluded donors with pre-aNRP alanine transaminase (ALT) levels above three times the upper limit of normal (ULN).[22]

Table 2. Criteria for consideration of aNRP and criteria for liver acceptance during aNRP

	Current criteria for consideration of aNRP				**Liver acceptance criteria during aNRP according to protocol**			
Author	**Donor age limit, years**	**CA limit, min**	**AVS limit, min**	**ALT, U/L**	**Macroscopic liver aspect**	**Microscopic criteria**	**ALT, U/L**	**Lactate**
uDCD								
Ghinolfi et al.[17, a]	70	45 [b]	170 [c]	No limit	Yes	Steatosis <30%, Fibrosis <F2, sever macroangiopathy	1000	Trend down
Justo et al.[20]	55	15	150	No limit	Yes	Steatosis <30%, Fibrosis	200 [j]	Trend down
Lazzeri et al.[19]	65	20	150 [d]	No limit	Yes	Steatosis <30%, Fibrosis <F2, Necrosis >5%	1000	Not used
De Carlis et al.[18]	65	n/a	160 [d]	No limit	Yes	Steatosis <30%, Fibrosis <F2	1000	Trend down
Champigneulle et al.[21]	55	15	120, 150 [e]	No limit	Yes	Steatosis <20%, Fibrosis	200	Not used
Fondevila et al.[22]	65	15	150	150 U/L [f]	Yes	Not used	200 [j]	Not used
Otero et al.[23]	50	15	120	No limit	Yes	Steatosis <30%, Necrosis	Not used	Not used
Author	**Donor age limit, years**	**fWIT criteria**	**fWIT limit, min**	**ALT, U/L**	**Macroscopic liver aspect**	**Microscopic criteria**	**ALT, U/L**	**Lactate**
cDCD								
De Carlis et al.[12]	75	SBP <50, sat <70%	60	No limit	Yes	Steatosis <30%, Fibrosis <F2	1000	Trend down or stable
Hessheimer et al.[11]	65, no limit [g]	SBP <60, sat <80%	30, no limit [g]	150, no limit [g]	Yes	Not used	200	Trend down
Muller et al.[10]	61, 66, 71 [h]	SBP <45	45	No limit	Not used	Steatosis <20%, Fibrosis < F2	200 [j]	Not used
Ghinolfi et al.[17, a]	70	SBP <50, sat <70%	120	No limit	Yes	Steatosis <30%, Fibrosis <F2, sever macroangiopathy	1000	Trend down
Muller et al.[13]	61, 66, 71 [h]	SBP <60	60	No limit	Not used	Steatosis <20%, Fibrosis < F2	200 [j]	Not used
Watson et al.[14]	n/a	-	No limit	No limit	Yes	Not used	200, 500 [i]	Trend down
Oniscu et al.[16]	n/a	SBP <50	30	150 [f]	Yes	Not used	200 [j]	Trend down
Rojas-Peña et al.[15]	65	-	No limit	No limit	Yes	Not used	Not used	Not used

[a] Article included in the cDCD and uDCD table
[b] Time of cardiac arrest (CA) and time of no touch time were combined
[c] Time of CA, time of AVS, and time of no touch were combined
[d] Time of CA and time of cardiopulmonary resuscitation were combined
[e] 150 minutes if mechanical ventilation was used
[f] 150 U/L is three times the upper limit of normal
[g] The criteria for consideration of aNRP were gradually expanded in the study period
[h] Donor age limit was modified from <61 years until 2018 to <71 years in 2020
[i] In the beginning of the aNRP experience a cutoff of 200 U/L was used; this later on increased to 500 U/L
[j] 200 U/L is four times upper limit of normal

Protocol acceptance criteria of the uDCD liver grafts during aNRP

Reported uDCD liver graft acceptance criteria consisted of macroscopic assessment, evaluation of ALT levels in blood, microscopic assessment, and lactate trend during aNRP (Table 2). All seven articles assessed gross appearance of the liver, colour of the liver and signs of congestion to evaluate cirrhosis, fibrosis, steatosis and perfusion of the liver.[17-23] Five articles (71%) assessed the perfusion of the liver [17-20,22], three articles (43%) assessed the vascularisation of the bile duct [19,20,22] and one article (14%) assessed perfusion of other abdominal organs, the small bowel in particular.[20]

In six articles (86%) ALT level in blood was included as parameter to asses liver graft quality.[17-22] Three out of these six articles reported ALT level below 200 U/L or four times the ULN as denominator for acceptance.[20-22] The other three articles accepted a maximum ALT level of 1,000 U/L. In these articles additional ex-vivo hypothermic or normothermic machine perfusion was undertaken before transplantation.[17-19]

In six articles (86%) routine microscopic evaluation was included in the protocol.[17-21,23] All six articles evaluated macrovesicular steatosis; one article accepted a maximum of 20% macrovesicular steatosis [13,21] and five articles accepted liver grafts with up to 30% of macrovesicular steatosis.[17-20,23] Five of the six protocols evaluated the amount of fibrosis [13,17-21], for which most protocols accepted no higher than an Ishak score of 2.[25]

Finally, only in three articles (43%) a protocol liver function-based organ assessment was mentioned, with lactate clearance indicating a well-functioning liver.[14,16,18] In these studies, lactate levels during NRP were supposed to demonstrated a downward trend. No cutoff values of minimum decrease in lactate were mentioned.

None of the included articles described evaluation of bile quality or bile production.

Liver graft utilisation in uDCD

Based on the donor consideration criteria mentioned previously, aNRP was initiated in 49% (36 - 95%; **Table 3**) of uDCD donors. Out of the non-proceeded aNRP candidates, in 32% (0 - 43%) there was no consent for donation, in 31% (0 - 41%) either the agonal phase, the CA or AVS phase was too long; and 27% (0 - 55%) of the uDCD donors was declined due to other medical contraindications for donation.

The liver graft acceptance rate after aNRP evaluation was 26% (14 - 100%; **Table 4**). This brings the total OUR to 16% (7 - 70%) for uDCD grafts. The main reason for decline of a graft in the uDCD cohort was technical or logistic failure, which occurred in 44% (0 - 50%). The main reasons for technical failure included artery dissection during cannulation, inadequate venous blood return, and insufficient persistent blood flow. Other reasons for decline of a graft based on acceptance criteria were ALT level outside the protocol limits in 31% (0 - 50%), followed by macroscopic aspect of the graft in 13% (0 - 20%), and microscopic evaluation in 8% (0 - 80%; **Table 4**). Decline of the liver graft based on a functional liver assessment such as lactate clearance was used in one study, occurring in 9%.

Table 3. Current criteria for consideration of aNRP

				Reason for donor ineligibility for proceeding to aNRP					
Author	**Potential donor**	**aNRP initiated**	**Not eligible for aNRP**	**No consent** [b]	**Agonal period**	**Pre-aNRP liver assessment**	**Age**	**Contraindication for donation**	**Other**
uDCD									
Ghinolfi et al.[17,a]	20	95%	1	0%	0%	0%	100%	0%	0%
Justo et al.[20]	-	-	-	-	-	-	-	-	-
Lazzeri et al.[19]	37	81%	7	43%	29%	0%	0%	29%	0%
De Carlis et al.[18]	-	-	-	-	-	-	-	-	-
Champigneulle et al.[21]	209	36%	133	32%	41%	0%	0%	14%	12%
Fondevila et al.[22]	400	50%	199	33%	25%	0%	0%	36%	6%
Otero et al.[23]	20	70%	6	0%	0%	0%	0%	0%	100%
Total uDCD	*686*	*49%*	*346*	*32%*	*31%*	*0%*	*0%*	*27%*	*10%*
				Reason for donor ineligibility for proceeding to aNRP					
Author	**Potential donor**	**aNRP initiated**	**Not eligible for aNRP**	**No consent**	**(f)WIT period**	**Pre-aNRP liver assessment**	**Age**	**Contraindication for donation**	**Other**
cDCD									
De Carlis et al.[12]	-	-	-	-	-	-	-	-	-
Hessheimer et al.[11]	1165	97%	413	0%	2%	0%	0%	4%	94% [c]
Muller et al.[10]	125	70%	37	0%	8%	0%	0%	16%	76% [d]
Ghinolfi et al.[17,a]	14	93%	1	0%	100%	0%	0%	0%	0%
Muller et al.[13]	251	90%	25	0%	100%	0%	0%	0%	0%
Watson et al.[14]	70	81%	13	0%	54%	15%	0%	31%	0%
Oniscu et al.[16]	36	56%	16	0%	81%	0%	6%	0%	13%
Rojas-Peña et al.[15]	50	74%	21	0%	85%	0%	0%	0%	15%
Total cDCD	*1711*	*90%* [d]	*526*	*0%*	*49%*	*1%*	*1%*	*21%*	*28%* [e]

[a] Article included in the cDCD and uDCD table
[b] No consent includes no consent from relatives or judicial reasons
[c] In 390 grafts static cold storage was performed
[d] 26 donors were considered as kidney-only donors without description
[e] from Hessheimer (2021) the 390 liver grafts transplanted after static cold storage are excluded from this calculation.

Table 4. Liver acceptance criteria during aNRP

				Reason for rejecting a graft during aNRP						
Author	**aNRP initiated**	**Transplanted**	**Rejected during aNRP**	**Macroscopic**	**Microscopic**	**ALT**	**Lactate**	**Technical or logistic failure**	**Other**	**OUR**
uDCD										
Ghinolfi et al.[17,a]	19	53%	9	9%	33%	15%	9%	11%	22%	50%
Justo et al.[20]	-	-	-	-	-	-	-	-	-	-
Lazzeri et al.[19]	30	33%	20	0%	30%	50%	0%	10%	10%	31%
De Carlis et al.[18]	19	74%	5	0%	80%	20%	0%	0%	0%	-
Champigneulle et al.[21]	76	14%	65	2%	12%	37%	0%	48%	2%	7%
Fondevila et al.[22]	201	17%	167	20%	0%	28%	0%	50%	1%	13%
Otero et al.[23]	14	100%	0	-	-	-	-	-	-	70%
Total uDCD	*359*	26%	*266*	*13%*	*8%*	*31%*	*0%*	*44%*	*3%*	*16%*
				Reason for rejecting a graft during aNRP						
Author	**aNRP initiated**	**Transplanted**	**Rejected during aNRP**	**Macroscopic**	**Microscopic**	**ALT**	**Lactate**	**Technical or logistic failure**	**Other**	**OUR**
cDCD										
De Carlis et al.[12]	52	87%	7	14%	43%	14%	29%	0%	0%	-
Hessheimer et al.[11]	752	72%	207	73%	5%	10%	0%	8%	4%	72% [b]
Muller et al. [10]	88	66%	30	13%	27%	13%	0%	47%	0%	48%
Ghinolfi et al.[17,a]	13	62%	5	20%	20%	40%	0%	0%	20%	57%
Muller et al.[13]	226	70%	67	0%	52%	18%	0%	30%	0%	63%
Watson et al.[14]	57	75%	14	36%	0%	36%	0%	0%	29%	61%
Oniscu et al.[16]	20	55%	9	22%	0%	44%	0%	22%	11%	31%
Rojas-Peña et al.[15]	29	45%	16	44%	0%	0%	0%	50%	6%	26%
Total cDCD	*1,247*	*71%*	*355*	*48%*	*16%*	*14%*	*1%*	*17%*	*4%*	*64%*

[a] Article included in the cDCD and uDCD table
[b] All livers that are selected for the static cold arm are excluded from this analysis

Recipient results from uDCD grafts transplanted after aNRP

The complication incidence rates of the uDCD grafts are described in **Figure 2** and **Supplementary Table 2**. PNF occurred in uDCD in 13% (95% CI: 6 - 25%; **Figure 2A**). NASs were seen in 6% (95% CI: 2 - 12%; **Figure 2B**) of the cases. One-year graft survival was 75% (95% CI: 66 - 82%; **Figure 2C**) and 1-year patient survival was 82% (95% CI: 75 - 88%; **Figure 2D**).

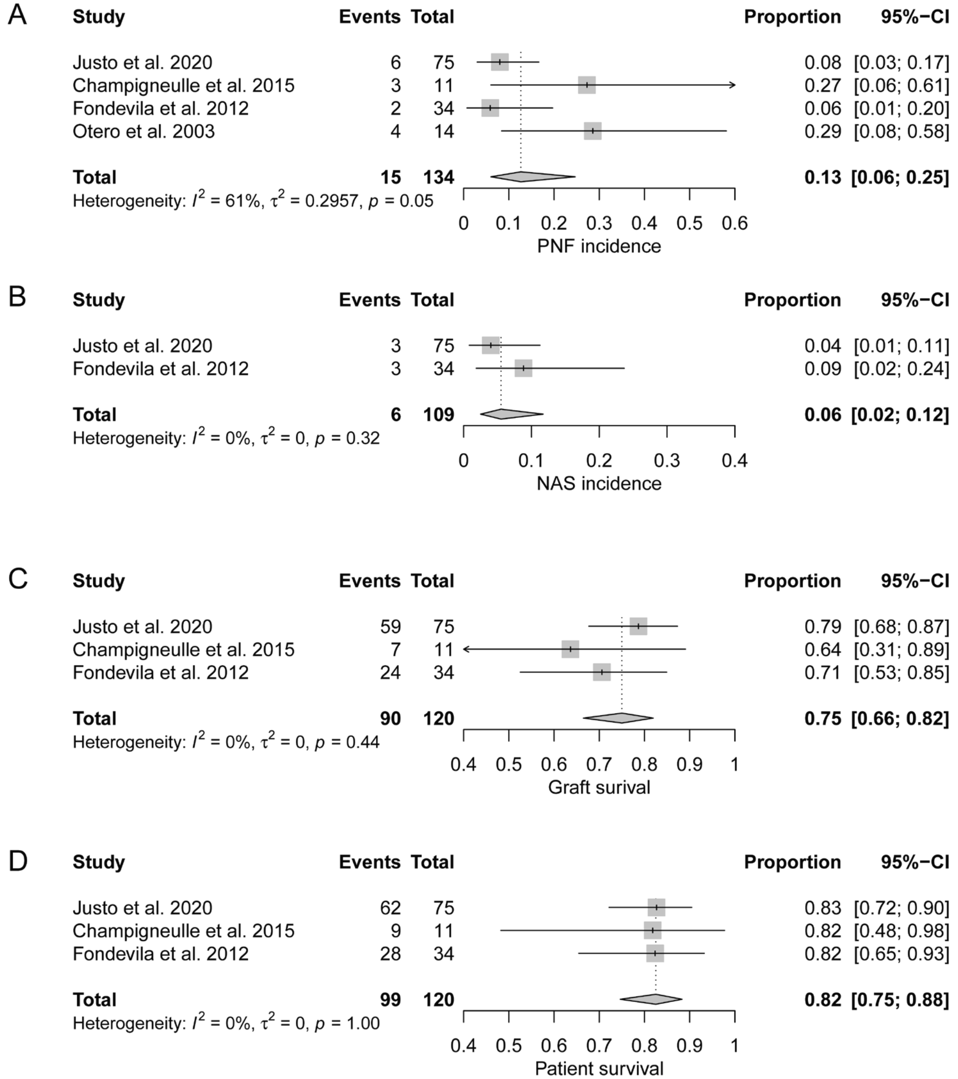

Figure 2. Post transplantation results after aNRP of the uDCD grafts. Studies that used combined machine perfusion techniques are excluded from these analyses. (A) The occurrence of PNF. (B) The occurrence of NAS. (C) The 1-year graft survival. (D) The 1-year patient survival.

Current criteria for consideration of aNRP in cDCD

In total, eight articles that reported cDCD donation are included in this section.[10-17] Five articles (63%) reported restrictions of the fWIT, although definition of the start of this fWIT varied widely between studies (**Table 2**). The time limitation of the length of the fWIT ranged between 30 and 120 minutes (**Table 2**).[10,13,16-18] Two articles (25%) used time from withdrawal of treatment to start of aNRP below 90 minutes as consideration criterion.[14,15] One article neither used fWIT nor time from withdrawal of treatment as restriction criterion.[11] Donor age limitation varied from 65 years up to no limit.[10-17] Compared to uDCD protocols, restrictions were less strict in cDCD. The ALT level of the donor above three times the ULN before aNRP was a consideration criterion in one study (13%)[16], while the other seven articles (88%) did not select based on pre-aNRP ALT level.[10-15,17]

Protocol acceptance criteria of the cDCD liver grafts during aNRP

During aNRP, reported liver graft acceptance criteria consisted of macroscopic assessment, evaluation of ALT levels in blood, microscopic assessment, and lactate trend (Table 2).

In seven articles (88%) ALT levels in blood were mentioned as a parameter to assess graft quality.[10-14,16,17] Four out of eight articles reported ALT levels below 200 U/L or four times the ULN as denominator for acceptance.[10,11,13,16] Three studies accepted higher ALT levels in the blood, with a maximum of 500 U/L [14] and 1000 U/L.[17,18] The protocols that accepted ALT levels to a maximum of 1,000 U/L performed additional ex-vivo hypothermic or normothermic machine perfusion before transplantation.[17,18]

In six articles (75%) macroscopic assessment was mentioned to decide whether a donor liver was transplantable (**Table 2**).[11,12,14-17] All six articles assessed cirrhosis, fibrosis, steatosis and perfusion of the liver. Two articles (25%) assessed perfusion of other abdominal organs, the small bowel in particular [15,16], and one article assessed the vascularisation of the bile duct.[16]

In five articles (63%) lactate clearance was mentioned as a parameter to assess liver function.[11,12,14,16,18] A downward lactate trend indicated a well-functioning liver.[11,12,14,16,18] However, Watson et al.[14] noted that lactate leaking back from nonperfused areas in the donor to the circuit decreases the reliability of the lactate trend as an indicator of liver function.

Finally, in only four articles (38%) the protocol mentioned routine microscopic evaluation.[10,12,13,17] All four articles evaluated macrovesicular steatosis; two articles accepted a maximum of 20% macrovesicular steatosis [10,13] and two articles accepted liver grafts with up to 30% of macrovesicular steatosis.[17,18] All four articles evaluated the amount of fibrosis[10,13,17,18], with all protocols accepting no higher than an Ishak score of 2.[25]

None of the included articles described evaluation of bile quality or bile production.

Liver graft utilisation in cDCD

In the cDCD donors, the aNRP initiation rate was 90% (56 - 97%; **Table 3**). The main reason for ending a cDCD procedure was extended fWIT, which occurred in 49% (0 - 100%) of the donors. Another 21% (0 - 31%) of the cDCD donation was not initiated because of absolute contraindication for donation, such malignancy or active infection.

The liver graft acceptance rate after aNRP evaluation was 71% (45 - 87%; **Table 4**) and the OUR was 64% (26 - 72%). The main reported reason for decline of a cDCD liver graft during aNRP evaluation was the macroscopic aspect in 48% (0 - 73%), followed by microscopic aspect in 16% (0 - 52%), ALT level outside protocol limits in 14% (0 - 44%), and lactate clearance outside protocol in 1% (0 - 29%); **Table 4**). Technical or logistic failure was the reason for declining the liver graft in 17% (0 - 50%). Reasons for technical failure consisted of donor vasculature being incompatible with establishing the NRP circuit, cannulation problems, and ability to reach adequate blood flow.

Recipient results from cDCD grafts transplanted after aNRP

The complication incidences of the cDCD grafts are stated in **Figure 3** and **Supplementary Table 2**. PNF was seen in 3% (95% CI: 2 - 4%; **Figure 3A**). NAS occurred in 2% (95% CI: 1 - 4%; **Figure 3B**). The 1-year graft survival was 91% (95% CI: 89 - 93%; **Figure 3C**) and 1-year patient survival was 93% (95% CI: 91 - 94%; **Figure 3D**)

PNF occurred significantly more frequently in uDCD compared to cDCD ($p < 0.001$). Furthermore, the 1-year graft and 1-year patient survival were significantly lower in the uDCD compared with the cDCD ($p < 0.001$ in both). No significant differences regarding NAS were seen ($p = 0.06$) between these groups.

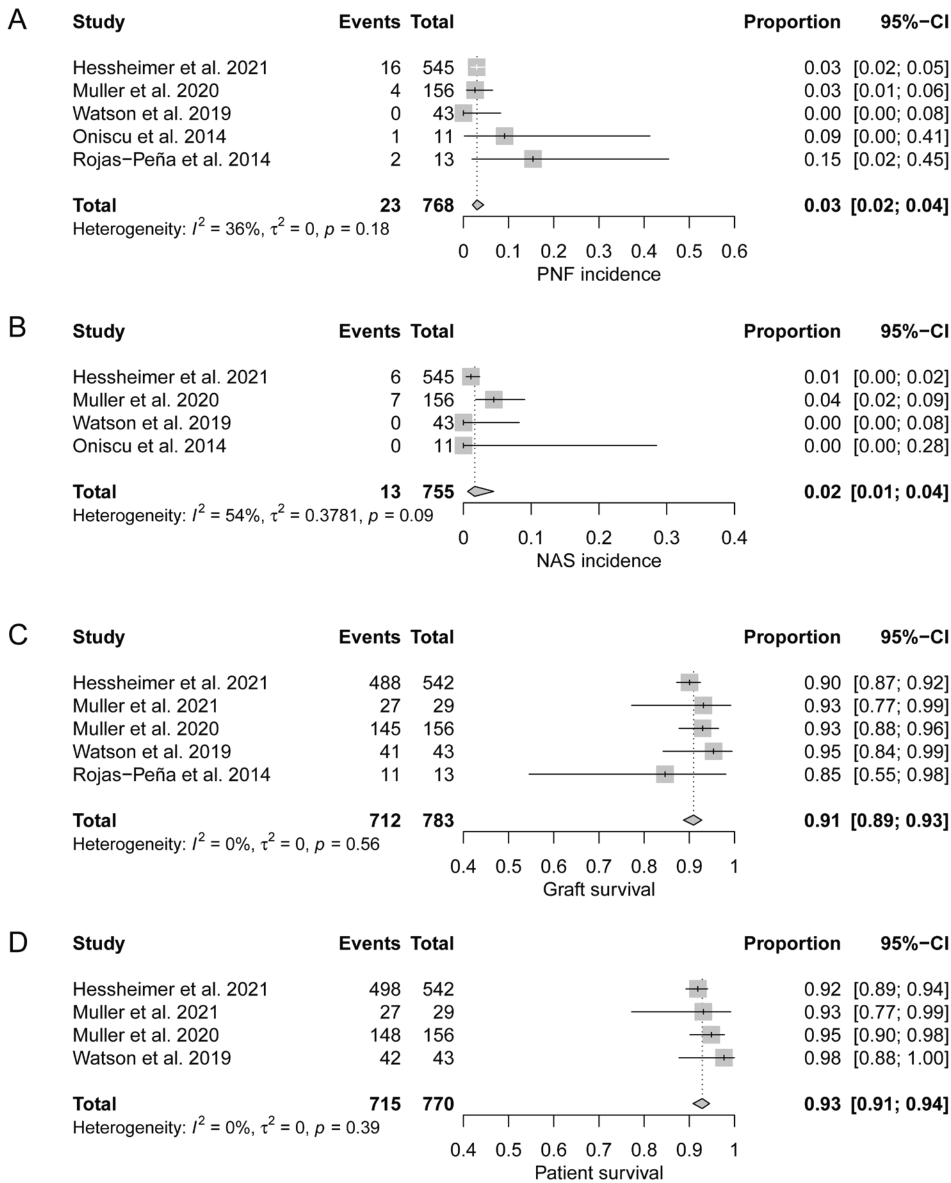

Figure 3. Post transplantation results after aNRP of the cDCD grafts. Studies that used combined machine perfusion techniques are excluded from these analyses. (A) The occurrence of PNF. (B) The occurrence of NAS. (C) The 1-year graft survival. (D) The 1-year patient survival.

Discussion

This report investigates different protocols for evaluation of liver grafts during aNRP and identifies the primary determinants for graft decline. The acceptance criteria during aNRP vary largely between protocols and between uDCD and cDCD, and there are differences in the importance of discard determinators.

For uDCD, the most important pre-aNRP parameter to exclude grafts from aNRP was extended agonal phase. During aNRP, the most important evaluation determinator to not transplant the graft was ALT level. Furthermore, the technical failure rate was noticeably high (44%), much higher than in other machine perfusion techniques. One of the reasons for the high complication rate might be the learning curve of the aNRP programs. Looking at the uDCD post transplantation results, we found an unacceptably high PNF incidence of 13% and a remarkably low NAS incidence of 6%, balanced against a modest OUR of 16%. In contrary to uDCD, in cDCD judgment of the macroscopic aspect is the main determinator of acceptance for transplantation over more objective criteria. The pooled complication incidence in cDCD is extraordinarily low and is comparable with the best DBD outcomes in terms of PNF (3%), NAS (2%), and 1-year graft survival (91%)[4]. However, aNRP failed to increase the OUR in cDCD (64%) to the level of DBD (82%).[1] Therefore, the focus in the uDCD cohort should be predominantly on prevention of PNF, while in the cDCD cohort the excellent results need to be preserved when expanding the OUR.

Prevention of PNF starts with a critical assessment of the acceptance criteria. None of the current evaluation criteria demonstrated to be able to completely avoid PNF; macroscopic and microscopic appearance mainly evaluate the pre-agonal status of the liver (e.g., amount of steatosis) and do not evaluate the effect of the agonal phase. ALT levels reflect liver injury, but they fall short on assessing remaining liver function, which will ultimately determine occurrence of PNF in the recipient. As a more functional analysis of remaining liver capacity, lactate clearance is frequently used. However, as lactate clearance is a very basic intrinsic liver function that will be supported by the hepatocytes almost until liver failure, debate remains whether lactate levels can discriminate differences in higher liver function.[26] Clearly, there is a need for a more objective indicator during aNRP, that reliably predicts liver function after transplantation.

The other point at issue is how to further increase the OUR in cDCD. With the current evaluation criteria, the macroscopic aspect -especially hepatic steatosis- is the leading denominator, rather than assessing functional reserve of the donor liver. Advanced level of steatosis is known to be associated with increased ischaemia-reperfusion injury; however, hepatic steatosis is reversible after transplantation. Especially the combination of an excessively steatotic (>30%) cDCD graft with high donor age is a risk factor for postoperative complications, as postreperfusion syndrome, EAD, acute kidney injury and NAS.[27,28] Therefore, usually a maximum of 30% steatosis is accepted in cDCD liver grafts. The same cutoff value is adopted in many of the aNRP protocols. In DBD liver grafts however, up to 60% of steatosis is now accepted, especially using ex-vivo machine perfusion.[29] As the quality of aNRP grafts resembles that of DBD liver grafts, the cutoff value for steatosis in cDCD liver grafts might be expanded.[30] Ideally, remaining liver function is assessed instead of the surrogate marker (microscopic or macroscopic) steatosis, as hepatic steatosis is reversible. Until it is possible to analyse true liver function during aNRP, the grafts that are rejected based on suboptimal macroscopic appearance could benefit from ex-vivo normothermic machine perfusion in a back-to-base strategy to safely extent the OUR.

How then to reliably analyse liver function during aNRP? As discussed earlier, the standard 'point of care' measurements are not reliable, because aNRP is a relatively open circuit with anoxic blood from non-perfused tissue leaking back to the circuit, thereby altering the normal values. An alternative approach to analyse residual liver function might be to implement a substrate-based liver function test in the aNRP setting. The concept is that all livers are exposed to a comparable dosage of substrate which is metabolised by the liver, indicating liver function. A potential substrate test might be the maximum liver function capacity (LiMAx) test, of which our research group already demonstrated the feasibility during normothermic machine perfusion.[31] Another approach is to assess real time the integrity of the liver graft during aNRP with the use of Raman microspectroscopy. Ember et al.[32] demonstrated that microvascular damage could be detected and potentially could assist the decision making during aNRP.

This systematic review has its limitations. At the moment aNRP protocols are heterogeneous, resulting in a bias when comparing post transplantation results between studies. This heterogeneity is not surprising as aNRP is rapidly developing in different countries, with different legislation and novel techniques. As the technique is maturing, the time has come to internationally standardise the procedure and evaluation criteria to increase comparability of different cohorts.

In conclusion, the currently used assessment criteria almost exclusively consists of macroscopic aspect and transaminase levels. This tends to overestimate suitability for transplantation in uDCD livers at the cost of high PNF rates, but tends to underestimate suitability for transplantation in cDCD livers at the cost of a low organ utility rate. Therefore, aNRP protocols should be tailored for the DCD donor type, being more stringent in uDCD donation and more liberal in cDCD donation. cDCD-aNRP would benefit from an additional assessment tool that better predicts posttransplant liver function to increase organ utilisation, while preserving the excellent results of cDCD-aNRP liver transplantation.

Acknowledgments

We thank W.M. Bramer and S.T.G. Gunput, clinical librarians, for assistance with the literature search.

Supplemental Digital Content

SDC, Material and Methods, Appendix S1. Complete literature search strategy. The final search was performed on the 2nd of January 2022.

Database	Search Strategy	Number of references	Number of unique references
Embase.com	('liver transplantation'/exp OR (((liver* OR hepat*) NEAR/6 (transplant* OR graft* OR allotransplant* OR allograft* OR donor* OR donat* OR recipient*))):ab,ti) AND ('regional perfusion'/de OR (((normothermic*) NEAR/3 (recirculat* OR membrane*-oxygenat*)) OR (regional* NEAR/3 perfusion*) OR ((extracorpor*) NEAR/3 support)):ab,ti) NOT ([Conference Abstract]/lim OR [Letter]/lim OR [Note]/lim OR [Editorial]/lim) AND [english]/lim	394 regular references	140 regular references
Medline Ovid	(Liver Transplantation/ OR (((liver* OR hepat*) ADJ6 (transplant* OR graft* OR allotransplant* OR allograft* OR donor* OR donat* OR recipient*))).ab,ti.) AND (regional perfusion/ OR (((normothermic*) ADJ3 (recirculat* OR membrane*-oxygenat*)) OR (regional* ADJ3 perfusion*) OR ((extracorpor*) ADJ3 support)).ab,ti.) NOT (letter* OR news OR comment* OR editorial* OR congres* OR abstract* OR book* OR chapter* OR dissertation abstract*).pt. AND english.la.	277	277
Cochrane CENTRAL	((((liver* OR hepat*) NEAR/6 (transplant* OR graft* OR allotransplant* OR allograft* OR donor* OR donat* OR recipient*))):ab,ti) AND ((((normothermic*) NEAR/3 (recirculat* OR membrane* next oxygenat*)) OR (regional* NEAR/3 perfusion*) OR ((extracorpor*) NEAR/3 support)):ab,ti)	20	9
Web of Science	TS=(((((liver* OR hepat*) NEAR/5 (transplant* OR graft* OR allotransplant* OR allograft* OR donor* OR donat* OR recipient*)))) AND ((((normothermic*) NEAR/2 (recirculat* OR membrane*-oxygenat*)) OR (regional* NEAR/2 perfusion*) OR ((extracorpor*) NEAR/2 support)))) AND LA=(english)	284	130
Google Scholar	"liver|hepatic transplantion|graft|allotransplantion|allograft|donor|donation|recipient" "normothermic recirculation|membrane"|"regional perfusion"|"extracorporeal support"|"extracorporeal*support"	100	74
Total		**1175**	**630**

Table S1. Determinants of criterium "macroscopic assessment"

Author	Cirrhosis	Fibrosis	Steatosis	Perfusion of the liver	Bile duct vascularization	Perfusion of other abdominal organs
De Carlis et al.[12]	X	X	X	X		
Hessheimer et al.[11]	X	X	X	X		
Muller et al.[10]						
Ghinolfi et al.[17]	X	X	X	X		
Justo et al.[20]	X	X	X	X	X	X
Lazzeri et al. [19]	X	X	X	X	X	
Muller et al.[13]						
Watson et al.[14]	X	X	X			
De Carlis et al.[18]	X	X	X	X		
Champigneulle et al.[21]	X	X	X			
Oniscu et al.[16]	X	X	X	X	X	X
Rojas-Peña et al.[15]	X	X	X	X		X
Fondevila et al.[22]	X	X	X	X	X	
Otero et al.[23]	X	X	X			

Table S2. Complete overview recipient outcome

uDCD								
Author	**Transplanted**	**PNF**	**EAD**	**Biliary complication**	**NAS**	**Graft survival**	**Patient survival**	**Follow-up time**
Ghinolfi et al.[17, A, B]	10	0%	20%	10%	10%	90%	90%	1-year
Justo et al. [20]	75	8%	n/a	31%	4%	78%	83%	1-year
Lazzeri et al.[19, B]	10	n/a	n/a	n/a	n/a	80%	90%	1-year
De Carlis et al.[18, B]	14	14%	21%	21%	7%	79%	91%	1-year
Champigneulle et al. [21]	11	27%	n/a	n/a	n/a	64%	82%	1-year
Fondevila et al.[22]	34	6%	47%	12%	8%	70%	82%	1-year
Otero et al.[23]	14	28%	n/a	28%	n/a	42%	72%	2-year
cDCD								
Author	**Transplanted**	**PNF**	**EAD**	**Biliary complication**	**NAS**	**Graft survival**	**Patient survival**	**Follow-up time**
De Carlis et al.[12, B]	45	5%	n/a	78%	2%	91%	98%	1-year
Hessheimer et al.[11]	545	3%	15%	12%	1%	90%	92%	1-year
Muller et al.[10,C]	58	n/a	n/a	n/a	n/a	93%	93%	1-year
Ghinolfi et al.[17, A, B]	8	0%	38%	13%	0%	100%	100%	1-year
Muller et al.[13]	156	2%	20%	n/a	5%	93%	95%	1-year
Watson et al.[14]	43	0%	12%	n/a	0%	96%	98%	1-year
Oniscu et al.[16]	11	9%	36%	18%	0%	n/a	n/a	n/a
Rojas-Peña et al.[15]	13	14%	n/a	14%	n/a	86%	n/a	1-year

A Article included in the cDCD and uDCD table, B Article performed after the aNRP hypothermic or normothermic machine perfusion. *The graft survival is death censored; C only 35 transplantation are included in the analyses.

References

1. Thuong M, Ruiz A, Evrard P, Kuiper M, Boffa C, Akhtar MZ, et al. New classification of donation after circulatory death donors definitions and terminology. Transplant International. 2016;29(7):749-59.
2. Lomero M, Gardiner D, Coll E, Haase-Kromwijk B, Procaccio F, Immer F, et al. Donation after circulatory death today: an updated overview of the European landscape. Transplant International. 2020;33(1):76-88.
3. O'Neill S, Roebuck A, Khoo E, Wigmore SJ, Harrison EM. A meta-analysis and meta-regression of outcomes including biliary complications in donation after cardiac death liver transplantation. Transplant international. 2014;27(11):1159-74.
4. Kalisvaart M, de Haan JE, Polak WG, Metselaar HJ, Wijnhoven BPL, JNM IJ, et al. Comparison of Postoperative Outcomes Between Donation After Circulatory Death and Donation After Brain Death Liver Transplantation Using the Comprehensive Complication Index. Ann Surg. 2017;266(5):772-8.
5. Kalisvaart M, Schlegel A, Umbro I, de Haan JE, Scalera I, Polak WG, et al. The Impact of Combined Warm Ischemia Time on Development of Acute Kidney Injury in Donation After Circulatory Death Liver Transplantation: Stay Within the Golden Hour. Transplantation. 2018;102(5):783-93.
6. van de Leemkolk FEM, Schurink IJ, Dekkers OM, Oniscu GC, Alwayn IPJ, Ploeg RJ, et al. Abdominal Normothermic Regional Perfusion in Donation After Circulatory Death: A Systematic Review and Critical Appraisal. Transplantation. 2020;104(9):1776-91.
7. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS medicine. 2009;6(7):e1000097.
8. Olthoff KM, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. Liver Transpl. 2010;16(8):943-9.
9. DerSimonian R, Laird N. Meta-analysis in clinical trials. Controlled clinical trials. 1986;7(3):177-88.
10. Muller X, Rossignol G, Damotte S, Gregoire A, Matillon X, Morelon E, et al. Graft utilization after normothermic regional perfusion in controlled donation after circulatory death—a single-center perspective from France. Transplant International. 2021;34(9):1656-66.
11. Hessheimer AJ, de la Rosa G, Gastaca M, Ruíz P, Otero A, Gómez M, et al. Abdominal normothermic regional perfusion in controlled donation after circulatory determination of death liver transplantation: Outcomes and risk factors for graft loss. American Journal of Transplantation. 2021.
12. De Carlis R, Schlegel A, Frassoni S, Olivieri T, Ravaioli M, Camagni S, et al. How to preserve liver grafts from circulatory death with long warm ischemia? A retrospective Italian cohort study with normothermic regional perfusion and hypothermic oxygenated perfusion. Transplantation. 2021;105(11):2385-96.
13. Muller X, Mohkam K, Mueller M, Schlegel A, Dondero F, Sepulveda A, et al. Hypothermic Oxygenated Perfusion Versus Normothermic Regional Perfusion in Liver Transplantation From Controlled Donation After Circulatory Death: First International Comparative Study. Ann Surg. 2020;272(5):751-8.
14. Watson CJE, Hunt F, Messer S, Currie I, Large S, Sutherland A, et al. In situ normothermic perfusion of livers in controlled circulatory death donation may prevent ischemic cholangiopathy and improve graft survival. Am J Transplant. 2019;19(6):1745-58.

15. Rojas-Pena A, Sall LE, Gravel MT, Cooley EG, Pelletier SJ, Bartlett RH, et al. Donation after circulatory determination of death: the university of michigan experience with extracorporeal support. Transplantation. 2014;98(3):328-34.
16. Oniscu GC, Randle LV, Muiesan P, Butler AJ, Currie IS, Perera MT, et al. In situ normothermic regional perfusion for controlled donation after circulatory death--the United Kingdom experience. Am J Transplant. 2014;14(12):2846-54.
17. Ghinolfi D, Dondossola D, Rreka E, Lonati C, Pezzati D, Cacciatoinsilla A, et al. Sequential Use of Normothermic Regional and Ex Situ Machine Perfusion in Donation After Circulatory Death Liver Transplant. Liver Transpl. 2020.
18. De Carlis R, Di Sandro S, Lauterio A, Botta F, Ferla F, Andorno E, et al. Liver Grafts From Donors After Circulatory Death on Regional Perfusion With Extended Warm Ischemia Compared With Donors After Brain Death. Liver Transpl. 2018;24(11):1523-35.
19. Lazzeri C, Bonizzoli M, Fulceri GE, Guetti C, Ghinolfi D, Li Marzi V, et al. Utilization rate of uncontrolled donors after circulatory death-a 3-year single-center investigation. Clin Transplant. 2020;34(8):e13896.
20. Justo I, Nutu A, Garcia-Conde M, Marcacuzco A, Manrique A, Calvo J, et al. Incidence and risk factors of primary non-function after liver transplantation using grafts from uncontrolled donors after circulatory death. Clin Transplant. 2020;35(1):e14134.
21. Champigneulle B, Fieux F, Cheisson G, Dondero F, Savier E, Riou B, et al. French survey of the first three-years of liver transplantation activity from uncontrolled donors deceased after cardiac death. Anaesth Crit Care Pain Med. 2015;34(1):35-9.
22. Fondevila C, Hessheimer AJ, Flores E, Ruiz A, Mestres N, Calatayud D, et al. Applicability and results of Maastricht type 2 donation after cardiac death liver transplantation. Am J Transplant. 2012;12(1):162-70.
23. Otero A, Gomez-Gutierrez M, Suarez F, Arnal F, Fernandez-Garcia A, Aguirrezabalaga J, et al. Liver transplantation from Maastricht category 2 non-heart-beating donors. Transplantation. 2003;76(7):1068-73.
24. Fondevila C, Hessheimer AJ, Ruiz A, Calatayud D, Ferrer J, Charco R, et al. Liver transplant using donors after unexpected cardiac death: novel preservation protocol and acceptance criteria. Am J Transplant. 2007;7(7):1849-55.
25. Ishak K. Histological grading and staging of chronic hepatitis. J hepatol. 1995;22:696-9.
26. Watson CJE, Kosmoliaptsis V, Pley C, Randle L, Fear C, Crick K, et al. Observations on the ex situ perfusion of livers for transplantation. American journal of transplantation. 2018;18(8):2005-20.
27. Schlegel A, Scalera I, Perera MTPR, Kalisvaart M, Mergental H, Mirza DF, et al. Impact of donor age in donation after circulatory death liver transplantation: is the cutoff "60" still of relevance? Liver Transplantation. 2018;24(3):352-62.
28. Croome KP, Mathur AK, Mao S, Aqel B, Piatt J, Senada P, et al. Perioperative and long-term outcomes of utilizing donation after circulatory death liver grafts with macrosteatosis: a multicenter analysis. American Journal of Transplantation. 2020;20(9):2449-56.
29. Mergental H, Laing RW, Kirkham AJ, Perera M, Boteon YL, Attard J, et al. Transplantation of discarded livers following viability testing with normothermic machine perfusion. Nat Commun. 2020;11(1):2939.
30. Ruiz P, Valdivieso A, Palomares I, Prieto M, Ventoso A, Salvador P, et al. Similar results in liver transplantation from controlled donation after circulatory death donors with normothermic regional perfusion and donation after brain death donors: a case-matched single-center study. Liver Transplantation. 2021;27(12):1747-57.

31. Schurink IJ, de Haan JE, Willemse J, Mueller M, Doukas M, Roest H, et al. A proof of concept study on real-time LiMAx CYP1A2 liver function assessment of donor grafts during normothermic machine perfusion. Scientific reports. 2021;11(1):1-12.
32. Ember KJI, Hunt F, Jamieson LE, Hallett JM, Esser H, Kendall TJ, et al. Noninvasive detection of ischemic vascular damage in a pig model of liver donation after circulatory death. Hepatology. 2021;74(1):428-43.