A Systematic Review and Meta-analysis of Cold In Situ Perfusion and Preservation of the Hepatic Allograft: Working Toward a Unified Approach

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The efficacy of cold in situ perfusion and static storage of the liver is a possible determinant of transplantation outcomes. The aim of this study was to determine whether there is evidence to substantiate a preference for a particular perfusion route (aortic or dual) or perfusion/preservation solution in donation after brain death (DBD) liver transplantation. The Embase, MEDLINE, and Cochrane databases were used (1980-2017). Random effects modeling was used to estimate effects on transplantation outcomes based on (1) aortic or dual in situ perfusion and (2) the use of University of Wisconsin (UW), histidine tryptophan ketoglutarate (HTK), Celsior, and/or Institut Georges Lopez–1 (IGL-1) solutions for perfusion/preservation. A total of 22 articles were included (2294 liver transplants). The quality of evidence ranged from very low to moderate Grading of Recommendations, Assessment, Development and Evaluations score. Meta-analyses were conducted for 14 eligible studies. Although there was no difference in the primary nonfunction (PNF) rate, a higher peak alanine aminotransferase (ALT) was recorded in dual compared with aortic-only UW-perfused livers (standardized mean difference, 0.24; 95% confidence interval, 0.01-0.47); a back-table portal venous flush was undertaken in the majority of aortic-only perfused livers. There were no relevant differences in peak enzymes, PNF, thrombotic graft loss, biliary complications, or 1-year graft survival in comparisons between dual-perfused livers using UW, HTK, Celsior, or IGL-1. In conclusion, there is no significant evidence that aortic-only perfusion of the DBD liver compromises transplantation outcomes, and it may be favored because of its simplicity. However, there is currently insufficient evidence to advocate for the use of any particular perfusion/preservation fluid over the others.

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Cold in situ perfusion and subsequent cold static storage (CS) of the liver is the most commonly pursued approach prior to transplantation. Across different jurisdictions internationally, there are many differences in protocols for the composition and route of administration of perfusion/preservation fluid.(1-3) Perfusion fluid(s) used in this process vary by composition, viscosity, and volumes administered; most commonly, University of Wisconsin (UW) or histidine tryptophan ketoglutarate (HTK) solutions are used.(4-6) In situ perfusion can be instituted via cannulation of the aorta alone, with or without additional access to the portal venous system to achieve "dual" perfusion. A back-table flush is then often performed via the portal vein (PV) and/or hepatic artery (HA) in the donor center before the liver is stored in the same solution for transportation.

One reason for inconsistency between guidelines is the conflicting evidence with respect to perfusion fluid

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; CIT, cold ischemia time; CS, cold static storage; DBD, donation after brain death; DCD, donation after circulatory death; HA, hepatic artery; HTK, histidine tryptophan ketoglutarate; IGL-1, Institut Georges Lopez–1; ITBLs, ischemic-type biliary lesions; GRADE, Grading of Recommendations, Assessment, Development and Evaluations; MH, Mantel–Haenszel; MELD, Model for End-Stage Liver Disease; NR, not recorded; PNF, primary nonfunction; PV, portal vein; RCT, randomized control trial; RR, risk ratio; SMD, standardized mean difference; UW, University of Wisconsin; WIT, warm ischemia time.

composition. Analysis of European and American registry data suggests an association between the use of HTK and hepatic allograft loss.[7,8] However, a systematic review and meta-analysis by O'Callaghan et al. found no significant outcome differences between UW, Celsior, or HTK.[9] Moreover, there is a paucity of data regarding the route or volume of in situ perfusion, in particular aortic-only compared with dual perfusion. Indeed, an important unknown is whether both in situ perfusion and subsequent CS preservation impact transplantation outcomes, rather than just the preservation fluid itself during transportation.

In this systematic review and meta-analysis, we analyzed published data pertaining to outcomes of liver transplantation after procurement from donation after brain death (DBD) donors, with the aim of identifying evidence supporting a specific perfusion route, volume(s), and/or fluid(s).

Methods

The protocol for this systematic review was prospectively registered with PROSPERO (registration number CRD42016038993).[10] The review was undertaken with adherence to the Preferred Reporting Items for Systematic Reviews and Meta-analyses statement and Meta-analysis of Observational Studies in Epidemiology guidelines.[11,12]

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Potential conflict of interest: Nothing to report.

STUDY SELECTION AND ELIGIBILITY

Both English and non-English language randomized control trials (RCTs) and observational studies were included. Study inclusion mandated information with respect to in situ perfusion route(s) and volume(s), with at least 10 transplants in each study group. UW, HTK, Celsior, or Institut Georges Lopez–1 (IGL-1) solution(s) must have been used as the final perfusion/CS solution in included articles, with comparisons either between these perfusion solutions, or between aortic and dual perfusion, preflush versus no preflush, or variable perfusion volumes. All pediatric and experimental studies were excluded, in addition to studies using machine perfusion preservation of the liver. Living donor data were not included in analyses. A uniform lack of perfusion data and poor study quality necessitated the exclusion of conference abstracts/proceedings. Only DBD donor data were included and analyzed here because it became apparent after an extensive literature search that there was insufficient published literature comparing in situ perfusion solution(s) and/or route(s) for donation after circulatory death (DCD) hepatic allografts.

LITERATURE SEARCH STRATEGY

Two independent researchers reviewed (A.H. and W.H.) the Embase, MEDLINE, and Cochrane databases, including in-process and Epub ahead of print citations (January 1980 to February 2017). Supporting Table 1 outlines the search strategy. Reference lists from full-text articles of relevance were subsequently manually searched to help include all available studies.

DATA EXTRACTION

A template was derived prior to the extraction of study data by 2 independent reviewers for the following parameters.

Baseline Data

Baseline data included the following: author(s); study date and period; center(s); donor patients/transplants; donor cardiac arrest and vasopressor/inotrope requirements; donor intensive care unit stay; donor liver function tests, cause of death, split-liver utilization and allocation region;[13] donor and recipient age; recipient Model for End-Stage Liver Disease (MELD) or

Child-Pugh score at transplant; procurement technique (classic or rapid);[14,15] cold ischemia time (CIT) and warm ischemia time (WIT); aortic or dual perfusion (flush); use of pre-flush (defined as an in situ perfusion fluid used prior to the final perfusion fluid) and type; use of back-table perfusion and its type and route; perfusion volume(s); and perfusion (preservation) solution(s) used.

Outcome Data

Primary study outcomes extracted included the following: peak posttransplant aspartate aminotransferase (AST) and alanine aminotransferase (ALT), graft loss after arterial thrombosis, and graft primary nonfunction (PNF).

Secondary study outcomes included the following: ischemic biliary complications and graft survival (1 year). Ischemic biliary complications were defined as biliary strictures/stenosis in the absence of graft vessel thrombosis and/or rejection.[16] Initial poor function, a commonly used definition for which is provided by Ploeg et al.,[17] was not considered in the analysis due to insufficient data and variable definitions among the different studies.

Data Synthesis and Statistics

Meta-analyses for risk ratios (RRs), mean difference, or standardized mean difference (SMD), where applicable, were calculated using a random effects model in all cases. If necessary prior to meta-analysis, continuous variables initially underwent SMD calculations between study groups using an online calculator.[18] Meta-analyses were conducted using Comprehensive Meta-Analysis, version 2.2 (Biostat, Inc., Englewood, NJ). Funnel plots were created for assessment of publication bias, where appropriate. Heterogeneity was estimated using the I^2 statistic, with a value $\geq$50% representing a high level of heterogeneity.

Risk of Bias Assessment

RCTs included in meta-analyses were assessed for bias by using the Cochrane Collaboration's assessment tool, whereas cohort/observational studies were subjected to the Newcastle-Ottawa scale.[19,20]

Quality of Evidence

The Grading of Recommendations, Assessment, Development and Evaluations (GRADE) guidelines were used to derive overall evidence quality for meta-analyses.[21]

Results

STUDY SELECTION

Figure 1 outlines the study selection process. There were 22 articles included in the systematic review, which were combined into 19 data sets after accounting for overlapping data. RCTs or quasi-RCTs accounted for 9 data sets, whereas 6 and 4 data sets were from retrospective and prospective cohort studies, respectively.[16,22-41] Fourteen articles were eligible for meta-analyses.

BIAS ASSESSMENT

The Cochrane Collaboration's tool was used for bias assessment of RCTs. Overall, selection bias and attrition bias were minimal, as evidenced by a low risk of bias for a majority of studies with regards to random sequence generation and incomplete outcome data presentation, respectively. There was a high risk of performance bias as it is extremely difficult if not impossible to blind surgical/perfusion staff. The remaining domains presented a mixed bias risk and/or were difficult to assess due to a lack of appropriate information (see Table 1; Supporting Table 2).

Cohort study bias assessment is presented in Supporting Table 3. Study cohort comparability was established in 78.6% of studies, especially with regards to organ CITs and donor and recipient ages. Less than 60% of the articles had adequate follow-up. The nature of outcome assessment by study personnel (ie, independent blind assessment and/or record linkage) was not specified in 57.1% of cases.

There were too few studies within each parameter analysis to enable the appropriate interpretation of any funnel plots.

BASELINE STUDY CHARACTERISTICS

Summary information regarding liver perfusion articles is provided in Table 1. Overall, there were 2294 liver transplants, with a median CIT of 8.2 hours. The comparison between UW and HTK was the most common (6 studies), followed by UW and Celsior (4 studies). The majority of article data sets used dual perfusion alone (12 of 19, 63.2%). Where specified, a rapid

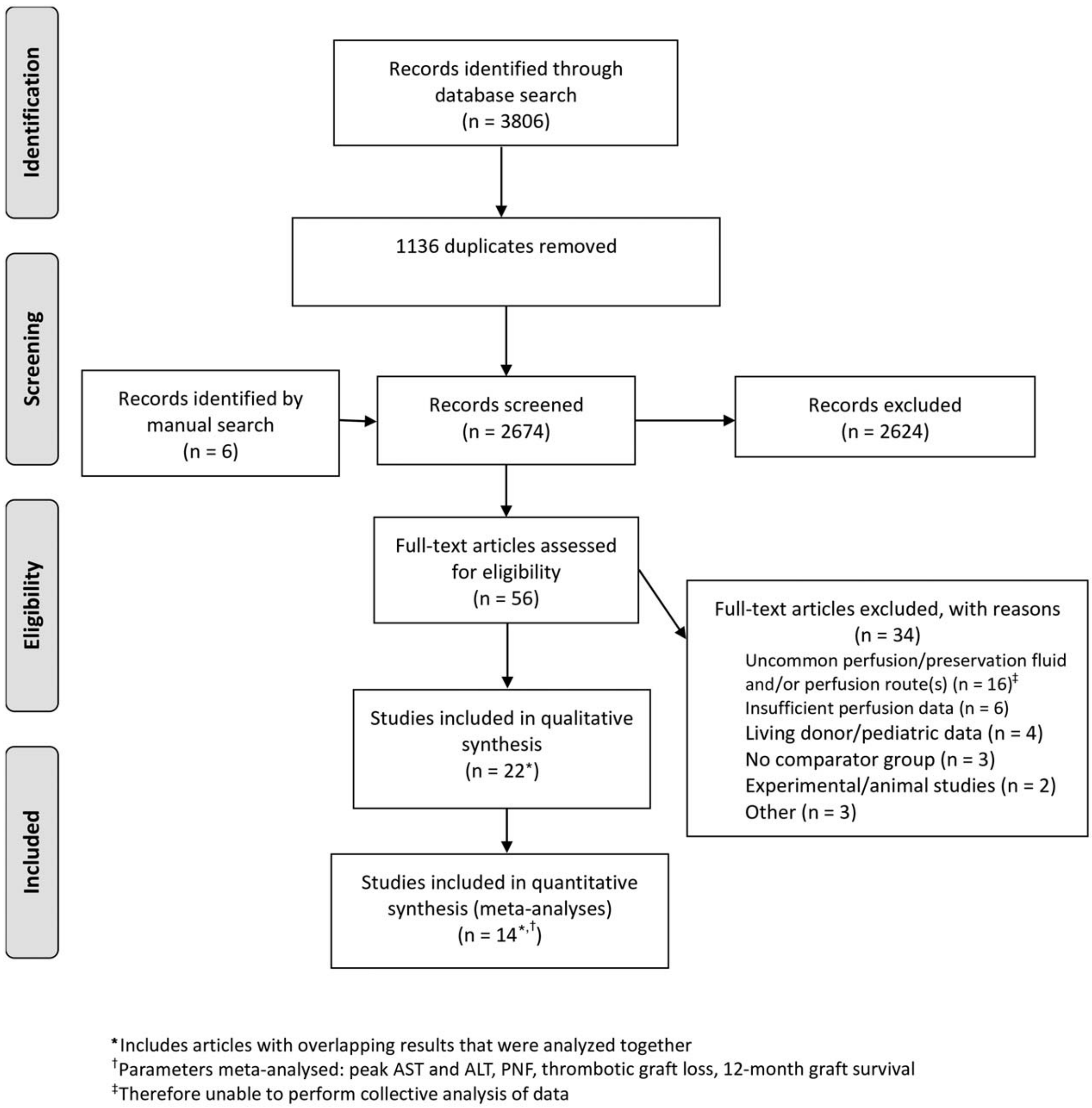

FIG. 1. Study selection flow diagram.

retrieval technique was explicitly employed in 7 studies,[15] whereas a mixture of rapid and classic procurement techniques were specified in 5 studies.[14] The different study comparator groups are also compared with respect to other donor and recipient characteristics, such as cause of death, graft steatosis, graft peak transaminases, split-liver utilization, and recipient sex and hepatitis virus status (Supporting Table 4). Where reported, the vast majority of donor deaths were secondary to trauma or a cerebrovascular accident; in general, whole livers with mild steatosis or less were employed, with normal donor transaminases (donor AST and/or ALT was reported in 7 studies, out of which it was only elevated in 10% of patients from 1 study).[30]

PERFUSION CHARACTERISTICS

UW solution was the most commonly employed perfusion and preservation solution. None of the included

TABLE 1. Summary Liver Perfusion and Preservation Study Characteristics

Liver Studies	Study Type	Study Period	Comparator Groups	Number Per Group	Donor Age	Recipient Age	Recipient MELD (mean) or Child Score	CIT (hours)	Aortic or Dual Perfusion	Total Perfusion (Flush) Volume (L)*	Back-table Flush HA/PV (L)
Anthuber et al.[22] (1993)	Retrospective	1989-1993	UW perfusion/CS	74	31.1	48.6	NR	8.9	Aortic	NR	0/1
			UW perfusion/CS	57	33.7	47.5	NR	8.7	Dual	4	NR
Avolio et al.[23] (2006)	Retrospective	NR	UW perfusion/CS	22	NR	52	Child C – 31.8%	9.8	Aortic	5.5	0/1
			HTK perfusion/CS	17	NR	44	Child C – 29.4%	10.7	Aortic	9	0/1
Boillot et al.[42] (1993)	Retrospective	1990-1992	UW perfusion/CS	33	29.3	39.0	NR	11.0	Aortic	NR†	NR
			UW perfusion/CS	28	30.8	39.5	NR	10.8	Dual	NR†	NR
Cavallari et al.[24] (2003)	RCT	1999-2001	UW perfusion/CS	90	49	49	Child C – 68.3%	7.3	Dual	4.2‡	0.3/0.7
			Celsior perfusion/CS	83	45	50	Child C – 66.7%	7.4	Dual	6.3‡	0.3/0.7
Chui et al.[25] (1998)	RCT	1994-1995	Marshall (Ross) preflush + UW perfusion/CS§	20	36.6	46.7‖	NR	9.7¶	Aortic	6	0/0.2
			Marshall (Ross) preflush + UW perfusion/CS§	20	35.1	38.2‖	NR	8.9¶	Dual	6	0/0.2
de Ville de Goyet et al.[26] (1994)	Retrospective	1990-1991	UW perfusion/CS	76	22	23	NR	13.3¶	Aortic	3	0/0.25
			UW perfusion/CS	64	22	24	NR	12.9¶	Dual	3.3	0/0
Dondéro et al.[39] (2010)	RCT	2007-2009	UW perfusion/CS	92	54	52	MELD – 15	< 8	Dual	4	0/0.75
			IGL-1 perfusion/CS	48	59	51	MELD – 17	< 8	Dual	4	0/0.75
Erhard et al.[27] (1994)	RCT	1990-1992	UW perfusion/CS	30	31.4#	41.4	NR	9.4	Dual	4	0/0.25
			HTK perfusion/CS	30	37.5#	43.5	NR	9.7	Dual	20	0/0.5
Gäbel et al.[41] (2001)	Retrospective	NR	UW perfusion/CS	22	51‖	51	NR	NR**	Aortic	3	NR
			UW perfusion/CS	22	41‖	52	NR	NR**	Dual	4	NR
García-Gil et al.[28] (2006); García-Gil et al.[29] (2011)	RCT	2001-2003	UW perfusion/CS	51	50.7	52.5	MELD – 15.7	6.6	Dual	5	0/1
			Celsior perfusion/CS	51	47.7	53.4	MELD – 15.3	6.4	Dual	6	0/1
Hatano et al.[30] (1997)	Prospective	NR	UW perfusion/CS	18	42.1	44.3	NR	12	Dual	4	NR
			HTK perfusion/CS	30	39.2	44.9	NR	10.2	Dual	20	NR
Lopez-Andujar et al.[31] (2009)	Quasi-RCT††	2003-2005	UW perfusion/CS	104	51.4	52.9	Child C – 51.9%	6	Dual	4.4	0/0
			Celsior perfusion/CS	92	54.3	53.1	Child C – 48.9%	5.4	Dual	4.5	0/0
Mangus et al.[35] (2008);¶¶ Mangus et al.[36] (2006)	Prospective¶¶	2001-2006	UW perfusion/CS	98	38	49	MELD – 17	8#	Aortic	3.2‡‡	NR
			HTK perfusion/CS	111	38	51	MELD – 18	6#	Aortic	3.8‡‡	NR
Meine et al.[32] (2006)	RCT	2003-2004	UW perfusion/CS	65	38.1#	49.9	Child C – 44.4%	9.7	Dual	3	0.5/0.5
			HTK perfusion/CS	37	44.6#	51.4	Child C – 44.4%	8.8	Dual	5	0.5/0.5

TABLE 1. *Continued*

Liver Studies	Study Type	Study Period	Comparator Groups	Number Per Group	Donor Age	Recipient Age	Recipient MELD (mean) or Child Score	CIT (hours)	Aortic or Dual Perfusion	Total Perfusion (Flush) Volume (L)*	Back-table Flush HA/PV (L)
Meine et al.[37] (2015)	Prospective	2009-2014	HTK perfusion/CS	65	45.4	53.3**	26	8.2	Dual	6	NR
			IGL-1 perfusion/CS	113	44.6	64.1**	22	8.2	Dual	4	NR
Moench and Otto[16] (2006)	Prospective	1997-2005	UW perfusion/CS	268	47.3	50.9	NR	9.7#	Dual	5	0.3/0§§
			HTK perfusion/CS	32	51.8	50.3	NR	11.0#	Dual	12.5	NR
Nardo et al.[34] (2001); Nardo et al.[40] (2004)	RCT	NR	UW perfusion/CS	60	52.9	51.0	Child C – 53.3%	7.3	Dual	4.5	NR
			Celsior perfusion/CS	53	51.0	50.0	Child C – 43.4%	7.0	Dual	6.5	NR
Nardo et al.[33] (2005)	RCT	NR	HTK perfusion/CS	20	57.9	51.3	Child C – 60%	7.5	Dual	10.5‖‖	NR
			Celsior perfusion/CS	20	64.0	55.1	Child C – 70%	7.6	Dual	6.3‖‖	NR
Wiederkehr et al.[38] (2014)	Retrospective	2008-2013	HTK perfusion/CS	125	43.4#	54.9#	MELD – 17.5	7.4#	Dual	3	0.5/0.5
			IGL-1 perfusion/CS	53	35.4#	51#	MELD – 19.9	5.4#	Dual	3	0.5/0.5
Summary Data	Prospective: 4; Retrospective: 6; RCT: 9	Range: 1989-2014	UW versus HTK: 6 UW versus Celsior: 4 HTK versus IGL-1: 2 Other solution comparisons: 7 Use of preflush: 1	Total: 2294	Median: 45 Range: 22-64	Median: 50.9 Range: 23-64.1	NA	Median: 8.2 Range: 5.4-13.3	Aortic perfusion alone: 2 Dual perfusion alone: 12 Aortic versus dual perfusion: 5	NA	NA

NOTE: No significant differences between parameters in comparator groups unless otherwise indicated.
*Does not include back-table flush volume, unless otherwise indicated.
†In situ perfusion ceased when "liver was palpably cold and free of blood."
‡Estimate based on 60 mL/kg for UW and 90 mL/kg for Celsior.
§Four liters of Ross preflush was given, followed by 2 L of UW flush, in both study groups.
‖Significance not specified.
¶Total ischemia time.
#$P < 0.05$.
**Article states no statistically significant difference between each group.
††Pseudo-randomized.
‡‡Includes back-table flush volume, given via the PV.
§§Given in 74 patients.
‖‖Estimate based on 150 mL/kg for HTK and 90 mL/kg for Celsior.
¶¶Only standard criteria donor data from Mangus et al.[35] (2008) included; perfusion details used from Mangus et al.[36] (2006).

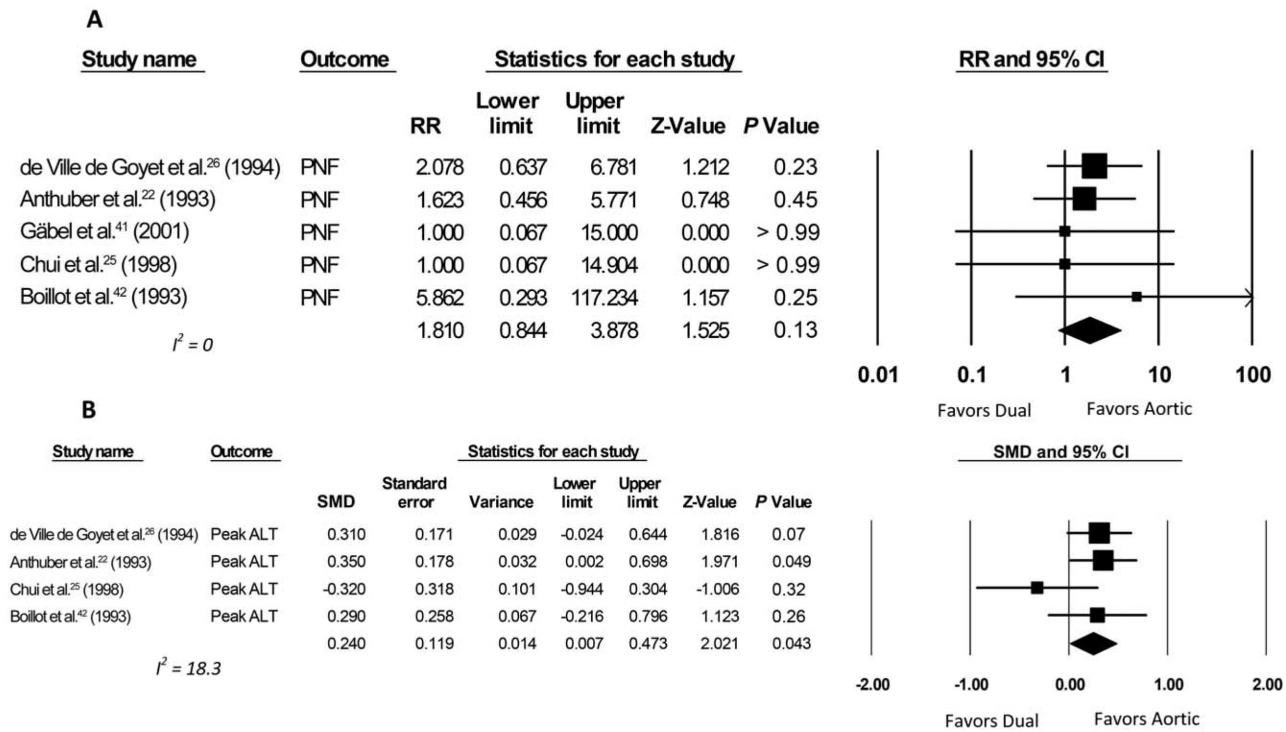

FIG. 2. Forest plots for (A) PNF and (B) peak ALT after in situ aortic or dual UW perfusion and preservation of the liver.

studies described the use of 1 fluid for perfusion and another for CS. Preflush was only used in 1 study.[25] UW dual perfusion was undertaken at lower volumes (median, 4.4 L; range, 3.0-5.0 L; n = 12 studies) compared with HTK (median, 6 L; range, 3.0-20.0 L; n = 7 studies) and Celsior (median, 6.3 L; range, 4.5-6.3 L; n = 5 studies), but not IGL-1 (median, 4.0 L; range, 3.0-4.0 L; n = 3 studies). Median volumes for aortic-only UW and HTK perfusion were 3.2 L (range, 3.0-5.5 L; n = 4 studies) and 3.8 L (range, 3.8-9.0 L; n = 2 studies), respectively.

A median of 1.0 L of perfusion fluid was used on the back-table for each of the UW (range, 0.25-1.0 L; n = 10 studies), HTK (range, 0.5-1.0 L; n = 5 studies), Celsior (n = 2 studies), and IGL-1 (n = 2 studies) groups. When the back-table perfusion route is stratified by perfusion fluid, the PV was solely used in 5 of 10 studies employing UW, compared with 1 study that only used the HA and 3 studies that undertook back-table perfusion via the PV and HA or bile duct. A total of 3 studies utilizing HTK (out of 5) undertook backtable perfusion via the PV alone, whereas both the PV and HA were used in a further 2 studies. One study each using Celsior employed solely the PV or both the PV and HA, whereas both IGL-1 articles used mixed back-table perfusion. Importantly, all studies that employed aortic-only in situ perfusion did so in conjunction with back-table portal perfusion, with the exception of 1 article in which the utilization of back-table perfusion was not specified.[41]

META-ANALYSES

Aortic Versus Dual Perfusion (University of Wisconsin)

Overall study quality was very low (see Table 1; Supporting Table 5). Two parameters were eligible for meta-analysis: peak ALT and graft PNF. There were no significant differences between aortic or dual UW perfusion with respect to PNF rates (Fig. 2). Peak ALT after transplantation was, however, significantly lower in the aortic-only perfusion group (SMD, 0.24; 95% confidence interval [CI], 0.01-0.47; $P = 0.04$).

University of Wisconsin Versus Histidine Tryptophan Ketoglutarate Dual Perfusion

Study quality, as derived using the GRADE guidelines, was once again very low (Supporting Table 5).

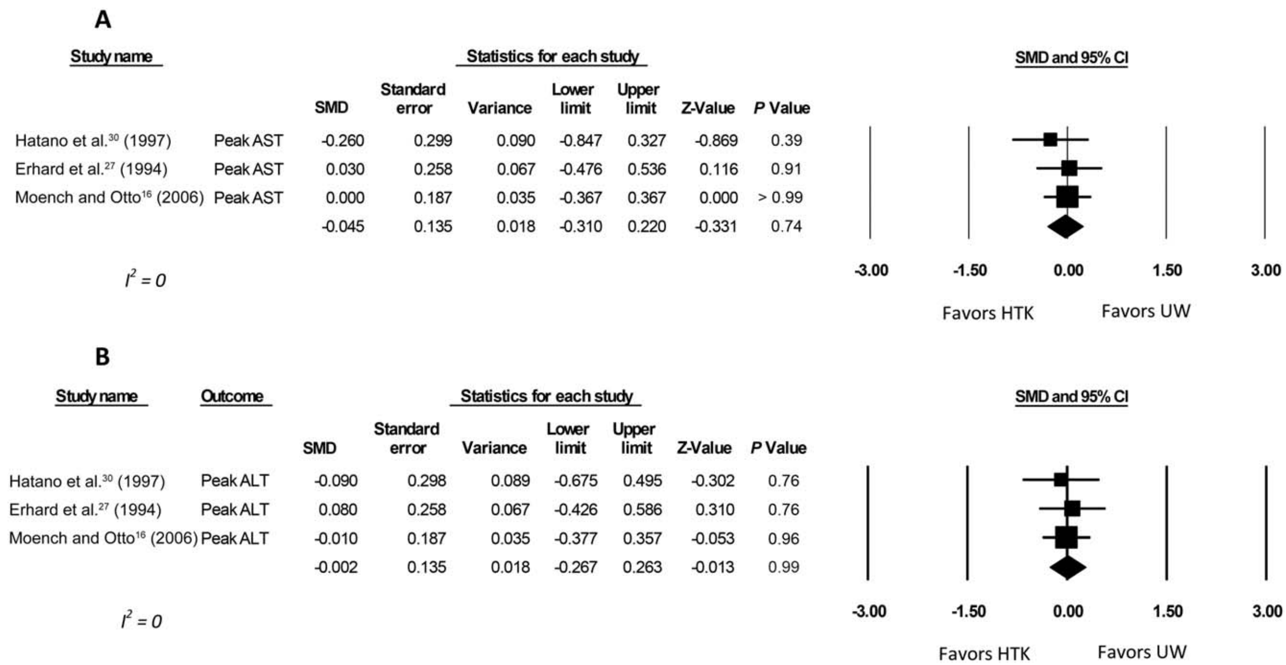

FIG. 3. Forest plots for (A) peak AST and (B) peak ALT after in situ dual perfusion and preservation of the liver with UW or HTK.

There were no significant differences in peak posttransplantation ALT or AST upon UW or HTK dual perfusion and preservation (Fig. 3).

University of Wisconsin Versus Celsior Dual Perfusion

Study quality, based on the GRADE guidelines, was moderate (Supporting Table 5). Thrombotic graft loss/retransplantation and PNF rates, in addition to 1-year graft survival, were not significantly different for either perfusion/preservation solution (Fig. 4).

OTHER COMPARISONS

Thrombotic Graft Loss

Three studies compared graft loss secondary to HA thrombosis after UW aortic-only versus dual perfusion.[26,41,42] In the aortic-only perfusion groups, rates were 3.9% (3 patients),[26] 0%,[42] and 4.5% (1 patient),[41] whereas in the respective dual-perfused groups, thrombotic graft loss occurred in 6.3% (4 patients),[26] 0 patients,[42] and 0 patients[41] ($P > 0.05$).

Graft loss secondary to hepatic arterial thrombosis in the various study groups was generally sparsely reported. For studies employing aortic-only in situ perfusion, data were available only for UW perfusion/CS (median, 3.9%; range, 0%-4.5%; n = 131 patients, 3 studies). In the dual-perfused groups, UW-perfused/CS livers had a median hepatic arterial thrombotic graft loss rate of 1.0% (range, 0%-6.3%; n = 359, 6 studies), compared with 3.1% (range, 0%-3.1%; n = 85, 2 studies), 2.0% (range, 0%-2.4%; n = 246, 4 studies), and 0.9% (n = 113, 1 study) for HTK, Celsior, and IGL-1, respectively.

Ischemic Anastomotic and Nonanastomotic Biliary Complications (Ischemic-Type Biliary Lesions)

One article reported biliary stenosis/ischemic-type biliary lesions (ITBLs) after utilization of aortic-only perfusion and hepatic preservation.[23] Multiple intrahepatic stenosis occurred in none of the patients receiving a graft perfused with UW compared with 1 (5.9%) from the HTK-perfused recipient cohort, with up to 6 months follow-up ($P > 0.05$). All patients in this study underwent PV back-table perfusion at the donor center but not HA back-table perfusion.

Biliary complication rates after in situ liver dual perfusion/CS using UW were available from 5 articles.

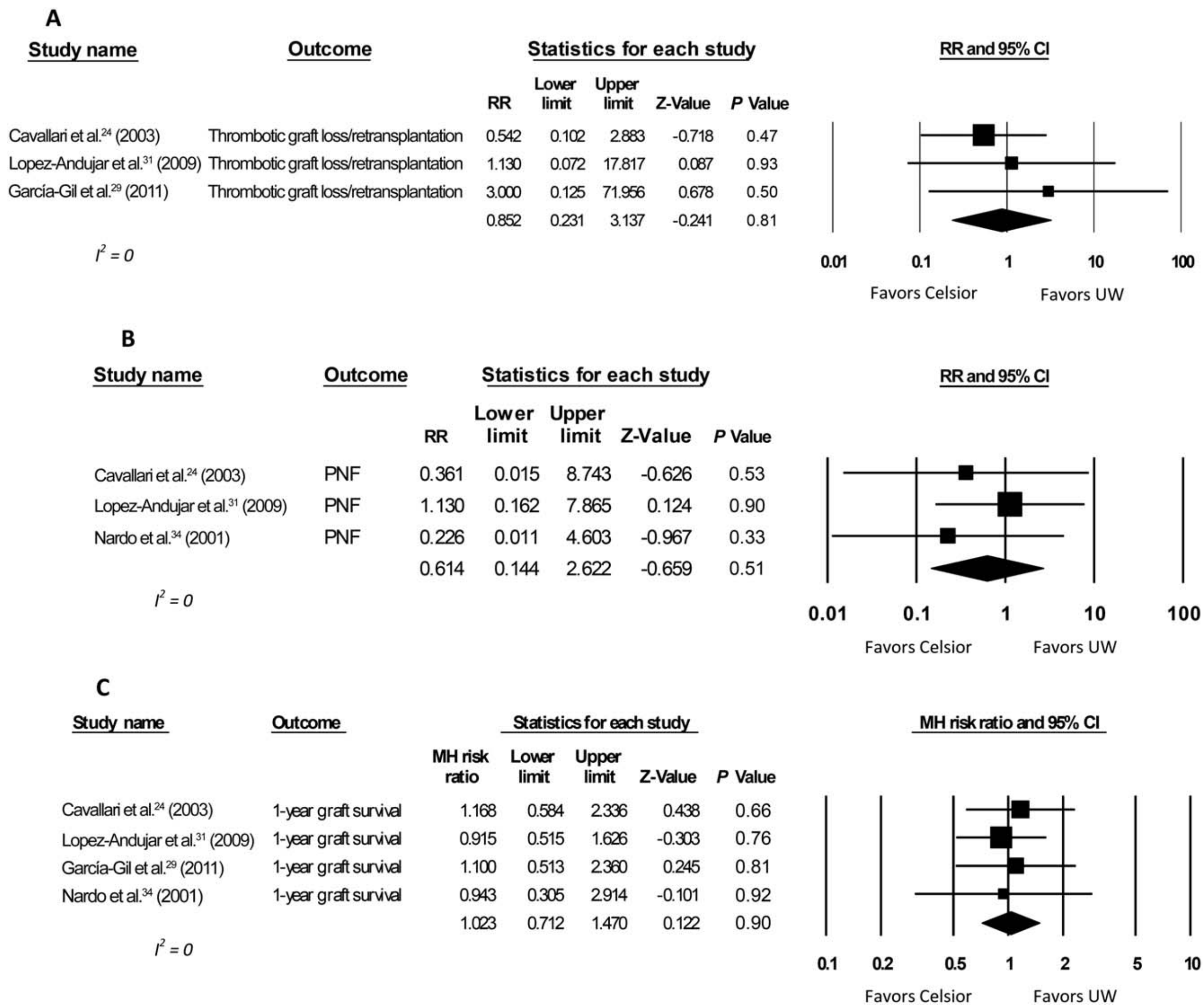

FIG. 4. Forest plots for (A) thrombotic graft loss/retransplantation, (B) PNF, and (C) 1-year graft survival after in situ dual perfusion and preservation of the liver with UW or Celsior.

Comparative anastomotic and/or nonanastomotic stricture rates between UW and Celsior in the study by Lopez-Andujar et al. were 3.9% (4/103) versus 2.2% (2/92), respectively, and 11.8% (6/51) versus 15.7% (8/51) in another study ($P > 0.05$ for both studies).[29,31] Dondéro et al. compared UW and IGL-1, with nonanastomotic stricture rates of 3.3% (3/92) versus 2.1% (1/48), respectively ($P > 0.05$).[39] Hepatic arterial back-table perfusion was not used in any of these studies. UW was compared with HTK by both Moench and Otto and Meine et al., with no significant differences in ischemic biliary complications between both perfusion/preservation fluids in either study.[16,32] Notably, Moench and Otto suggested that ITBL rates were significantly lower in UW-perfused and preserved livers that underwent high-pressure arterial back-table perfusion compared with UW perfusion without this (2.7% compared with 21.1%; $P < 0.001$).[16]

Graft Survivals

Meta-analyses were not possible for graft survival comparisons in a majority of cases, with the exception of UW versus Celsior dual perfusion (Fig. 4). There was no 1-year graft survival data for aortic or dual perfusion using IGL-1, aortic-only perfusion using Celsior, or aortic versus dual UW perfusion/CS. Aortic compared with dual UW perfusion/CS survivals were available, however, after 20 months in 1 study: 72.9% (62/85 patients) versus 61.5% (48/78), respectively ($P > 0.05$).[26]

One-year graft survivals were available from 1 study for UW (n = 98 patients) compared with HTK aortic-only (n = 98 patients) liver perfusion; respective survivals were 83.7% and 86.5% ($P > 0.05$).[35] UW dual perfusion yielded a median 1-year graft survival of 85.0% (range, 80.0%-93.8%; n = 370 patients, 5 studies), compared with 83.0% for Celsior dual perfusion (range, 78.4%-90.6%; n = 299, 5 studies). One-year graft survival after HTK dual perfusion was 94.0% (range, 75.0%-94.0%; n = 2 studies), although this analysis only included data from a total of 57 patients.

Discussion

This systematic review has attempted to analyze the data in the literature regarding the ideal perfusion route (aortic-only or dual), volume(s), and solution(s) for DBD liver transplantation. In situ liver perfusion using UW is the most common occurrence in the literature. UW appears to be perfused via the aortic and portal routes in a majority of studies and at lower volumes compared with HTK and Celsior. Although the overall quality of included articles was either low or moderate, the most important finding of this study is the lack of a significant beneficial effect to the use of dual perfusion over aortic-only perfusion with respect to early and 1-year graft outcomes. Furthermore, after stratifying by in situ perfusion routes, we were unable to show significant differences in posttransplantation outcomes including thrombotic graft loss, graft survival and ITBL for grafts that underwent UW, HTK, Celsior, or IGL-1 perfusion and subsequent CS. This latter observation should, however, be interpreted in the context of insufficient study data for these parameters in the majority of perfusion fluid and/or route comparisons, thereby preventing further statistical analyses.

Dual perfusion during procurement entails cannulation and fluid perfusion via both the aorta and PV, and necessarily requires more preparation time and dissection in comparison to aortic-only perfusion. Furthermore, dual perfusion poses added potential risks when the pancreas is to be retrieved, due to potential blockage of pancreas perfusate outflow and subsequent pancreatic congestion.[43-45] Although the dual perfusion technique should theoretically achieve more comprehensive liver perfusion and cooling, at a faster rate, final liver temperature appears to be very similar to that achieved via aortic-only cooling.[25] Perhaps of more significance than the rate at which an organ is cooled is its rate of rewarming, which may partially explain the advantages of controlled rewarming and/or subnormothermic machine perfusion.[46,47] Furthermore, aortic-only cooling also indirectly provides a portal flush through the mesenteric venous outflow.[25] An additional consideration that possibly explains the equivalence of the 2 techniques is the use of a portal venous back-table flush in at least 5 of 7 articles using aortic-only in situ perfusion. Meta-analyses in this study showed a higher graft peak ALT but not PNF after aortic-only versus dual perfusion, and there was no evidence of impaired graft survival. The impact of possible confounding factors such as donor liver steatosis, elevated donor enzymes, and split-liver utilization could not be reliably assessed due to insufficient available data (Supporting Table 4). Nevertheless, the overall outcome data from this systematic review and meta-analysis does not support the additional time and complexity of establishing dual perfusion in situ compared with aortic-only perfusion.

The only objective evidence in favor of dual perfusion in the literature to our knowledge is provided by D'Amico et al., who compared aortic with dual perfusion using Celsior for "suboptimal" liver procurement, without associated pancreas retrieval.[48] This study was excluded from our analyses because it employed a modified portal perfusion technique using Celsior with simultaneous tourniquet clamping of splenomesenteric inflow, and focused on suboptimal grafts. D'Amico et al. included data from a total of 35 patients, and although not statistically significant, the aortic-flush group here had a trend toward greater CITs and donor hemodynamic compromise, and a higher proportion of recipients with hepatitis C as the reason for transplantation. Use of dual perfusion in suboptimal/expanded criteria livers in preference to aortic-only perfusion is not supported by other major studies, and as such, this remains an area for further investigation. Moreover, some authors also recommend dual perfusion during DCD liver retrieval.[49] Similarly, this recommendation is not supported by any significant evidence in the literature and requires additional research.

Multiple abdominal organ perfusion and preservation fluids are available, with differing viscosities, electrolyte compositions, and other mediators. Although previous systematic reviews have attempted to compare hepatic allograft outcomes stratified by preservation fluid, the in situ perfusion routes were altogether ignored; it is highly likely that final graft outcomes are related not only to organ preservation during transportation per se, but also to the period of in situ perfusion.[9,50] From our findings, there appears to be no difference in at least short-term liver transplant outcomes when DBD grafts are perfused and subsequently stored in UW, HTK, Celsior, or IGL-1. Survival data were limited and far from conclusive for 1 fluid over another. However, in a recent multicenter European database analysis, Adam et al. suggested lower 3-year graft survivals in HTK-preserved grafts, including split livers, in comparison with UW, IGL-1, and Celsior.[8] The possible deleterious effect of HTK may be related to CITs and donor status, with Stewart et al. showing a further increase in graft loss for HTK livers compared with UW when DCD livers and/or livers with CITs more than 8 hours were transplanted.[7]

ITBLs present a significant complication of liver transplantation that can potentially be targeted by alterations in perfusion fluids and techniques. Indeed, Eurotransplant guidelines recommend high-pressure arterial perfusion of the hepatic graft on the back-table to prevent ITBL based on the work of Moench et al.[3,51] The theoretical basis for this is provided by the apparent impairment in perfusion of small vessels supplying the biliary tree if higher viscosity fluids such as UW are employed; this may be negated by high pressure perfusion via the aorta or on the back-table via HA.[16,51,52] The corollary of this is that the use of HTK itself may reduce intrahepatic biliary strictures when compared with UW, especially in DCD donors, due to its lower viscosity.[35,53,54] Data from the studies included in this review do not appear to support these assertions,[16,32] although this may have been impacted by the fact that only DBD donor data were included. Furthermore, back-table hepatic arterial perfusion was not used in multiple studies, seemingly without deleterious consequences to biliary luminal integrity.

Procurement costs are an important consideration in most parts of the world, and have in some cases driven research into alternative flush and perfusion strategies. The majority of articles comparing perfusion economics analyze alternatives to the relatively higher cost UW solution: 1 L of UW costs \$300 to \$500 US dollars.[55-57] Adam et al. in France substituted UW dual liver perfusion with Euro-Collins aortic perfusion/UW portal perfusion, demonstrating savings of \$750 per case, and perhaps even improved immediate graft parameters.[58] A potential area of cost-saving may also be provided by switching from dual to aortic-only UW perfusion, with lower UW volumes used in aortic-only perfusion, although this remains to be formally proven. Considering that cumulative evidence does not seem to support dual liver perfusion, a cost advantage here may provide further impetus to use the single route.

Results presented in this systematic review and meta-analysis must be interpreted cautiously. In particular, overall study quality, as determined by the GRADE assessment, was mostly very low, and at best moderate (Supporting Table 5). Selection bias also needs to be considered as much of the study data are derived from recipient liver transplantation outcomes, and as such is confounded by the omission of grafts that may have been discarded. Heterogeneity, small study sample sizes, inadequate patient follow-up in some studies, and a significant proportion of observational studies all introduced further biases to overall effect estimates, necessitating the use of random effects models in all meta-analyses. With respect to the RCTs alone, blinding of research personnel was of concern, although this is to be expected in studies of this nature; furthermore, a significant proportion of domains could not be assessed due to a lack of appropriate information. In addition, we could not formulate conclusions regarding optimal volumes of preservation solution during in situ perfusion due to a paucity of relevant data.

Overall, we have attempted to correlate liver transplantation outcomes with the initial route of in situ cold perfusion, in addition to the preservation solution used for this perfusion and subsequently also for static cold storage. Because it is extremely difficult, if not impossible, to tease out the individual effects of in situ perfusion and then later CS preservation, study groups have been analyzed with both factors in mind.

We have shown that despite the ubiquity of dual perfusion in the literature and guidelines, its utilization has not been supported by better outcomes in comparison to aortic-only perfusion for DBD liver transplantation. It should, however, be noted that aortic-only perfusion is usually accompanied by a portal venous back-table flush. There are insufficient data to draw robust conclusions about the outcome associated with the use of different perfusion/preservation fluids, especially with regards to graft survivals, ITBL rates, and thrombotic graft loss rates. Outcome data are also lacking regarding the utilization of an in situ preflush, optimal perfusion volumes, perfusion in DCD donors, appropriate protocols for back-table perfusion, and the use of dual perfusion in suboptimal donors. Additional appropriately powered RCTs focusing on these specific issues are required to resolve these questions. If aortic-only perfusion is indeed proven to be cheaper and not deleterious in comparison to dual perfusion, including in the DCD and expanded criteria donor setting, this may influence procurement surgeons toward the utilization of a more unified retrieval approach.

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