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BRIEF COMMUNICATION

HLA Immune Response Genetics WILEY

Ellipro scores of donor epitope specific HLA antibodies are not associated with kidney graft survival

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In kidney transplantation, donor HLA antibodies are a risk factor for graft loss. Accessibility of donor eplets for HLA antibodies is predicted by the ElliPro score. The clinical usefulness of those scores in relation to transplant outcome is unknown. In a large Dutch kidney transplant cohort, Ellipro scores of pre-transplant donor antibodies that can be assigned to known eplets (donor epitope specific HLA antibodies [DESAs]) were compared between early graft failure and long surviving deceased donor transplants. We did not observe a significant Ellipro score difference between the two cohorts, nor significant differences in graft survival between transplants with DESAs having high versus

Abbreviations: DESA, donor epitope specific antibodies; HLA, anti-human leucocyte antigen; PI, protrusion index; SAB, single antigen bead.

For affiliations refer to page 5

low total Ellipro scores. We conclude that Ellipro scores cannot be used to identify DESAs associated with early versus late kidney graft loss in deceased donor transplants.

KEYWORDS

DESA, Ellipro scores, kidney transplantation

Kidney transplantation is the ultimate treatment for patients with end-stage kidney failure. The presence of donor epitope specific antibodies (DESAs) has been found to be associated with inferior graft survival.[1,2] For detection and interpretation of DESAs, solid-phase single antigen bead (SAB) luminex assay in combination with high-resolution anti-human leucocyte antigen (HLA) typing data are used. Eplets are a cluster of polymorphic amino acid configurations within a 3.0–3.5 Å radius that is hypothesized to be sufficient to induce an antibody response, when mismatched in a transplant setting.[3,4] Various tools are available to determine eplets mismatches, including the ElliPro epitope predictor program. This online algorithm combines a modified version of Thornton's method, that identifies structural epitopes based on protrusion from the antigen surface,[5] with a residue clustering algorithm.[6] The ElliPro score is the protrusion index (PI) of an amino acid residue, which is defined as the percentage of the protein atoms enclosed in the ellipsoid at which the residue first become lying outside the ellipsoid (ElliPro: Antibody Epitope Prediction [iedb.org]). The ElliPro score could be a useful tool to predict the antigenicity of eplets, since it outperformed other structure-based methods,[6] and can be used to distinguish HLA Class I eplets for which no specific antibodies have been found (might be classified as non-eplets) from antibody-verified eplets which have amino acid residues with higher ElliPro scores.[7] However, it is unknown whether (antibody-verified) donor eplets predicted with the ElliPro program and recognized by pre-transplant DESA are associated with transplant outcome. Furthermore, not all DESA-positive patients have a poor survival rate, suggesting that donor eplets do not all share the same clinical relevance in relation to transplant outcome. Therefore, we evaluated the clinical relevance of the ElliPro score by evaluating long-term graft outcome in kidney transplant recipients with pretransplant DESA against donor eplets separated into high versus low ElliPro scores.

In a large cohort consisting of 3235 deceased-donor kidney recipients transplanted in the Netherlands between 1995 and 2005, we screened for the presence of pre-transplant HLA Class I and/or Class II antibodies using LifeCodes SAB assay Class I and/or II kits. Informed consent for use of their clinical data was obtained from all subjects. The study protocol was approved by the Biobank Research Ethics Committee of the UMC Utrecht (Tc Bio 13-633). To upscale the available donor and recipient low resolution HLA typing data to high resolution, we used National Marrow Donor Program (NMDP) Haplostats tool with the haplotype frequency tables.[8] DESAs were defined as antibodies against eplets, listed in the HLA Eplet Registry (epregistry.com.br, accessed December 2, 2022), present in the donor and not in the recipient and defined positive exclusively by the relevant HLA Class I and/or Class II (except HLA-DP) in the SAB assay. 315 deceased-donor transplants had one or more DESAs and were included in further analysis.

The Ellipro scores of each of those donor eplets were assessed on ElliPro: Antibody Epitope Prediction (iedb.org). Next, we selected the transplants in which the graft failed within the first year after transplantation ($n = 61$), and compared those to transplants in which the graft survived >12 years after transplantation ($n = 66$). There are no significant differences of baseline characteristics between both cohorts, except for a decreased donor age in the long surviving transplants (Table 1). We looked at the distribution of the Ellipro scores (very low, low, intermediate, and high) of all donor eplets, and normalized it to the number of eplets per transplant. The median number of DESA is 4 (range 1–35) in early graft failure patients, and 5 (range 2–15) in the long surviving patients. Most donor eplets have intermediate to high Ellipro scores (Figure 1, left). Compared to the distribution of scores in transplants in which the graft survived >12 years after transplantation ($n = 66$, Figure 1, right), we did not observe differences compared to patients suffering from graft loss within 1 year (Mann–Whitney U test), indicating that a higher Ellipro score is not associated with graft failure. In addition, MFI values (interquartile range) of DESAs found in patients in which the kidney graft has failed within 1 year after transplantation were comparable to MFI values in patients in which the graft survived >12 years (2710 [3954] and 2580 [3748], respectively) (Supplemental Figure S1).

Next, we analyzed the distribution of the total Ellipro scores of all DESAs per transplant (weighted score)

TABLE 1 Patient, donor, and transplant characteristics.

Characteristics	Early failure Tx (<1 year) $n = 61$	Surviving Tx (>12 years) $n = 66$	p-value
Patient			
Age at transplant, y, mean ± SD	45.8 ± 13.8	42.6 ± 13.8	0.206[a]
Female sex, *n* (%)	35 (57.4)	32 (48.5)	0.409[b]
PRA at time of transplant, %, mean ± SD	32.8 ± 35.2	30.1 ± 36.0	0.667[a]
Highest PRA, % mean ± SD	54.5 ± 37.6	51.3 ± 38.9	0.643[a]
Dialysis			
No, *n* (%)	1 (1.6)	4 (6.1)	0.256[b]
Yes-hemodialysis, *n*	39 (63.9)	39 (59.1)	
Yes-peritoneal dialysis, *n*	17 (27.9)	22 (33.3)	
Unknown, *n* (%)	4 (6.6)	1 (1.5)	
Time on dialysis, y, mean ± SD	4.0 ± 2.4	3.4 ± 3.1	0.239[a]
Donor			
Donor age, y, mean ± SD	46.1 ± 16.6	38.2 ± 15.7	0.007[a]
Donor female sex, *n* (%)	28 (45.9)	23 (34.8)	0.276[b]
Type of donor			
Deceased-DBD, *n* (%)	47 (77.0)	58 (87.9)	0.169[b]
Deceased-DCD, *n* (%)	14 (23.0)	8 (12.1)	
Cold ischemia time			
Deceased donors, h, mean ± SD	24.3 ± 7.0	22.0 ± 6.8	0.059[a]
Transplant			
Retransplant, *n* (%)	37 (60.7)	35 (53.0)	0.492[b]
HLA-A/B/DR broad mismatches, mean ± SD	2.0 ± 1.2	2.0 ± 1.1	0.823[a]
Induction therapy			
IL-2 receptor blocker, *n* (%)	12 (19.7)	9 (13.6)	0.499[b]
T cell-depleting antibody,[d] *n* (%)	4 (6.6)	9 (13.6)	0.307[b]
Initial immunosuppression, *n* (%)			
Steroids	58 (95.1)	66 (100.0)	0.108[c]
MMF/azathioprine	45 (73.8)	49 (74.2)	1.000[b]
Cyclosporine/tacrolimus	53 (86.9)	60 (90.9)	0.660[b]
Sirolimus	3 (4.9)	2 (3.0)	0.671[c]
Other	5 (8.2)	10 (15.2)	0.348[b]
Unknown	2 (3.3)	0 (0.0)	0.229[c]

Abbreviations: DBD, donation after brain death; DCD, donation after cardiac death; IL, interleukin; MMF, mycophenolate mofetil; Tx, transplantation.
[a]Two-sample *t*-test for continuous variables.
[b]Chi-square test for categorical variables.
[c]Fisher's exact test for categorical variables ($n < 5$).
[d]T cell-depleting antibody therapy: ALG, ATG, OKT3 monoclonal antibodies.

within both cohorts. The total score per transplant takes into account both the number of DESA and the strength of each score. It has been calculated as follows: the number of DESA multiplied by the strength of each score: very low 0, low 1, intermediate 2, and high 3. Median weighted scores in the early failure transplants, for example, failed within 1 year post transplantation, and in the surviving transplants, for example, survived more than 12 years post transplantation did not differ significantly (9.0 and 9.5, respectively; $p = 0.63$, Mann–Whitney *U*-test, two-tailed) (Figure 2). Furthermore, the weighted Ellipro scores are not significantly correlated

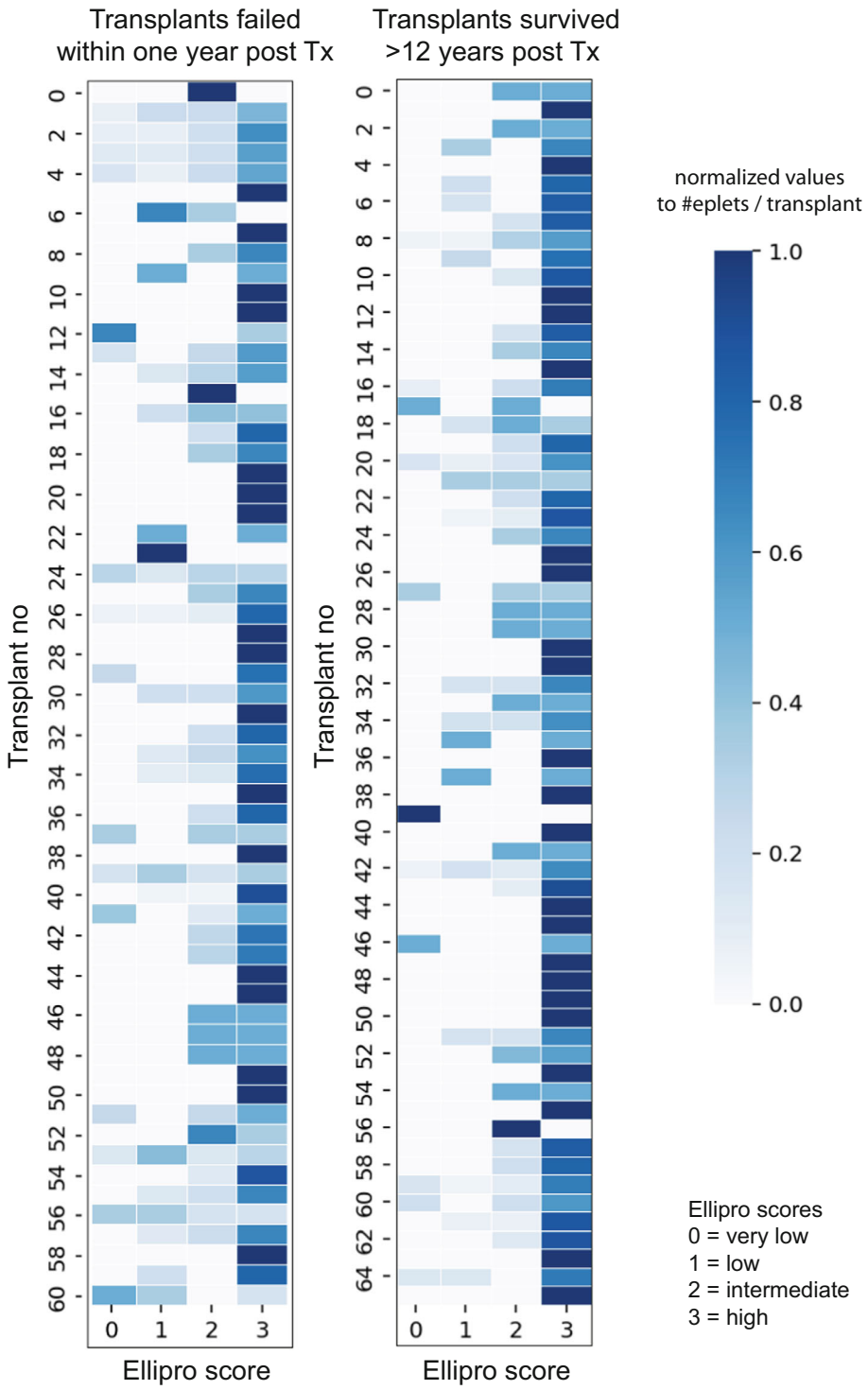

FIGURE 1 Distribution of Ellipro scores of donor eplets per transplant. The Ellipro scores of donor epitopes were assessed on ElliPro: Antibody Epitope Prediction (iedb.org), and normalized to the number of eplets per transplant (range 0 [light blue] to 1 [dark blue]). Examples of normalized values are: transplant X had 2 donor epitope specific antibodies (DESAs) with an intermediate Ellipro score, and 8 DESAs with a high score (in total 10 DESAs), resulting in normalized values of 0.2 (2/10) for category 2 and 0.8 (8/10) (for category 3). Transplant Y had in total 7 DESAs, of which 2 with a very low Ellipro score (2/7 = 0.29), 1 with a low score (1/7 = 0.14), 2 with an intermediate score (2/7 = 0.14), and 2 with a high Ellipro score (2/7 = 0.29). Each row represents a transplant. The distribution is showed for the transplants in which the kidney graft failed within the first year after transplantation (left), and for the transplants in which the kidney graft survived for more than 12 years after transplantation (right): 0 = very low, 1 = low, 2 = intermediate, 3 = high Ellipro score. In 315 deceased donor transplants in which DESA were positive, 82 donor eplets had very low Ellipro scores, 110 eplets had low Ellipro scores, 182 eplets had intermediate Ellipro scores, and 296 donor eplets had high Ellipro scores.

to graft loss. The weighted scores per transplants were divided into two groups: a high score group (total weighted score >20), and a low score group (total weighted score <20). Kaplan–Meier estimates for transplants with DESAs having high or low scores were plotted for the early failure transplants and for the long surviving transplants, and showed no difference in graft survival rates ($p = 0.9737$ and $p = 0.8789$, respectively) (Figure S2).

Although high ElliPro scores seemed to be linked to the antibody binding properties of eplets,[7] higher scores are not directly associated with inferior graft survival. It could be because the ElliPro score calculates the PI of a residue, but does not take into account their physiochemical and structural properties (e.g., polarity, hydrophobicity, and surface charge), which in part also determine the antigenicity of an eplet.[9–11] Recent literature showed that

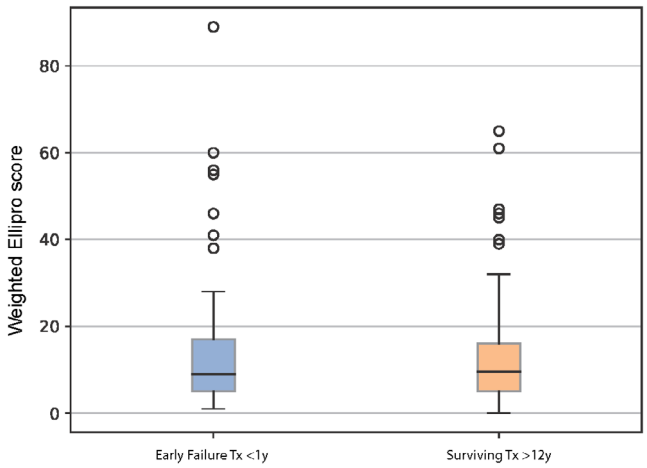

FIGURE 2 Distribution of the total Ellipro score per transplant (weighted score) in early failure and long surviving transplants. The total Ellipro score has been calculated by multiplying the number of donor epitope specific antibodies (DESAs) by the strength of each score: very low 0, low 1, intermediate 2, and high 3. The distribution is showed for the transplants in which the kidney graft failed within the first year after transplantation (blue), and for the transplants in which the kidney graft survived for more than 12 years after transplantation (orange). Significance has been determined using a Mann–Whitney *U* test, two-tailed.

a single antibody-verified eplet mismatch is already a risk factor for kidney graft loss. However, the relative hazard is increased looking per 10 eplet mismatches.[12] In this study, we evaluated only the weighted Ellipro score of all DESAs per transplant in relation to graft failure, but did not taken into account whether graft survival rates would be different per 5 or 10 mismatches.

HLA typing data available at serological split level has been imputed to 2-field high resolution data. Imputation could result in lower accuracy. Literature showed that imputation accuracy using the NMDP Haplostats tool is between 60% and 70% for Caucasians.[13–16] However, for HLA Class II, only 35% agreement has been found between real and imputed high-resolution data.[17] For the interpretation of the data, lower imputation accuracy should be taken into account especially for HLA Class II and in a multi-ethnic population.

In previous studies it has been shown that donor-specific HLA antibodies in living donors have differential effects on graft survival than in deceased donors.[18] Due to the low prevalence of DESA in living donors (9 transplants with graft failure within 1 year post transplantation) we cannot perform statistical analysis for the living donors.

In summary, although most donor eplets recognized by pretransplant antibodies have an intermediate to high ElliPro score in deceased donor transplants that failed within 1 year, there are no significant differences in graft survival compared to transplants that survived >12 years post transplantation. Although ElliPro scores cannot be used for defining DESAs associated with early versus late graft failure in deceased donor transplants, they could be useful for identifying antigenic eplets.

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CONFLICT OF INTEREST STATEMENT

The authors have declared no conflicting interests to disclose.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

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SUPPORTING INFORMATION

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

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