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Pretransplant virtual PRA and long-term outcomes of kidney transplant recipients

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Summary

Virtual panel-reactive antibodies (vPRA) have been implemented to gauge sensitization worldwide. It is unclear how it associates with long-term outcomes, and its correlation with peak (pPRA) or actual (aPRA) has not been studied. We retrospectively reviewed data from 18- to 65-year-old kidney-only transplant patients during 1.1.1996–31.7.2011 in our center. PRAs were calculated based on solid-phase techniques. Of the 726 qualified cases, regardless of the PRA type, sensitized patients (PRA > 5%) had more females and previous transplant. Highly sensitized (HS, PRA > 50%) had longer waiting time, lower transplant rate, less living donor, more delayed graft function, and acute rejection. The conformity between vPRA and pPRA in HS was 75%, 57% between pPRA and aPRA. Forty-three percent (61/142) patients whose pPRA was >5% had no detectable aPRA and maintained similar outcomes as sensitized patients. Multivariate analysis showed consistently lower death-censored graft survival in HS defined by vPRA [HR 2.086 (95% CI 1.078–4.037), $P < 0.05$] and pPRA [HR 2.139 (95% CI 1.024–4.487), $P < 0.05$]. Both vPRA and pPRA provided reliable way estimating sensitization and predicting long-term graft survival, while aPRA might underestimate true sensitization. vPRA might be the most objective parameter to gauge sensitization.

Introduction

Panel-reactive antibody (PRA) has been used to measure pretransplant sensitization against HLA for decades [1–3]. However, it remains to be a mystifying parameter. When expressed as the percentage of positive cross-matches against a laboratory-specific panel of peripheral blood cells representative of the HLA-antigen frequency of the regional donor pool, it have been indicated as unreliable by laboratory quality controls [4,5]. The detection methods might be different from study to study, and the PRA threshold to define sensitization varies from 0 to 50% in the literatures [1,6–10]. It is also unclear to what extent higher PRA bears higher immunological risks, and whether or not a quantifiable relationship exists. In addition, it is debatable which PRA level, the pretransplant peak PRA (pPRA) or the actual PRA (aPRA) at the time of transplant, is more relevant [11–13] to graft survival.

With the introduction of solid-phase assays [6,14], the sensitivity of antibody specification has greatly improved, especially when using the Luminex Single Antigen Bead (SAB) test kits. From the HLA-antigen specificities obtained with this test, virtual PRA (vPRA), or calculated PRA (cPRA), has been used to predict the chance of having a positive cross-match. It is based on the antibody specification and reflects the antigen frequency in the

entire donor pool. Because of the potential of being more objective and reliable, it is being implemented worldwide. There has been no study to describe clinical characteristics related to vPRA, its association with sensitization events, or its predicting value in evaluating long-term outcomes comparing with the traditional PRAs. Therefore, we performed this retrospective study to explore the utility of vPRA in defining sensitization, its correlation with historically used PRAs, and its relationship with clinical outcomes.

Subjects and methods

Subjects

All kidney-only transplant patients, 18–65 years old at the time of transplantation, who were transplanted during January 1, 1996–July 31, 2011 in our center, were included. Strict avoidance of repeated mismatches and unacceptable antigens, defined by complement-dependant cytotoxicity assay (CDC) and solid-phase assays, was applied to all recipients [5,15]. All laboratory, clinical and medication data were recorded in our transplant database (TBase) [16,17]. Each graft was treated as a separate case.

PRA measurement, calculation, and terminology definition

For this study, only results of solid-phase analysis were used as the detection technique for PRA. This was performed by ELISA (Lambda Antigen Tray, One Lambda, Canoga Park, CA, USA) from 1996 to 2006. Since 2006, Luminex has become the main method, using either phenotype antigen beads or single antigen beads (LabScreen PRA or LabScreen Single Antigen, One Lambda, Canoga Park, CA, USA). Antibody specification prior to 2006 was carried out by CDC and ELISA, by CDC and Luminex SAB thereafter.

To calculate vPRA, all antibody specification results available within the study period were entered into the online vPRA calculator at Eurotransplant website (http://www.etrl.org/Virtual%20PRA/Default.aspx). With ELISA and Luminex phenotype beads, PRA was calculated as the percentage of positive reaction against a panel of blood donor HLA-antigens based on combined HLA class I (A and B) plus II (DR) specificities according to Eurotransplant HLA point calculation requirements.

We use the following PRA terms for subsequent analysis: vPRA was the highest value when using all antibody specification information, which might be based on different techniques depends on when the test was performed; pPRA was defined as the maximum PRA value of all pretransplant PRA measurements; aPRA was the PRA value immediately before transplant.

Clinical and laboratory data collection

Data retrieved from TBase included the following: gender, date of birth, date of initiating dialysis which was used to calculate waiting time, date of transplant, donor type (living vs. deceased), donor age, initial immunosuppression, development of delayed graft function (DGF), defined as needing dialysis within 7 days after transplant surgery, all episodes of biopsy-proven acute rejection (BPAR), first date of positive donor-specific antibody (DSA), date of returning to dialysis, and date of death.

Serum creatinine and 24-hour urine total protein at 3 months, 6 months, 1, 3, and 5 years after transplantation were collected. The four-variable Modification of Diet in Renal Disease (MDRD) equation [18] was used to calculate estimated glomerular filtration rate (eGFR).

Outcome measures

The outcome measures of interest were as follows: (1) patient survival, taken from the time of transplantation until death or end of observation; (2) death-censored graft survival, defined as re-initiation of dialysis, taken from the time of transplantation until returning to dialysis or end of observation; and (3) graft survival, defined as the composite end-point of patient death and graft failure, taken from the time of transplantation until death or returning to dialysis or end of observation. Outcome was followed completely, either through clinic visit in our center, or by communication with other centers if patient relocated [17]. End of observation date is November 25, 2011.

Statistics

All data were analyzed using SPSS version 20 (SPSS Inc., Chicago, IL, USA). Continuous variable was shown as mean $\pm$ standard deviation (SD). Group comparisons were made using ANOVA among more than three groups. Homogeneity of variables was tested using Levene analysis. *Post hoc* subgroup comparison between nonhomogenous variables was performed using Tamhane analysis; comparison between homogenous variables was performed by Bonferroni analysis. Student's *t*-test was used for 2-group comparison. Categorical variables were expressed as proportion compared with chi square test. A type I error rate below 5% ($P < 0.05$) was considered significant.

Kaplan–Meier survival curves were plotted for the effect of categorical variables on time of event. For this purpose, categorical transformations of the following continuous variables were performed using their median as cut-off value: recipient age at transplant, donor age, waiting time, and transplant period. Log-rank test was used to determine the significance between survival curves. Variables applied

to univariate analysis included the following: gender, donor type, previous transplant, DGF, BPAR, DSA, as well as enrollment in Eurotransplant Acceptable Mismatch (AM) program. Only variables with significant association ($P < 0.2$) in univariate analysis were then entered into multivariate analysis. All outcome measures were also analyzed in transplants performed before and after 2006 to evaluate potential era-effect of technique update. Separate models, each containing confounding covariates with a PRA factor (either vPRA, pPRA, or aPRA) fit to the data, were used.

Receiver operating characteristic (ROC) curves fitting was used to find the possible predictive cut-off points for vPRA, pPRA, aPRA, and change of PRA (ΔPRA) which was the difference between pPRA and aPRA, on outcome measures.

Results

We identified 726 18- to 65-year-old kidney-only transplants during January 1, 1996 to July 31, 2011 in our center with available pretransplant PRA, including vPRA, pPRA, and aPRA, results.

Demographics features

Patients were grouped into nonsensitized (NS, <5%), low (LS, 5–50%), and highly sensitized (HS, >50%) groups based on their vPRA levels. The demographics features of each group are shown in Table 1.

In general, sensitized groups, including both LS and HS, had more females and more with previous transplant. HS group has longer waiting time, lower transplant rates at least for the first 5 years, and received less living donation. More sensitized patient received induction with IL-2 inhibitors and had tacrolimus, rather than cyclosporine, initially. Waiting time and transplant rates were similar when patients were grouped based on aPRA or pPRA (data not shown), although the case number in each group was slightly different.

Table 1. Demographic features of 726 18- to 65-year-old kidney-only transplant† grouped by peak virtual panel-reactive antibody.

			Groups			
		Total	NS (<5%)	LS (5–50%)	HS (>50%)	*P*‡
N (%)		726	606 (83.5)	45 (6.2)	75 (10.3)	
Male *N* (%)		449 (61.8)	392 (64.7)	24 (53.3)*	33 (44)*	0.001
Age at transplant (years)§		44.3 ± 12.3	44.6 ± 12.3	44.2 ± 12.5	42.7 ± 11.4	0.358
Living donation (%)		253 (34.8)	231 (38.3)	13 (28.9)	8 (10.7)*,Δ	<0.001
Donor Age (years)§		47.6 ± 13.1	47.7 ± 13.2	46.9 ± 11.8	47.1 ± 13.7	0.866
Previous transplant	0	612 (84.3)	567 (93.6)	20 (44.4)*	25 (33.3)*,Δ	<0.001
	1	98 (13.5)	35 (5.8)	22 (48.9)*	41 (54.7)*,Δ	
	2	15 (2.1)	4 (0.7)	3 (6.7)*	8 (10.7)*,Δ	
	3	1 (0.1)	0	0	1 (1.3)	
Transplant	Waiting time (years)§	4.2 ± 0.1	4.1 ± 0.1	4.0 ± 0.5	5.5 ± 0.5*,Δ	0.008¶
Accumulated transplant rate (%)	1-year	47.7	52.5	35.6	16	
	3-year	69.4	73.8	64.4	37.3	
	5-year	81.1	83.5	75.6	65.3	
Mismatch	Broad§	2.4 ± 1.6	2.4 ± 1.6	2.1 ± 1.6	2.4 ± 1.4	0.581
	Split§	1.4 ± 1.1	1.4 ± 1.1	1.3 ± 1.1	1.3 ± 1.2	0.718
Initial Immunosuppression**	ATG	8 (1.1)	7 (1.2)	1 (2.3)	0 (0)	0.529
	IL-2 inhibitors	359 (49.4)	288 (46.6)	31 (70.5)*	40 (62.5)*	0.001
	Cyclosporine	392 (54)	360 (58.3)	17 (38.6)*	15 (23.4)*	<0.001
	Tacrolimus	261 (36)	213 (34.8)	18 (40.9)	30 (46.9)*	0.112
	mTOR inhibitors	49 (6.7)	44 (7.1)	1 (2.3)	4 (6.3)	0.458
	MPA	569 (78.4)	488 (79)	35 (79.5)	46 (71.9)	0.415
	Other	94 (12.9)	84 (13.6)	4 (9.1)	6 (9.4)	0.465
Follow-up time (years)§		5.5 ± 3.9	5.8 ± 3.9	4.4 ± 3.3*	4.5 ± 3.4*,Δ	0.004

HS, high sensitization; LS, low sensitization; NS, nonsensitization; ATG, antithymoglobulin; IL-2, interlukin-2; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin.

*$P < 0.05$ comparing with NS.

Δ$P < 0.05$ comparing with LS.

†Results presented as "case number (percentage of the group)" unless indicated otherwise.

‡Comparison made among <5, 5–50 and >50% groups using ANOVA unless otherwise indicated.

§Results presented as mean±Standard Deviation.

¶Results are from Kaplan–Meier log-rank analysis.

**Steroids usage was 100% in each group and was therefore not included.

Delayed graft function (DGF)

NS had lower incidence of DGF (17.5%, $P < 0.001$) compared with LS (31.4%) and HS (36%) group. Grouping with aPRA (30.6% in LS, 33.3% in HS vs. 18.8% in NS, $P = 0.018$) and pPRA (36.5% in LS, 34.3% in HS vs. 16.6% in NS, $P < 0.001$) rendered similar result. No difference between LS and HS group.

Biopsy-proven acute rejection (BPAR) and development of post-transplant Donor-specific antibody (DSA)

BPAR in both LS and HS were significantly higher than NS (17.8% in LS, 14.7% in HS vs. 6.8% in NS, $P < 0.001$), although the time to the first BPAR episode was not different among these groups (overall mean 22 ± 33.1 months, median 4.3 months. 27 ± 36 months for NS, 12.6 ± 22 months for LS, 15.8 ± 30.6 months for HS, $P = 0.4$). HS had significantly higher rate of positive DSA (33.3%) after transplantation, comparing with LS (17.8%, $P = 0.03$) and NS (14.9%, $P < 0.001$). Time to first detected DSA were shorter for HS (17.9 ± 4.8 months) and LS (13.6 ± 13.1 months) comparing with NS (46.3 ± 4.3 months, $P < 0.001$. Overall, mean 41.4 ± 42.7 months, median 25.3 months). These differences remained when using pPRA and aPRA.

Laboratory results

As shown in Table 2, there was no difference in eGFR among groups. When proteinuria was categorized into <500, 500–1000 and >1000 mg/day, only LS group at 5-year and HS group at 3-month had relatively more patients with proteinuria 500–1000 mg/day and >1000 mg/day (Table 3). In general, however, most detected proteinuria was mild at any time point without notable differences among groups. These trends were similar in aPRA- and pPRA-based groups.

Case number difference when using different PRA terms

As shown in Table 4, the nonsensitized population defined was highly constant with all PRA terms. The conformity between vPRA and pPRA or aPRA in HS patients was in high 70%, however, relatively low between pPRA and aPRA. Of the 142 sensitized patients according to pPRA, 69 (43%) had no detectable sensitization immediately before transplant.

Survival

Of the 726 transplants, 98 returned to dialysis; 83 died, 60 (72.3%) died with a functioning graft. Survival for groups based on vPRA, pPRA, and aPRA were analyzed first. The effect of conversion, that is those who were "nonsensitized" in the immediate pretransplant serum (aPRA < 5%) but had a history of sensitization (pPRA > 5%), was then examined to assess the relevance of aPRA vs. pPRA. Kaplan–Meier survival and log-rank test results for vPRA and the conversion effect on outcomes were summarized in Fig. 1. Surviving curves for aPRA and pPRA were similar to vPRA and were not shown.

Patient survival (Fig. 1–a1) was not affected by vPRA-based sensitization, however, statistically inferior in the LS group when using pPRA, which persisted even when they were converted to NS immediately before transplant (Table 5). Both LS and HS had lower rate of death-censored graft survival (Fig. 1–a2) and graft survival (Fig. 1–a3) with shorter time to event when using vPRA and pPRA.

Interestingly, the converters with a pretransplant aPRA <5% and a history of sensitization (pPRA > 5%) had the same death-censored graft survival (Fig. 1–b2) and graft survival rates (Fig. 1–b3) as those who remained sensitized, both were significantly lower comparing with patients whose PRA were persistently <5%. Analysis of actual vPRA levels (vPRA at time of transplant) provided similar results. Patients with vPRA converted from sensitized to nonsensitized had the same graft survival and death-censored graft survival (data not shown).

Table 2. Estimated GFR at different time point post-transplant.

Time post-transplant†	Group	*N*	eGFR (ml/min)‡	*P*§
1-month	NS (<5%)	560	46 ± 24.2	0.646
	LS (5–50%)	39	42.7 ± 21.5	
	HS (>50%)	71	39.2 ± 23.3	
3-month	NS (<5%)	557	50.5 ± 21.6	0.494
	LS (5–50%)	36	49.7 ± 23.2	
	HS (>50%)	71	44.9 ± 22.9	
6-month	NS (<5%)	563	52.2 ± 22.4	0.392
	LS (5–50%)	39	49.9 ± 25.3	
	HS (>50%)	72	46.7 ± 21.2	
1-year	NS (<5%)	540	53.3 ± 21	0.987
	LS (5–50%)	35	48.5 ± 20.9	
	HS (>50%)	64	47.2 ± 20.3	
3-year	NS (<5%)	430	50.9 ± 20.4	0.244
	LS (5–50%)	23	44 ± 25.4	
	HS (>50%)	46	41.4 ± 20.8*	
5-year	NS (<5%)	303	49.2 ± 18.7	0.096
	LS (5–50%)	16	40 ± 23.2	
	HS (>50%)	24	40 ± 19.4	

eGFR, estimated glomerular filtration rate; HS: high sensitization; LS: low sensitization; NS: nonsensitization.

*$P < 0.05$ compared with <5 group in post hoc analysis.

†Results beyond 5-year were not compared due to limited availability in LS and HS.

‡Results presented as mean ± standard deviation.

§Comparison made among all three groups.

Table 3. Case distribution in proteinuria category of groups based on virtual PRA at different time point post-transplant.

Time post-transplant†	Virtual PRA Group	*N*	<500 mg/day *N* (%)‡	500–1000 mg/day *N* (%)‡	>1000 mg/day *N* (%)‡	*P*§
3-month	NS (<5%)	414	358 (86.5)	38 (9.2)	18 (4.3)	0.302
	LS (5–50%)	28	23 (82.1)	3 (10.7)	2 (7.1)	
	HS (>50%)*,Δ	53	40 (75.5)	9 (17)	4 (7.5)	
6-month	NS (<5%)	458	402 (87.8)	39 (8.5)	17 (3.7)	0.351
	LS (5–50%)	35	30 (85.7)	2 (5.7)	3 (8.6)	
	HS (>50%)	62	50 (80.6)	8 (12.9)	4 (6.5)	
1-year	NS (<5%)	418	370 (88.5)	30 (7.2)	18 (4.3)	0.202
	LS (5–50%)	30	23 (76.7)	3 (10)	4 (13.3)	
	HS (>50%)	54	47 (87)	3 (5.6)	4 (7.4)	
3-year	NS (<5%)	325	277 (85.2)	24 (7.4)	24 (7.4)	0.255
	LS (5–50%)	17	12 (70.6)	3 (17.6)	2 (11.8)	
	HS (>50%)	42	32 (72.1)	4 (9.5)	6 (14.3)	
5-year	NS (<5%)	252	220 (87.3)	16 (6.3)	16 (6.3)	<0.001
	LS (5–50%)*	11	4 (36.4)	2 (18.2)	3 (27.3)	
	HS (>50%)	22	17 (77.3)	2 (9.1)	3 (13.6)	

PRA: panel-reactive antibody; HS: high sensitization; LS: low sensitization; NS: nonsensitization.
*$P < 0.05$ comparing with NS.
Δ$P < 0.05$ comparing with LS.
†Results beyond 5-year were not compared due to limited availability in LS and HS.
‡Results presented as "case number (percentage of the group)".
§Comparison made among all three groups.

Table 4. (A) Case Number Difference in groups based on virtual PRA and peak PRA. (B) Case Number Difference in groups based on virtual PRA and actual PRA. (C) Case Number Difference in groups based on peak PRA and actual PRA.

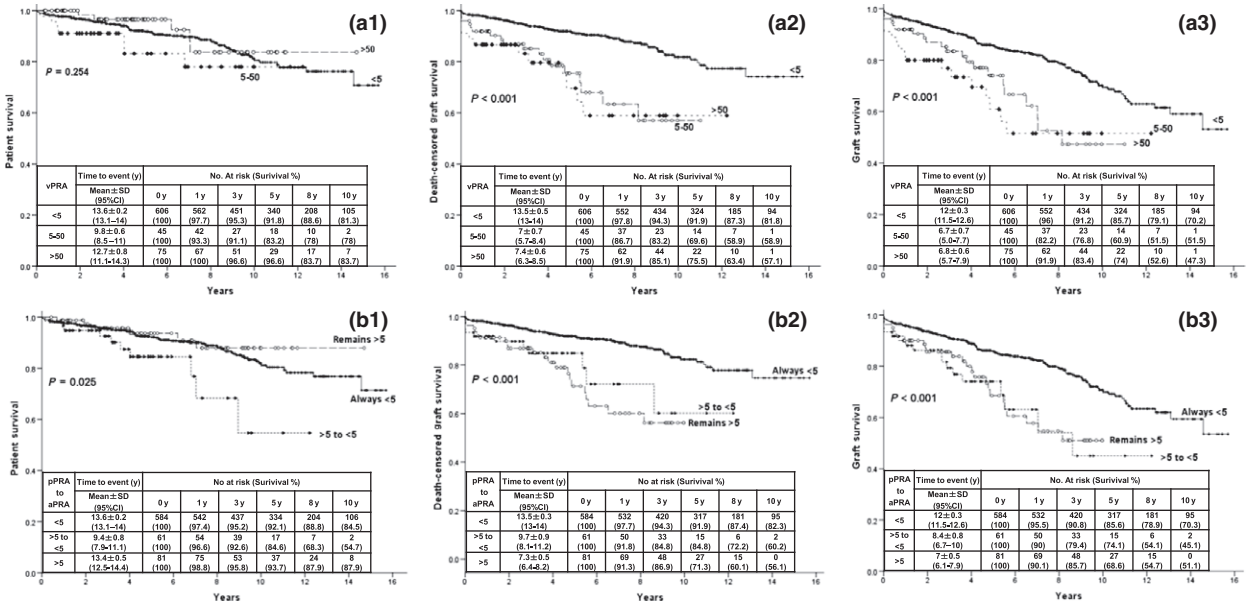
		Virtual PRA groups			
		<5	5–50	>50	Total
(A)					
Peak PRA groups	<5	584 (100%)	0 (0%)	0 (0%)	584
	5–50	21 (33%)	26 (41%)	16 (25%)	63
	>50	1 (1%)	19 (24%)	59 (75%)	79
Total		606	45	75	726
(B)					
Actual PRA groups	<5	597 (92.6%)	21 (3.3%)	27 (4.2%)	645
	5–50	9 (25%)	14 (38.9%)	13 (36.1%)	36
	>50	0 (0%)	10 (22.2%)	35 (77.8%)	45
Total		645	36	45	726
		Actual PRA groups			
(C)					
Peak PRA groups	<5	584 (100%)	0 (0%)	0 (0%)	584
	5–50	33 (52%)	30 (48%)	0 (0%)	63
	>50	28 (35%)	6 (8%)	45 (57%)	79
Total		645	36	45	726

PRA, panel-reactive antibody.

Patients enrolled in the AM program were not analyzed separately with only two deaths and four graft losses.

Multivariate outcome analysis

Table 5 summaries all significant confounding factors in either univariate or multivariate analysis. HS group defined by vPRA or pPRA, but not aPRA, rendered significantly higher risk for death-censored graft failure than NS after multivariate analysis, although the hazard ratio (HR) was only 1/10 of BPAR. The LS group defined by pPRA was associated with a higher risk of death and was the only group significant for graft failure, along with older age, DGF and BPAR.



vPRA	Time to event (y) Mean±SD (95%CI)	No. At risk (Survival %) 0 y	1 y	3 y	5 y	8 y	10 y
<5	13.6±0.2 (13.1–14)	606 (100)	562 (97.7)	451 (95.3)	340 (91.8)	208 (88.6)	105 (81.3)
5–50	9.8±0.6 (8.5–11)	45 (100)	42 (93.3)	27 (91.1)	18 (83.2)	10 (78)	2 (78)
>50	12.7±0.8 (11.1–14.3)	75 (100)	67 (100)	51 (96.6)	29 (96.6)	17 (83.7)	7 (83.7)

vPRA	Time to event (y) Mean±SD (95%CI)	No. At risk (Survival %) 0 y	1 y	3 y	5 y	8 y	10 y
<5	13.5±0.5 (13–14)	606 (100)	552 (97.8)	434 (94.3)	324 (91.9)	185 (87.3)	94 (81.8)
5–50	7±0.7 (5.7–8.4)	45 (100)	37 (86.7)	23 (83.2)	14 (69.6)	7 (58.9)	1 (58.9)
>50	7.4±0.6 (6.3–8.5)	75 (100)	62 (91.9)	44 (85.1)	22 (75.5)	10 (63.4)	1 (57.1)

vPRA	Time to event (y) Mean±SD (95%CI)	No. At risk (Survival %) 0 y	1 y	3 y	5 y	8 y	10 y
<5	12±0.3 (11.5–12.6)	606 (100)	552 (96)	434 (91.2)	324 (85.7)	185 (79.1)	94 (70.2)
5–50	6.7±0.7 (5.0–7.7)	45 (100)	37 (82.2)	23 (76.8)	14 (60.9)	7 (51.5)	1 (51.5)
>50	6.8±0.6 (5.7–7.9)	75 (100)	62 (91.9)	44 (83.4)	22 (74)	10 (52.6)	1 (47.3)

pPRA to aPRA	Time to event (y) Mean±SD (95%CI)	No at risk (Survival %) 0 y	1 y	3 y	5 y	8 y	10 y
<5	13.6±0.2 (13.1–14)	584 (100)	542 (97.4)	437 (95.2)	334 (92.1)	204 (88.8)	106 (84.5)
>5 to <5	9.4±0.8 (7.9–11.1)	61 (100)	54 (96.6)	39 (92.6)	17 (84.6)	7 (68.3)	2 (54.7)
>5	13.4±0.5 (12.5–14.4)	81 (100)	75 (98.8)	53 (95.8)	37 (93.7)	24 (87.9)	8 (87.9)

pPRA to aPRA	Time to event (y) Mean±SD (95%CI)	No at risk (Survival %) 0 y	1 y	3 y	5 y	8 y	10 y
<5	13.5±0.3 (13–14)	584 (100)	532 (97.7)	420 (94.3)	317 (91.9)	181 (87.4)	95 (82.3)
>5 to <5	9.7±0.9 (8.1–11.2)	61 (100)	50 (91.8)	33 (84.8)	15 (84.6)	6 (72.2)	2 (60.2)
>5	7.3±0.5 (6.4–8.2)	81 (100)	69 (91.3)	48 (86.9)	27 (71.3)	15 (60.1)	6 (56.1)

pPRA to aPRA	Time to event (y) Mean±SD (95%CI)	No. At risk (Survival %) 0 y	1 y	3 y	5 y	8 y	10 y
<5	12±0.3 (11.5–12.6)	584 (100)	532 (95.5)	420 (90.8)	317 (85.6)	181 (78.9)	95 (70.3)
>5 to <5	8.4±0.8 (6.7–10)	61 (100)	50 (90)	33 (79.4)	15 (74.1)	6 (54.1)	2 (45.1)
>5	7±0.5 (6.1–7.9)	81 (100)	69 (90.1)	48 (85.7)	27 (68.6)	15 (54.7)	6 (51.1)

Figure 1 Kaplan–Meier survival curves of outcomes. The *X*-axis is the time to event expressed in years. *Y*-axis is the cumulative survival rate. Panel a (upper panel). Analysis on groups based on peak virtual PRA level. Groups are: <5%, 5–50%, >50%; Panel b (lower panel). Analysis on groups based on group conversion from peak PRA to actual PRA. Groups are: always <5%, >5 to <5%, remained >5%. a–1. Patient survival in peak vPRA-based groups; a–2. Death-censored graft survival in peak vPRA-based groups; a–3. Graft survival in peak vPRA-based groups. b–1. Patient survival in groups based on PRA conversion; b–2. Death-censored graft survival in groups based on PRA conversion; b–3. Graft survival in groups based on PRA conversion. PRA: panel-reactive antibody; pPRA: peak panel-reactive antibody; vPRA: virtual panel-reactive antibody; aPRA: actual panel-reactive antibody; *N*: total number; SD: standard deviation; CI: confidence interval; y: year.

To analyze the effect of the introduction of the Luminex technology in 2006, we also analyzed outcomes for cases before and after 2006. There were 385 transplants performed before 2006 in our cohort, 341 after 2006. Both LS and HS defined by vPRA in transplant after 2006 were associated with increased risk of death-censored graft failure comparing with NS (LS vs. NS: HR 4.5 (1.3–15.6); HS vs. NS: HR 3.5 (1.3–9.7), $P < 0.05$ for both).

Receiver operated cut-off (ROC) analysis

To determine a potential PRA cut-off that separates patients with inferior outcomes, we performed ROC analysis for each outcome using different PRAs. There was no significant predictive threshold of any PRA for any of the outcomes measured in ROC curve fitting, with all area under the curves (AUC) close to 0.5.

Discussion

Here, we present a study evaluating the clinical suitability of vPRA, examining its association with other historically used PRA terms, and comparing their value in predicting long-term patient and graft outcomes, with extra emphasis to clarify the terminology and to eliminate confusion. To our knowledge, this is the first effort to portrait the clinical picture, immunological characteristics and risks, and to assess whether the recently introduced virtual PRA value is a clinically meaningful parameter on which organ allocation can be based.

We first assessed whether or not vPRA was able to reveal past exposure and manifest immunological risks. Sensitized patients based on vPRA, including both LS and HS, were mostly females and with history of transplant, in agreement with observations when using other PRAs [6,11,19]. Other features, such as higher percentage of DGF, needing for stronger immunosuppression, higher percentage of DSA as well as more BPAR episodes, were also similar with different PRA methods. As expected, highly sensitized patients experienced significantly longer waiting time and lower transplant rate, reflecting the challenge in finding a suitable match. PRA does not have a simple linear association with waiting time, which is not surprising given the large donor pool in Eurotransplant and an allocation policy which aims to counteract such differences (e.g. points on mismatch probability and acceptable mismatch program). In our cohort, the transplant rate in all waitlisted patients with comparable sensitization was similar to the Eurotransplant region [20].

Although it is expected to see differences in PRA levels when using different detection methods, the scope of this

Table 5. Estimates of confounding predictors* for outcomes using Cox proportional hazards model.

		Univariate analysis		Multivariate analysis	
Outcomes	Predictors	HR (95% CI)	*P*	HR (95% CI)	*P*
Patient Survival	vPRA	–	0.264	–	0.245
	5–50 vs. <5	1.8 (0.8–3.8)	0.160	1.8 (0.8–3.9)	0.144
	>50 vs. <5	0.7 (0.3–1.8)	0.472	0.7 (0.3–1.8)	0.493
	Age at transplant, >46 vs. <46†	2.9 (2.2–3.8)	<0.001	3.7 (2.3–6.1)	<0.001
	Donor-specific antibody†	3.0 (1.3–6.9)	0.009	0.5 (0.2–1.1)	0.076
	Deceased vs. living donor†	1.7 (1.1–2.8)	0.022	1.2 (0.7–2.0)	0.449
	Delayed graft function†	2.0 (1.2–3.2)	0.006	2.0 (1.2–3.4)	0.009
	pPRA	–	0.006	–	0.018
	5–50 vs. <5	2.6 (1.4–5.1)	0.003	2.3 (1.2–4.5)	0.012
	>50 vs. <5	0.6 (0.3–1.5)	0.309	0.6 (0.3–1.6)	0.327
	aPRA	–	0.274	–	0.334
	5–50 vs. <5	1.4 (0.5–3.8)	0.535	1.4 (0.5–3.9)	0.516
	>50 vs. <5	0.4 (0.1–1.4)	0.144	0.4 (0.1–1.6)	0.192
Death-censored Graft Survival	vPRA	–	0.000	–	0.078
	5–50 vs. <5	3.5 (1.9–6.5)	0.000	2.1 (0.8–5.0)	0.111
	>50 vs. <5	3.2 (1.9–5.4)	0.000	2.1 (1.1–4.0)	0.029
	Previous transplant†	1.9 (1.2–2.9)	0.003	0.8 (0.4–1.5)	0.469
	Donor-specific antibody	2.3 (1.5–3.4)	<0.001	0.8 (0.5–1.4)	0.491
	Deceased versus living donor†	1.7 (1.1–2.6)	0.016	1.3 (0.8–2.1)	0.249
	Delayed graft function†	1.6 (1.0–2.6)	0.04	1.4 (0.8–2.5)	0.209
	Biopsy-proven acute rejection†	21.8 (14.8–32.2)	<0.001	20.9 (13.7–31.9)	<0.001
	pPRA	–	0.000	–	0.065
	5–50 vs. <5	3.1 (1.7–5.6)	0.000	2.0 (1.0–3.9)	0.050
	>50 vs. <5	3.1 (1.9–5.2)	0.000	2.1 (1.0–4.5)	0.044
	aPRA	–	0.000	–	0.738
	5–50 vs. <5	2.3 (1.1–5.0)	0.036	1.1 (0.5–2.8)	0.785
	>50 vs. <5	3.7 (2.1–6.4)	0.000	1.4 (0.6–2.9)	0.436
Graft Survival	vPRA	–	0.000	–	0.083
	5–50 vs. <5	2.7 (1.6–4.6)	0.000	2.0 (1.0–4.1)	0.050
	>50 vs. <5	2.2 (1.3–3.4)	0.001	1.7 (1.0–3.0)	0.067
	Age at transplant, >46 vs. <46†	1.8 (1.1–2.2)	<0.001	2.0 (1.4–2.7)	<0.001
	Previous transplant†	1.5 (1.1–2.1)	0.021	0.9 (0.5–1.5)	0.642
	Deceased versus living donor†	1.8 (1.3–2.5)	0.001	1.3 (0.9–1.9)	0.179
	Delayed graft function†	1.8 (1.3–2.6)	0.001	1.6 (1.1–2.5)	0.019
	Biopsy-proven acute rejection†	7.1 (5.4–9.4)	<0.001	9.9 (7.0–14.0)	<0.001
	pPRA	–	0.000	–	0.101
	5–50 vs. <5	2.5 (1.5–4.1)	0.000	1.8 (1.0–3.1)	0.045
	>50 vs. <5	2.2 (1.4–3.4)	0.001	1.6 (0.9–2.9)	0.136
	aPRA	–	0.002	–	0.812
	5–50 vs. <5	1.6 (0.8–3.2)	0.226	1.0 (0.5–2.2)	0.986
	>50 vs. <5	2.4 (1.5–4.0)	0.001	1.2 (0.6–2.3)	0.535

CI: confidence interval; PRA: panel-reactive antibody; pPRA: peak panel-reactive antibody; vPRA: virtual panel-reactive antibody; y: year.
*Variables applied to univariate analysis included: recipient age at transplant, donor age, waiting time, transplant period, gender, donor type, previous transplant, DGF, BPAR, DSA. Variables with significant association in univariate analysis were then entered into multivariate analysis. Only factors that were significant in either univariate or multivariate analysis are included in this table.
†Values are from the univariate and multivariate analysis when using vPRA. They are slightly different when using aPRA and pPRA; however, the statistical significance does not change.

problem has not been investigated so far. We observed that nonsensitized patients, when set as PRA < 5%, were reliably defined regardless of the PRA, and had the best outcomes. The overall prevalence of sensitization was similar, ranging from 11% to 18%, consistent with the entire Eurotransplant area, which is 14.3% [20]. For sensitized patients, they could belong to HS or LS when using different terms. This denotes the fluctuation of the antibody titer itself and differences in the detection and calculation methods. While aPRA is always less or equal to pPRA based on the definition we used, the relationship between pPRA and vPRA is less predictable. Using a panel to estimate PRA results in variation and poor reproducibility across different laboratories [4]. In contrast, vPRA is the antigen frequency against the entire donor pool. Once the antibody specification information is available, vPRA can be obtained, and its level only changes if the antibody profile changes, therefore more consistent and dependable. The introduction of more advanced antibody technique, such as SAB, gives more precision in antibody detection nowadays, making vPRA and the final virtual cross-match more reliable. Due to the potential $100,000 a year saving in preliminary screening, virtual cross-match protocol is being applied widely [21].

The change of pPRA to aPRA on long-term outcome is controversial [12,22]. While a low aPRA might reflect relative quiescent immune activity for the time being, pPRA records historical sensitizing events that could lead to immunological memory [23,24]. This might explain that patients who converted from sensitized to nonsensitized in our cohort still had similar outcomes as the sensitized patients. Multicenter studies and the increasingly popular registries-based data analysis tend to use aPRA [25,26]. Based on our findings, we believe that using aPRA as the sole measure may underestimate true sensitization and post-transplant immunological risks.

Despite the long use of PRA [8,27,28], and a commonly embraced belief that higher PRA is associated with worse long-term outcomes, there are surprisingly limited and inconsistent results on how the degree of PRA is associated with outcomes. The OPTN/UNOS registry showed that the 1-year and 5-year graft survival rates were the same for PRA 0, 1–49 and 50–100% in adults [6]. The USRDS data exhibited a graded association between PRA at the time of transplant and adjusted risk of death-censored graft failure, death with functioning graft, and the combined event of graft failure and death [26]. A multicenter study found inferior short- and long-term graft outcomes with increasing PRA level [25]. Meanwhile, PRA > 40% in pediatric population had a lower 5-year graft survival [10]. Mai, *et al.* [8] achieved equivalent 3-year graft survival in two sensitized cohorts with PRA > 20% and patients with PRA < 20%. It is difficult to compare results from studies of different patient populations, interventions, immunosuppression regimens, as well as differently defined PRA regarding its detection technique, pretransplant time point and cut-offs.

We identified significant association of sensitization with inferior death-censored graft survival in HS, but not LS group, when using vPRA or pPRA after multivariate analysis. The association between vPRA and death-censored graft failure was more prominent after 2006 when Luminex technology was introduced. This may suggest improved predictive value of vPRA over time. Important clinical trait such as BPAR, which was associated with an overwhelmingly high risk for graft loss, was the same in HS and LS. Similarly, the development of DSA after transplantation was statistically higher in HS group. However, a threshold could not be identified in ROC analysis. When using vPRA as continuous variable for multivariate analysis, HR only increases 1.5% for every one point increase of vPRA (data not shown). It is therefore prudent not to consider LS as harmless until larger study clearly demonstrate clinically meaningful differences in outcome based on sensitization status.

In our cohort, older age and DGF were associated with higher risk of death. Sensitization was irrelevant, as with most of other studies except for very few [26,29]. LS group defined by pPRA did show higher risk of death. The reason is not obvious by this study. One possible explanation is that other factors not discussed in our study, such as baseline disease, comorbidities, etc. might have played a role given that the demographic features in HS and LS were similar even when using different PRA terms. Overall, graft survival certainly reflected the combination effect of patient survival and death-censored graft survival.

The significance of aPRA for graft survival in univariate analysis did not sustain, again reflecting that aPRA might underestimate the true sensitization, a notion worth remembering when referring to certain study or large-scaled national registries. Cautions should be taken when relying on aPRA solely for sensitization evaluation, and converting from being sensitized to nonsensitized ought not to imply reduced immunological risks. Despite our strict policy to avoid all known antigens, we could not avoid unknown donor antigens. In Eurotransplant donor typing for DQ and DP was not performed until recently, when DQ typing became mandatory. Our results confirm the hypothesis that history of pretransplant DSA is important and all DSA should be avoided in light of extensive donor typing.

There are several limitations of our study. This is a retrospective observational study not designed to address risk factors of each outcome. Donor selection and immunosuppression were likely more rigorous in highly sensitized patients, two factors that could markedly improve graft

survival. Not all confounding factors for outcome were included, for example, other immunological factors such as DP and DQ antibody, comorbidities or baseline disease, change in practise pattern or policies, etc. Therefore, we are unable to propose a pathological cause of the outcomes, or to comment on the complex association between PRA and clinical events or outcomes. A possible era-effect due to progress in laboratory technique, medication availability, or revision in guidelines is difficult to be completely eliminated for subjects spanned more than a decade. A transplant performed after 2006 could have pPRA and vPRA based on measurement before 2006; however, the significance of our results should remain since the CDC-based calculation might give falsely low level. The sample size of the sensitized subgroups was small especially if using a more comprehensive grouping. Larger study free of variation in laboratory methods and terminology confusion is required to firmly estimate whether or not there is a pro rata increase for any of the outcomes with a higher level of PRA/vPRA. As we could not find a cut-off in any PRA method, and AUC values in the ROC analysis were around 0.5, this effect might be smaller than anticipated. The promising value of vPRA in outcome prediction certainly needs to be validated with more homogeneous data.

Our cohort was less diverse ethnically as compared to other populations studied [6,8,10], and patients who were enrolled in the Eurotransplant Senior Program were excluded. As our standard practice, we emphasize on thorough characterization of potential harmful HLA-antibodies, strict sensitization prevention, as well as avoidance of unacceptable antigens and repeated mismatch. CDC crossmatch is always performed before transplant. We do not perform desensitization because our dialysis survival was good [30] although waiting time was slightly longer. As only well-matched organs are transplanted, all sensitized patients are receiving basiliximab induction and standard triple immunosuppression. Until we have solid evidence, it is our belief that, to reach the most long-term benefit of renal transplants, it is critical to practise sensitization prevention [5]. Therefore, strengths of our study include a uniform allocation policy with strict avoidance of unacceptable antigens, complete follow-up, a standardized induction and maintenance immunosuppression protocol, and uniform post-transplant monitoring, in contrast to previous reports from registry data or single centers, where heterogeneous pre-transplant histocompatibility testing and induction immunosuppression protocol were unavoidable.

In conclusion, sensitized patients defined by peak, actual, or vPRA all demonstrated more past sensitisation, additional difficulty in receiving a transplant, and higher rate of post-transplant immunological complications. High sensitization, defined by either vPRA or pPRA, correlates to inferior graft outcome, and aPRA may underestimate true sensitization. vPRA appears to be the overall most attractive parameter to gauge sensitization in that it provides a more reproducible way of measuring and reporting, gives more accurate estimate on the match probability, and might be a reliable measure to predict long-term graft survival.

Authorship

LH: performed study, collected data, analyzed data, wrote the paper. NL: designed study, performed study, analyzed data, edited the paper. MN: performed study, collected data, analyzed data, edited the paper. MN: analyzed data, edited the paper. LL, PG, DS, FH, JW, SB and HHN: collected data, edited the paper. CS: designed study, analyzed data, edited the paper. KB: designed study, collected data, analyzed data, wrote the paper.

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