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The Molecular Diagnosis Might Be Clinically Useful in Discrepant Kidney Allograft Biopsy Findings: An Analysis of Clinical Outcomes

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Background. The Molecular Microscope Diagnostic System (MMDx) may overcome histology shortcomings. Previous studies have simply examined discrepant findings but have not attempted to determine clinical endpoints. To measure performance, clinical outcomes are strongly required. **Methods.** This single-center cohort study described discrepancies between MMDx and histology from 51 kidney transplant recipients (KTRs) and analyzed 72 indication biopsies, including 21 follow-up biopsies. Clinical performance was assessed by a combined endpoint of graft failure, rejection on follow-up biopsy, de novo donor-specific antibody, and improvement of kidney allograft function upon antirejection treatment. **Results.** MMDx agreed in 33 (65%) and differed in 18 (35%) of 51 KTRs. Most discrepancies occurred in biopsies called no rejection by MMDx and rejection by histology (15/24, 63%). In contrast, in biopsies called rejection by MMDx, 3 were classified as no rejection by histology (3/27, 11%). Discrepant findings between MMDx and histology occurred following delayed graft function and MMDx from biopsies with a low percentage of cortex. Among 15 biopsies classified as no rejection by MMDx but rejection by histology, the clinical course suggested no rejection in 9 cases. Six KTRs reached the endpoint, showing predominant $t \geq 2$ lesions. **Conclusions.** The most often occurring discrepancy is rejection by histology but no rejection by MMDx. As more KTRs do not meet the combined endpoint for rejection, MMDx might be clinically useful in these discrepant cases. Although strong histological findings have priority in indicating the treatment, clinical implementation of MMDx could strengthen treatment strategies.

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INTRODUCTION

Histologic evaluation of kidney biopsy tissue and classification of rejection (R) according to the Banff classification criteria into antibody-mediated rejection (AMR) and T cell–mediated rejection (TCMR) still represents the standard approach for evaluation of allograft injury.[1-3] However, histologic evaluation of biopsy tissue has several limitations. The diagnostic rules defined by the biannually updated Banff consensus report[1,3] derive from expert opinion without external standards for validation rather than from unambiguous data. Histologic classification relies on arbitrary semiquantitative scoring of 6 canonical lesions (ie, i-score and t-score for TCMR; ptc-score, g-score, and cg-score for AMR, whereas v-score can be seen in both), leading to high interobserver variability even for clear histologic rejection categories.[4,5]

Furthermore, consensus guidelines are often built upon questionable rules; hence, the classification of AMR considering only histology is still impossible when microvascular inflammation is present, but donor-specific antibodies (DSAs) are absent, and C4d staining is lacking.[3] These limitations explain the need to improve the

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precision (ie, reproducibility) of histologic diagnoses and question their accuracy (ie, correct diagnosis). The recent Banff consensus report acknowledges these limitations and states that molecular diagnostics could help clarify ambiguous cases.[3] The Molecular Microscope Diagnostic System (MMDx) measuring global gene expression in biopsy tissue has the potential to overcome these unmet needs.[6] MMDx outputs are continuous rather than semi-quantitative and objective and allow insights into the pathophysiological mechanisms of disease states based on gene expression patterns.[6-9]

Moreover, only little tissue (average length of 3 mm) is required for diagnosis, and MMDx can read cortex and medulla.[10] For assigning a molecular diagnosis, MMDx combines supervised and unsupervised analysis. In the former, conventional histologic phenotypes and lesions were used to develop molecular classifiers by applying machine learning-based algorithms to detect gene expressions associated with phenotypes.[11] In unsupervised archetypical analysis, rejection archetypes are based on patterns in the molecular data alone without the need for external histological phenotypic information.[11] Hence, an MMDx biopsy report has 2 components: (i) an automated report that combines rejection-related scores, that is, archetypes, together with a graphical representation of the biopsy in a principal component analysis plot based on the molecular classifiers and (ii) a categorical interpretation assigned by an expert or automated algorithm.[11,12]

Several studies comparing molecular and histology diagnoses on kidney allograft biopsies have shown a disagreement between MMDx and histology in 24%–37% of biopsies.[11,13] Among MMDx diagnoses, discrepancies were seen in all histologic diagnoses.[13] In a previous study, clinicians disagreed with histology more often and indicated that MMDx would give them more confidence in clinical management.[14] However, which diagnostic system is "more correct" is unclear. Data on clinical outcomes could clarify whether MMDx provide clinically useful information in cases of a discrepancy from histology. This study analyzed in unselected indications biopsies discrepant cases where the MMDx diagnosis differed from the histologic diagnosis. This analysis aimed (i) to assess the discrepancy rate and type between MMDx and histology, (ii) to identify clinical and biopsy-related factors associated with discrepancies, and (iii) to provide insights into the diagnostic performance of MMDx through clinical follow-up, including follow-up biopsies.

MATERIALS AND METHODS

Study Population

We conducted a single-center study at the University Hospital of Zurich. We studied 51 kidney transplant recipients (KTRs), transplanted between January 7, 1974, and May 11, 2020, who underwent a total of 72 first or follow-up indications (n = 70) or protocol biopsies (n = 2, in ABOi transplants) between July 2018 and June 2020. A total of 21 follow-up biopsies were performed among 15 KTRs. Biopsies were analyzed by histology and MMDx, and KTRs were clinically followed for at least 6 mo. Biopsies with missing data among the elementary Banff lesions were excluded.[1] The study was approved by the cantonal ethics commission review board of Zurich, Switzerland (KEK-ZH-Number 2020-02817) and has been conducted in compliance with the Declaration of Helsinki.

Immunosuppressive Therapy

Primary immunosuppression was a triple-drug regimen with a calcineurin inhibitor (tacrolimus or cyclosporine), mycophenolic acid, and steroids. KTRs received induction either with an interleukin-2R antagonist or a T cell–depleting agent.

Patient Care and Clinical Outcome on Follow-up

After the initial hospital stay, all KTRs received follow-up visits at the following time points: weekly at week 2–6, biweekly at week 6–12, monthly at month 3–6, and bimonthly at months 6–12. After that, quarterly aftercare was provided in collaboration with local nephrologists and at least yearly in our outpatient clinic.

At each visit, kidney function measures, urinary sediment, quantification of proteinuria, and urinary decoy-cell shedding were performed. Screening for BK virus (BKV)– and cytomegalovirus-DNAemia was conducted at months 1–6, 8, 10, 12, and 18 and at any unclear deterioration of kidney function or clinical suspicion.

Regular surveillance for anti-HLA antibodies by Luminex mix screening assay is provided at months 3, 6, 12, and at each annual visit. In case of a positive Luminex mix screening assay, a Luminex single antigen bead assay is added to detect DSAs.

Transplant biopsies are performed upon the indication of deteriorating graft function with elevated serum-creatinine, increasing proteinuria, or the appearance of de novo DSA. Protocol biopsies are done for ABO-incompatible transplantation at months 3 and 12 only. TCMR is treated by pulse steroids. No exact protocol exists for treating borderline rejections; cases are treated with steroids or left untreated. Acute AMR is treated with pulse steroids, plasmapheresis with intravenous immunoglobulin, and rituximab. In chronic AMR, immunosuppression is intensified to ensure a triple regimen consisting of tacrolimus, mycophenolic acid, and prednisone.

After indication biopsy, a clinical combined endpoint within a follow-up of 6 mo was recorded combining graft failure, R in the follow-up biopsy, development of de novo DSAs, or improvement of kidney allograft function >30% upon antirejection treatment at +6 mo after the baseline biopsy. Baseline creatinine before the biopsy was defined as the mean of the 3 lowest creatinine values in the 6 mo before.

Biopsy Evaluation

Biopsy cores were evaluated at the bedside by trained pathology technicians for sample accuracy, that is, sufficient cortex in the biopsy core. The local pathologist with special training for evaluating kidney biopsies assigned histologic diagnoses of allograft biopsies. Histological diagnoses were classified following a 2018 Reference Guide to the Banff Classification[2] and The Banff 2019 Kidney Meeting Repor.[3] Staining for C4d by immunofluorescence, SV40 by immunohistochemistry, and analysis by electron microscopy were performed on all biopsies. Simultaneously, all biopsies were analyzed by the Molecular Microscope Diagnostic System (MMDx; One

Lambda, Canoga Park, CA). For MMDx diagnostics, a small portion of a 16-gauge biopsy core was immediately placed into RNA*later* and stored at room temperature until shipped to ATAGC/TSI (Edmonton, Canada), as described previously.[12] Briefly, each MMDx report gives a score of 6 archetypes (R1 no rejection, R2 TCMR, R3 Mixed AMR/TCMR, R4 early stage AMR, R5 fully developed AMR, R6 late-stage AMR). The visual presentation of the biopsy in a principal component analysis plot is based on 7 classifiers (TCMR, AMR, g > 0, cg > 0, ptc > 0, i > 0, t > 1) together with a categorical automated interpretative MMDx report combining the molecular measurements based upon the archetypes.[12] To compare discrepancies between MMDx and histology, we used the output classes from the automated MMDx report.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Version 25 (SPSS, Chicago, IL). Two-sided Kruskal-Wallis test and Mann-Whitney *U* test were used for nonparametric independent samples for comparisons of different groups. Clinical characteristics were compared across groups using Fisher's exact test for categorical variables. Outcomes were measured with Kaplan-Meier models and were compared by log-rank tests.

RESULTS

Clinical Characteristics

In 51 KTRs, 72 biopsies were analyzed by MMDx and histology between July 2018 and June 2020. The median time of biopsy posttransplantation was 12 mo (range: 0–543 mo; Figure 2A, B). A subgroup of 15 KTRs underwent 21 follow-up biopsies; the median time from the first biopsy to the first follow-up biopsy was 4 mo (range: 1–7 mo). Pre- and posttransplant basic clinical characteristics and biopsy-related features are shown in Tables 1–3. KTRs were followed for a median time of 17 mo (range: 1–27 mo) after the first indication or protocol biopsy. During this follow-up period, no KTR died, and 6 KTRs returned to dialysis (5 KTRs due to AMR, 1 KTR due to TCMR). Figure 1 shows a flow diagram of discrepant and nondiscrepant cases.

Discrepancy Rate and Type Between the MMDx and Histologic Diagnosis

Discrepancies between the MMDx and the histologic diagnosis regarding rejection (R) and type of R are shown in Table 4, **Table S1** (**SDC,** http://links.lww.com/TP/C508). Distribution of biopsies over time posttransplant with MMDx rejection case mix and discrepancies, where MMDx differed from histology, are shown in Figure 2A,B.

In 33 of 51 first biopsies (65%), MMDx confirmed the histologic diagnosis, with 24 of 33 cases (73%) of confirmed R and 9 of 33 cases (27%) of confirmed NR. Only 2 of 24 cases (8%) showed a discrepancy in the R type between TCMR and ABMR (**Table S1, SDC,** http://links.lww.com/TP/C508).

Twelve repeat biopsies were performed in 7 of the 24 KTRs with confirmed R (all AMR). These follow-up biopsies again agreed between MMDx and histology diagnosis of R. Among 2 of 9 KTRs with confirmed NR, 4 follow-up biopsies also confirmed NR. Among 9 KTRs with

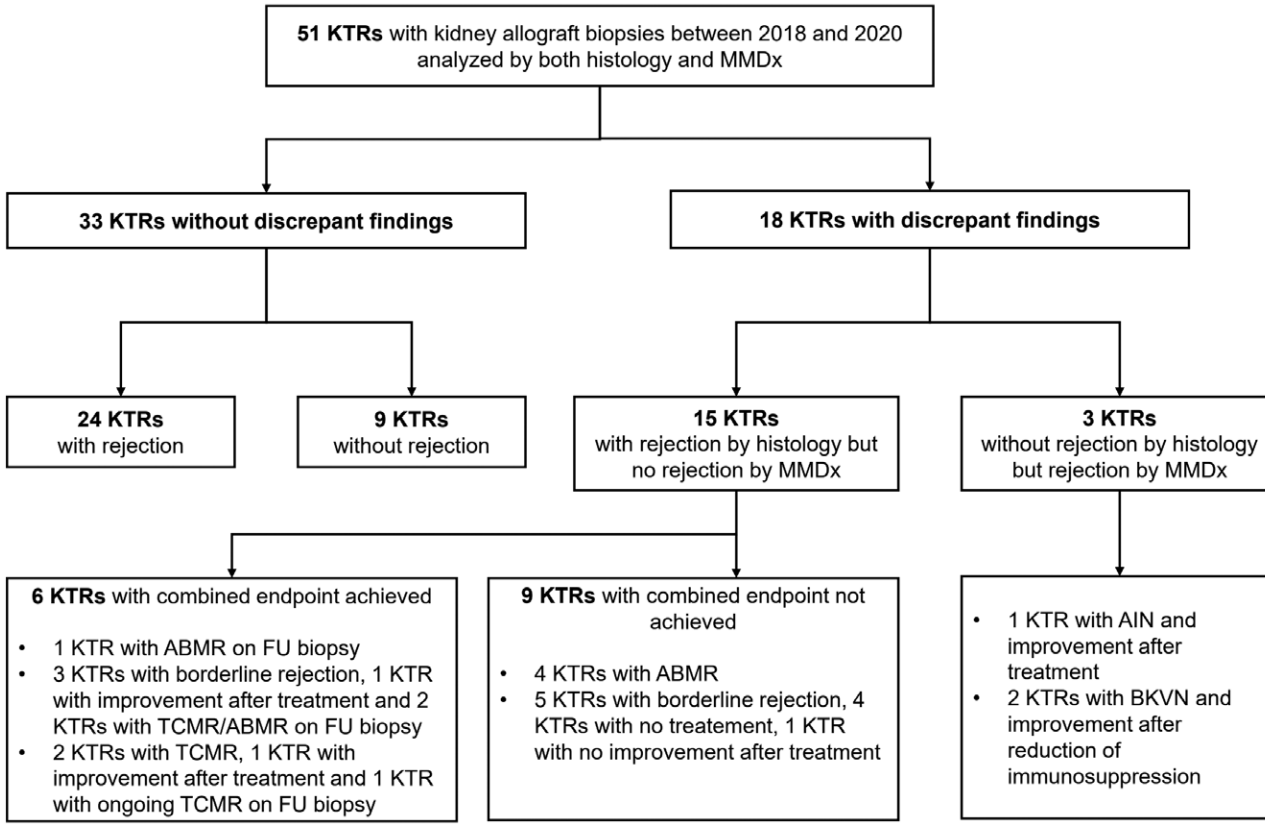

FIGURE 1. Flow diagram on discrepant and nondiscrepant cases and whether the combined endpoint was met with or without treatment. AMR, antibody-mediated rejection; AIN, acute interstitial nephritis; BKVN, BK virus nephropathy; FU, follicular unit; KTR, kidney transplant recipient; MMDx, Molecular Microscope Diagnostic System; TCMR, T cell–mediated rejection.

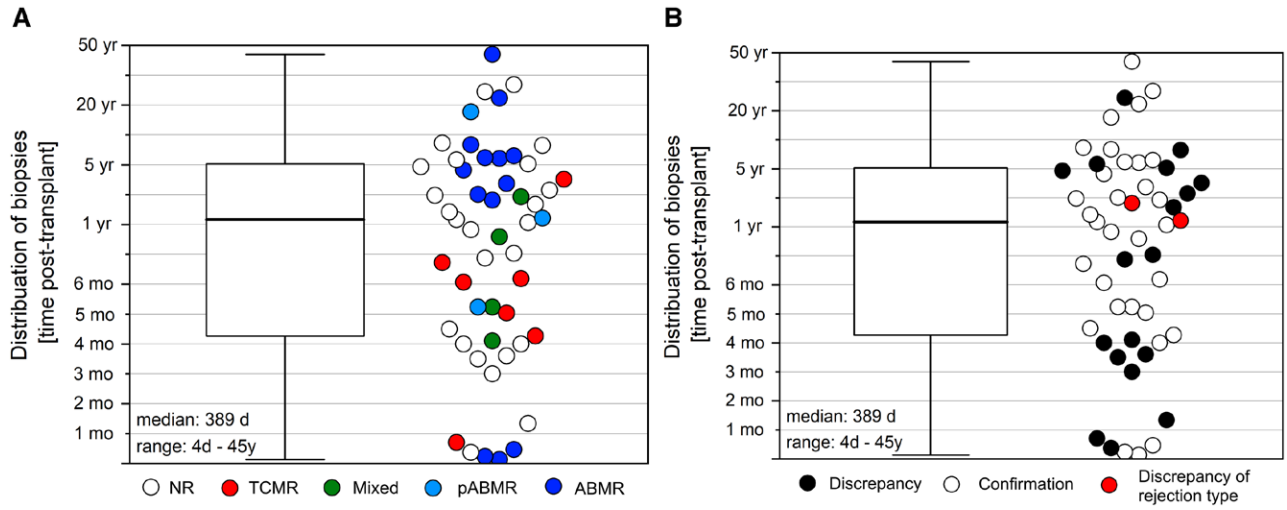

FIGURE 2. (A) Distribution of biopsies over time posttransplant with MMDx rejection case mix. (B) Distribution of biopsies over time posttransplant with discrepancies where MMDx differed from histology. AMR, antibody-mediated rejection; MMDx, Molecular Microscope Diagnostic System; NR, no rejection; pABMR, pathologic antibody-mediated rejection; TCMR, T cell–mediated rejection.

TABLE 1.
Pretransplant basic characteristics

	Study cohort (n = 51)
Recipient age at transplantation, y[a]	49 (23–73)
Recipient age at biopsy, y[a]	53 (26–77)
Recipient male sex, n (%)	37 (73)
Donor age, y[a]	51 (19–76)
Donor male sex, n (%)	27 (53)
KDPI of deceased donors[a]	57 (3–100)
Deceased donation, n (%)	29 (57)
DCD	5 (10)
Living donation, n (%)	22 (43)
ABO-compatible	18 (35)
ABO-incompatible	4 (8)
Number of kidney transplantation, n (%)	
1	48 (94)
≥2	3 (6)
Causes of ESRD, n (%)	
Glomerulonephritis	14 (27)
Diabetic nephropathy	3 (6)
Hypertensive nephropathy	3 (6)
Polycystic kidney disease	9 (18)
Uropathy (incl. CAKUT)	6 (12)
Other/undetermined	16 (31)
Preformed DSA before biopsy, n (%)	10 (20)
HLA-class 1	4 (8)
HLA-class 2	10 (20)

[a]Median (range).
CAKUT, congenital anomalies of kidney and urinary tract; DCD, donation after circulatory death; DSA, donor-specific antibodies; ESRD, end-stage renal disease.

TABLE 2.
Posttransplant basic characteristics

	Study cohort (n = 51)
Delayed graft function, n (%)	10 (20)
Maintenance immunosuppression at biopsy, n (%)	
Calcineurin inhibitor	48 (94)
Tacrolimus	38 (75)
Ciclosporin	10 (20)
Sirolimus	2 (4)
Belatacept	1 (2)
Antimetabolite	50 (98)
MMF/MPA	47 (92)
Azathioprine	3 (6)
Steroids	39 (76)
Concomitant CMV-replication, n (%)	5 (10)
Concomitant BKV-replication, n (%)	10 (20)
De novo DSA before biopsy, n (%)	14 (27)
HLA-class 1	3 (6)
HLA-class 2	14 (27)
De novo DSA after biopsy, n (%)	5 (10)
HLA-class 1	1 (2)
HLA-class 2	5 (10)

[a]Median (range).
BKV, BK virus; CMV, cytomegalovirus; DSA, donor-specific antibodies; MMF, mycophenolate mofetil; MPA, mycophenolic acid.

NR, the following histologic diagnoses were detected: 3 cases of normal kidney allograft histology (33%), 3 cases of calcineurin inhibitor toxicity (33%), 1 case of polyomavirus-associated nephropathy, 1 case of mesangial hypercellularity indicating recurrence of the primary IgA nephropathy, and 1 case of widespread tubulointerstitial calcifications of unknown origin.

In 18 of 51 cases (35%), MMDx differed from the histologic diagnosis. Fifteen of eighteen cases (83%) showed histologic R (but NR in the MMDx diagnosis), and 3 of 18 cases (17%) showed histologic NR (but R in the MMDx diagnosis). Among 5 of 15 KTRs with histologic R (but NR in the MMDx), 4 follow-up biopsies confirmed R in the MMDx diagnosis, and 1 follow-up biopsy showed NR in the MMDx and histologic R. Among 15 KTRs with histologic R (but NR in the MMDx), histology showed 10 cases of Borderline/TCMR (67%) and 5 cases of AMR (33%). Among 3 KTRs with histologic NR (but TCMR in the MMDx diagnosis), histology showed the following: 1 case of acute granulomatous interstitial nephritis attributed to cotrimoxazole with full

TABLE 3.
Biopsy-related characteristics

	Study cohort (n = 51)
Time of biopsy posttransplant, mo[a]	12 (0–543)
Early biopsies (<1 y)	26 (51)
Late biopsies (≥1 y)	25 (49)
Indication for biopsy, n (%)	
eGFR	39 (76)
Proteinuria	6 (12)
de novo DSA	4 (8)
Protocol biopsy, n (%)	2 (4)
MMDx percent cortex, %[a]	71 (0–96)
Baseline serum-creatinine before biopsy, µmol/L[a]	146 (80–550)
Serum-creatinine at biopsy, µmol/L[a]	180 (83–587)
Δ serum-creatinine at biopsy >30%	17 (33)
eGFR CKD-Epi at biopsy, mL/min[a]	36 (6–72)
Baseline proteinuria before biopsy, mg/mmol[a]	250 (0–8120)
Proteinuria ≥ 50 mg/mmol	20 (39)
Proteinuria at biopsy, mg/mmol[a]	370 (0–7770)
Histological diagnosis, n (%)	
No rejection	12 (24)
TCMR	18 (35)
Boderline	9 (18)
TCMR IA/IB	3 (6)
TCMR IIA/IIB	6 (12)
Mixed AMR/TCMR	1 (2)
AMR	20 (39)
Active AMR	3 (6)
Chronic-active AMR	17 (33)
Banff scores[b]	
t	1.1 (1.0)
i	0.7 (0.9)
v	0.4 (0.6)
ptc	0.8 (1.0)
g	0.9 (1.0)
cg	0.8 (1.2)
SV40 positivity, n (%)	5 (10)
Treatment, n (%)	
Steroids alone	13 (25)
IVIG alone	2 (4)
Plasma exchange alone	1 (2)
Steroids + plasma exchange	3 (6)
Steroids + IVIG	3 (6)
Steroids + plasma exchange + IVIG	1 (2)
No treatment	28 (55)

[a]Median (range)
[b]Mean (standard deviation).
AMR, antibody-mediated rejection; CKD, chronic kidney disease; DSA, donor-specific antibodies; eGFR, estimated glomerular filtration rate; IVIG, intravenous immunoglobulin; TCMR, T cell–mediated rejection.

recovery of kidney function after treatment with 1 mg per kg body weight of oral prednisone and 2 cases of polyomavirus-associated nephropathy with full recovery of kidney function after reduction of maintenance immunosuppression.

Clinical and Biopsy-related Factors Associated With Discrepancies Between the MMDx and Histologic Diagnosis

Pre- and posttransplant basic clinical characteristics and biopsy-related features of 15 KTRs with discrepant findings (MMDx NR/histology R), 9 KTRs with confirmed NR, and 24 KTRs with confirmed R are shown in Tables 5–7.

Discrepancies were more common among KTRs with donation after circulatory death (DCD), delayed graft function, and indication biopsy within the first posttransplant year. A biopsy-related factor associated with discrepant findings (MMDx NR/Histology R) is the percentage of cortex available for the MMDx diagnosis. Discrepant findings showed a median percentage of cortex of 15% (6 of 15 biopsies (40%) with all medulla) compared with 80% in concordant findings (6 of 33 biopsies [18%] with all medulla).

Clinical Evaluation of Discrepancies Between the MMDx and the Histologic Diagnosis

The 15 KTRs with discrepant findings (MMDx NR/histology R) were clinically evaluated using the combined endpoint in the 6 mo follow-up period: graft failure, R in the repeat biopsy, development of de novo DSA, or improvement of kidney allograft function >30% upon antirejection treatment at +6 mo after the baseline biopsy (Figure 1). The Kaplan-Meier curve for the combined endpoint is shown in Figure 3 and **Figure S1** (**SDC,** http://links.lww.com/TP/C508) (excluding borderline rejection cases). Nine KTRs with discrepant findings did not reach the endpoint, whereas 6 KTRs reached the combined endpoint (Figure 1). In 1 MMDx NR/histology rejection case, AMR was confirmed in the follow-up biopsy also in the MMDx. Among 5 KTRs with MMDx NR/histology borderline/TCMR, 3 KTRs showed an improvement in kidney function following treatment of borderline/TCMR, 2 KTRs displayed TCMR in the follow-up biopsy this time confirmed with the MMDx diagnosis, 1 KTR additionally developed de novo DSA, and 1 KTR showed resolution of TCMR in the follow-up biopsy (**Table S3, SDC,** http://links.lww.com/TP/C508).

The 15 KTRs with MMDx NR/histology R and the 3 KTRs with MMDx R/histology NR, the MMDx diagnosis, and the histology diagnosis reach an accurate clinical performance in 9 of 18 KTRs (50%) according to the combined clinical endpoint.

Pre- and posttransplant clinical and biopsy-related characteristics of 6 KTRs with discrepant findings (MMDx NR/histology R), who reached the combined endpoint, and 9 KTRs with discrepant findings (MMDx NR/histology R), who did not reach the combined endpoint, are shown in **Table S2A–C** (**SDC,** http://links.lww.com/TP/C508). The course of kidney allograft function over the observation period is shown in **Figure S2** (**SDC,** http://links.lww.com/TP/C508). Delayed graft function and indication biopsy within the first posttransplant year were more common among KTRs who reached the combined endpoint. Besides, t-lesion ≥2 was found among KTRs who reached the combined endpoint.

DISCUSSION

Although MMDx has emerged as a promising diagnostic platform and the number of publications discussing the advantages of MMDx has evolved,[6,10-14] no study has addressed the question regarding clinical performance in cases of a discrepancy using clinical benchmarks such as treatment response or change in function.

First, we further confirmed an overall discrepancy rate of 35% between the MMDx and histologic diagnosis,

TABLE 4.

Discrepancy rate between the MMDx and histologic diagnosis

		Histologic diagnosis			
		Rejection	No rejection	Row totals	No. discrepancies per row (%)
Automated MMDx diagnosis	Rejection	24	3[a]	27	3/27 (11)
	No rejection	15	9	24	15/24 (63)
Colum totals		39	12	51	
No. discrepancies per columns (%)		15/39 (38)	3/12 (25)		18/51 (35)

[a]Discrepancies explained by the histologic diagnosis.
MMDx, Molecular Microscope Diagnostic System.

TABLE 5.

Pretransplant basic characteristics of 15 KTRs (Discrepancy: MMDx NR/Histology R) vs 9 KTRs (confirmed NR) vs 24 KTRs (confirmed R)

	NR/R (n = 15)	NR/NR (n = 9)	R/R (n = 24)	*P*
Recipient age at transplantation, y[a]	42 (26–70)	47 (39–61)	55 (23–73)	0.368
Recipient age at biopsy, y[a]	49 (27–71)	58 (41–68)	59 (26–77)	0.214
Recipient male sex, n (%)	11 (73)	3 (33)	20 (83)	0.022
Donor age, y[a]	51 (27–76)	48 (43–55)	52 (19–70)	0.514
Donor male sex, n (%)	10 (67)	6 (67)	11 (46)	0.461
KDPI of deceased donors[a]	61 (16–100)	50 (49–54)	64 (3–95)	0.882
Deceased donation, n (%)	12 (80)	4 (44)	12 (50)	0.120
DCD	4 (27)	0 (0)	1 (4)	0.077
Living donation, n (%)	3 (20)	5 (56)	12 (50)	0.120
ABO-compatible	3 (20)	3 (33)	11 (46)	0.186
ABO-incompatible	0 (0)	2 (22)	1 (4)	
Number of kidney transplantation, n (%)				
1	13 (87)	9 (100)	23 (96)	0.573
≥2	2 (13)	0 (0)	1 (4)	
Causes of ESRD, n (%)				–
Glomerulonephritis	5 (33)	1 (11)	8 (33)	
Diabetic nephropathy	1 (7)	0 (0)	1 (4)	
Hypertensive Nephropathy	1 (7)	1 (11)	2 (8)	
Polycystic kidney disease	1 (7)	3 (33)	5 (21)	
Uropathy (incl. CAKUT)	2 (13)	0 (0)	3 (13)	
Other/undetermined	5 (33)	4 (44)	6 (25)	
Preformed DSA before biopsy, n (%)	3 (20)	1 (11)	5 (21)	0.896
HLA-class 1	2 (13)	0 (0)	2 (8)	0.652
HLA-class 2	3 (20)	1 (11)	5 (21)	0.457

[a]Median (range).
CAKUT, congenital anomalies of kidney and urinary tract; DCD, donation after circulatory death; DSA, donor-specific antibodies; ESRD, end-stage renal disease; KTRs, kidney transplant recipients; MMDx, Molecular Microscope Diagnostic System.

which is well supported in the literature.[13] Our analysis indicates that a diagnosis of rejection by MMDx is mainly associated with a diagnosis of R in histology. Only a few discrepancies were found when MMDx assigned a diagnosis of R, and histology showed no rejection. Here the clinical courses in 2 cases of polyomavirus-associated nephropathy and 1 case of acute granulomatous interstitial nephritis tend to support a false-positive MMDx diagnosis and the accuracy of the histologic diagnosis based on the response to treatment. The MMDx diagnosis of TCMR in BKV-associated nephropathy always acknowledges that TCMR may be directed at viral antigens, alloantigens, or both and that the changes may resolve without treatment when the virus clears. However, simultaneous TCMR molecular changes due to virus and alloantigens, according to the MMDx result, cannot be excluded either.[15,16] These discrepancies underline the lack of specificity of TCMR classifiers. Furthermore, MMDx cannot give a detailed picture in cases of nonrejection where histology further describes diagnosis signs of potential therapeutic relevance, such as calcineurin inhibitor toxicity.

The major discrepancy rate as high as 83% was seen between NR reported by MMDx and R by histology. Although half of these discrepancies were because of borderline rejection, with questionable therapeutic relevance,[17] in the other half, histology suggested treatment of TCMR or AMR. In line with the literature, borderline rejection, AMR suspicious, and transplant glomerulopathy

TABLE 6.

Posttransplant basic characteristics of 15 KTRs (Discrepancy: MMDx NR/Histology R) vs 9 KTRs (confirmed NR) vs 24 KTRs (confirmed R)

	NR/R (n = 15)	NR/NR (n = 9)	R/R (n = 24)	*P*
Delayed graft function, n (%)	6 (40)	0 (0)	4 (17)	0.060
Maintenance immunosuppression, n (%)				
Calcineurin Inhibitor	15 (100)	9 (100)	22 (92)	–
Tacrolimus	12 (80)	7 (78)	17 (71)	1
Ciclosporin	3 (20)	2 (22)	5 (21)	–
Sirolimus	0 (0)	1 (11)	1 (4)	–
Belatacept	0 (0)	0 (0)	1 (4)	–
Antimetabolite	15 (100)	7 (78)	24 (100)	1
MMF/MPA	14 (93)	7 (78)	22 (92)	0.510
Azathioprine	1 (7)	0 (0)	2 (8)	
Steroids	13 (87)	6 (67)	19 (79)	
Concomitant CMV-replication, n (%)	3 (20)	0 (0)	2 (8)	0.384
Concomitant BKV-replication, n (%)	3 (20)	1 (11)	5 (21)	0.896
De novo DSA before biopsy, n (%)	2 (13)	1 (11)	10 (42)	0.121
HLA-class 1	1 (7)	0 (0)	2 (8)	1
HLA-class 2	2 (13)	1 (11)	10 (42)	0.121
De novo DSA after biopsy, n (%)	2 (13)	0 (0)	4 (17)	0.635
HLA-class 1	1 (7)	0 (0)	0 (0)	0.500
HLA-class 2	2 (13)	0 (0)	4 (17)	0.718

[a]Median (range).

BKV, BK virus; CMV, cytomegalovirus; DSA, donor-specific antibodies; KTRs, kidney transplant recipients; MMDx, Molecular Microscope Diagnostic System; MMF, mycophenolate mofetil; MPA, mycophenolic acid

have been defined as ambiguous histology categories with high discrepancies.[13] Because intensification of immunosuppression, especially in older and vulnerable KTRs, can have far-reaching consequences, especially difficult-to-control bacterial and viral infections, which can adversely affect patient survival, graft survival, and graft function, the accuracy of an R is highly critical. This is where MMDx is suggested to overcome some limitations of histology. Although clinicians reported agreement with MMDx more often than with histology, a positive bias motivated by the novelty of MMDx cannot be entirely excluded.[14] Therefore, it seems all the more essential to evaluate the clinical performance of the R diagnosis in cases of discrepancy between MMDx and histology by the clinical course of KTRs.

Our approach in this comparative study was to investigate the diagnostic performance of histology compared with MMDx. Only the retrospect evaluation of the clinical course and response to treatment allows the claim for a good diagnostic performance. Hence, we defined a combined clinical endpoint occurring within a follow-up period of 6 mo consisting of graft failure, R in a follow-up biopsy, development of de novo DSA, or improvement of kidney allograft function to evaluate the clinical performance of R diagnosis. Sixty percent of KTRs with NR by MMDx but R by histology did not meet the clinical endpoint, indicating potentially useful information of MMDx.

The borderline category represents a particular challenge and reflects, in some cases, early TCMR progressing to fully developed TCMR, whereas others reflect acute kidney injury. Although the MMDx has not been trained on borderline rejection, it has been previously shown that MMDx reclassifies one-third of borderline cases showing molecular features of TCMR classifiers.[18] In our hands, 30% of borderline cases met our endpoint endpoint suggesting a diagnosis of rejection. In comparison, 70% did not reach the endpoint, which might be a positive bias toward MMDx as the latter has not been trained initially on considering borderline cases. Moreover, we know that MMDx does not appear to be an appropriate biomarker to predict the risk of future TCMR/AMR since borderline rejection may progress very slowly or not. Recently, donor-derived cell-free DNA has been suggested to predict adverse clinical outcomes among KTRs with borderline rejection.[19,20] Additionally, our work suggested that a high number of epitope mismatches puts KTRs with borderline rejection at an increased risk of progressing to TCMR.[21]

There are also cases where the MMDx contradicts the histological diagnosis of AMR in KTRs with DSA.[22] Whether this putative superiority of MMDx is real in these cases or whether the clinical endpoint is just not met because of the limited treatment options for AMR cannot be assessed. Biopsies classified as AMR by histology but with negative AMR scores in the MMDx diagnosis have been described previously.[7] These discrepancies have been suggested to represent false-positive histology, false-positive DSA, or false-negative MMDx, which may be attributed to the heterogeneity of AMR itself. AMR-positive MMDx scores correlated with peritubular capillaritis, glomerulitis, double contours, DSA, and C4d staining, but less with arterial fibrosis and arthritis.[14] Hence a lack of cortex in the analyzed tissue fragments might explain the discrepancy in AMR cases. Additionally, the fact that MMDx is not performed on the same sample tissue needs to be considered as an additional potential source for the observed discrepancies. Since MMDx is independent of DSA, it may clarify the ambiguous cases of AMR when the DSA is uncertain.[23,24] However, treatment effect in these

TABLE 7.

Biopsy-related characteristics of 15 KTRs (discrepancy: MMDx NR/Histology R) vs 9 KTRs (confirmed NR) vs 24 KTRs (confirmed R)

	NR/R (n = 15)	NR/NR (n = 9)	R/R (n = 24)	*P*
Time of biopsy posttransplant, mo[a]	8 (0–296)	12 (4–342)	23 (0–543)	0.685
Early biopsy (<1 y)	8 (53)	5 (56)	11 (46)	0.864
Late biopsy (≥1 y)	7 (47)	4 (44)	13 (54)	
Indication for biopsy, n (%)				
eGFR	13 (87)	6 (67)	18 (75)	0.912
Proteinuria	1 (7)	1 (11)	3 (13)	0.032
de novo DSA	1 (7)	0 (0)	3 (13)	
Protocol biopsy, n (%)	0 (0)	2 (22)	0 (0)	
MMDx percent cortex, %[a]	15 (0–88)	80 (2–95)	80 (0–96)	0.094
Baseline serum-creatinine before biopsy, μmol/L[a]	162 (91–228)	120 (80–230)	151 (101–550)	0.085
Serum-creatinine at biopsy, μmol/L[a]	190 (91–377)	133 (83–292)	190 (95–587)	0.059
Δ Serum-creatinine at biopsy >30%	5 (33)	2 (22)	7 (29)	0.918
eGFR CKD-Epi at biopsy, mL/min[a]	33 (12–60)	39 (14–72)	32 (6–66)	0.193
Baseline proteinuria before biopsy, mg/mmol[a]	255 (53–1147)	190 (50–8120)	277 (0–1600)	0.645
Proteinuria ≥500 mg/mmol	6 (40)	1 (11)	12 (50)	
Proteinuria at biopsy, mg/mmol[a]	255 (70–4222)	320 (70–7770)	530 (90–2000)	0.137
Histological diagnosis, n (%)				–
No rejection		9 (100)		
TCMR	10 (67)	–	8 (33)	
Boderline	8 (53)	–	1 (4)	
TCMR IA/IB	0 (0)	–	3 (13)	
TCMR IIA/IIB	2 (14)	–	4 (17)	
Mixed AMR/TCMR	0 (0)	–	1 (4)	
AMR	5 (33)	–	15 (63)	
active AMR	0 (0)	–	3 (13)	
Chronic-active AMR	5 (33)	–	12 (50)	
Banff scores[b]		–		
t	0.9 (0.7)	0.3 (0.7)	1.5 (1.0)	–
i	0.4 (0.5)	0.2 (0.4)	0.9 (1.1)	
v	0.3 (0.4)	0.0 (0.0)	0.6 (0.6)	
ptc	0.3 (0.6)	0.0 (0.0)	1.4 (1.0)	
g	0.6 (1.0)	0.3 (0.7)	1.4 (1.0)	
cg	1.1 (1.4)	0.1 (0.3)	1.0 (1.3)	
SV40 positivity, n (%)	0 (0)	1 (11)	4 (17)	
Treatment, n (%)				–
Steroids alone	4 (27)	0 (0)	8 (33)	
IVIG alone	0 (0)	0 (0)	1 (4)	
Plasma exchange alone	0 (0)	0 (0)	0 (0)	
Steroids + plasma exchange	1 (7)	0 (0)	1 (4)	
Steroids + IVIG	0 (0)	0 (0)	3 (13)	
Steroids + plasma exchange + IVIG	0 (0)	0 (0)	3 (13)	
No treatment	10 (67)	9 (100)	8 (33)	

[a]Median (range).
[b]Mean (standard deviation).
AMR, antibody-mediated rejection; DSA, donor-specific antibodies; eGFR, estimated glomerular filtration rate; KTRs, kidney transplant recipients; MMDx, Molecular Microscope Diagnostic System; TCMR, T cell–mediated rejection.

discrepant cases needs to be determined through a prospective study with random assignment to defined treatments.

Overall, our study showed that 40% of KTRs with discrepant findings of MMDx NR/histology R reached the combined endpoint, primarily observed in early biopsies within the first posttransplant year and when histology assigned a t-lesion score of ≥2 or v-lesion score of ≥1 with a clear diagnosis of TCMR.[1,2] Hence, especially in cases of histologic diagnosis of borderline/TCMR rejection category, higher Banff t lesions scores are calling for therapeutic intervention. In line herewith, TCMR-positive MMDx scores have been shown to correlate mainly with interstitial infiltrates and tubulitis but less with arteritis.[25,26] The finding that mainly early biopsies reached the endpoint accounts for more reversible conditions in the early posttransplant period, whereas late biopsies often show

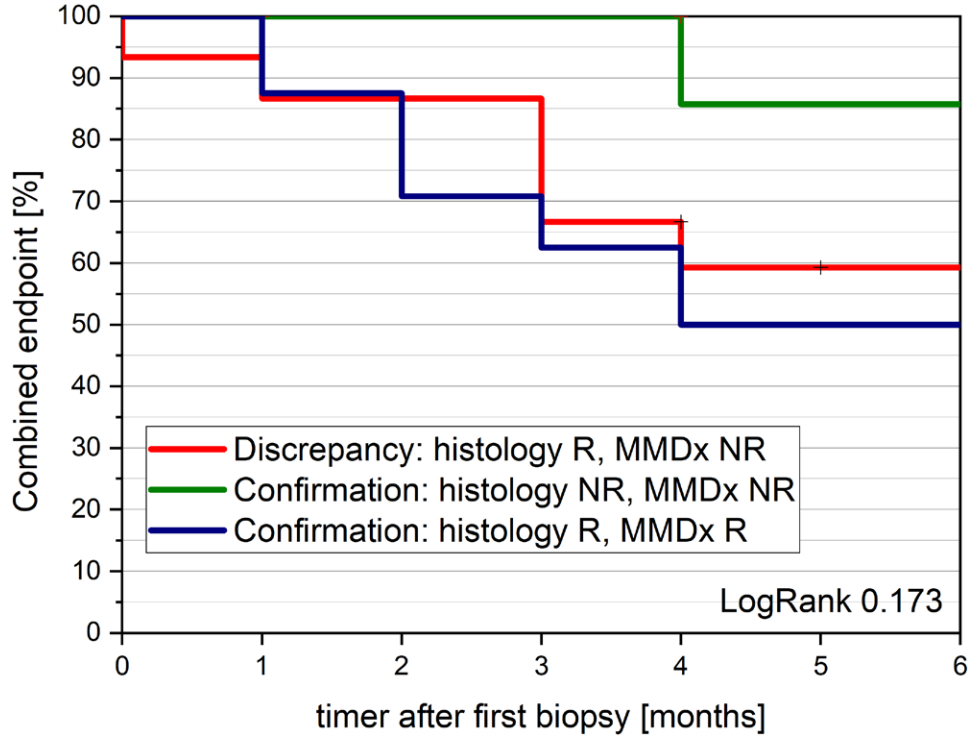

FIGURE 3. Combined endpoint of (1) kidney allograft loss, (2) development of de novo DSA, (3) confirmation of R on follow-up biopsy, or (4) improvement of kidney allograft function upon antirejection treatment between 15 KTRs (discrepant R [histology]/NR [MMDx]) vs 9 KTRs (confirmed NR) vs 24 KTRs (confirmed R). DSA, donor-specific antibody; KTR, kidney transplant recipient; MMDx, Molecular Microscope Diagnostic System; NR, no rejection; R, rejection.

untreatable conditions, particularly AMR. At least, the lack of cortex is suspicious to explain the cases in which MMDx missed the diagnosis of AMR in the first biopsy but confirmed it in the follow-up biopsy. Although the MMDx is supposed to read medulla biopsy, all medulla samples have been suggested to undergo adjusted interpretation and warrant caution in clinical practice.[10] Hence, according to our data, 3-mm tissue fragments are sufficient for accurate diagnosis in case of a high percentage of cortex, but not if only presenting medulla, especially concerning diagnosing AMR. This limitation of MMDx could be circumvented relatively easily in clinical practice. Biopsies that are very small or predominantly medulla should be avoided because they increase the risk of sampling error.

Limitations of our study are the relatively small number of biopsies and repeat biopsies and the retrospective character. Yet, our data's high granularity with thorough follow-up of each KTR and the availability of repeat biopsies in a relevant number of patients are clear strengths. Our approach reflects the day-to-day care of transplant patients. In difficult and complex situations where the "troubled" physician must decide whether to increase, decrease, or maintain the immunosuppression, we added the MMDx results to the standard histology reading. In this clinical context, the useful clinical information of the molecular read-out often confirmed the histology reading and the treatment strategy. In case of discrepancies, the molecular diagnosis prompted an intense discussion and reevaluation with the pathologist again in the clinical context and course. Hence, this integrated approach of histology and molecular reading is a real benefit for the patient and explains why the physician and the pathologist ask for diagnosis provided by the MMDx technology, acknowledging the diagnostic challenge of biopsy readings and treatment decisions.

The definition of diagnostic criteria, which help decide whether MMDx or histology is correct in discrepancies, is crucial in clinical patient care. We propose the following approach regarding the use of MMDx in clinical practice: in late posttransplant biopsies with a histologic diagnosis of borderline rejection, we suggest adding an analysis by MMDx, which may help clarify the need for treatment. This reclassification of borderline lesions into NR, TCMR, or AMR is an unmet issue that has been discussed extensively[22] and might be solved by MMDx.[23] However, in biopsies during the first posttransplant year showing high Banff t lesions scores, the histologic diagnosis of rejection should govern the treatment and optional testing by MMDx.

Ideally, a clinical trial randomly assigning KTRs with a discrepancy in the diagnosis of AMR, the diagnosis of borderline rejection, and possibly TCMR with stable graft function would help further investigate the diagnostic performance of MMDx compared with histology concerning clinical endpoints. A clinical trial randomly assigning KTRs to treatment seems reasonable based on the evidence.

Our work cannot conclusively assess the benefit of follow-up biopsies, including MMDx. Especially in cases of confirmed R, it remains open whether follow-up biopsies with MMDx can further improve treatment decision-making and prognosis. Since 1 primary goal of MMDx is to overcome subjective judgment, required expert opinion, and poor interobserver reproducibility of histologic assessment, our single-center study is clearly influenced by our

expert pathologists' work, which may be another reason for the suspected overdiagnosis by histologic assessment.

In summary, discrepancies between the MMDx and the histologic diagnosis arise most likely in cases of MMDx showing NR and histology diagnosis of R. These discrepant findings appear relevant not only in cases of histological borderline rejection but also in AMR and TCMR. Our clinical follow-up suggests that MMDx might provide clinically useful information to the histologic diagnosis in discrepant cases, supporting the further implementation of MMDx in clinical practice. However, strong histological findings with clear Banff t-score lesions have priority to indicate the treatment even in discrepant situations.

REFERENCES

1. Racusen LC, Solez K, Colvin RB, et al. The Banff 97 working classification of renal allograft pathology. *Kidney Int*. 1999;55:713–723.
2. Roufosse C, Simmonds N, Clahsen-van Groningen M, et al. A 2018 reference guide to the banff classification of renal allograft pathology. *Transplantation*. 2018;102:1795–1814.
3. Loupy A, Haas M, Roufosse C, et al. The Banff 2019 Kidney Meeting Report (I): Updates on and clarification of criteria for T cell- and antibody-mediated rejection. *Am J Transplant*. 2020;20:2318–2331.
4. Furness PN, Taub N, Assmann KJ, et al. International variation in histologic grading is large, and persistent feedback does not improve reproducibility. *Am J Surg Pathol*. 2003;27:805–810.
5. Furness PN, Taub N; Convergence of European Renal Transplant Pathology Assessment Procedures (CERTPAP) Project. International variation in the interpretation of renal transplant biopsies: report of the CERTPAP Project. *Kidney Int*. 2001;60:1998–2012.
6. Halloran PF, Famulski KS, Reeve J. Molecular assessment of disease states in kidney transplant biopsy samples. *Nat Rev Nephrol*. 2016;12:534–548.
7. Famulski KS, de Freitas DG, Kreepala C, et al. Molecular phenotypes of acute kidney injury in kidney transplants. *J Am Soc Nephrol*. 2012;23:948–958.
8. Einecke G, Reeve J, Sis B, et al. A molecular classifier for predicting future graft loss in late kidney transplant biopsies. *J Clin Invest*. 2010;120:1862–1872.
9. Reeve J, Böhmig GA, Eskandary F, et al; INTERCOMEX MMDx-Kidney Study Group. Generating automated kidney transplant biopsy reports combining molecular measurements with ensembles of machine learning classifiers. *Am J Transplant*. 2019;19:2719–2731.
10. Madill-Thomsen KS, Wiggins RC, Eskandary F, et al. The effect of cortex/medulla proportions on molecular diagnoses in kidney transplant biopsies: rejection and injury can be assessed in medulla. *Am J Transplant*. 2017;17:2117–2128.
11. Reeve J, Böhmig GA, Eskandary F, et al; MMDx-Kidney study group. Assessing rejection-related disease in kidney transplant biopsies based on archetypal analysis of molecular phenotypes. *JCI Insight*. 2017;2:94197.
12. Halloran PF, Reeve J, Akalin E, et al. Real time central assessment of kidney transplant indication biopsies by microarrays: the INTERCOMEX study. *Am J Transplant*. 2017;17:2851–2862.
13. Madill-Thomsen K, Perkowska-Ptasińska A, Böhmig GA, et al; MMDx-Kidney Study Group. Discrepancy analysis comparing molecular and histology diagnoses in kidney transplant biopsies. *Am J Transplant*. 2020;20:1341–1350.
14. Reeve J, Sellarés J, Mengel M, et al. Molecular diagnosis of T cell-mediated rejection in human kidney transplant biopsies. *Am J Transplant*. 2013;13:645–655.
15. Pesavento TE. The Use of molecular techniques to distinguish BK nephropathy from acute rejection: close but not quite. *Transplantation*. 2021;105:2346–2347.
16. Halloran PF, Madill-Thomsen KS, Böhmig GA, et al; INTERCOMEX Investigators. A 2-fold approach to polyoma virus (BK) nephropathy in kidney transplants: distinguishing direct virus effects from cognate T cell-mediated inflammation. *Transplantation*. 2021;105:2374–2384.
17. de Freitas DG, Sellarés J, Mengel M, et al. The nature of biopsies with "borderline rejection" and prospects for eliminating this category. *Am J Transplant*. 2012;12:191–201.
18. Gupta G, Moinuddin I, Kamal L, et al. Correlation of donor-derived cell-free DNA with histology and molecular diagnoses of kidney transplant biopsies. *Transplantation*. 2022;106:1061–1070.
19. Stites E, Kumar D, Olaitan O, et al. High levels of dd-cfDNA identify patients with TCMR 1A and borderline allograft rejection at elevated risk of graft injury. *Am J Transplant*. 2020;20:2491–2498.
20. Lezoeva E, Nilsson J, Wüthrich R, et al. High PIRCHE scores may allow risk stratification of borderline rejection in kidney transplant recipients. *Front Immunol*. 2022;13:788818.
21. Halloran PF, Chang J, Famulski K, et al. Disappearance of T cell-mediated rejection despite continued antibody-mediated rejection in late kidney transplant recipients. *J Am Soc Nephrol*. 2015;26:1711–1720.
22. Einecke G, Reeve J, Gupta G, et al; INTERCOMEX investigators. Factors associated with kidney graft survival in pure antibody-mediated rejection at the time of indication biopsy: Importance of parenchymal injury but not disease activity. *Am J Transplant*. 2021;21:1391–1401.
23. Senev A, Coemans M, Lerut E, et al. Histological picture of antibody-mediated rejection without donor-specific anti-HLA antibodies: Clinical presentation and implications for outcome. *Am J Transplant*. 2019;19:763–780.
24. Snijders MLH, Varol H, van der Zwan M, et al. Molecular analysis of renal allograft biopsies: Where do we stand and where are we going? *Transplantation*. 2020;104:2478–2486.
25. Hruba P, Madill-Thomsen K, Mackova M, et al. Molecular patterns of isolated tubulitis differ from tubulitis with interstitial inflammation in early indication biopsies of kidney allografts. *Sci Rep*. 2020;10:22220.
26. Hruba P, Madill-Thomsen K, Mackova M, et al. Three-month course of intragraft transcriptional changes in kidney allografts with early histological minimal injury—a cohort study. *Transpl Int*. 2021;34:974–985.