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Review

Donor-specific ELISPOT assay for predicting acute rejection and allograft function after kidney transplantation: A systematic review and *meta*-analysis

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ABSTRACT

Acute rejection remains an important problem after kidney transplantation. Enzyme-linked immunosorbent spot (ELISPOT) assay has been investigated extensively and has shown promising results as a predictor of allograft rejection. The objective of this study was to systematically review and analyze the predictive value of the donor-specific ELISPOT assay to identify recipients at risk for acute rejection. Electronic databases were searched for studies reporting donor-specific ELISPOT and kidney transplantation outcomes. Odds ratio (OR) for acute rejection was calculated, along with standardized mean difference (SMD) of cytokine producing-cells between recipients with and without acute rejection. Pooled estimates were calculated using random-effect models. The positive ELISPOT cutoff frequencies were extracted from each study. From 665 articles found, 32 studies were included in the *meta*-analysis. IFN-γ was the most investigated cytokine (30 out of 32 studies). Patients with positive pre-transplantation donor-reactive IFN-γ ELISPOT had an OR of 3.3 for acute rejection (95%-CI 2.1 to 5.1), and OR of 6.8 (95%-CI 2.5 to 18.9) for post-transplantation ELISPOT. Recipients with rejection had significantly higher frequencies of pre- and post-transplantation cytokine producing-cells (SMD 0.47, 95%-CI 0.07 to 0.87 and SMD 3.68, 95%-CI 1.04 to 6.32, respectively). Pre-transplantation ELISPOT had a positive predictive value of 43% and a negative predictive value of 81% for acute rejection. A positive ELISPOT result was associated with a lower estimated glomerular filtration rate (SMD −0.59, 95%-CI −0.83 to −0.34). In conclusion, patients with a high frequency of donor-reactive IFN-γ ELISPOT are at higher risk for acute rejection. The donor-specific IFN-γ ELISPOT assay can serve as an immune-monitoring tool in kidney transplantation.

1. Introduction

Acute kidney transplant rejection remains a major barrier to allograft longevity [1–3]. With improvements in immunosuppressive treatment, the incidence of acute rejection in the first year after transplantation has decreased to 10–20%, depending on the recipient's immunological risk [2,4–6]. Acute rejection is associated with poor outcomes, including an increased risk of acute graft loss, *de novo* donor-specific anti-human leukocyte antigen (HLA) antibody (DSA) formation, and allograft loss in the long-term [1,2,4,7].

An immune-monitoring tool that reliably predicts an individual patient's rejection risk would allow clinicians to intervene earlier and to personalize immunosuppressive therapy. Patients with a high-immunological risk profile could receive more potent immunosuppression, for example with T lymphocyte-depleting agents, whereas patients with a low risk of rejection could be given standard or less intense immunosuppression. This would prevent over-immunosuppression and may therefore reduce complications such as malignancy and infection [8,9].

In the acute rejection process, foreign (*i.e.,* donor-derived) HLA is

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presented to recipient T and B lymphocytes by antigen-presenting cells (APCs). APCs can present donor antigens in 3 ways, the so-called direct, indirect, and semi-direct pathway. Direct antigen presentation occurs in the early post-transplantation period, when intact donor HLA molecules on the surface of donor APCs are recognized directly by recipient T lymphocytes. In indirect antigen presentation, donor HLA molecules are internalized and processed in recipient APCs, and are then presented as peptide fragments in the context of recipient HLA. The semi-direct pathway is the process in which intact donor HLA is acquired on the surface of recipient APCs [10]. The presentation of intact donor HLA or fragments thereof, leads to an aggressive effector T lymphocyte response, which includes $CD8^+$ cytotoxic T lymphocytes (CTL) and $CD4^+$ T helper lymphocytes (Th) [11,12]. Upon alloantigen activation, these effector T lymphocytes produce large amounts of pro-inflammatory cytokines, such as interferon-γ (IFN-γ), interleukin (IL)-17 and IL-21. In brief, IFN-γ is a pleiotropic cytokine which enhances both innate and adaptive immune responses against the donor organ, by increasing the expression of donor HLA, promoting leukocyte migration to the allograft, and modulating the cytotoxic functions of T lymphocytes and NK cells [13–15]. IL-17 is the hallmark cytokine of Th17 lymphocytes, and stimulates and recruits neutrophils and monocytes to the site of inflammation, thereby facilitating the acute rejection process [16–19]. IL-21 stimulates the expansion of $CD8^+$ T lymphocytes and enhances their cytolytic potential [20,21]. Moreover, both IL-17 and IL-21 are involved in germinal center formation and modulate antibody production, which is associated with antibody-mediated rejection (ABMR) [22–25]. In addition to the aforementioned pro-inflammatory cytokines, the recipient's immune cells also release IL-10, a cytokine that has anti-inflammatory and immunosuppressive functions, and inhibits the release of pro-inflammatory cytokines from antigen-presenting cells [26,27].

The enzyme-linked immunosorbent spot (ELISPOT) assay is a highly sensitive and specific test that quantifies cytokine-producing T lymphocytes reactive to a specific antigen. In the setting of organ transplantation, peripheral blood mononuclear cells (PBMCs) of the transplant recipient are co-cultured with donor cells (either donor PBMCs or spleen cells). These donor cells present antigens via the direct antigen presentation pathway. The recipient PBMCs respond by producing cytokines which can be captured in an ELISPOT plate pre-coated with an anti-cytokine antibody. Subsequently, the cells are lysed and washed away. Areas in which cytokine has been captured are detected by a biotinylated anti-cytokine detecting and staining procedure (Fig. 1). This method allows the quantitative measurement of cytokine-producing cells at the single cell level [28–30].

Previous ELISPOT studies reported that kidney transplant recipients with high numbers of donor-reactive, cytokine-producing cells were at high risk of developing acute rejection and had inferior allograft function [31–34]. However, these studies differed in terms of the type of cytokine measured, the timing of measurement (pre-transplantation or post-transplantation), and the type of acute rejection (acute T cell-mediated rejection (aTCMR) *versus* ABMR). Moreover, a previous *meta*-analysis included only pre-transplantation ELISPOT studies and IFN-γ was the only cytokine of interest [35].

The objective of this study was to determine the extent to which donor-specific ELISPOT assays can be used to predict the risk of acute rejection and graft function after kidney transplantation. A systematic review with *meta*-analysis was performed of all studies using the ELISPOT assay as an immune-monitoring tool in kidney transplantation. ELISPOT studies before and after transplantation, and measuring all types of cytokines were included.

2. Methods

2.1. Data sources and searches

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement was used for this systematic review [36]. The search was performed in Scopus, MEDLINE, and the Cochrane Central Register of Controlled Trials to identify eligible studies on 28 December 2020. The references listed in the retrieved articles were also reviewed and manually added if deemed appropriate. The following search term was used for Scopus: TITLE-ABS-KEY (ELISPOT AND Transplantation), and the Medical Subject Heading (MeSH) terms ("Enzyme-Linked Immunospot Assay"[MeSH]) AND "Kidney Transplantation"[MeSH] were used in the MEDLINE search. The MeSH descriptors which explored all trees of [Enzyme-Linked Immunospot Assay] and [Kidney Transplantation] were applied to the Cochrane Central register of Controlled Trials.

2.2. Study selection

Prospective and retrospective studies of the donor-specific ELISPOT

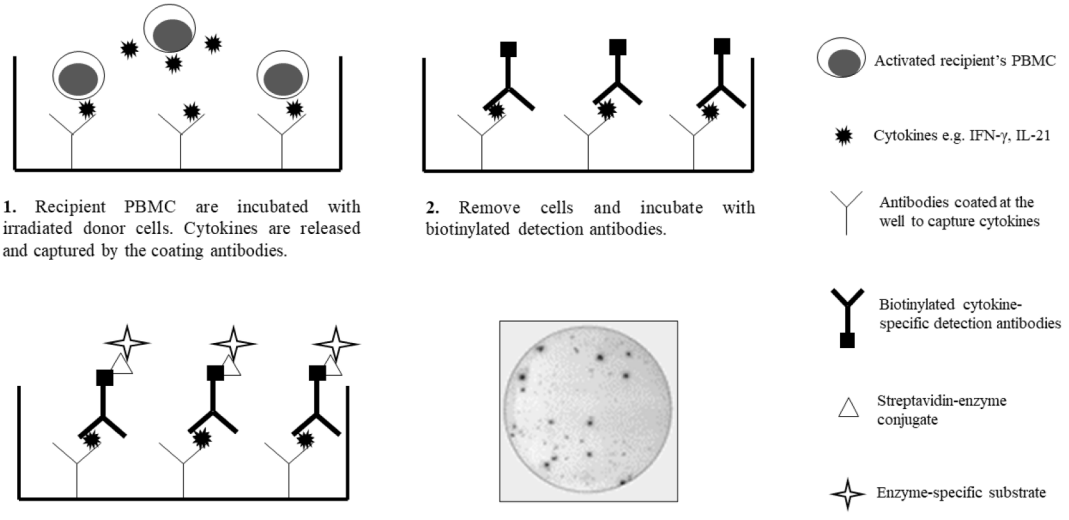

Fig. 1. ELISPOT method.

assay for cytokine-producing cells were included. Regarding the ELISPOT assay procedure, the included studies had to use donor PBMCs or spleen cells and incubate these with recipient PBMCs, and had to measure the frequency of cytokine-producing T lymphocytes. This systematic review and *meta*-analysis included all cytokines measured and ELISPOT assays performed both the pre- and post-transplantation phase. Only studies that reported the association or correlation between the ELISPOT test results and acute rejection in kidney transplant recipients were selected; studies had to provide detailed information about the number of patients with positive and negative ELISPOT results, or the actual frequencies of the cytokine-producing T lymphocytes, in the rejection and non-rejection group. In addition to the risk of acute rejection, we investigated the association between ELISPOT, *de novo* DSA formation, and kidney allograft function. The Standards for Reporting of Diagnostic Accuracy Studies (STARD) 2015 were followed as a guidance for study reviews [37]. Two authors (S.U. and S.K.) independently screened the titles and abstracts from the electronic databases, and full-text articles were retrieved for comprehensive review. Disagreement was resolved through the consensus and judgement by C. B. and D.H.

2.3. Data extraction and quality assessment

The following information was extracted from each study: authors' name, year of publication, country of origin, timing of the ELISPOT assay (before or after transplantation), cytokine measured, total patients included in the study, the number of patients with acute rejection, and the type of acute rejection. If the studies did not distinguish between TCMR and ABMR, the total number of rejections was used for the analysis. ELISPOT cutoff values that discriminated between patients having either a positive or negative ELISPOT result were obtained as presented in each study. These threshold values varied according to each study's protocol. The actual frequencies of donor-specific cytokine-producing cells in the ELISPOT assay were also extracted, if available. For studies that measured ELISPOT assays at multiple time points post-transplantation, the mean ± standard deviation (SD) of the post-transplantation ELISPOT frequencies was used as a representative value. Kidney allograft function was extracted in the studies and recorded as estimated glomerular filtration rate (eGFR). The risk of bias was assessed by the Quality Assessment of Diagnostic Accuracy Studies tool (QUADAS-2) [38]. This tool evaluates 4 domains including patient selection, index test, reference standard, and flow and timing. These domains are used to classify the risk of bias and applicability to the population of interest, and can be categorized into "low", "high", and "unclear".

2.4. Data synthesis and analysis

The pooled diagnostic OR of predicting acute rejection was calculated from patients with positive donor-specific ELISPOT and compared with patients with a negative ELISPOT test result. A continuity correction was applied to all cells in studies with any zero-cell count [39]. Pooled sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were analyzed using bivariate models that account for the correlation between these parameters. SMD was analyzed to represent the difference between the frequencies of donor-specific cytokine-producing cells between patients with and without acute rejection. In an effort to standardize the different number of recipient PBMCs used in the ELISPOT assay in each study, the cytokine-producing cells were normalized to 3×10^5 recipient PBMCs. Allograft function is presented as eGFR and was compared between those who had a positive or negative ELISPOT assay result. The mean and SD were estimated by the method of Wan *et al.* if not provided in the study [40]. All pooled estimates were calculated using random-effects models. A funnel plot was used to investigate publication bias, and Egger's test was used to test for asymmetry of the funnel plot [41]. The existence of heterogeneity among study effect sizes was analyzed using Cochrane's Q-test and the I^2 index. A low Q-test p-value indicates the presence of heterogeneity. An I^2 index higher than 75% indicates high heterogeneity. The analyses were performed using Stata Statistical Software (Release 16.1) with the user written commands midas, metandi, and metadta (StataCorp LLC, College Station, TX) and GraphPad Prism version 9.0.0 for Windows (GraphPad Software, San Diego, CA).

2.5. Ethical considerations

This *meta*-analysis and systematic review did not directly obtain data from human or animal subjects. All of the included studies' information was published in scientific journals without the possibility to identify individual patients.

3. Results

3.1. Characteristics of the studies

A total of 665 citations was identified in the initial search. Duplicate and irrelevant studies were excluded, leaving 71 studies for full-text review, of which 32 studies were included in the *meta*-analysis [31–34,42–69]. The flow diagram of study selection is depicted in Fig. 2. The summary of study characteristics is illustrated in Table 1. In brief, 16 studies investigated pre-transplantation donor-specific ELISPOT [33,34,42,46,50–52,54,56,58,60–62,66,67,69], 8 studies described post-transplantation ELISPOT [43,45,49,53,55,59,63,64], and 8 studies investigated ELISPOT both before and after transplantation [31,32,44,47,48,57,65,68]. The exact timing of the post-transplantation ELISPOT measurements varied between studies, ranging from an exact time point in the first few months after transplantation, whereas in other studies, the ELISPOT assay was measured at non-fixed time points. With regard to the cytokine measured by ELISPOT, most studies measured IFN-γ-producing cells, three studies measured IL-10-producing cells, one study measured IL-17-producing cells, and one study evaluated IL-21-producing cells. Each study used different cutoff values to discriminate between a positive and negative ELISPOT test result (Table 1).

Fourteen studied did not report the timing of acute rejection. The other 18 studies reported that acute rejection occurred from week 2 to month 60 post-transplantation. For the calculation of the OR, 26 studies provided sufficient information on the patients with positive and negative ELISPOT results and the incidence of rejection [31–34,42–46,48–53,56,58,60,61,63–69]. Eighteen studies presented the actual values of cytokine producing-cells to allow the calculation of the SMD [31,34,42–45,47–49,51,53,54,58,59,62,63,65,68]. Finally, 10 studies were examined for the association between ELISPOT results and allograft function. Six studies used the 6-variable MDRD equation [33,44,46,51,52,58], 1 study used the simplified 4-variable MDRD equation [60], and 3 studies did not specify which MDRD equation was used [32,53,63]. The assessment of the risk of bias and applicability concerns, evaluated by QUAD-2, is shown in Supplementary Table S1.

3.2. Diagnostic performance of donor-specific ELISPOT assay for predicting acute rejection

The pooled diagnostic OR is illustrated in Fig. 3. A positive pre-transplantation IFN-γ ELISPOT predicted acute rejection with an OR of 3.29 (95%-CI 2.11 to 5.13; p-value < 0.001, I^2 47.9%, Q-test p-value $= 0.009$), and a positive post-transplantation IFN-γ ELISPOT was associated with acute rejection with an OR of 6.84 (95%-CI 2.48 to 18.89; p-value < 0.001, I^2 64.6%, Q-test p-value $= 0.013$). There was only one study that associated rejection with a pre-transplantation and post-transplantation IL-21 ELISPOT assay (OR 11.0, 95%-CI 2.26 to 53.64; $p = 0.003$, and OR 8.46, 95%-CI 1.87 to 38.31; $p = 0.006$), respectively. After combining the OR of all cytokines, patients with a positive pre-transplant donor-specific assay were at a 3.50-fold higher risk for

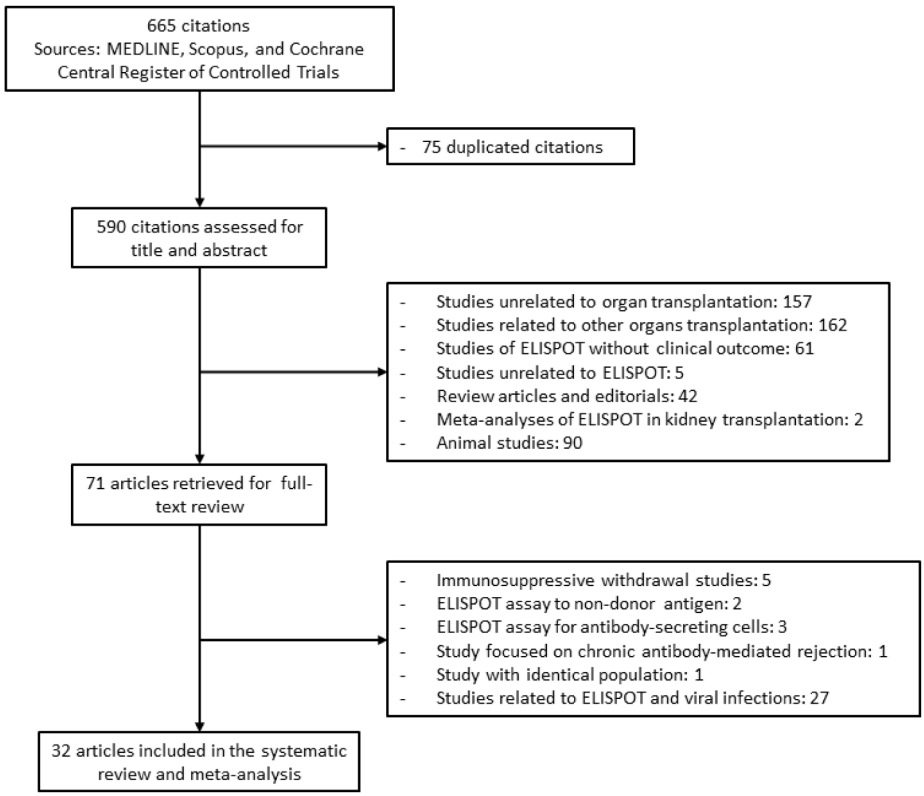

Fig. 2. Flow diagram of study selection.

acute rejection (95%-CI 2.25 to 5.45; p -value < 0.001, I^2 49.6%, Q-test p-value = 0.007) compared with patients with negative ELISPOT. In the same way, patients with a post-transplant donor-specific ELISPOT had a 9.20-fold higher risk for acute rejection (95%-CI 3.47 to 24.41; p-value < 0.001, I^2 67.3%, Q-test p-value = 0.004). When sub-grouped into studies that reported aTCMR only (*i.e.,* excluding mixed-type rejection and ABMR), the pooled OR of patients with a positive ELISPOT was 2.81 (95%-CI 1.90 to 4.16; p-value < 0.001, I^2 25.2%, Q-test p-value = 0.045) for the pre-transplant ELISPOT assay, and 12.65 (95%-CI 6.59 to 24.28; p-value < 0.001, I^2 0%, Q-test p-value = 0.930) for the post-transplant ELISPOT (Supplementary Figure S1).

The donor-specific ELISPOT assay was unable to differentiate patients with and without ABMR (pooled OR 0.79, 95%-CI 0.27 to 2.28; p-value = 0.665, I^2 0%, Q-test p-value = 0.532, Supplementary Figure S2), or patients with and without *de novo* DSA (pooled OR 1.56, 95%-CI 0.09 to 26.64; p-value = 0.757, I^2 82.5%, Q-test p-value = 0.017, Supplementary Figure S3).

Studies of donor-specific IFN-γ ELISPOT contained sufficient information to be combined into a pooled sensitivity and specificity analysis as shown in Table 2. The sensitivity and specificity of the ELISPOT test was at its highest when used in the post-transplantation period for aTCMR (sensitivity 0.81, 95%-CI 0.69 to 0.89; specificity 0.74, 95%-CI 0.59 to 0.85). The timing of post-transplantation ELISPOT in each study varied but was most frequently tested somewhere in the period between 3 and 6 months after transplantation, and associated with acute rejection up to 12 months post-transplantation (Table 1). In contrast, the pre-transplantation or post-transplantation IFN-γ ELISPOT assay had poor diagnostic performance for ABMR. However, only 379 patients were included in the analysis of the association between ELISPOT and ABMR, compared with 1,599 patients in the studies of ELISPOT and aTCMR (Table 2). The PPV and NPV of the IFN-γ ELISPOT assay depend on the prevalence of acute rejection, which was 32% (95%-CI 25% to 39%) in this *meta*-analysis. The pooled PPV and NPV of the pre-transplantation IFN-γ ELISPOT was 43% (95%-CI 36% to 50%) and 81% (95%-CI 74% to 88%), respectively. For the post-transplantation IFN-γ ELISPOT, the pooled PPV was 54% (95%-CI 45% to 63%) and the pooled NPV was 79% (95%-CI 70% to 87%). The nomograms for the PPV and NPV of the pre-transplantation and post-transplantation IFN-γ ELISPOT for acute rejection are plotted in Supplementary Figure S4.

The funnel plot of the log OR for acute rejection is depicted in Supplementary Figure S5, and demonstrates incomplete symmetry. This indicates the possibility of some publication bias due to under-reporting of negative studies. The p-value from Egger's test was 0.085 for pre-transplantation ELISPOT and 0.117 for post-transplantation ELISPOT.

3.3. *Difference of actual ELISPOT frequencies in patients with and without rejection*

Fig. 4 displays the SMD between patients with and without rejection. Patients with rejection had higher donor-specific ELISPOT frequencies compared with patients without rejection (SMD 1.71, 95%-CI 0.57 to 2.86; p-value = 0.003, I^2 98.0%, Q-test p-value < 0.001). This analysis was only possible for the total rejection incidence due to the lack of ELISPOT frequencies reported for the subgroups of aTCMR and ABMR. To illustrate the actual frequencies of the ELISPOT assay for each type of cytokine, the frequencies of cytokine-producing cells were pooled (Fig. 5). The mean ± SD of IFN-γ-producing cell frequencies in patients with and without acute rejection was 176 ± 287 *versus* 86 ± 172 producing cells per 3 × 10^5 PBMCs pre-transplantation (p-value = 0.033), and 246 ± 256 *versus* 77 ± 87 producing-cells per 3 × 10^5 PBMCs post-transplantation (p-value = 0.015). IL-17-producing-cells from patients with and without rejection were significantly different in the post-transplantation period (161 ± 7 *versus* 51 ± 18 producing-cells per 3 × 10^5 PBMCs; p-value < 0.001). The frequencies of IL-21-producing cells in the pre-transplantation and post-transplantation ELISPOT were significantly different in patients with and without acute rejection (35 ± 38 *versus* 12 ± 10 producing-cells per 3 × 10^5 PBMCs; p-value = 0.011 in pre-transplantation, and 60 ± 69 *versus*. 25 ± 33 producing-cells per 3 ×

Table 1
Summary characteristics of included studies.

References	Authors and year of publication	Country of origin	Pre-transplantation ELISPOT	Post-transplantation ELISPOT	Cytokine measured	Cutoff of ELISPOT after subtraction of negative control	Total patients with available ELISPOT results	Patients with acute rejection	Timing of acute rejection	Patients with aTCMR	Patients with ABMR
42	Heeger et al. 1999	USA	Yes	No	IFN-γ	20 pc per 3 × 10^5 PBMCs	19	7	N/A	7	0
43	Najafian et al. 2002	USA	No	Randomly after 6 months post-KT	IFN-γ	60 pc per 10^6 PBMCs	27	15	N/A	15	0
31	Hricik et al. 2003	USA	Yes	Randomly in the first 6 months post-KT	IFN-γ	10 pc per 3 × 10^5 PBMCs	55	5	Up to 6 months pot-KT	5	0
44	Nickel et al. 2004	Germany	Yes	Randomly in the first 6 months post-KT	IFN-γ	200 pc pre-KT and 10 pc post-KT per 3 × 10^5 PBMCs	52	18	Up to 6 months pot-KT	18	0
45	Poggio et al. 2004	USA	No	Randomly post-KT	IFN-γ	15 pc per 3 × 10^5 PBMCs	20	11	N/A	11	0
46	Augustine et al. 2005	USA	Yes	No	IFN-γ	25 pc per 3 × 10^5 PBMCs	37	11	N/A	11	0
47	Bellisola et al. 2006	Italy	Yes	Protocol: 5 times in 2 months post-KT	IFN-γ	N/A (per 2 × 10^5 PBMCs)	8	3	N/A	3	0
48	Nather et al. 2006	Germany	Yes	Protocol: 2 times in 6 months post-KT	IFN-γ	21 pc pre-KT and 13 pc post-KT per 2 × 10^5 PBMCs	23	12	N/A	12	0
49	Van Den Boogaardt et al. 2006	Netherlands	No	Randomly in the first 4 months post-KT	IFN-γ, IL-10	150 pc pre-KT and 30 pc post-KT per 1.5 × 10^5 PBMCs	16	8	Up to 4 months post-KT	8	0
50	Augustine et al. 2007	USA	Yes	No	IFN-γ	25 pc per 3 × 10^5 PBMCs	100	21	Up to 12 months post-KT	21	0
51	Kim et al. 2007	South Korea	Yes	No	IFN-γ	12 pc per 2 × 10^5 PBMCs	45	11	N/A	N/A	N/A
52	Augustine et al. 2008	USA	Yes	No	IFN-γ	25 pc per 3 × 10^5 PBMCs	130	24	N/A	24	0
53	Bestard et al. 2008	Germany	No	Randomly after 24 months post-KT	IFN-γ	20 pc per 3 × 10^5 PBMCs	34	17	N/A	N/A	N/A
54	Reinsmoen et al. 2008	Germany	Yes	No	IFN-γ	N/A (per 2 × 10^5 PBMCs)	30	22	N/A	18	4
55	Kim et al. 2009	South Korea	No	Protocol: 3 times in 2 months post-KT	IL-10	N/A (per 2 × 10^5 PBMCs)	42	11	Up to 2 weeks post-KT	N/A	N/A
56	Koscielska-Kasprzak et al. 2009	Poland	Yes	No	IFN-γ	N/A	53	14	Up to 12 months post-KT	N/A	N/A
57	Cherkassky et al. 2011	USA	Yes	Protocol: 3 times in 6 months post-KT	IFN-γ	N/A (per 2 × 10^5 PBMCs)	31	1	Up to 5 months post-KT	1	0
58	Kim et al. 2012	South Korea	Yes	No	IFN-γ	12 pc per 2 × 10^5 PBMCs	154	18	Up to 12 months post-KT	15	5
32	Bestard et al. 2013	Spain	Yes	Protocol: at 6 months post-KT	IFN-γ	25 pc per 3 × 10^5 PBMCs	60	14	Up to 12 months post-KT	13	1
33	Hricik et al. 2013	USA	Yes	No	IFN-γ	25 pc per 3 × 10^5 PBMCs	118	25	Up to 12 months post-KT	25	0
59	Nazari et al. 2013	Iran	No	Protocol: at 3 months post-KT	IFN-γ	N/A (per 1 × 10^5 PBMCs)	30	10	N/A	N/A	N/A

(*continued on next page*)

Table 1 (*continued*)

References	Authors and year of publication	Country of origin	Pre-transplantation ELISPOT	Post-transplantation ELISPOT	Cytokine measured	Cutoff of ELISPOT after subtraction of negative control	Total patients with available ELISPOT results	Patients with acute rejection	Timing of acute rejection	Patients with aTCMR	Patients with ABMR
34	Crespo et al. 2015	Spain	Yes	No	IFN-γ	25 pc per 3 × 10^5 PBMCs	157	42	N/A	37	5
60	Hricik et al. 2015	USA	Yes	No	IFN-γ	25 pc per 3 × 10^5 PBMCs	176	15	Up to 6 months post-KT	15	0
61	Hricik et al. 2015	USA	Yes	No	IFN-γ	25 pc per 3 × 10^5 PBMCs	15	6	Up to 16 months post-KT	6	0
62	Slavcev et al. 2015	Czech	Yes	No	IFN-γ	N/A (per 5 × 10^4 PBMCs)	47	22	Up to 12 months post-KT	17	5
63	Crespo et al. 2017	Spain	No	Protocol: at 3 and 6 months post-KT	IFN-γ	19 pc per 3 × 10^5 PBMCs	161	31	Up to 6 months post-KT	24	7
64	Crespo et al. 2017	Spain	No	Protocol: at 6 months post-KT	IFN-γ	25 pc per 3 × 10^5 PBMCs	75	22	Up to 6 months post-KT	17	5
65	Mohammadi et al. 2017	Iran	Yes	Protocol: 3 times in 3 months post-KT	IFN-γ, IL-10, IL-17	N/A (per 1 × 10^5 PBMCs)	57	12	Up to 12 months post-KT	N/A	N/A
66	Schachtner et al. 2017	Germany	Yes	No	IFN-γ	25 pc per 3 × 10^5 PBMCs	15	7	N/A	7	0
67	Gandolfini et al. 2018	Spain	Yes	No	IFN-γ	25 pc per 3 × 10^5 PBMCs	168	15	N/A	14	1
68	Van Besouw et al. 2019	Netherlands	Yes	Protocol: at 6 months post-KT	IL-21	18 pc pre-KT and 62 pc post-KT per 3 × 10^5 PBMCs	81	28	Pre-KT: up to 6 months Post-KT: up to 60 months	26	10
69	Schachtner et al. 2020	Germany	Yes	No	IFN-γ	25 pc per 3 × 10^5 PBMCs	150	36	Up to 12 months post-KT	33	3

ABMR, antibody-mediated rejection; aTCMR, acute T cell-mediated rejection; N/A, not available; pc, producing cells; PBMCs, peripheral blood mononuclear cells;

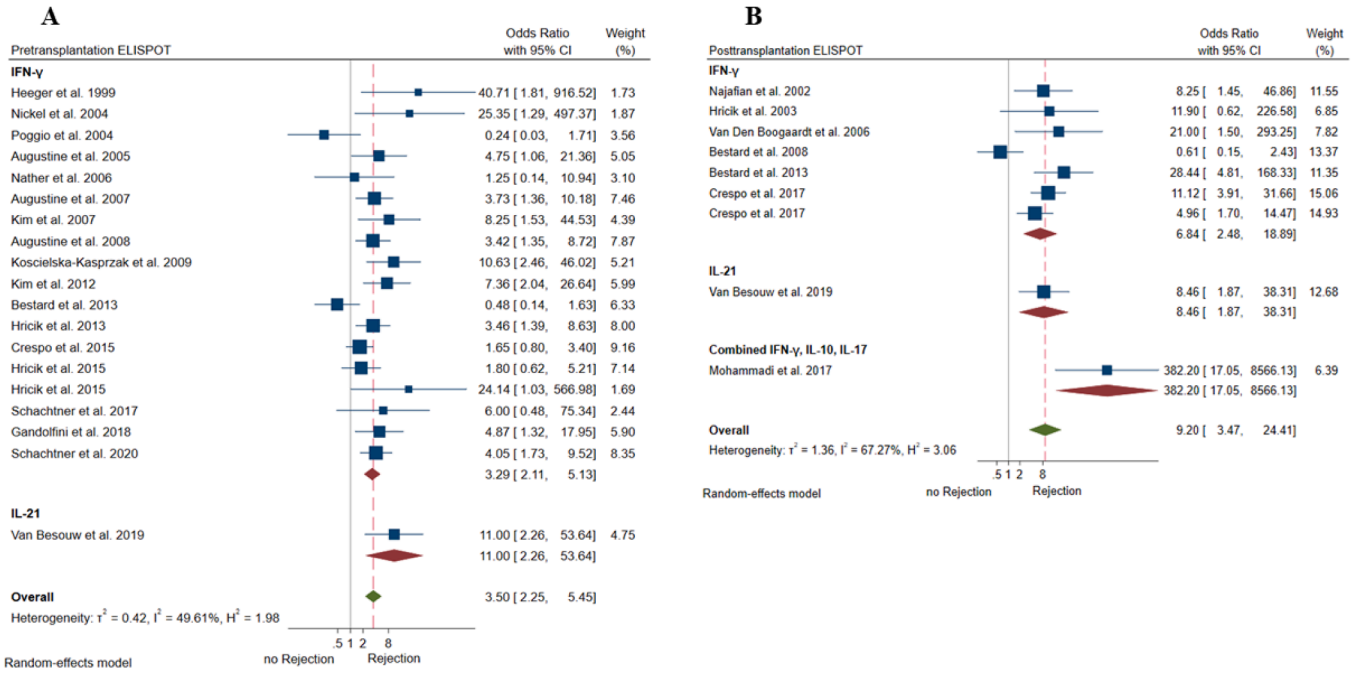

Fig. 3. Forest plot of OR for acute rejection in patients with positive pre-transplantation ELISPOT assay (A) and post-transplantation ELISPOT assay (B).

Table 2
Sensitivity and specificity of IFN-γ ELISPOT assay for acute rejection.

Total rejection	Number of patients reported	Pooled sensitivity	95%-CI	Pooled specificity	95%-CI
Pre-KT ELISPOT	1,485	0.63	0.54–0.71	0.65	0.55–0.75
Post-KT ELISPOT	414	0.73	0.62–0.82	0.69	0.51–0.83
aTCMR	**Number of patients reported**	**Pooled sensitivity**	**95%-CI**	**Pooled specificity**	**95%-CI**
Pre-KT ELISPOT	1,219	0.60	0.51–0.68	0.65	0.52–0.77
Post-KT ELISPOT	380	0.81	0.69–0.89	0.74	0.59–0.85
ABMR	**Number of patients reported**	**Pooled sensitivity**	**95%-CI**	**Pooled specificity**	**95%-CI**
Pre-KT ELISPOT	304	0.11	0.01–0.63	0.50	0.40–0.60
Post-KT ELISPOT	75	0.20	0.01–0.70	0.57	0.45–0.69

ABMR, antibody-mediated rejection; aTCMR, acute T cell-mediated rejection.

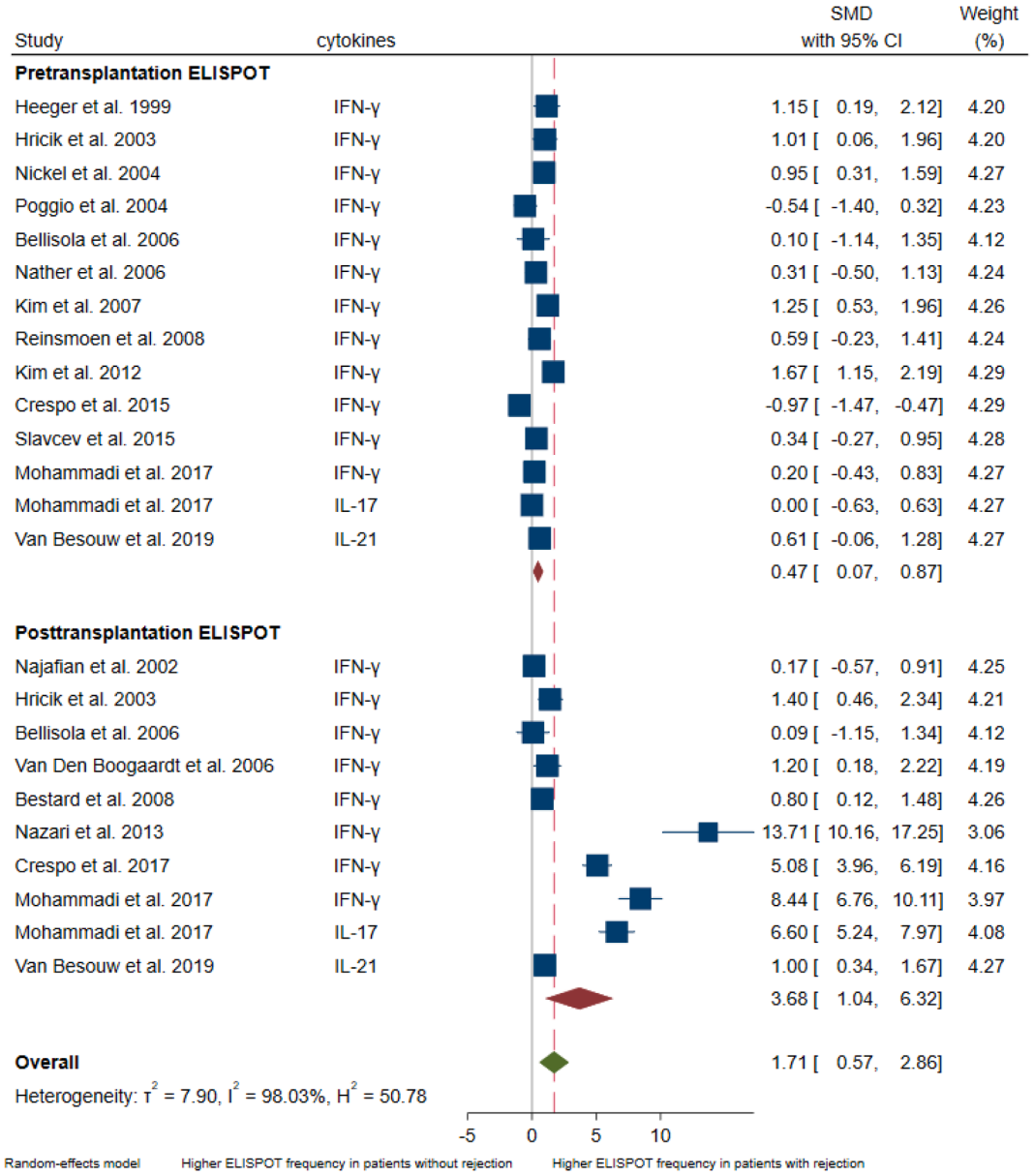

Fig. 4. Forest plot of SMD showing the difference of ELISPOT frequencies between patients with and without rejection.

10^5 PBMCs; p-value = 0.02 in the post-transplantation phase). IL-10 was not included in the analysis of SMD due to its distinctive feature as an anti-inflammatory and regulatory cytokine [27,70]. However, the actual frequencies of pre-transplantation and post-transplantation donor-specific IL-10-producing cells were significantly different between patients with and without rejection (1,246 ± 51 *versus* 1,161 ± 34 producing cells per 3 × 10^5 PBMCs pre-transplantation; p-value < 0.001, and 501 ± 517 *versus* 863 ± 593 producing cells per 3 × 10^5 PBMCs post-

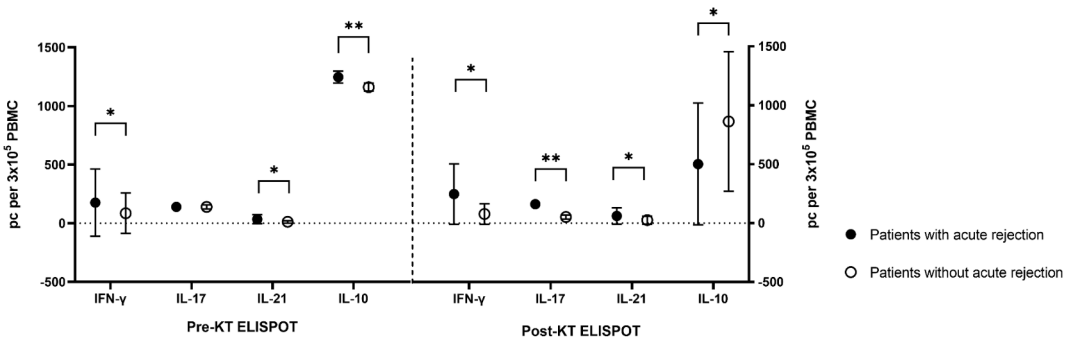

Fig. 5. Pooled actual frequencies of cytokine-producing cells (with 95%-CI) for patients with and without acute rejection, categorized by the timing of ELISPOT assay (*, p-value < 0.05; **, p-value < 0.00; pc, producing-cells).

transplantation; p-value = 0.025).

3.4. The association between kidney allograft function and donor-specific ELISPOT

The SMD of eGFR between patients with a positive and negative ELISPOT is shown in Fig. 6. Patients with a positive donor-specific ELISPOT had a significantly lower eGFR both at month 6 and month 12 after transplantation (pooled SMD −0.59, 95%-CI −0.83 to −0.34; p-value < 0.001, I^2 73.3%, Q-test p-value < 0.001). The actual eGFR values in patients with a positive ELISPOT were significantly lower compared with patients with negative ELISPOT, regardless of the timing of the ELISPOT measurement or the timing of the eGFR measurement (Fig. 7). There was not enough data to perform a *meta*-analysis of the association between serum creatinine and ELISPOT result, since only few studies reported serum creatinine as an endpoint.

4. Discussion

The results of this systematic review and *meta*-analysis demonstrate that ELISPOT is a useful immune-monitoring tool that can assist clinicians in stratifying the risk for acute rejection. Moreover, patients with a positive ELISPOT result were at higher risk for inferior kidney allograft function at 6 and 12 months after transplantation. Risk stratification using the donor-reactive ELISPOT assay can therefore guide personalization of an individual patient's immunosuppressive therapy.

The immune-monitoring tools currently in routine use for identifying rejection risk in kidney transplant recipients have poor predictive values. Only surrogate biomarkers such as immunosuppressive drug concentrations and the formation and titer of DSA have been implemented in routine diagnostics. None of these biomarkers evaluate the actual ongoing *in vivo* (or *in vitro*) interaction between the recipient's immune system and donor antigens, which should be the phenomenon of interest when assessing and monitoring rejection risk [30,71,72]. As a test that evaluates donor-reactive T lymphocyte-mediated immune responses (via the direct antigen presentation pathway), the ELISPOT assay determines the frequency of cytokine-producing cells and has been extensively investigated in relation to acute rejection [73]. In addition, evidence from previous studies showed that the results of the donor-reactive ELISPOT assay are dynamic and relate to the state of

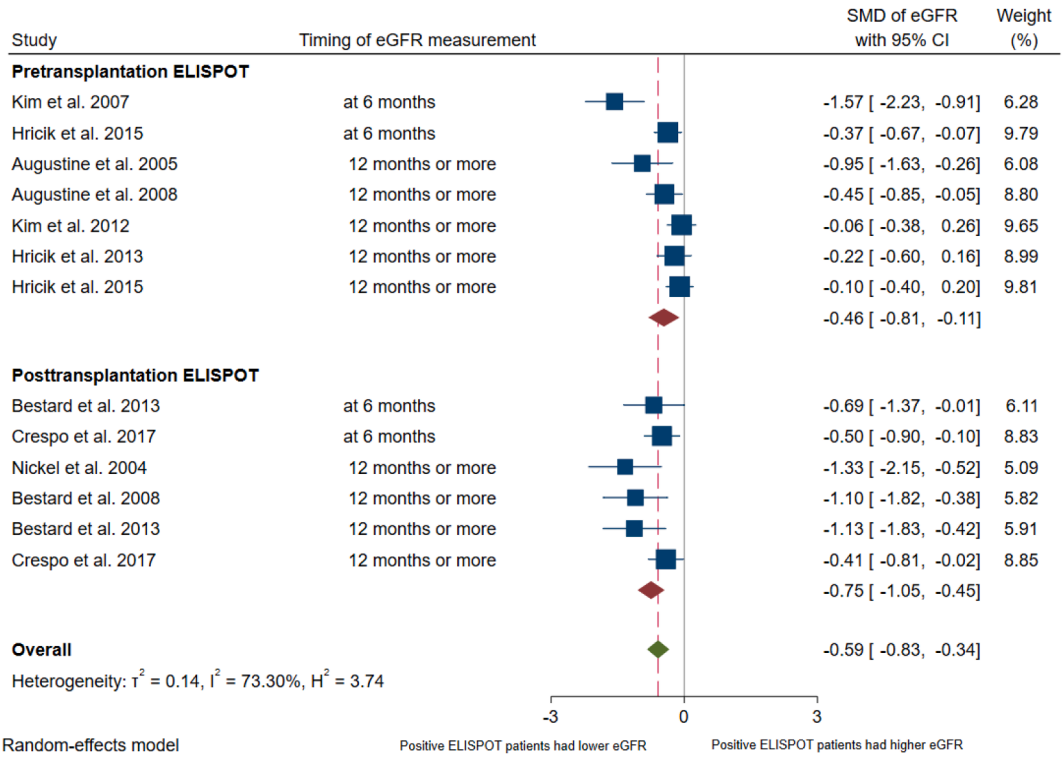

Fig. 6. Forest plot of SMD showing the difference of eGFR between patients with positive and negative ELISPOT results.

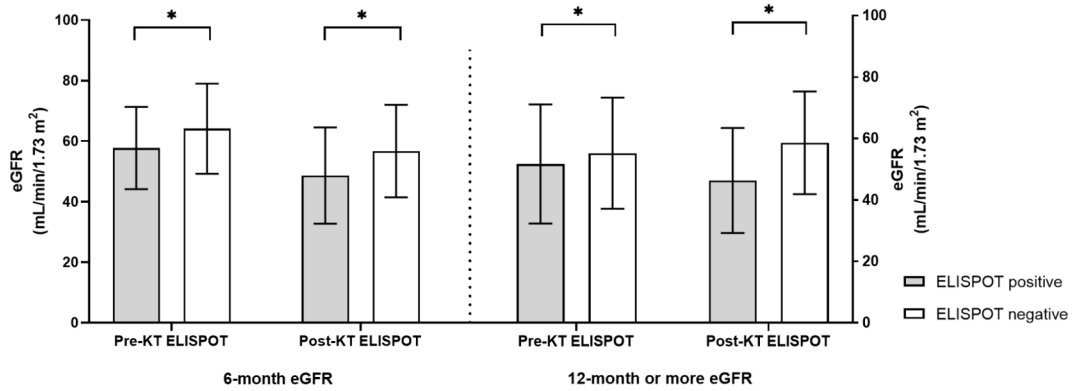

Fig. 7. Pooled eGFR (with 95%-CI) of patients with positive and negative ELISPOT results, categorized by the timing of ELISPOT assay and timing of eGFR measurement (*, p-value < 0.05).

immunosuppression of an individual patient, and may assist with the adjustment of immunosuppressive medication doses [32,34,52,57,58].

Our *meta*-analysis reported several cytokines used in the ELISPOT assay, including IFN-γ, IL-17, IL-21, and IL-10. Most studies reported results for IFN-γ, and demonstrated that this cytokine is useful as a predictor of acute rejection. We cannot draw conclusions about the diagnostic performance of IL-17 and IL-21, because only one study investigated each cytokine [65,68]. Interestingly, the frequency of IL-10-producing cells was higher in patients with acute rejection compared to patients without acute rejection [74]. As IL-10 is an anti-inflammatory and regulatory cytokine, a high number of IL-10-producing cells in patients with acute rejection likely reflects the response to inflammation and rejection in the allograft, rather than being the cause [75–78]. In contrast, the post-transplantation IL-10 ELISPOT results showed lower levels of IL-10-producing cells in patients with acute rejection compared with patients without acute rejection. However, as the 95%-CI of the post-transplantation IL-10 ELISPOT frequency analysis was exceptionally wide, this indicates a high degree of heterogeneity. Moreover, IL-10 can be secreted from many cells that are included in recipient PBMCs fractions during the ELISPOT procedure [26,79]. There might thus be significant variation in the source of IL-10, that at least partially contributes to the inconsistency of ELISPOT frequencies observed. Future studies could examine this possibility by purifying PBMCs cell subtypes to investigate ELISPOT responses in distinct T lymphocyte subsets.

For the type of acute rejection, sensitivity and specificity were best when the IFN-γ ELISPOT assay was used for the diagnosis of aTCMR, reflecting that alloreactive T lymphocytes mostly contribute to aTCMR rather than ABMR [80]. However, the number of patients included in the studies of ELISPOT dedicated to ABMR were limited and therefore the performance characteristics in predicting this outcome could not be fully evaluated. Interestingly, other studies that were not included in this *meta*-analysis, showed the potential of the ELISPOT assay in detecting donor-specific antibody-secreting cells, which might be a more relevant predictive biomarker for ABMR [81–83]. A next step in ELISPOT research could be the development of an assay that simultaneously measures the effector cytokines IFN-γ, IL-17, and IL-21. This might increase the sensitivity and specificity of the ELISPOT assay for predicting acute rejection after transplantation.

Both the pre-transplantation and the post-transplantation ELISPOT assay had a good NPV (81% and 79%, respectively) but a poor to moderate PPV (43% and 54%, respectively) for acute rejection. This indicates that the pre-transplantation donor-specific ELISPOT assay is an immune stratification tool that reliably predicts the absence of acute rejection. The high NPV of the ELISPOT assay suggests that the assay may best be used to identify patients with a low risk of rejection and in whom immunosuppression can thus be safely minimized, rather than use the test to identify high-risk patients who may require more intensive immunosuppression. However, the PPV and NPV are not intrinsic properties of the test and depend on the prevalence of acute rejection. The PPV and NPV of the ELISPOT assay are thus subject to change when applied in different populations.

As one might expect, kidney allograft function, represented by eGFR, was inferior in patients with positive ELISPOT compared with the negative ELISPOT patients. This finding supports the association of an alloimmune response that contributes to acute rejection, and may result in poor allograft function [1,2,84]. However, the inferior allograft function observed in this *meta*-analysis is not necessarily the result of acute rejection only. Patients with a positive donor-reactive ELISPOT can also experience a more subtle alloimmune response, so called chronic ABMR or chronic TCMR, which causes a slow decline of kidney allograft function [85–87]. Moreover, other post-transplant complications can contribute to renal allograft dysfunction such as calcineurin inhibitor-mediated nephrotoxicity, BK virus nephropathy, or recurrent glomerular disease [88,89]. Apart from acute rejection, clinicians should be aware of all possible causes of allograft dysfunction, even in the presence of a positive ELISPOT result.

With regard to personalized immunosuppressive therapy, we propose that the ELISPOT assay can inform treatment decisions by 3 distinct approaches. The first approach regards the pre-transplantation donor-reactive ELISPOT. It may inform the clinician about the recipient's immune status, and guide initial immunosuppression and choice of induction therapy. Patients with high levels of pre-transplant ELISPOT frequencies might need more potent immunosuppressive therapy such as T lymphocyte-depleting agents, while patients with a low level of donor-reactivity may only require an IL-2 receptor antagonist with standard low-exposure maintenance immunosuppression. Second, ELISPOT assay results could assist clinicians in appropriately adjusting immunosuppressive regimens. A standardized protocol for repeat ELISPOT testing, *e.g.,* every 3–6 months in the first year after transplantation, might aid in discriminating patients in whom immunosuppression can be safely reduced *versus* those in whom tapering of immunosuppression should be avoided. Third, the ELISPOT results could potentially guide the treatment of patients who are diagnosed histologically with borderline acute cellular rejection. There is ongoing uncertainty whether borderline acute rejection represents true rejection (and should thus be treated) or that it merely reflects harmless infiltration of the graft, or even immunoregulation, and is therefore best left untreated [87,90]. The role of ELISPOT in individualizing immunosuppression is being studied in an ongoing randomized, controlled trial (ClinicalTrials.gov identifier: NCT03465397).

Our *meta*-analysis has strengths and limitations. This is the first *meta*-analysis that includes both pre- and post-transplantation donor-specific ELISPOT studies and investigates several cytokines, other than IFN-γ. We demonstrated a consistent association between ELISPOT results and allograft function. However, some included studies did not clearly

mention the type of rejection nor the timing of acute rejection, complicating interpretation of the temporal relationship between the ELISPOT diagnostic performance and acute rejection. This issue is particularly important for the post-transplantation ELISPOT. Not every study clearly described the exact timing of the ELISPOT measurement in relation to the timing of acute rejection (see Table 1). While the pre-transplantation ELISPOT appears to predict acute rejection, the findings from post-transplantation ELISPOT should be more cautiously interpreted as they often represent an association rather than a true prediction. Moreover, we found significant variation in ELISPOT cutoff frequencies that were used to identify positive and negative ELISPOT results in each study. This variation could be due in part to differences in the source of the donor antigens, which included both donor PBMCs and spleen cells. In studies investigating living donor kidney transplantation, PBMCs were used for the ELISPOT assay, whereas spleen cells of the donor were used in case of deceased donor kidney transplantation. In almost every study, both types of donor (and thus both PBMCs and splenocytes) were used, and no study reported the ELISPOT results based on the donor source. To the best of our knowledge, no studies have systematically investigated the effect of the type of stimulator cell on ELISPOT results. In addition, differences in the responder cell fractions (*e.g.* PBMCs *versus* purified T-lymphocytes), and the sensitivity of the ELISPOT reader might also contribute to the ELISPOT results. Future studies should consider these factors in the design and analysis of ELISPOT assay studies.

In summary, donor-specific ELISPOT assays for cytokine-producing cells are useful tools to identify patients at risk for acute rejection, and may allow stratification of patients into high and low immunological risk kidney transplant recipients and guide immunosuppressive therapy. Further optimization of the ELISPOT technique and a standardization of the timing and use in clinical practice may lead to an improvement in its diagnostic performance.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

[1] Y. Bouatou, D. Viglietti, D. Pievani, et al., Response to treatment and long-term outcomes in kidney transplant recipients with acute T cell-mediated rejection, Am. J. Transplant. 19 (7) (2019) 1972–1988.
[2] P.A. Clayton, S.P. McDonald, G.R. Russ, S.J. Chadban, Long-Term Outcomes after Acute Rejection in Kidney Transplant Recipients: An ANZDATA Analysis, J. Am. Soc. Nephrol. 30 (9) (2019) 1697–1707.
[3] A. Hart, J.M. Smith, M.A. Skeans, et al., OPTN/SRTR 2017 Annual Data Report: Kidney, Am. J. Transplant. 19 (Suppl 2) (2019) 19–123.
[4] A.J. Matas, K.J. Gillingham, W.D. Payne, J.S. Najarian, The impact of an acute rejection episode on long-term renal allograft survival (t1/2), Transplantation 57 (6) (1994) 857–859.
[5] A.O. Ojo, J.M. Morales, M. González-Molina, et al., Comparison of the long-term outcomes of kidney transplantation: USA versus Spain, Nephrol. Dial. Transplant. 28 (1) (2013) 213–220.
[6] M. Lorent, Y. Foucher, K. Kerleau, et al., The EKiTE network (epidemiology in kidney transplantation - a European validated database): an initiative epidemiological and translational European collaborative research, BMC Nephrol. 20 (1) (2019) 365.
[7] A.J. Matas, A. Humar, W.D. Payne, et al., Decreased acute rejection in kidney transplant recipients is associated with decreased chronic rejection, Ann Surg. 230 (4) (1999) 493–498, discussion 8-500.
[8] D.C. Dafoe, E. Tantisattamo, U. Reddy, Precision Medicine and Personalized Approach to Renal Transplantation, Semin. Nephrol. 38 (4) (2018) 346–354.
[9] G. Zaza, S. Granata, P. Tomei, A. Dalla Gassa, A. Lupo, Personalization of the immunosuppressive treatment in renal transplant recipients: the great challenge in "omics" medicine, Int. J. Mol. Sci. 16 (2) (2015) 4281–4305.
[10] J.H.Y. Siu, V. Surendrakumar, J.A. Richards, G.J. Pettigrew, T cell Allorecognition Pathways in Solid Organ Transplantation, Front. Immunol. 9 (2018) 2548.
[11] F. Salcido-Ochoa, S.S. Hue, S. Peng, et al., Histopathological analysis of infiltrating T cell subsets in acute T cell-mediated rejection in the kidney transplant, World J Transplant. 7 (4) (2017) 222–234.
[12] M.M. D'Elios, R. Josien, M. Manghetti, et al., Predominant Th1 cell infiltration in acute rejection episodes of human kidney grafts, Kidney Int. 51 (6) (1997) 1876–1884.
[13] L.G. Hidalgo, P.F. Halloran, Role of IFN-gamma in allograft rejection, Crit. Rev. Immunol. 22 (4) (2002) 317–349.
[14] K. Schroder, P.J. Hertzog, T. Ravasi, D.A. Hume, Interferon-gamma: an overview of signals, mechanisms and functions, J. Leukoc. Biol. 75 (2) (2004) 163–189.
[15] J.R. Schoenborn, C.B. Wilson, Regulation of interferon-gamma during innate and adaptive immune responses, Adv. Immunol. 96 (2007) 41–101.
[16] B.E. Burrell, D.K. Bishop, Th17 cells and transplant acceptance, Transplantation 90 (9) (2010) 945–948.
[17] V. Gorbacheva, R. Fan, X. Li, A. Valujskikh, Interleukin-17 promotes early allograft inflammation, Am. J. Pathol. 177 (3) (2010) 1265–1273.
[18] L. Ma, H. Zhang, K. Hu, et al., The imbalance between Tregs, Th17 cells and inflammatory cytokines among renal transplant recipients, BMC Immunol. 16 (2015) 56.
[19] N.M. van Besouw, K. Caliskan, A.M. Peeters, et al., Interleukin-17-producing CD4(+) cells home to the graft early after human heart transplantation, J. Heart Lung Transplant. 34 (7) (2015) 933–940.
[20] C.C. Baan, A.H. Balk, I.E. Dijke, et al., Interleukin-21: an interleukin-2 dependent player in rejection processes, Transplantation 83 (11) (2007) 1485–1492.
[21] X. Shi, R. Que, B. Liu, et al., Role of IL-21 signaling pathway in transplant-related biology, Transplant Rev (Orlando). 30 (1) (2016) 27–30.
[22] F. Abadja, B. Sarraj, M.J. Ansari, Significance of T helper 17 immunity in transplantation, Curr Opin Organ Transplant. 17 (1) (2012) 8–14.
[23] Y. Wu, N.M. van Besouw, Y. Shi, M.J. Hoogduijn, L. Wang, C.C. Baan, The Biological Effects of IL-21 Signaling on B-Cell-Mediated Responses in Organ Transplantation, Front. Immunol. 7 (2016) 319.
[24] K. de Leur, F.J. Dor, M. Dieterich, L.J. van der Laan, R.W. Hendriks, C.C. Baan, IL-21 Receptor Antagonist Inhibits Differentiation of B Cells toward Plasmablasts upon Alloantigen Stimulation, Front. Immunol. 8 (2017) 306.
[25] G.A. Böhmig, Z. Kikic, M. Wahrmann, et al., Detection of alloantibody-mediated complement activation: A diagnostic advance in monitoring kidney transplant rejection? Clin. Biochem. 49 (4) (2016) 394–403.
[26] W. Ouyang, A. O'Garra, IL-10 Family Cytokines IL-10 and IL-22: from Basic Science to Clinical Translation, Immunity 50 (4) (2019) 871–891.
[27] E.H. Steen, X. Wang, S. Balaji, M.J. Butte, P.L. Bollyky, S.G. Keswani, The Role of the Anti-Inflammatory Cytokine Interleukin-10 in Tissue Fibrosis, Adv Wound Care (New Rochelle). 9 (4) (2020) 184–198.
[28] E. Crespo, O. Bestard, Biomarkers to assess donor-reactive T-cell responses in kidney transplant patients, Clin. Biochem. 49 (4–5) (2016) 329–337.
[29] M. Salvadori, A. Tsalouchos, Biomarkers in renal transplantation: An updated review, World J. Transplant. 7 (3) (2017) 161–178.
[30] M. Quaglia, G. Merlotti, G. Guglielmetti, G. Castellano, V. Cantaluppi, Recent Advances on Biomarkers of Early and Late Kidney Graft Dysfunction, Int. J. Mol. Sci. 21 (15) (2020).
[31] D.E. Hricik, V. Rodriguez, J. Riley, et al., Enzyme linked immunosorbent spot (ELISPOT) assay for interferon-gamma independently predicts renal function in kidney transplant recipients, Am. J. Transplant. 3 (7) (2003) 878–884.
[32] O. Bestard, J.M. Cruzado, M. Lucia, et al., Prospective assessment of antidonor cellular alloreactivity is a tool for guidance of immunosuppression in kidney transplantation, Kidney Int. 84 (6) (2013) 1226–1236.
[33] D.E. Hricik, E.D. Poggio, K.J. Woodside, et al., Effects of cellular sensitization and donor age on acute rejection and graft function after deceased-donor kidney transplantation, Transplantation 95 (10) (2013) 1254–1258.
[34] E. Crespo, M. Lucia, J.M. Cruzado, et al., Pre-transplant donor-specific T-cell alloreactivity is strongly associated with early acute cellular rejection in kidney transplant recipients not receiving T-cell depleting induction therapy, PLoS ONE 10 (2) (2015).
[35] N. Montero, S. Farouk, I. Gandolfini, et al., Pretransplant Donor-specific IFNgamma ELISPOT as a Predictor of Graft Rejection: A Diagnostic Test Accuracy Meta-analysis, Transplant Direct. 5 (5) (2019), e451.

[36] A. Liberati, D.G. Altman, J. Tetzlaff, et al., The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration, BMJ 339 (2009), b2700.
[37] P.M. Bossuyt, J.B. Reitsma, D.E. Bruns, et al., STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies, BMJ 351 (2015), h5527.
[38] P.F. Whiting, A.W. Rutjes, M.E. Westwood, et al., QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies, Ann. Intern. Med. 155 (8) (2011) 529–536.
[39] M.J. Sweeting, A.J. Sutton, P.C. Lambert, What to add to nothing? Use and avoidance of continuity corrections in meta-analysis of sparse data, Stat. Med. 23 (9) (2004) 1351–1375.
[40] X. Wan, W. Wang, J. Liu, T. Tong, Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range, BMC Med. Res. Method. 14 (2014) 135.
[41] M. Egger, G. Davey Smith, M. Schneider, C. Minder, Bias in meta-analysis detected by a simple, graphical test, BMJ 315 (7109) (1997) 629–634.
[42] P.S. Heeger, N.S. Greenspan, S. Kuhlenschmidt, et al., Pretransplant frequency of donor-specific, IFN-gamma-producing lymphocytes is a manifestation of immunologic memory and correlates with the risk of posttransplant rejection episodes, J. Immunol. 163 (4) (1999) 2267–2275.
[43] N. Najafian, A.D. Salama, E.V. Fedoseyeva, G. Benichou, M.H. Sayegh, Enzyme-linked immunosorbent spot assay analysis of peripheral blood lymphocyte reactivity to donor HLA-DR peptides: Potential novel assay for prediction of outcomes for renal transplant recipients, J. Am. Soc. Nephrol. 13 (1) (2002) 252–259.
[44] P. Nickel, F. Presber, G. Bold, et al., Enzyme-linked immunosorbent spot assay for donor-reactive interferon-gamma-producing cells identifies T-cell presensitization and correlates with graft function at 6 and 12 months in renal-transplant recipients, Transplantation 78 (11) (2004) 1640–1646.
[45] E.D. Poggio, M. Clemente, J. Riley, et al., Alloreactivity in renal transplant recipients with and without chronic allograft nephropathy, J. Am. Soc. Nephrol. 15 (7) (2004) 1952–1960.
[46] J.J. Augustine, D.S. Siu, M.J. Clemente, J.A. Schulak, P.S. Heeger, D.E. Hricik, Pre-transplant IFN-γ ELISPOTs are associated with post-transplant renal function in African American renal transplant recipients, Am. J. Transplant. 5 (8) (2005) 1971–1975.
[47] G. Bellisola, G. Tridente, F. Nacchia, F. Fior, L. Boschiero, Monitoring of Cellular Immunity by Interferon-Gamma Enzyme-Linked Immunosorbent Spot Assay in Kidney Allograft Recipients: Preliminary Results of a Longitudinal Study, Transpl. Proc. 38 (4) (2006) 1014–1017.
[48] B.J. Näther, P. Nickel, G. Bold, et al., Modified ELISPOT technique - Highly significant inverse correlation of post-Tx donor-reactive IFNγ-producing cell frequencies with 6 and 12 months graft function in kidney transplant recipients, Transpl. Immunol. 16 (3–4) (2006) 232–237.
[49] D.E.M. Van Den Boogaardt, P.P.M.C. Van Miert, Y.J.H. De Vaal, J.W. De Fijter, F.H. J. Claas, D.L. Roelen, The ratio of interferon-γ and interleukin-10 producing donor-specific cells as an in vitro monitoring tool for renal transplant patients, Transplantation 82 (6) (2006) 844–848.
[50] J.J. Augustine, E.D. Poggio, M. Clemente, et al., Hemodialysis vintage, black ethnicity, and pretransplantation antidonor cellular immunity in kidney transplant recipients, J. Am. Soc. Nephrol. 18 (5) (2007) 1602–1606.
[51] S.H. Kim, E.J. Oh, M.J. Kim, et al., Pretransplant Donor-Specific Interferon-γ ELISPOT Assay Predicts Acute Rejection Episodes in Renal Transplant Recipients, Transpl. Proc. 39 (10) (2007) 3057–3060.
[52] J.J. Augustine, E.D. Poggio, P.S. Heeger, D.E. Hricik, Preferential benefit of antibody induction therapy in kidney recipients with high pretransplant frequencies of donor-reactive interferon-γ enzyme-linked immunosorbent spots, Transplantation 86 (4) (2008) 529–534.
[53] O. Bestard, P. Nickel, J.M. Cruzado, et al., Circulating alloreactive T cells correlate with graft function in longstanding renal transplant recipients, J. Am. Soc. Nephrol. 19 (7) (2008) 1419–1429.
[54] N.L. Reinsmoen, K.M.M. Cornett, R. Kloehn, et al., Pretransplant donor-specific and non-specific immune parameters associated with early acute rejection, Transplantation 85 (3) (2008) 462–470.
[55] S.H. Kim, E.J. Oh, J.Y. Ghee, et al., Clinical significance of monitoring circulating CD4+CD25+ regulatory T cells in kidney transplantation during the early posttransplant period, J. Korean Med. Sci. (2009;24(SUPPL.1):S135–S42.).
[56] K. Koscielska-Kasprzak, D. Drulis-Fajdasz, D. Kaminska, et al., Pretransplantation Cellular Alloreactivity Is Predictive of Acute Graft Rejection and 1-Year Graft Function in Kidney Transplant Recipients, Transpl. Proc. 41 (8) (2009) 3006–3008.
[57] L. Cherkassky, M. Lanning, P.N. Lalli, et al., Evaluation of alloreactivity in kidney transplant recipients treated with antithymocyte globulin versus IL-2 receptor blocker, Am. J. Transplant. 11 (7) (2011) 1388–1396.
[58] Y. Kim, K.H. Park, B.H. Chung, et al., Pretransplant IFN-γ ELISPOT assay as a potential screening test to select immunosuppression protocols for patients receiving basiliximab induction therapy, Translational Research. 160 (3) (2012) 230–236.
[59] B. Nazari, A. Amirzargar, B. Nikbin, et al., Comparison of the Th1, IFN-γ secreting cells and FoxP3 expression between patients with stable graft function and acute rejection post kidney transplantation, Iranian Journal of Allergy, Asthma and Immunology. 12 (3) (2013) 262–268.
[60] D.E. Hricik, J. Augustine, P. Nickerson, et al., Interferon gamma ELISPOT testing as a risk-stratifying biomarker for kidney transplant injury: Results from the CTOT-01 multicenter study, Am. J. Transplant. 15 (12) (2015) 3166–3173.
[61] D.E. Hricik, R.N. Formica, P. Nickerson, et al., Adverse outcomes of tacrolimus withdrawal in immune-quiescent kidney transplant recipients, J. Am. Soc. Nephrol. 26 (12) (2015) 3114–3122.
[62] A. Slavcev, K. Rybakova, E. Svobodova, et al., Pre-transplant donor-specific Interferon-gamma-producing cells and acute rejection of the kidney allograft, Transpl. Immunol. 33 (2) (2015) 63–68.
[63] E. Crespo, P. Cravedi, J. Martorell, et al., Posttransplant peripheral blood donor-specific interferon-gamma enzyme-linked immune spot assay differentiates risk of subclinical rejection and de novo donor-specific alloantibodies in kidney transplant recipients, Kidney Int. 92 (1) (2017) 201–213.
[64] E. Crespo, S. Roedder, T. Sigdel, et al., Molecular and Functional Noninvasive Immune Monitoring in the ESCAPE Study for Prediction of Subclinical Renal Allograft Rejection, Transplantation 101 (6) (2017) 1400–1409.
[65] F. Mohammadi, G. Solgi, M. Tajik, et al., Enzyme-Linked Immunosorbent Spot (ELISpot) monitoring of cytokine-producing cells for the prediction of acute rejection in renal transplant patients, Eur. Cytokine Netw. 28 (3) (2017) 93–101.
[66] T. Schachtner, M. Stein, P. Reinke, Increased alloreactivity and adverse outcomes in obese kidney transplant recipients are limited to those with diabetes mellitus, Transpl. Immunol. 40 (2017) 8–16.
[67] I. Gandolfini, E. Crespo, M. Baweja, et al., Impact of preformed T-cell alloreactivity by means of donor-specific and panel of reactive T cells (PRT) ELISPOT in kidney transplantation, PLoS ONE 13 (7) (2018), e0200696.
[68] N.M. Van Besouw, L. Yan, R. De Kuiper, et al., The number of donor-specific IL-21 producing cells before and after transplantation predicts kidney graft rejection, Front. Immunol. 10 (APR) (2019).
[69] T. Schachtner, M. Stein, N.M. Otto, P. Reinke, Preformed donor-reactive T cells that persist after ABO desensitization predict severe T cell-mediated rejection after living donor kidney transplantation – a retrospective study, Transpl. Int. 33 (3) (2020) 288–297.
[70] K.N. Couper, D.G. Blount, E.M. Riley, IL-10: the master regulator of immunity to infection, J. Immunol. 180 (9) (2008) 5771–5777.
[71] H. Waldmann, Mechanisms of immunological tolerance, Clin. Biochem. 49 (4) (2016) 324–328.
[72] C. Crescioli, Chemokines and transplant outcome, Clin. Biochem. 49 (4) (2016) 355–362.
[73] O. Millán, M. Brunet, Cytokine-based immune monitoring, Clinical Biochem. 49 (4) (2016) 338–346.
[74] C. Neumann, A. Scheffold, S. Rutz, Functions and regulation of T cell-derived interleukin-10, Semin. Immunol. 44 (2019), 101344.
[75] R. Weimer, S. Zipperle, V. Daniel, S. Carl, G. Staehler, G. Opelz, Pretransplant CD4 helper function and interleukin 10 response predict risk of acute kidney graft rejection, Transplantation 62 (11) (1996) 1606–1614.
[76] J.T. Fitzgerald, J.R. Johnson, R.V. Perez, Pre-transplant elevations of interleukin-12 and interleukin-10 are associated with acute rejection after renal transplantation, Clin. Transplant. 18 (4) (2004) 434–439.
[77] G.W. Burke, G. Ciancio, R. Cirocco, et al., Association of interleukin-10 with rejection-sparing effect in septic kidney transplant recipients, Transplantation 61 (7) (1996) 1114–1116.
[78] A. Asderakis, D. Sankaran, P. Dyer, et al., Association of polymorphisms in the human interferon-gamma and interleukin-10 gene with acute and chronic kidney transplant outcome: the cytokine effect on transplantation, Transplantation 71 (5) (2001) 674–677.
[79] L.M. Williams, G. Ricchetti, U. Sarma, T. Smallie, B.M. Foxwell, Interleukin-10 suppression of myeloid cell activation–a continuing puzzle, Immunology 113 (3) (2004) 281–292.
[80] L.E. Becker, C. Morath, C. Suesal, Immune mechanisms of acute and chronic rejection, Clin. Biochem. 49 (4) (2016) 320–323.
[81] M. Lucia, S. Luque, E. Crespo, et al., Preformed circulating HLA-specific memory B cells predict high risk of humoral rejection in kidney transplantation, Kidney Int. 88 (4) (2015) 874–887.
[82] S. Luque, M. Lúcia, E. Melilli, et al., Value of monitoring circulating donor-reactive memory B cells to characterize antibody-mediated rejection after kidney transplantation, Am. J. Transplant. 19 (2) (2019) 368–380.
[83] M. Nam, S. Shin, K.U. Park, et al., Association of Antibody-Secreting Cells With Allograft Rejection After Renal Transplantation, Transplant Proc. 52 (6) (2020) 1729–1733.
[84] A. Lindholm, S. Ohlman, D. Albrechtsen, G. Tufveson, H. Persson, N.H. Persson, The impact of acute rejection episodes on long-term graft function and outcome in 1347 primary renal transplants treated by 3 cyclosporine regimens, Transplantation 56 (2) (1993) 307–315.
[85] R.B. Mannon, Immune monitoring and biomarkers to predict chronic allograft dysfunction, Kidney Int. 78 (SUPPL. 119) (2010) S59–S65.
[86] N. Safinia, B. Afzali, K. Atalar, G. Lombardi, R.I. Lechler, T-cell alloimmunity and chronic allograft dysfunction, Kidney Int. 78 (2010) S2–S12.
[87] C. Wiebe, D.N. Rush, I.W. Gibson, et al., Evidence for the alloimmune basis and prognostic significance of Borderline T cell-mediated rejection, Am. J. Transplant. 20 (9) (2020) 2499–2508.
[88] S.A. Joosten, Y.W.J. Sijpkens, C. van Kooten, L.C. Paul, Chronic renal allograft rejection: Pathophysiologic considerations, Kidney Int. 68 (1) (2005) 1–13.
[89] S. Udomkarnjananun, N. Townamchai, S.J. Kerr, et al., The First Asian Kidney Transplantation Prediction Models for Long-term Patient and Allograft Survival, Transplantation 104 (5) (2020) 1048–1057.
[90] T. Shimizu, Clinical and Pathological Analyses of Borderline Changes Cases after Kidney Transplantation, Nephron. 144 (1) (2020) 91–96.