ORIGINAL ARTICLE

A kidney discard decision strategy based on zero-time histology analysis could lead to an unjustified increase in the organ turndown rate among ECD

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SUMMARY

The utility of zero-time kidney biopsies (KB) in deciding to accept expanded criteria donor (ECD) kidneys remains controversial. However, zero-time histology is one of the main causes for discarding kidneys in the United States. In a single-centre study, we examined the utility and impact on outcome of the use of frozen section zero-time KB among ECD. Ninety-two zero-time KB were analysed for accept/discard decision between 2005 and 2015 among ECD. 53% of kidneys were rejected after zero-time KB analysis; there was no difference in individual clinical and biological data between accepted/rejected groups. However, histology of rejected kidneys showed more sclerotic glomeruli (20% vs. 8%; $P < 0.001$), increased interstitial fibrosis (1.25 ± 0.12 vs. 0.47 ± 0.09; $P < 0.0001$), more arteriosclerosis (2.14 ± 0.17 vs. 1.71 ± 0.11; $P = 0.0032$) and arteriolar hyalinosis (2.15 ± 0.12 vs. 1.55 ± 0.11; $P = 0.0006$). Using propensity score matching, we generated a group of 42 kidney allograft recipients who received a transplant matched for donor zero-time histology and clinical characteristics with donors whose kidneys were rejected. Interestingly, their 1- and 5-year graft survival and function were similar to the global cohort of ECD recipients. In conclusion, when performed, zero-time KB was a decisive element for kidney discard decision. However, adverse zero-time histology was not associated with poorer graft survival and kidney function among ECD.

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Key words
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Introduction

Kidney transplantation is a cost-saving treatment that extends the lives of patients with end-stage renal disease (ESRD) [1]. Because of organ shortage and population aging, kidney transplants from extended criteria donors (ECD) (donors aged ≥60 years or aged 50–59 years with vascular comorbidities [2]) are an increasing source of organs for treating ESRD [3].

However, whereas ECD represent about 40–50% of deceased kidney transplants in France, they represent <20% in the United States. Moreover, nearly 40% of procured ECD organs in the United States are ultimately refused by transplant teams and discarded [4]. This decision-making is complex and multifactorial. In the United States, the use of biopsy is very frequent in the evaluation of ECD kidneys, in contrast to the evaluation of non-ECD kidneys and zero-time biopsy findings are an important correlate of ECD kidney discard. Indeed, about 80% of ECD kidneys are biopsied in the United States compared with 20% of non-ECD kidneys [5]. In addition to conventional criteria such as donor age, hypertension/diabetes status or serum creatinine, biopsy findings are one of the most frequently cited reasons for the discard of recovered ECD kidneys even following the introduction of the Kidney Donor Profile Index (KDPI) [6] / Kidney Donor Risk Index (KDRI). Organ Procurement and Transplantation Network policies in the United States currently recommend preimplantation biopsy for all kidneys with a KDPI >85% or at the surgeon's request [7]. In France, the transplantation allocation system follows the rules of the French national agency for organ procurement (Agence de la Biomédecine) and ECD represent a high proportion of kidney donors. Kidneys from ECD are preferentially offered to older recipients, and donors older than 65 years with an eGFR <60 ml/min/1.73 m^2 may be offered for dual kidney transplantation to recipients older than 65 years. The French transplant allocation system does not use the KDPI score or systematic zero-time donor kidney biopsies in organ acceptance decisions. Zero-time biopsies are only used for acceptance decision in a subset of ECD at the request of the nephrologist, based on particular pejorative clinical or biological data.

Yet, the importance of donor histology is controversial. For example, glomerulosclerosis percentage was associated with graft failure risk and graft function in several studies [5,8]. However, recent data showed that circulating anti-HLA donor specific antibodies (DSA) and cold ischaemia time were the main independent determinants of outcome following ECD transplantation rather than zero-time histology [9]. Moreover, a recent study from the American Scientific Registry of Transplant Recipients analysing 36 700 discarded kidneys among 212 305 deceased donor kidneys procurements, revealed a large overlap in the quality of discarded and transplanted kidneys [6]. Other data suggest that performing procurement biopsies for decisions on kidney discard could lead to increased kidney discard rates [5,10]. Thus, the added benefits of zero-time biopsy of a potential kidney donor to aid in the decision-making process, on top of standard donor biological and clinical data, remain unclear [11,12].

We retrospectively studied our strategy of using frozen section zero-time kidney biopsy for the decision to accept or reject potential ECD kidneys and determined its utility and impact on kidney transplantation outcome.

Methods

Study design

We included all patients who underwent kidney transplantation from a deceased expanded criteria donor (donation after brain death exclusively) in Necker Hospital (Paris, France) between November 2005 and January 2015 ('global ECD Cohort'). In France, according to the Agence de la Biomédecine legislation, kidneys from ECD donors older than 65-year-old with an eGFR <60 ml/min/1.73 m^2 are offered for dual kidney transplantation (DKT). Kidney allocation policies are described in details in https://www.agence-biomedecine.fr/.

All the recipients had a negative lymphocyte IgG cytotoxicity crossmatch. Post-transplant immunosuppression was standardized according to the immunological risk. There was no patient involvement in this study.

In our centre, a zero-time biopsy is performed in all deceased donor kidneys that are transplanted; histology is made available post-transplant and is not used to influence the decision to accept or reject the organ except for a small subset of donors with unfavorable clinical, biological or radiological characteristics, where a frozen section is analysed before transplantation to help in the decision to accept or reject the kidney.

We focused on the ECD who had a zero-time kidney biopsy used for acceptance decision. After frozen section kidney biopsy analysis, one group of kidneys was used for transplantation ('accepted ECD') whereas the other group of ECD kidneys was rejected for transplantation ('rejected ECD').

Using a propensity score, we created a third group ('matched ECD cohort') extracted from our 'global ECD cohort' of 622 recipients transplanted during the study period. This third group of recipients was matched on rejected donors characteristics based on clinical (age and hypertension history), biological (serum creatinine) and zero-time histological characteristics according to the international Banff criteria (percentage of glomerulosclerosis, ci (interstitial fibrosis), ct (tubular atrophy) and cv (vascular fibrous intimal thickening) scores) in order to estimate the 'virtual' graft survival and kidney function when using this type of kidneys (Figure 1).

Clinical data

We obtained clinical data from recipients and donors from two national registries: Données Informatiques Validées en Transplantation (DIVAT) and CRISTAL database from the Agence de la Biomédecine. Both registries are prospectively filled. Scaled donor-based KDRI was calculated retrospectively assuming multiracial origins for all the donors as in France ethnicity is not available in medical records. eGFR on transplanted organs was measured only in those with functioning grafts.

Kidney biopsies

As aforementioned, for all the global ECD cohort, a zero-time needle 16-Gauge core kidney allograft biopsy was performed in the implanting centre by the surgeon. If fast analysis for discard/acceptance decision was not needed, that biopsy was fixed in FAA (a solution of alcohol, formalin, and acetic acid), and subsequently embedded in paraffin. The biopsy sections (4 μm thick) were stained with periodic acid-Schiff, Masson's trichrome, Jones methenamine silver and haematoxylin and eosin. The allograft paraffin-embedded kidney biopsies were scored and graded according to the international Banff for kidney allograft transplantation. The report on the paraffin-embedded biopsy did not affect acceptance decision.

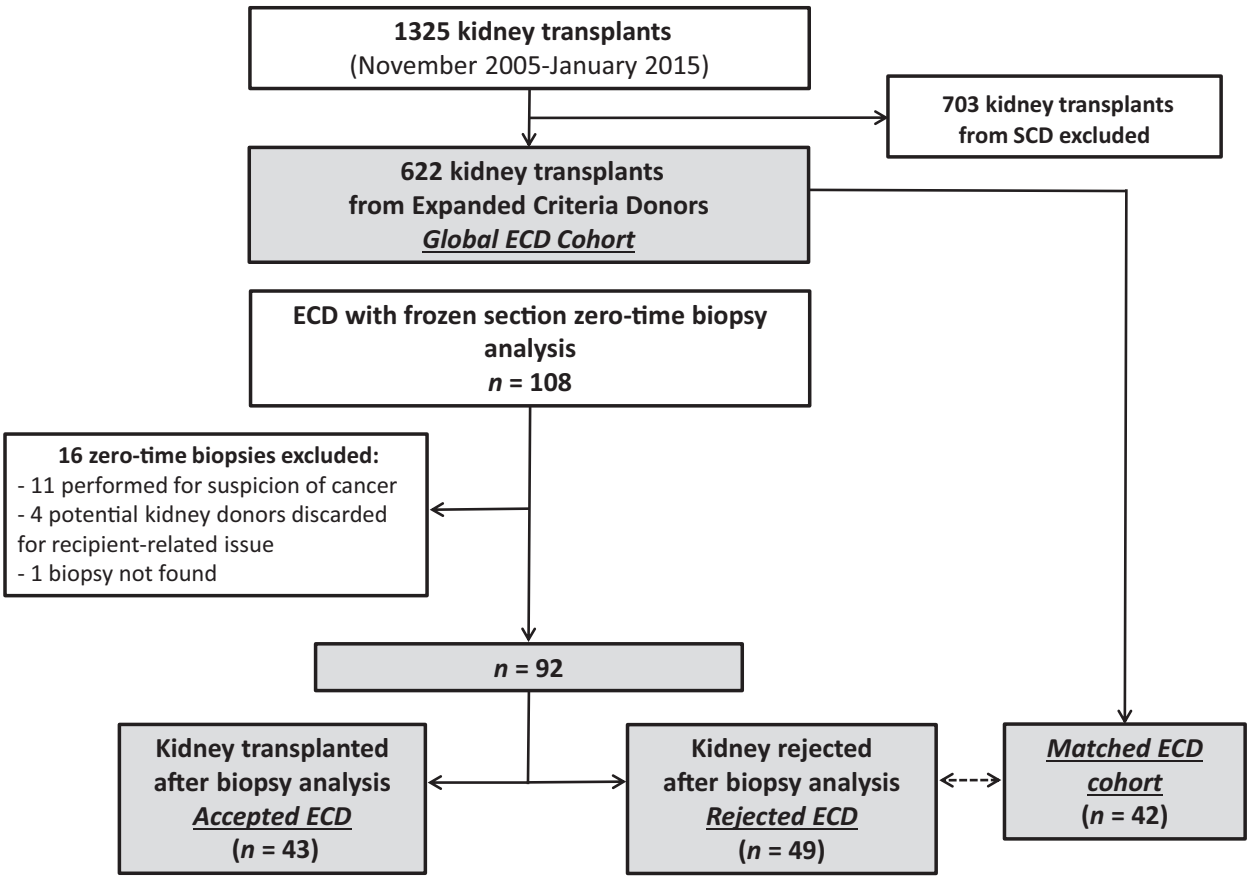

Figure 1 Study flow chart. Based on clinical radiological and biological data from the ECD donors, nephrologists decide whether to perform or not a frozen section zero-time kidney biopsy analysis. After the pathologist evaluation, kidneys are accepted or rejected. A paraffin-embedded biopsy sample analysis is performed in all deceased donors with a result 48 hours after the transplantation, therefore not influencing the decision to accept or reject the kidney.

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If fast histological analysis was needed for acceptance/discard decision, the zero-time biopsy was immediately frozen and stained with haematoxylin and eosin. We will define them in the manuscript as 'frozen section zero-time kidney biopsies'. The pathologist scored glomerulosclerosis, interstitial fibrosis and tubular atrophy, arteriosclerosis and arteriolar hyalinosis according to the international Banff criteria. The report was transmitted to the nephrologist in order to accept or reject the kidney for transplantation. This decision was made by a senior transplant nephrologist. Rejected kidneys were offered to another transplant centre until acceptance, otherwise discarded. All the frozen section zero-time kidney biopsies were re-analysed for the study by the same pathologist (MR). After frozen section analysis, the biopsy was fixed in FAA, embedded in paraffin and re-scored by the pathologist.

All the kidney allograft biopsies were read by a trained transplant pathologist (MR, JPDVH, LG).

Statistical analysis

Continuous variables were expressed as median (interquartile range) and categorical variables as numbers (percentages).

A comparison by the Wilcoxon's test or the Fisher's exact test was performed between transplantation for ECD without vs. frozen section zero-time kidney biopsy analysis, respectively, for continuous or categorical variables.

Then, among all frozen section zero-time kidney biopsy analysis, we compared clinical, biological and histological characteristics according to the final decision of transplantation or not. The same tests were used as previously described.

Then, we used a propensity score to match discarded kidney based on frozen section zero-time kidney biopsy analysis with the global ECD cohort. The propensity score was estimated using a logistic regression model that contained as predictors: donor age, history of arterial hypertension in donor, last serum creatininemia measured in donor and histological findings in zero-time kidney biopsies. Histological matching was performed on frozen section scores from 'rejected ECD' to FAA-fixed scores of 'global ECD cohort'. Subjects were 1:1 matched without replacement by the estimated propensity score using nearest neighbor matching with a caliper of 0.2SD of the logit of the propensity score. Standardized differences were determined to ascertain balance between the propensity-matched groups. The difference in survival curves between matched and non-matched patients was evaluated using the log-rank test.

All analyses were carried out using R 3.1.1 (R foundation for Statistical Computing Vienna, Austria).

Ethics statement

The study was conducted in accordance with the ethical guidelines from the Assistance Publique – Hôpitaux de Paris. No institutional review board approval was necessary at the time of the study as it was a retrospective study involving no intervention. The study conducted according with the ethical standards of the 2000 Declaration of Helsinki as well as the Declaration of Istanbul 2008. Each recipient from the present study was given written informed consent to be included in the DIVAT and CRISTAL database networks that were used for the study.

Results

Population

Among 1325 kidney transplants performed in our centre during the 10-year study period, 622 (47%) were from ECD.

At the request of the nephrologist, a frozen section zero-time kidney biopsy analysis to help guide the organ allocation decision was performed in 108 donors (Figure 1). Ninety-two biopsies analysed for ECD organ acceptance decision were included in the study. Biopsies were excluded if they were performed for cancer suspicion ($n = 11$), when transplantation was not performed for recipient issues ($n = 4$) (i.e. for positive cytotoxicity crossmatch) or when the zero-time biopsy was not found ($n = 1$). After analysis of the frozen section of zero-time biopsies by a senior pathologist, 46.7% ($n = 43$) of organs were transplanted whereas 53.3% ($n = 49$) were rejected. Only 6 rejected organs were transplanted at another centre.

Baseline characteristics of ECD with frozen section zero-time kidney biopsy analysis for organ allocation decision

Frozen section zero-time kidney biopsy analysis for allocation decision was performed on donors with the following characteristics: median age 72 years [64–79], median BMI of 25.8 [23.4–29.7], hypertension history in 67 donors (72.8%), history of diabetes mellitus in 14 (15.2%), cerebrovascular cause of death in 68 (73.9%), trauma cause of death in 15 (16.3%), anoxia cause of death in 7 (7.6%), proteinuria in 57 (61.9%) and

median serum creatinine level of 100 µmol/l [78–128] (Table 1).

Compared to the global ECD cohort, donors with a frozen section zero-time kidney biopsy analysis had a similar age, BMI, frequency of diabetes mellitus history and death from cerebrovascular cause rate. However, frozen section zero-time kidney biopsy was more frequently requested in donors with higher KDRI scores (2.41 [1.93; 2.85] vs. 2.15 [1.77; 2.67], $P = 0.01$), with a history of hypertension ($n = 67$ (72.8%) vs. $n = 362$ (59.7%), $P = 0.02$), with higher serum creatinine (100 [78–128] vs. 79 [61–101] µmol/L, $P < 0.001$) and when anoxia was the cause of death ($n = 7$ (7.6%) vs. $n = 15$ (2.4%), $P < 0.001$) (Table 1).

Interestingly, zero-time kidney biopsy analysis to help guide the allocation decision did not significantly increase the median cold ischaemia time of transplantation compared with ECD global cohort data (20.7 hours [16.8–26.5] vs. 21.3 hours [17.3–27.5], P = ns).

Discard decision after zero-time kidney biopsy analysis was associated with poor histological findings

Out of the 92 donors with frozen section zero-time kidney biopsy analysis, 43 transplantations were performed (46.7%) whereas 49 kidney donors (53.3%) were not transplanted and either discarded ($n = 43$) or transferred to another transplant centre ($n = 6$). We compared clinical and biological characteristics between rejected kidneys and accepted kidneys in our frozen section zero-time kidney biopsy cohort. Age (68 vs. 74 years), BMI (25.9 [23.9–29.9] vs. 25.7 [23.0–29.7]), hypertension (67.4% vs. 77.5%), diabetes mellitus history (10.0% vs. 24.5%) and cerebrovascular cause of death (67.4% vs.79.6%) were similar between these two groups (P = ns). Serum creatinine was also comparable (96 vs.101 µmol/l, P = ns) (Table 2). However, kidneys rejected after zero-time kidney biopsy analysis had a higher KDRI score (2.84 [2.48–3.15] vs. 2.35 [1.93–2.94], $P = 0.02$).

Interestingly, we found that poor histological findings were significantly more frequent in rejected ECD kidneys (Table 3). Kidneys rejected after zero-time kidney biopsy analysis had a higher Remuzzi score (6.2 ± 0.33 vs. 3.4 ± 0.27, $P < 0.0001$), a higher median glomerulosclerosis percentage (20 [15–33] vs. 8% [0–15], $P < 0.0001$), a higher mean ci score (1.25 ± 0.12 vs. 0.47 ± 0.09, $P < 0.0001$) and ct score (1.19 ± 0.13 vs. 0.44 ± 0.08, $P < 0.0001$), more severe arteriosclerosis (2.14 ± 0.17 vs. 1.71 ± 0.11, $P = 0.0032$) and arteriolar hyalinosis score ah

Table 1. Baseline characteristics of the global ECD cohort and the ECD with frozen section zero time kidney biopsy analysis for organ acceptance decision.

	Global ECD cohort $n = 622$	Frozen section zero-time kidney biopsy analysis $n = 92$	*P* value
Donor variables			
Donor age, years (median, range)	70 [62–77]	72 [64–79]	0.11
Weight, kg (median, range)	72 [62–81]	72 [63–83]	0.40
Height, cm (median, range)	165 [160–175]	165 [160–174]	0.95
Body Mass index, kg/m^2 (median, range)	25.6 [23–29]	25.8 [23.4–29.7]	0.75
Hypertension, *n* (%)	362 (59.7)	67 (72.8)	**0.02**
Diabetes mellitus, *n* (%)	83 (16.0)	14 (15.2)	0.30
Tobacco use, *n* (%)	50 (25.6)	24 (26.1)	0.88
Cause of death, *n* (%)			
Anoxia	15 (2.4)	7 (7.6)	**<0.001**
Cerebrovascular disease	477 (76.8)	68 (73.9)	
Trauma	89 (14.3)	15 (16.3)	
Other	41 (6.6)	2 (2.2)	
Serum creatinine, µmol/L (median, range)	79 [61–101]	100 [78–128]	**<0.001**
Proteinuria, *n* (%)	386 (62)	57 (61.9)	0.92
KDRI (median, range)	2.15 [1.77;2.67]	2.41 [1.93;2.85]	**0.01**
Recipients variables			
Graft rank >1, *n* (%)	117 (17.6)		
Recipient gender (male), *n* (%)	405 (61)		
Recipient age	62 [54–68]		
Dual kidney transplantation, *n* (%)	140 (21.1)		

Significant statistical results are in bold (when $P < 0.05$).

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Table 2. Baseline donor characteristics of kidneys accepted or rejected after frozen section zero-time biopsy analysis.

	Frozen section zero-time kidney biopsy analysis $n = 92$		
	Accepted ECD $n = 43$	Rejected ECD $n = 49$	*P* value
Donor variables			
Donor age, years (median, range)	68 [62–79]	74 [67–79]	0.16
Weight, kg (median, range)	71 [66–90]	71 [60–82]	0.28
Height, cm (median, range)	166 [160–175]	165 [160–173]	0.50
Body Mass index, kg/m^2 (median, range)	25.9 [23.9–29.9]	25.7 [23.0–29.7]	0.33
Hypertension, *n* (%)	29 (67.4)	38 (77.5)	0.35
Diabetes mellitus, *n* (%)	2 (10.0)[†]	12 (24.5)	0.50
Tobacco use, *n* (%)	11 (25.6)	13 (26.5)	0.98
Cause of death, *n* (%)			
Anoxia	4 (9.3)	3 (6.1)	0.42
Cerebrovascular disease	29 (67.4)	39 (79.6)	
Trauma	8 (18.6)	7 (14.3)	
Other	2 (4.7)	0	
Serum creatinine, µmol/l (median, range)	96 [82–124]	105 [72–128]	0.85
Proteinuria, *n* (%)	31 (79.5)	26 (59.1)	0.06
KDRI (median, range)	2.35 [1.93–2.94]	2.84 [2.48–3.15]	**0.02**
Recipients variables			
Graft rank >1, *n* (%)	2 (4.7)		
Recipient gender (male), *n* (%)	30 (69.8)		
Recipient age	62 [52–70]		
Dual kidney transplantation, *n* (%)	10 (23.3)		

Significant statistical results are in bold (when $P < 0.05$).

[†]Percentage calculated on 20 available datas.

(2.5 ± 0.12 vs.1.55 ± 0.11, $P = 0.0006$) (Table 3 and Figure S1). Biopsy quality, estimated by the number of glomeruli used to assess the degree of glomerulosclerosis, was similar in both accepted and discarded groups (11 [9–15] vs. 13 [9–21], $P = 0.31$).

An ECD cohort matched with the rejected kidney donors characteristics has a comparable graft survival and kidney function than the global ECD cohort

Our findings showed that 53% of kidneys were rejected after frozen section zero-time biopsy analysis showing poor histological findings. As there are conflicting data on the prognostic value of zero-time kidney biopsy findings, we wanted to know if our strategy led to the discard of potentially useful organs. To answer this, we selected, in our global ECD kidney transplant recipients cohort, a matched-ECD cohort with similar donor clinical (age and hypertension history), biological (serum creatinine) and zero-time histological characteristics (glomerulosclerosis percentage, ci, ct and cv scores) as the rejected kidneys, using a propensity score-based strategy. Histological matching was performed on frozen section scores from 'rejected ECD' to FAA-fixed scores of 'global ECD cohort'. The correlation between 'frozen' scores and FAA-fixed scores in our centre was highly significant (e.g. glomerulosclerosis scoring, Spearman $r = 0.66$, $P < 0.0001$). We then compared their graft function and graft survival to the rest of the global ECD cohort and to the 43 transplanted kidneys after frozen section zero-time kidney biopsy analysis.

We found a matched cohort of 42 recipients in the same period who were transplanted with an ECD kidney with similar characteristics compared to rejected kidneys (Table S1 and Figure S2). Percentage of DKT in the matched cohort was comparable to the percentage of DKT proposal in the rejected kidney cohort (51% vs 52%). Other characteristics are detailed in Table S2.

The distribution of propensity scores is shown in Figure S3.

Median follow-up after transplantation was 5.0 years [3–7.8] in the global ECD cohort and 4.2 [2.8–7.2] years in the matched ECD cohort. Interestingly, the overall and death-censored graft survival (Figure 2 and 3 respectively) of the matched ECD cohort were similar

Table 3. Histological characteristics of kidneys accepted or rejected after frozen section zero-time biopsy analysis.

	Zero-time frozen section analysis $N = 92$		
	Accepted ECD $n = 43$	Rejected ECD $n = 49$	*P* value
Glomeruli analysed per biopsy (mean, range)	11 [9–15]	13 [9–21]	0.31
Glomerulosclerosis (%, range)	8 [0–15]	20 [15–33]	**<0.0001**
ci (*n*, %)			
0	25 (58.1)	12 (25)	**<0.0001**
1	16 (37.2)	13 (26.5)	
2	2 (4.7)	22 (44.9)	
3	0 (0)	1 (2.0)	
ct, (*n*, %)			
0	25 (58.1)	14 (29.2)	**<0.0001**
1	17 (39.5)	12 (24.5)	
2	1 (2.3)	21 (42.9)	
3	0 (0)	1 (2.0)	
ah, (*n*, %)			
0	2 (4.7)	2 (4.1)	**0.0001**
1	19 (46.5)	8 (16.7)	
2	17 (39.5)	19 (38.8)	
3	4 (9.3)	19 (38.8)	
cv, (*n*, %)			
0	1 (2.3)	2 (4.1)	**0.009**
1	14 (32.6)	6 (12.2)	
2	22 (51.2)	20 (45.4)	
3	4 (9.3)	16 (36.3)	
ci score (mean, SEM)	0.47 ± 0.09	1.25 ± 0.12	**<0.0001**
ct score (mean, SEM)	0.44 ± 0.08	1.19 ± 0.13	**<0.0001**
cv score (mean, SEM)	1.71 ± 0.11	2.14 ± 0.17	**0.0032**
ah score (mean, SEM)	1.55 ± 0.11	2.15 ± 0.12	**0.0006**
Remuzzi score* (mean, SEM)	3.4 ± 0.27	6.2 ± 0.33	**<0.0001**

Significant statistical results are in bold (when $P < 0.05$).

SEM, standard error of the mean; ci, interstitial fibrosis; ct, tubular atrophy; ah, arteriolar hyalinosis; cv, vascular fibrous intimal thickening.

*The Remuzzi score was calculated as previously published [16].

to the global ECD cohort. At 1-year and 5-year post-transplantation, death-censored graft survival of the matched cohort and the global ECD cohort were 95.3% vs. 92.5% and 85.7% and 85.9%, respectively. Details are shown in Table 4.

Additionally, graft function, estimated with eGFR, was similar at 1-year and 5-year post-transplantation between the matched ECD cohort and global ECD cohort: 44 [30–52] vs. 47 [36–57] ml/min/1.73m^2 and 42 [36–52] vs. 43 [34–55] ml/min/1.73m^2, respectively, P = ns as showed in Figure 4.

Finally, the graft survival and 1-year or 5-year graft function of the 43 transplanted kidneys after frozen section zero-time biopsy analysis were similar to the two other groups (Figures 2–4).

Discussion

In this retrospective study from one of the main French kidney transplant centres, zero-time histology on frozen section was used on nephrologist demand for the decision-making process for acceptance of kidneys. This strategy was mainly performed in donors with higher serum creatinine levels and hypertension history rates compared to our global ECD cohort and led to 53% of kidneys being discarded.

Interestingly, we found that the discarded kidney donors had higher KDRI global scores and poorer histological findings with more glomerulosclerosis, interstitial fibrosis and vascular lesions. More interestingly, using a propensity score to retrospectively select a group of 42 kidney recipients matched on donor histology

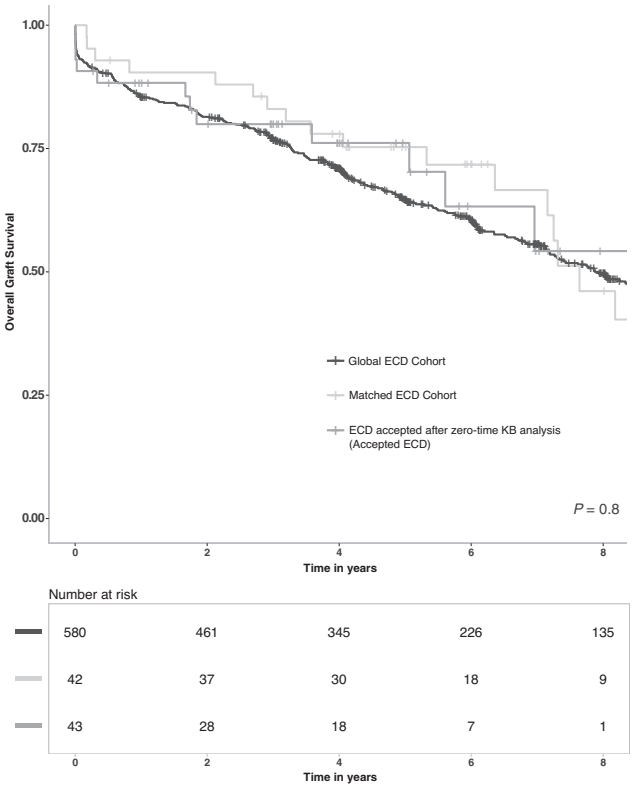

Figure 2 Overall graft survival. Kaplan–Meier curves of overall kidney allograft survival in the 'global ECD cohort', in the 'matched ECD cohort' and in the 'accepted ECD cohort' (transplanted ECD after frozen section zero-time kidney biopsy analysis).

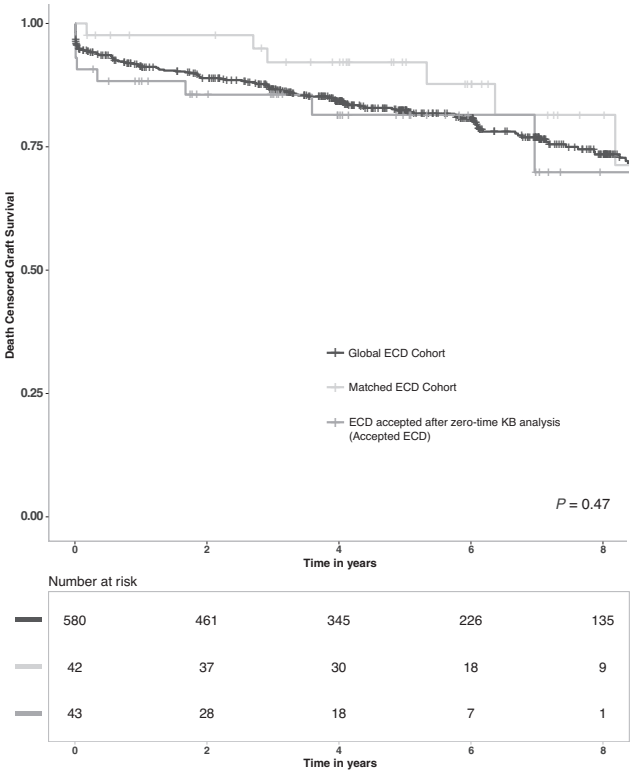

Figure 3 Death-censored graft survival. Kaplan–Meier curves of death-censored kidney allograft survival in the 'global ECD cohort', in the 'matched ECD cohort' and in the 'accepted ECD cohort' (transplanted ECD after frozen section zero-time kidney biopsy analysis).

(percentage of glomerulosclerosis, interstitial fibrosis and tubular atrophy, arteriosclerosis), age, serum creatinine and hypertension history of the donors whose kidney was rejected, we found that this group had a similar graft survival and kidney function at 1 and 5 years compared with the global ECD cohort. These results suggest that in ECD, zero-time histology may not be the major factor associated with graft survival. A kidney discard decision strategy based on zero-time histology analysis could lead to an unjustified increase in the organ turndown rate among ECD.

There are conflicting data on the impact of zero-time histology impact on graft survival and graft function [8,11–13] and it remains a matter of debate. UK transplant centres currently perform a prospective trial asking whether the introduction of a national, 24 h, digital histopathology service increases the number, and improves outcomes, of kidneys transplanted from older deceased donors [14]. Furthermore, the histological lesion best associated with graft dysfunction is not well determined: glomerulosclerosis and arteriosclerosis seem to be better associated with graft function than

Table 4. Overall survival, death-censored graft survival and losses to follow-up of the matched ECD cohort and the global ECD cohort.

	Matched ECD cohort $n = 42$	Global ECD cohort $n = 622$	P value
Death-censored graft loss (n, %)			
1 year post-transplant	2 (4.7%)	46 (7.5%)	0.52
2 years post-transplant	4 (9.5%)	59 (9.5%)	
3 years post-transplant	5 (11.9%)	71 (11.4%)	
5 years post-transplant	6 (14.3%)	88 (14.1%)	0.13
Deaths (n, %)			
1 year post-transplant	5 (11.9%)	35 (5.7%)	0.09
2 years post-transplant	5 (11.9%)	45 (7.2%)	
3 years post-transplant	6 (14.2%)	58 (9.3%)	
5 years post-transplant	12 (28.5%)	100 (16.1%)	**0.04**
Losses to follow-up (n, %)			
1 year post-transplant	0 (0%)	8 (1.2%)	0.45
2 years post-transplant	1 (2.3%)	12 (1.9%)	
3 years post-transplant	2 (4.8%)	34 (5.4%)	
5 years post-transplant	4 (9.5%)	115 (18.5%)	0.14

Significant statistical results are in bold (when $P < 0.05$).

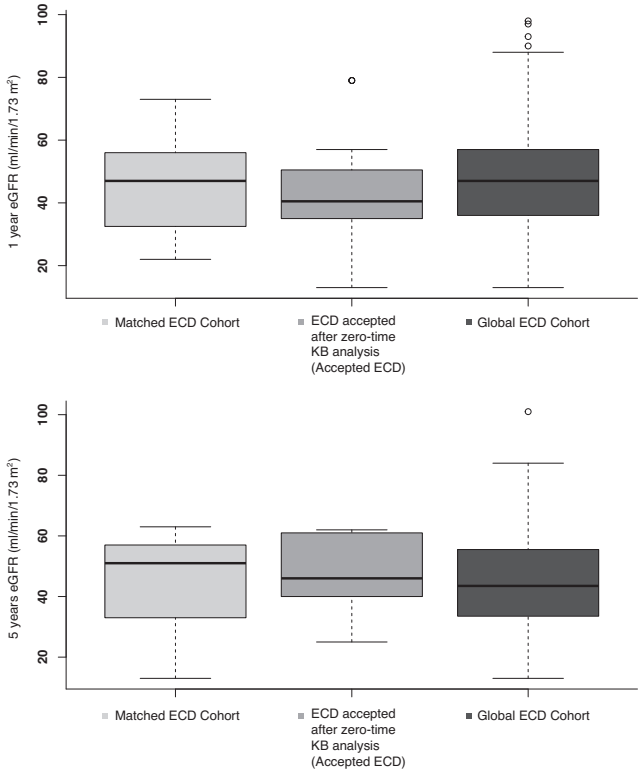

Figure 4 Kidney allograft estimated function (eGFR) at one year and five years post-transplant. Kidney allograft estimated function (eGFR) at one year and five years post-transplant in the global ECD cohort, in the matched ECD cohort and in the 'accepted ECD cohort' (transplanted ECD after frozen section zero-time kidney biopsy analysis).

interstitial fibrosis or arteriolar hyalinosis [5,8,15]. Even using composite histological scores, the association with graft failure was not consistent [16–18]. Two scores combining donor's clinical and histological data have been shown to be better associated with eGFR and graft survival but their predictive value was still moderate [19,20]. Importantly, the 2017 publication from the Banff working group on preimplantation biopsies did not recommend the use of unique rigid cut-offs, such as 20% of glomerulosclerosis, in decisions to discard kidneys [12]. Moreover, a large recent study among ECD showed no independent association between zero-time histology and graft survival whereas the presence of preformed DSA or cold ischaemia time were independent predictors of graft survival [9].

This lack of consistent data between zero-time kidney biopsy findings and graft outcome is of high clinical importance, since the decision-making process based on histology is used on a large scale in the United States, where the discard rate among kidneys from ECD is about 40% [5]. Even after the allocation policy changed and the introduction of the use of the KDRI/KDPI score, zero-time biopsy findings are one of the main reasons for kidney discard in the United States [21]. This high discard rate among ECD in the United States suggests that the transplant centres are very cautious on expanded criteria organs with poor histological findings. On the other hand, taking into account the organ shortage and the high mortality rates among patients on the waiting-list in the United States, kidney discard should be based on solid data for predicting graft survival.

The poor correlation between zero-time histology and graft survival could be explained by several factors. Timing, technique and pathologist experience [22] have an important impact on the quality of zero-time biopsy histological evaluation [11]. Indeed, a recent study[20] demonstrated that concordance between on-call pathologists and experienced renal pathologists was poor for several histological features, such as arterial intimal thickening, interstitial fibrosis, tubular atrophy and arteriolar hyalinosis. About twenty percent of kidneys discarded by on-call pathologists were considered acceptable by the experienced renal pathologists. In our centre, a specialized transplant pathologist analysed all the biopsies, decreasing the risk of over or underestimating lesions. However, frozen section analysis can be challenging due to images with a lower contrast and frost artefacts which could alter the tubulointerstitial compartment [19,23,24]. Indeed, rapid formalin fixation and paraffin embedding protocols are not widely available and frozen section analysis is generally used.

The type of biopsy can also affect the analysis. Our biopsies were performed by the surgeon with a core needle and some studies have shown that wedge biopsies could be more reliable and include more glomeruli [12]. However, other studies suggest that wedge biopsies overestimate glomerulosclerosis and discard rate due to the increase rate of sclerotic glomeruli in the superficial cortex [25,26]. The Banff working group on preimplantation biopsies also showed greater concordance in frozen wedge biopsies for the number of glomeruli, number of globally sclerosed glomeruli and interstitial inflammation compared with frozen core biopsies, and recommend the use of wedge biopsies.

It is important to notice that poor histological findings may have a great influence on nephrologist's kidney discard decision. This is suggested by other studies showing that glomerulosclerosis higher than 20% is associated with a 17-fold increased risk for discard [5] and by the discard strategy in the United States which is mainly driven by biopsy findings.

However, our study shows that the 'virtual' graft survival and function at one and five years post-

transplant of an ECD cohort matched on discarded kidneys donor characteristics was similar to the global ECD cohort, suggesting that zero-time histology may not be the major factor associated with graft survival and that strategy could lead to an unjustified increase in the organ turndown rate. That conclusion is also supported by a recent study evaluating zero-time histology in the United States and France [27]. Given these findings, our transplant centre stopped the practice of using zero-time biopsies to support the accept/decline decision.

We acknowledge the study's limitations. As it is a single-centre and retrospective study, we are unable to determine causation and the generalizability of our findings. Discard decision making is complex and multifactorial and even if the histological findings are strongly associated with ECD discard, other factors such as donor-recipient matching could have contributed to the final decision. With regards to propensitity score matching, we determined a matched cohort based on relevant clinical, histological and biological factors. Given the limited sample size, we were unable to include other potentially relevant factors. Finally, we acknowledge that with core needles the sample size is smaller compared to wedge biopsies which may affect histological score reliability. Twenty-five glomeruli were required in the original Remuzzi score, a number rarely obtained with core needles.

In conclusion, our findings do not encourage a discard decision based on frozen section zero-time kidney histology. As the benefit of kidney allocation based on zero-time histology is not conclusive, we think that this strategy could be deleterious for recipients on the waiting list. Many of these discarded kidneys could preclude some selected recipients from many years of dialysis-free survival, as recently highlighted by a study comparing discard strategies between France and the United States [28].

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Conflict of Interest

The authors of this manuscript declare no competing financial interest and no conflict of interests to disclose.

Authorship

YL and MR: designed the study. YL, MR, DA and AR: collected the data. YL, MJ, MR and OA: analysed the data. YL, MJ and MR: made the figures. YL and MR drafted the paper. YL, MJ, FM, LA, CT, LG, J-PDVH, FA, CL, DA and MR: revised the paper. All authors approved the final version of the manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Propensity score matching for rejected ECD.

Table S2 Supplemental characteristics of global ECD cohort, matched ECD cohort and accepted ECD.

Figure S1 Histological findings.

Figure S2 Matching strategy for survival study (propensity score).

Figure S3 Distribution of Propensity Scores.

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