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Combined Liver-Kidney Transplantation With Preformed Anti–human Leukocyte Antigen Donor-Specific Antibodies

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Introduction: The impact of preformed donor-specific anti–human leukocyte antigen (HLA) antibodies (pDSAs) after combined liver-kidney transplantation (CLKT) is still uncertain.

Methods: We conducted a retrospective study in 8 European high-volume transplant centers and investigated the outcome of 166 consecutive CLKTs, including 46 patients with pDSAs.

Results: Patient survival was lower in those with pDSAs (5-year patient survival rate of 63% and 78% with or without pDSA, respectively; $P = 0.04$). The presence of pDSAs with a mean fluorescence intensity (MFI) $\geq$ 5000 (hazard ratio 4.96; 95% confidence interval: 2.3–10.9; $P < 0.001$) and the presence of 3 or more pDSAs (hazard ratio 6.5; 95% confidence interval: 2.5–18.8; $P = 0.05$) were independently associated with death. The death-censored liver graft survival was similar in patients with or without pDSAs. Kidney graft survival was comparable in both groups. (The 1- and 5-year death-censored graft survival rates were 91.6% and 79.5%, respectively, in patients with pDSAs and 93% and 88%, respectively, in the donor-specific antibody [DSA]-negative group, $P =$ not significant). Despite a higher rate of kidney graft rejection in patients with pDSAs (5-year kidney graft survival rate without rejection of 87% and 97% with or without pDSAs, respectively; $P = 0.04$), kidney function did not statistically differ between both groups at 5 years post-transplantation (estimated glomerular filtration rate 45 $\pm$ 17 vs. 57 $\pm$ 29 ml/min per 1.73 m^2, respectively, in patients with and without pDSAs). Five recipients with pDSAs (11.0%) experienced an antibody-mediated kidney rejection that led to graft loss in 1 patient.

Conclusion: Our results suggest that CLKT with pDSAs is associated with a lower patients' survival despite good recipients', liver and kidney grafts' outcome.

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The pathogenicity of DSAs differs according to the transplanted organ. After kidney transplantation with pDSAs, antibody-mediated rejection (AMR) is a common complication, and long-term kidney allograft

survival is poorer compared with that observed in patients transplanted without pDSAs.[1,2] Conversely, the incidence of acute AMR after liver transplantation with pDSAs is relatively infrequent,[3] and early graft failure related to AMR is exceptional.[4] There is no consensual strategy regarding the clinical management of CLKT in patients with pDSAs. Considering that in this context hyperacute humoral rejection does not usually occur, the presence of pDSAs is not considered to be a contraindication for CLKT. Nevertheless, several studies have reported a lower kidney graft survival after CLKT in case of an initial positive crossmatch (XM).[5,6] Data concerning CLKT with pDSAs and negative XM are scarce. Moreover, few data are available concerning the use of induction therapy in this context. After HLA-incompatible kidney transplantation, most centers use rituximab and polyclonal antibodies.[7] After HLA-incompatible liver transplantation, a potential interest of polyclonal antibodies has been suggested from some[8] but not all studies,[3] whereas rituximab does not seem to improve the outcome.[3]

The aims of the present large retrospective, multicenter study were to assess the outcome of liver and kidney transplants in the setting of CLKT with pDSAs and to evaluate the impact of induction therapy.

PATIENTS AND METHODS

Study Population

All CLKTs with anti-HLA screening with the Luminex single antigen (Luminex SA, Canoga Park, CA) available at transplantation from the following 8 European transplant centers were included in this retrospective study ($N = 166$): Lyon University Hospital (2002–2018, $n = 41$), Toulouse University Hospital (2008–2019, $n = 34$), Montpellier University Hospital (2010–2015, $n = 22$), Bellvitge University Hospital (2002–2010, $n = 21$), Kremlin-Bicètre University Hospital (2010–2015, $n = 17$), Strasbourg University Hospital (2009–2018, $n = 15$), Tours University Hospital (2011–2016, $n = 14$), and Bordeaux University Hospital (2012–2015, $n = 2$). All transplants were from donation after brain death.

In addition to the comparison of the outcome of CLKT patients with or without pDSAs, we compared the rejection rate, kidney histologic features observed in protocol biopsies obtained at 1 year, and graft survival of CLKT patients with pDSAs to those observed in a group of kidney transplant alone (KTA) recipients with pDSAs who were grafted in Toulouse between 2008 and 2017 ($n = 86$) (Table 1). Furthermore, we compared patient and graft survival rates of CLKT patients with pDSAs with those observed in a group of liver transplant alone recipients with pDSAs who were grafted in Toulouse between 2008 and 2018 ($n = 38$) (Table 1).

According to French Law (loi Jardé), anonymous retrospective studies do not require institutional review board approval.

Immunologic Analysis

The presence of pDSAsM or *de novo* DSAs was tested using Labscreen Single Antigen technology (One Lambda, Canoga Park, CA) in all centers, except in Lyon. The Labscreen Single Antigen determined the specificity of class I HLAs in A/B and class II in DR/DQ IgG antibodies in the recipients' sera (centrifuged at 10,000*g* for 10 minutes) according to the manufacturer's instructions. The presence and specificity of antibodies were then detected using a Labscan 100 (One Lambda, Canoga Park, CA), and the mean fluorescence (baseline value) for each sample in each bead was evaluated. A baseline mean fluorescence intensity value of >1000 was considered positive. The immunodominant DSA was the DSA with the highest MFI at transplantation. The MFI sum was the sum of all A/B/DR/DQ MFI of the DSAs.

In Lyon, pDSAs were detected using the Lifecodes single-antigen technology (LMX Deluxe; Immucor, Norcross, GA). The Lifecodes single antigen (LSA class I/II) determined the specificity of class I HLAs in A/B and class II in DR/DQ IgG antibodies in the recipients' sera according to the manufacturer's instructions. The presence and specificity of antibodies were then detected, and the MFI for each sample in each bead was evaluated. An MFI value of >1000 was considered positive. In order to compare the MFI obtained with the Lifecodes single-antigen technology and those obtained with Labscreen Single Antigen technology, we doubled the MFI obtained with the Lifecodes SA because it was recently suggested in a recent publication.[9] All XMs were performed by lymphocytotoxicity.

Pathologic Analysis

All rejection episodes were biopsy proven and classified according to the liver or renal Banff classification.[9–11] The 1-year systematic kidney biopsies were analyzed and classified according to the renal Banff classification.[9]

Statistical Analyses

Reported values represent the means (± SD) or medians (ranges). Quantitative variables were compared using the Mann-Whitney nonparametric test. Categoric variables are expressed as percentages and compared between groups using the chi-square test or, if appropriate, the Fisher exact

Table 1. Patients' characteristics

Variables	CLKT recipients without pDSAs (n = 120)	CLKT recipients with pDSAs (n = 46)	P value (CLKT with vs. without DSAs)	KTA with pDSAs (n = 86)	P value (KTA vs. CLKT with pDSAs)	LTA with pDSAs (n = 38)	P value (LTA vs. CLKT with pDSAs)
Recipient's age, yr (mean)	51 ± 13	50 ± 13	0.58	49 ± 13	0.73	53 ± 10	0.70
Recipient's sex, male (%)	74 (62)	26 (57)	0.55	37 (43)	0.35	6 (16)	<0.001
Initial liver disease			0.10	—	—		<0.001
Alcohol	31 (26)	6 (13)				12 (32)	
Viral (HBV, HCV)	17 (14)	8 (17)				8 (21)	
PKD	23 (19)	14 (31)				0	
Autoimmune (PCS, AIH, PBC)	8 (7)	0				8 (21)	
Primitive hyperoxaluria	8 (7)	6 (13)				0	
Other	33 (27)	12 (26)				10 (26)	
MELD score at transplantation, median (minimum–maximum)	24 (6–40)	23 (6–40)	0.09	—	—	24 (6-40)	0.56
Liver retransplantation, yes (%)	17 (14)	5 (11)	0.76	—	—	5 (13)	0.62
Positive HBV-DNA at transplantation, yes (%)	1 (1)	0	>0.99	0	>0.99	0	>0.99
Positive HCV-DNA at transplantation, yes (%)	12 (10)	4 (9)	>0.99	0	0.01	6 (16)	0.34
Liver cold ischemia time (min), mean (± SD)	430 ± 180	400 ± 200	0.20			413 ± 146	0.62
Initial kidney disease (%)			0.25		<0.001	—	—
IgA nephropathy	23 (19)	6 (13)		9 (11)			
Diabetes	12 (10)	7 (15)		6 (7)			
PKD/urinary tract abnormalities	24 (20)	13 (28)		32 (37)			
Hyperoxaluria	13 (11)	8 (18)		0			
Vascular	0	0		8 (9)			
Unknown/other	48 (40)	12 (26)		31 (36)			
Kidney retransplantation, yes (%)	17 (14)	9 (20)	0.54	54 (63)	<0.001	—	—
Kidney cold ischemia time (minimum), mean (± SD)	738 ± 300	716 ± 253	0.40	191 ± 369	0.01	—	—
Donor age, yr (mean)	45 ± 16	48 ± 15	0.18	49 ± 15	0.22	54 ± 19	0.56
HLA mismatches							
Class I	3.1 ± 1.2	3.3 ± 1.1	0.18	4.4 ± 1.3	0.50	3.2 ± 1.0	0.50
Class II	2.5 ± 1.5	2.9 ± 1.3	0.15	2.0 ± 1.2	0.30	3.0 ± 0.9	0.45
Positive anti-HLA antibodies, yes (%)	27 (23)	46 (100)	<0.001	86 (100)	>0.99	38 (100)	>0.99
Positive pDSAs at transplantation, yes (%)	—	46 (100)	—	86 (100)	>0.99	38 (100)	>0.99
Anti–class I DSA	—	19 (41)		50 (58)	0.07	12 (32)	0.38
Anti–class II DSA	—	19 (41)		28 (33)	0.40	11 (29)	0.26
Anti–class I and II DSA	—	8 (18)		8 (9)	0.22	15 (39)	0.03
Number of pDSAs, median (minimum–maximum)	—	1 (1-7)		1 (1-3)	0.15	2 (1–7)	0.20
Mean MFI of the ID pDSA at Tx		6000 ± 5500		4100 ± 4000	0.77	9440 ± 6430	0.01
Mean sum of MFI pDSA at Tx	—	15,600 ± 26,700		5600 ± 8200	0.41	44,000 ± 50,000	<0.001
Positive LCT-XM at transplantation, y (%)	—	13 (28)		2 (2)	<0.001	10 (26)	>0.99
Positive T-cell crossmatch	—	0		0			
Positive B-cell crossmatch		1 (2)		0			
Positive T- and B-cell crossmatch		12 (26)		2 (2)	<0.001		
Induction therapy, yes (%)	95 (79)	44 (96)	0.01	86 (100)	0.11	35 (92)	0.65
Polyclonal antibodies	35 (29)	39 (85)	<0.001	86 (100)	<0.001	27 (71)	0.18
Anti-IL2R blockers	60 (50)	7 (15)	<0.001	0	0.003	8 (21)	0.57
Rituximab, y (%)	0	7 (15)	<0.001	64 (74)	<0.001	15 (39)	0.01
Apheresis sessions, yes (%)	0	5 (11)	<0.001	46 (53)	<0.001	5 (13)	0.75
i.v. Ig, yes (%)	0	10 (22)	<0.001	21 (24)	0.82	4 (10)	0.24

(Continued on following page)

Table 1. (Continued) Patients' characteristics

Variables	CLKT recipients without pDSAs (n = 120)	CLKT recipients with pDSAs (n = 46)	P value (CLKT with vs. without DSAs)	KTA with pDSAs (n = 86)	P value (KTA vs. CLKT with pDSAs)	LTA with pDSAs (n = 38)	P value (LTA vs. CLKT with pDSAs)
Initial immunosuppression							
CNI use, yes (%)	120 (100)	46 (100)	>0.99	86 (100)	>0.99	38 (100)	>0.99
Tacrolimus, yes (%)	95 (79)	36 (78)	>0.99	86 (100)	<0.001	37 (97)	0.01
Cyclosporin A, yes (%)	25 (21)	10 (22)	>0.99	0	<0.001	1 (3)	0.01
MPA, yes (%)	120 (100)	46 (100)	>0.99	86 (100)	>0.99	38 (100)	>0.99
Steroids, yes (%)	119 (99)	43 (100)	>0.99	86 (100)	>0.99	38 (100)	>0.99
1-year post-transplant immunosuppression							
CNI use, y (%)	102[a] (91)	39[b] (100)	0.33	73[c] (97)	0.83	31[d] (100)	>0.99
Tacrolimus, yes (%)	84 (82)	37 (94)	0.10	73 (97)	0.80	31 (100)	0.49
Cyclosporin A, yes (%)	18 (18)	2 (6)	0.10	0	0.19	0	0.49
MPA, yes (%)	95 (85)	28 (72)	0.66	65 (87)	0.33	31 (100)	0.001
mTORi, yes (%)	3 (8)	0	0.57	10 (13)	0.06	0	0
Azathioprine, yes (%)	3 (8)	0	0.57	0	>0.99	0	0
Belatacept, yes (%)	0	0	>0.99	2 (3)	>0.99	0	0
Steroids, yes (%)	80 (71)	18 (46)	0.006	75 (100)	<0.001	31 (100)	<0.001

AIH, autoimmune hepatitis; BPC, biliary primary cirrhosis; CKLT, combined liver-kidney transplantation; CNI, calcineurin inhibitor; DSA, donor-specific antibody; HBV, hepatitis B virus; HCV, hepatitis C virus; ID, immunodominant; KTA, kidney transplant alone; LCT-XM, lymphocytotoxicity crossmatch; MFI, mean fluorescence intensity; MPA, mycophenolic acid; mTORi, mTOR inhibitors; PCS, primary cholangitis sclerosis; PKD, polycystic kidney disease; TX, transplantation.
[a]Eight patients died during the first year post-transplant.
[b]Seven patients died during the first year post-transplant.
[c]Four patients died, and 7 additional patients returned to dialysis during the first year post-transplant.
[d]Seven patients died during the first year post-transplant.

test. A P value < 0.05 was considered statistically significant. The cumulative probability of patient or graft survival or acute rejection was calculated using the Kaplan-Meier method. A Cox proportional hazard analysis was used to identify predictive factors for recipient survival and acute kidney graft rejection. Variables with a P value < 0.10 in the univariate analysis as well as the transplant center, the persistence of pDSAs after transplantation, and factors known to be associated with the "acute rejection" and "recipient survival" outcomes (including the occurrence of biliary complications and liver retransplantation) were entered in the stepwise multivariable model with backward elimination. Statistical analyses were performed using XLSTAT software (Addisoft, Paris, France).

RESULTS

Study Population and Initial Immunosuppressive Strategy

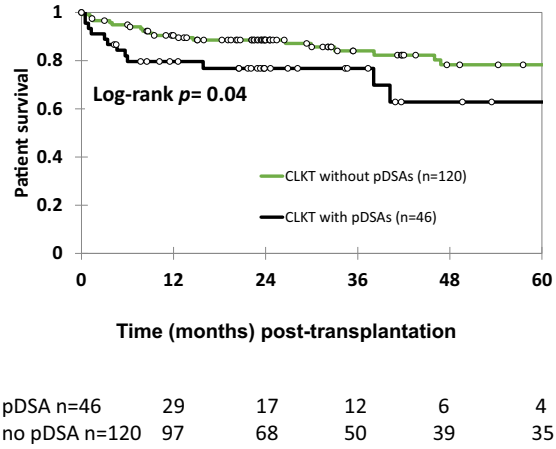
The main characteristics of the patients who were included are presented in Table 1. Forty-six of the 166 (28%) CLKT recipients included in the study presented with pDSAs at transplantation. Alcoholic liver disease was the main indication for transplantation in patients without pDSAs, whereas patients with pDSAs presented principally with polycystic kidney disease. Kidney retransplantations were more frequent in patients with pDSAs (26% vs. 12% in patients without pDSAs, $P = 0.008$).

Induction therapy was more frequently used in patients with pDSAs and was mainly based on the use of polyclonal antibodies, whereas anti–interleukin-2 receptor blockers were more frequently used in patients without pDSAs. The initial and maintenance immunosuppression was similar in both groups.

Compared with KTA recipients with pDSAs, CLKT recipients with pDSAS had a positive XM at transplantation significantly more often. Conversely, they received polyclonal antibodies, rituximab, and tacrolimus-based immunosuppression less often; had steroid withdrawal after transplantation; and had undergone apheresis less often.

Patient Survival According to the Presence of pDSAs

Fifteen patients (7 recipients with pDSAs [15.2%] and 11 recipients without pDSAs [9.2%]) died during the first year post-transplantation ($P = 0.40$). In patients with pDSAs, deaths were related to infection (bacterial pneumonia [$n = 2$], invasive aspergillosis [$n = 1$], and bile duct infection [$n = 2$]) and heart failure (related to pulmonary embolism [$n = 1$] and myocardial infarction [$n = 1$]). In patients without pDSAs, the causes of death were related to infection (bacterial pneumonia [$n = 1$], invasive aspergillosis [$n = 2$], and bile duct infection [$n = 4$]), cancer ($n = 1$), and heart failure (myocardial infarction [$n = 3$]). During the follow-up, patient survival was significantly lower in patients with pDSAs compared with patients without pDSAs (Figure 1). Among patients with pDSAs who died during the



Variable	HR	95% CI	p
pDSA with a MFI >5,000	4.96	2.3- 10.9	*<0.001*
3 or more pDSAs	4.7	1.6- 13.9	*0.005*

Figure 1. Patient survival according to the presence of preformed donor-specific anti–human leukocyte antigen antibodies (pDSAs). Kaplan-Meier recipient analysis and Cox proportional regression model according to the presence of pDSAs after combined liver-kidney transplantation (CLKT). CI, confidence interval; HR, hazard ratio.

follow-up, there was a trend of a higher rate of positive T- and/or B-cell XM at transplantation (6/12 vs. 7/34, $P = 0.07$), a higher number of pDSAs (median 1.5 [range 1–7] vs median 1 (range 1–5), $P = 0.07$), and a higher MFI of the immunodominant DSA (median 8000 [range 1200–17,000] vs. 4000 [1000–15,000], $P = 0.07$). However, we did not find any difference concerning the class of DSAs (i.e., 8/12 patients who died and 19/34 patients who did not for anti–class I DSAs [$P = 0.73$]; 8/12 patients who died and 19/34 patients who did not for anti–class II DSAs ($P = 0.73$]; and 4/34 patients who died and 4/12 patients who did not for both anti–class I and II DSAs [$P = 0.18$]). The sum of pDSA MFIs at transplantation was also similar in both groups (i.e., 9000 [range 1200–67,000] vs. 5000 [1000–94,000], $P = 0.18$).

We assessed the predictive factors for death by means of a Cox proportional regression model with backward elimination according to the presence of pDSAs, the transplant center, the persistence of pDSAs after transplantation, the XM (T and/or B cells) result at transplantation, the initial liver disease, the occurrence of kidney rejection after transplantation, kidney retransplantation, the occurrence of biliary complications after liver transplantation, and the presence of liver retransplantation. After adjusting for these factors, the presence of a pDSA with an MFI higher than 5000 (hazard ratio 4.96; 95% confidence interval: 2.3–10.9; $P < 0.001$) and a number of 3 or more pDSAs (hazard ratio 4.7; 95% confidence interval: 1.6–13.9; $P = .005$) were the predictive factors identified in this model.

Liver Transplant Outcome

Three liver transplant patients with pDSAs presented with end-stage liver failure 6 months (range 3–16 months) post-transplantation. The causes of liver failure were arterial thrombosis resulting in ischemic cholangitis in 3 cases. One patient was retransplanted, and 2 patients died while on the waiting list.

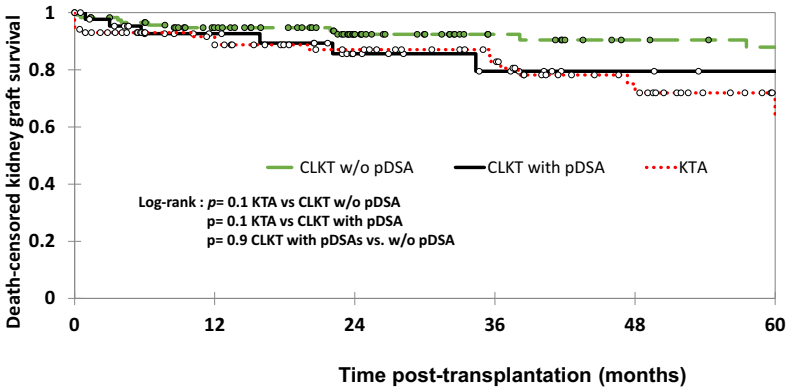
Four patients without pDSAs evolved to end-stage liver failure 37 months (range 4–92 months) post-transplantation. The causes of liver failure were ischemic cholangitis in 3 cases, which were successfully retransplanted, and the cause was unknown in the last recipient who rapidly died from sepsis.

The biopsy-proven liver rejection rate was similar in both groups (2% and 6.5%, respectively, at 5 years in patients with and without pDSAs; $P =$ not significant; Supplementary Figure S1). All of these cases were steroid sensitive. During the follow-up, no CLKT recipient presented histologic features of antibody-mediated liver rejection. No liver failure related to graft rejection was observed during the follow-up period.

Patients' survival did not differ after CLKT or liver transplant alone with pDSAs (Supplementary Figure S2). Ductal complications occurred in 7 (15%) patients with pDSAs and 20 (17%) patients without pDSAs ($P = 0.43$).

Kidney Transplant Outcome in CLKT recipients

During the follow-up, death-censored graft survival was similar in CLKT patients with or without pDSAs (Figure 2). At 12 months post-transplantation, kidney function was significantly better in patients without pDSAs (Figure 3). However, no difference in kidney function was observed at 24 and 60 months (Figure 3). Similarly, proteinuria did not differ in the 2 groups at 1 year post-transplantation (P = 0.26, Supplementary Figure S3). We assessed the predictive factors for kidney graft loss by means of a Cox proportional regression analysis, including the presence of pDSAs, the donor and recipient age, the use of polyclonal antibodies at induction, the presence of a positive XM at



CLKT with pDSA n=46	29	17	12	6	4
CLKT w/o pDSA n=120	97	68	50	39	35
KTA with pDSA n= 86	64	51	40	25	10

Figure 2. Death-censored kidney graft survival. CLKT, combined liver-kidney transplantation; KTA, kidney transplant alone; pDSA, preformed donor-specific anti–human leukocyte antigen antibody.

transplantation, and kidney retransplantation. We found no significant associated factor for kidney graft loss after CLKT.

The frequency of biopsy-proven kidney rejection episodes was significantly higher in patients with pDSAs (Figure 4). During the follow-up, 5 patients in each group had a biopsy-proven kidney rejection: 5 AMRs in CLKT recipients with pDSAs and 4 acute T-cell–mediated rejections and 1 AMR related to the occurrence of *de novo* DSAs in CLKT recipients without pDSAs. Hence, the incidence of AMR was significantly higher in patients with pDSAs (5/46 patients with pDSAs vs. 1/120 patients without pDSAs, $P = 0.007$).

Among patients with pDSAs, the rate of biopsy-proven kidney rejection in patients transplanted with a positive (T and/or B cells) XM was not significantly different compared with patients transplanted with a negative XM (3/13 vs 2/33, $P = 0.13$). Kidney graft survival was similar in patients with a positive or negative XM (3 patients in both groups reverted to dialysis), and kidney graft function was similar in both groups 1 and 5 years post-transplantation (53 $\pm$ 21 and 48 $\pm$ 27 ml/min per 1.73 m^2 in patients transplanted with a positive XM and 46 $\pm$ 20 and 44 $\pm$ 13 ml/min/1.73 m^2 in patients with a negative XM, P = not significant).

We assessed predictive factors for rejection by means of a Cox proportional regression analysis, including pDSAs, the use of polyclonal antibodies, the donor age, the class, and the number of DSAs at transplantation. We found no significant associated factor for kidney graft rejection after CLKT in this model.

All 5 rejections in patients with pDSAs were considered to be AMRs. All but 1 were treated with steroid pulses, plasma exchanges, rituximab perfusion (375 mg/m^2, 2 doses), and intravenous Igs (2 g/kg). The last patient received steroid pulses, eculizumab (900 mg/wk for 1 month and then 1200 mg every 2 weeks) for 6 months, and bortezomib (1.3 mg/m^2, twice weekly in 4 perfusions). At the last follow-up (i.e., 23 [range 4–133] months postrejection), 4 of the 5 patients had preserved kidney function. Only 1 patient, who experienced AMR and a recurrence of type I hyperoxaluria, reverted to dialysis 8 months post-transplantation (7 months after kidney rejection). Among patients without pDSAs, only 1 presented with an acute AMR with detectable anti–class II *de novo* anti-HLA DSAs and reverted to dialysis 4 months post-rejection despite treatment with plasma exchanges and steroid pulses. All 4 other rejections were considered to be steroid-sensitive, T-cell–mediated rejections and reserved a functional kidney transplant at the last follow-up (51 [range 22–102] months after kidney rejection).

Kidney Transplant Outcome in CLKT Versus KTA Recipients

Death-censored kidney graft survival did not differ between CLKT and KTA recipients with pDSAs (Figure 2). It did not statistically differ with that observed in CLKT without pDSAs. Kidney function was significantly better in CLKT without pDSAs at 1 year after the transplantation compared with both groups (CLKT and KTA) with pDSAs (Figure 3). However, no difference between all 3 groups was observed at 5 years post-transplantation (Figure 3). Conversely, the graft rejection rate was significantly higher in patients who received a KTA with pDSAs compared with patients who received a CLKT with or without pDSAs

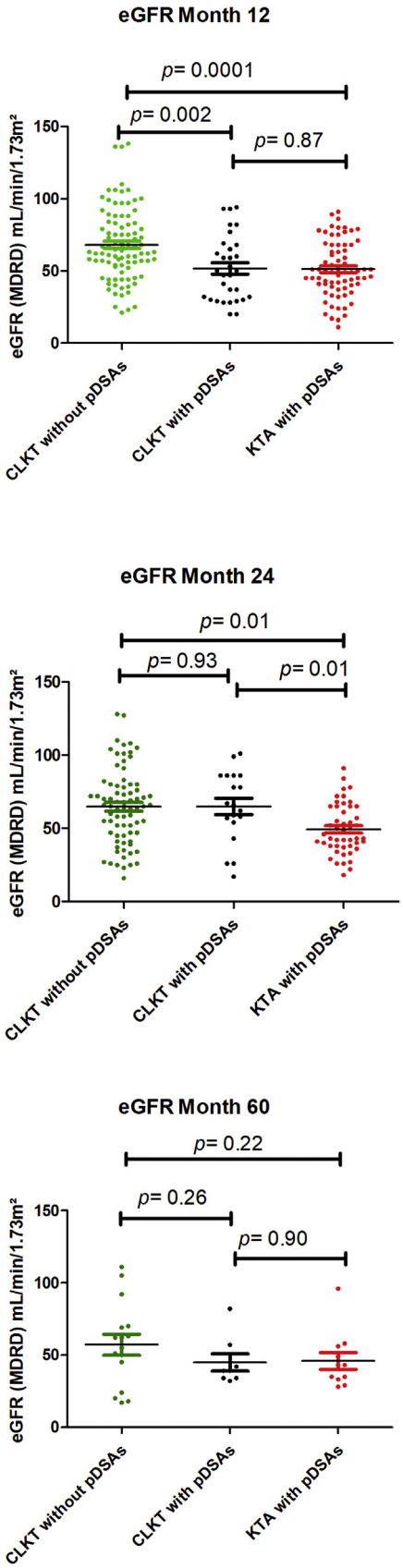


Figure 3. Modification of Diet in Renal Disease (MDRD) estimated glomerular filtration rate (GFR) at years 1, 2, and 5 post-transplantation.

(Figure 4). Moreover, the incidence of AMRs was significantly higher in KTA recipients with pDSAs (25/86 KTA with pDSAs [29%]) compared with CLKT with pDSAs (5/46 [11%], $P = 0.03$).

We analyzed kidney histologic features observed on 1-year protocol kidney biopsies. Microvascular inflammation, transplant glomerulopathy, and C4d positivity tended to be more prevalent in KTA recipients with pDSAs compared with CLKT with pDSAs (Supplementary Figure S4).

Immunologic Follow-Up

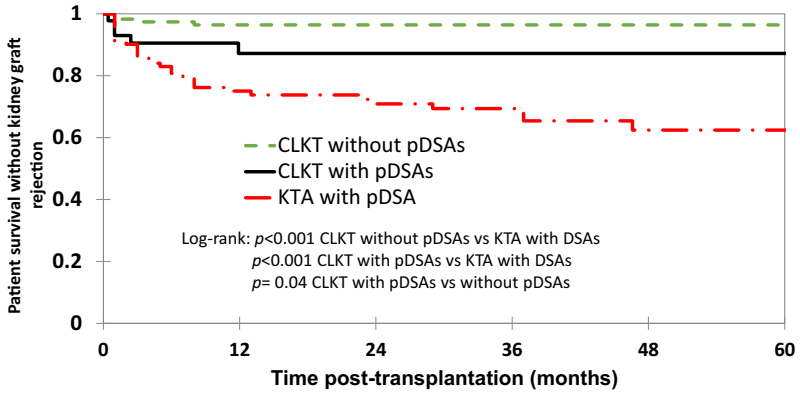
Sixteen of the 46 patients (35%) with pDSAs were tested for the presence of HLA antibodies 1 month post-transplantation. Four of the 6 patients (67%) with only anti–class I pDSA, 3 of the 5 (60%) patients with only anti–class II pDSAs, and 4 of the 5 (80%) patients with both anti–class I and II pDSAs still had detectable DSAs.

Among the 46 patients with pDSAs, 22 patients (48%) were retested for anti-HLA DSA at least once after transplantation. At the last anti-HLA screening (i.e., 17 [range 1–63] months post-transplantation), 3 of 9 (33%), 5 of 8 (63%), and 4 of 5 (80%) patients with anti–class I, anti–class II, and both anti–class I and II pDSAs, respectively, still had detectable DSAs.

De novo DSAs were detected in 10 of the 82 patients without pDSAs who were tested for anti-HLA antibodies after transplantation. Only 1 of these patients developed an AMR. Of note, none of the 21 recipients with pDSAs tested after transplantation developed *de novo* DSAs.

DISCUSSION

The number of CLKTs performed each year has increased over the past decades.[12] Therefore, the question of whether or not a double transplantation is acceptable for a recipient with pDSAs is not uncommon. In our series, 28% of the CLKT recipients presented with pDSAs at transplantation, a proportion that corresponds with previous reports.[13,14] The improvement in patient and graft survival in case of CLKT compared with sequential transplantations highlights the immunologic protective effect of the liver on kidney grafts.[15] However, the deleterious effect of pDSAs in sensitized recipients with a positive XM was previously raised by some registry analyses.[5,6] Our data are consistent with these results demonstrating a lower rate of recipient survival in case of pDSAs, but, interestingly, even if the presence of DSA with an MFI higher than 5000 and the presence of 3 or more pDSAs were associated with lower patient



CLKT with pDSAs n=46	29	17	12	6	4
CLKT w/o pDSAs n= 120	97	68	50	39	35
KTA with pDSAs n=86	64	51	40	25	10

Figure 4. Survival without kidney graft rejection. CLKT, combined liver-kidney transplantation; KTA, kidney transplant alone; pDSA, preformed donor-specific anti–human leukocyte antigen antibody.

survival, these factors were not associated with kidney rejection or liver failure. One could argue that patients with pDSAs received a high level of immunosuppression compared with other patients, leading to more infectious, cardiovascular, and neoplastic events. Recently, Kamal *et al.*[16] performed a registry analysis from the OPTN database to investigate the role of induction after CLKT. They did not find any effect of induction therapy on patients' and allografts' survivals. However, in patients who received T-cell–depleting induction therapy, CNI-based immunosuppression was associated with decreased patients' survival and both liver and kidney transplants' survival. This is in line with our study in which the large majority of CLKT recipients with pDSAs were given T-cell–depleting induction therapy and CNI-based maintenance immunosuppression. Further prospective studies are needed to assess the optimal induction therapy agent after CLKT. In a previous single-center retrospective analysis of 86 CLKT recipients (including 30 with pDSAs), O'Leary *et al.*[13] reported that anti–class II DSAs were associated with a poorer rate of patient survival and an increased risk of both liver and kidney graft losses. In our study, liver graft survival with pDSAs was excellent and was similar to CLKT patients without pDSAs and to liver transplant alone recipients with preformed DSAs. Moreover, the death-censored kidney graft survival did not differ according to the presence or absence of pDSAs. In addition, kidney AMR was infrequent after CLKT even in case of pDSA with a positive XM. The incidence of kidney graft failure related to AMR was rare. We observed only 1 case of graft failure in a patient who presented with an AMR associated with a recurrence of hyperoxaluria. Similarly, we observed no liver failure related to graft rejection. However, we were unable to assume that some of the cases of arterial thrombosis were associated with an alloimmune phenomenon. To note, in the O'Leary *et al.* study,[13] the reasons for liver transplantation were different from ours. In their study, a high proportion of patients were infected by hepatitis C virus.

We also compared the outcome of CLKT with pDSAs and kidney transplant patients alone with DSAs. Despite a higher proportion of positive XMs at transplantation and significantly lesser use of apheresis, rituximab, polyclonal antibodies, tacrolimus, and steroids in the CLKT group, kidney graft survival during the follow-up and graft function at 5 years after transplantation did not statistically differ between the groups. Furthermore, the AMR rate was significantly higher in patients who received a kidney transplant alone (29% vs. 11%, $P = 0.03$). This is in line with a recent study by Taner *et al.*[17] that described the outcome of 68 CLKTs (including 14 with pDSAs) and compared them with 136 kidney transplant recipients (including 28 patients with pDSAs). They found a higher AMR rate in patients with isolated kidney transplantation (46%) compared with CLKT recipients (7%). Thereafter, the same group described a unique evolution of the transcriptome in protocol kidney biopsies from CLKTs with pDSAs showing less inflammation or endothelial activation and an increase in the expression of genes related to tissue integrity compared with those observed in isolated kidney transplant patients with pDSAs[18] but also a donor-specific hyporesponsiveness after CLKT not observed in isolated kidney transplant recipients.[19] All of these data support the relative safety of CLKT with pDSAs in terms of liver and kidney graft survival.

In our study, the incidence of *de novo* DSAs was similar to that observed after isolated kidney or liver

transplantation.[20,21] However, the incidence of antibody-mediated liver or graft rejection was lower than in isolated transplantation because only 1 of the 10 recipients who developed *de novo* DSAs presented an AMR. Recently, in a cohort of 83 CLKTs, Parajuli *et al.*[22] reported that 23 of 83 patients developed *de novo* DSAs during the follow-up, but *de novo* DSA occurrence was not associated with graft failure of the kidney or liver. Further analyses are required to explain this difference. Interestingly, we also reported that no patients with pDSAs developed *de novo* DSAs in our cohort.

Our present study has several limitations. First, it is a retrospective study of 8 high-volume European transplant centers. Therefore, the management of these patients was quite different, and potential confounding factors such as the presence of residual renal function at transplantation or different local practice to propose isolated liver or kidney transplantation versus combined transplantation were not evaluated. Nevertheless, through this study, the outcome of CKLD in a real-life setting can be described. Second, DSAs were not tested with complement-binding tests or Ig subclasses, which are supposed to improve the specificity of DSAs after kidney and liver transplantation,[2,23] and we used the 2 different available Luminex single-antigen bead assays (Immucor Lifecodes used in Lyon Hospital and One Lambda Labscreen used in the other centers) to detect anti-HLA antibodies. However, it was previously shown that these assays lead to comparable results and that the MFI is 50% lower in the the Immucor assay compared with the One Lambda assay.[9] In the end, we only assessed the role of A/B/DR/DQ DSA in our cohort and did not evaluate the role of Cw or DP antibodies after CLKT. However, we excluded patients who had only anti-Cw or anti-DP antibodies from the analysis. Third, because all participating centers assessed rejection using kidney rather than liver biopsies, we only reported on isolated liver rejections, which could result in the underdiagnosis of the real incidence of liver graft rejection (and therefore, some liver AMR) in this cohort. Moreover, the absence of a difference in the liver transplant rejection rate and the histologic pattern observed in the 1-year biopsies after CLKT versus KTA with pDSAs could be related to the small number of patients. Notwithstanding, in light of the liver graft outcome, the impact of liver graft rejection related to the presence of pDSAs seems to be limited, and despite the small sample size, biopsies from KTA tended to show more antibody-related lesions in the pDSA group.

In conclusion, CLKT with pDSA seems to be associated with a higher mortality rate, even if the direct role of DSAs remains elusive. Nevertheless, this procedure is also associated with good mid- to long-term liver and kidney graft survival. The reasons for the high mortality rate as well as optimal induction and maintenance therapy in this context should be explored in further prospective large studies.

DISCLOSURE

All the authors declared no competing interests.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Survival without liver graft rejection. CLKT, combined liver-kidney transplantation; pDSAs, preformed donor-specific anti–human leukocyte antigen antibodies.

Figure S2. Patient survival after combined liver-kidney transplantation (CLKT) or liver transplantation alone (LTA) with preformed donor-specific anti–human leukocyte antigen antibodies (pDSAs).

Figure S3. Proteinuria/urine creatinine (P/C) ratio at 12 months post-transplantation.

Figure S4. One-year protocolar biopsies in combined liver-kidney transplantation (CLKT) with or without preformed donor-specific anti–human leukocyte antigen antibodies (pDSAs) and in kidney transplant alone (KTA) with pDSAs.

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