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Development and validation of a peripheral blood mRNA assay for the assessment of antibody-mediated kidney allograft rejection: A multicentre, prospective study

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ABSTRACT

Background: Antibody-mediated rejection, a leading cause of renal allograft graft failure, is diagnosed by histological assessment of invasive allograft biopsies. Accurate non-invasive biomarkers are not available.
Methods: In the multicentre, prospective BIOMARGIN study, blood samples were prospectively collected at time of renal allograft biopsies between June 2011 and August 2016 and analyzed in three phases. The discovery and derivation phases of the study ($N = 117$ and $N = 183$ respectively) followed a case-control design and included whole genome transcriptomics and targeted mRNA expression analysis to construct and lock a multigene model. The primary end point was the diagnostic accuracy of the locked multigene assay for antibody-mediated rejection in a third validation cohort of serially collected blood samples ($N = 387$). This trial is registered with ClinicalTrials.gov, number NCT02832661.
Findings: We identified and locked an 8-gene assay (*CXCL10, FCGR1A, FCGR1B, GBP1, GBP4, IL15, KLRC1, TIMP1*) in blood samples from the discovery and derivation phases for discrimination between cases with ($N = 49$) and without ($N = 134$) antibody-mediated rejection. In the validation cohort, this 8-gene assay discriminated between cases with ($N = 41$) and without antibody-mediated rejection ($N = 346$) with good diagnostic accuracy (ROC AUC 79·9%; 95% CI 72·6 to 87·2, $p < 0{\cdot}0001$). The diagnostic accuracy of the 8-gene assay was retained both at time of stable graft function and of graft dysfunction, within the first year and also later after transplantation. The 8-gene assay is correlated with microvascular inflammation and transplant glomerulopathy, but not with the histological lesions of T-cell mediated rejection.
Interpretation: We identified and validated a novel 8-gene expression assay that can be used for non-invasive diagnosis of antibody-mediated rejection.
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1. Introduction

Antibody-mediated rejection is recognized as a primary cause of graft failure after kidney transplantation [1–3]. It is hallmarked

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Research in context

Evidence before this study

Despite the use of powerful immunosuppression after kidney transplantation, allograft rejection, and most importantly antibody-mediated rejection, remains a strong predictor of graft failure. The diagnosis of antibody-mediated rejection is made on kidney allograft biopsies performed at the time of decline in glomerular filtration rate or appearance of proteinuria. Because antibody-mediated rejection can occur in the absence of immediate clinical signs or changes in these graft functional characteristics, some centers also perform kidney allograft biopsies at fixed time points (surveillance biopsies). Glomerular filtration rate and proteinuria are non-specific markers for antibody-mediated rejection, as many other immunological and non-immunological injuries can disturb graft function. More accurate non-invasive diagnostic markers are thus needed, with better sensitivity and specificity for antibody-mediated rejection than estimated glomerular filtration rate and proteinuria. We have systematically followed up and reviewed the scientific literature related to the development of non-invasive biomarkers for antibody-mediated rejection of kidney allografts. We focused on PubMed and Scopus for papers that report on non-invasive diagnostic markers for antibody-mediated kidney allograft rejection. We found only few independently validated non-invasive peripheral blood biomarkers for acute rejection. None of these markers were further developed or specific for diagnosis of antibody-mediated rejection.

Added value of this study

In this study, we identified and independently validated a novel non-invasive biomarker for antibody-mediated kidney allograft rejection in peripheral blood. To our knowledge, this is the first study to report on a biomarker with sufficiently good diagnostic accuracy for antibody-mediated rejection to be useful for clinical care of kidney transplant recipients. The diagnostic accuracy of the 8-gene assay for antibody-mediated rejection was superior to that of classical clinical indicators, and the assay offered additional benefit in clinical decision-making to perform or not to perform a biopsy for diagnosis of antibody-mediated rejection. We were able to show that the diagnostic accuracy of the 8-gene assay was retained in different clinical scenarios: early and late after transplantation, at time of stable graft function and at time of graft dysfunction.

Implications of all the available evidence

Our results indicate that kidney transplant recipients suffering from antibody-mediated rejection can be identified by our newly developed non-invasive 8-gene assay. The 8-gene assay can serve as a clinical decision aid whether or not to perform an invasive biopsy for confirmation of antibody-mediated rejection at times of clinical suspicion. In routine follow-up of stable kidney transplant patients, this biomarker shows potential for more timely detection of often-missed subclinical antibody-mediated rejection and prompt initiation of targeted therapies after confirmative histological diagnosis, before chronic damage has developed and before the disease process becomes irreversible.

histologically by inflammation and C4d deposition in peritubular capillaries, glomerulitis, intimal arteritis and expansion/duplication of the glomerular basement membrane [4].

Currently, the diagnosis of antibody-mediated rejection after kidney transplantation is made based on histological assessment of invasive kidney biopsies according to the regularly updated Banff international consensus [4]. Antibody-mediated rejection can be diagnosed in clinically indicated biopsies at time of graft functional problems (rise in serum creatinine or proteinuria), but can also occur subclinically, without changes in these graft functional parameters. Subclinical antibody-mediated rejection also associates with increased risk of graft failure [5] but often remains undetected, unless protocol-specified (surveillance) kidney biopsies are performed [2,6]. Such protocol-specified biopsies are routinely performed in some centres, but not all, at varying time after transplantation.

Based on the association between antibody-mediated rejection and kidney graft failure, and the impossibility to repeatedly perform invasive protocol-specified biopsies, non-invasive diagnostic markers are needed with better sensitivity and specificity than eGFR and proteinuria [2,5,7]. Other groups have suggested non-invasive markers for antibody-mediated rejection, primarily assessed in urine samples [8–13]. Additional validation of these markers is necessary to support their potential clinical value [14–16].

Kidney allograft rejection is associated with molecular changes in renal allograft tissue, which reflect transcription changes in resident cells (e.g. interferon-gamma inducible changes in the donor endothelium) or changes in cell populations, like infiltration and activation of effector T cells and macrophages in T- cell mediated rejection or margination and activation of natural killer cells in antibody-mediated rejection [17,18]. As these graft infiltrating cells are activated primarily in lymphoid organs before travelling and infiltrating the allograft [19], we hypothesized that the molecular changes that occur in renal allograft biopsies with antibody-mediated rejection could also be reflected by changes in circulating immune cells.

Given the lack of non-invasive, sufficiently validated markers for antibody-mediated rejection, we aimed to develop and validate an mRNA-based gene set in peripheral blood that is able to non-invasively rule out or detect ongoing antibody-mediated rejection after kidney transplantation.

2. Methods

2.1. Study design, patient population and sample collection

This part of the BIOMArkers of Renal Graft INjuries (BIOMARGIN) study (www.biomargin.eu) is a multicentre, prospective, multiphase study, performed in four European transplant centres (Hôpital Necker Paris, France; University Hospitals Leuven, Belgium; Medizinische Hochschule Hannover, Germany; and Centre Hospitalier Universitaire Limoges, France). Samples were prospectively and consecutively collected at time of renal allograft biopsies, between June 2011 and August 2016. In the four clinical centres, protocol renal allograft biopsies were performed at 3, 12, and sometimes at 24 months after transplantation, according to local centre practice, in addition to clinically indicated biopsies (biopsies at time of graft dysfunction). All adult patients who had received a single kidney allograft at these institutions and who provided written informed consent, were eligible. Recipients of combined transplantations were excluded. All transplantations were performed with negative complement-dependent cytotoxicity cross-matches. Institutional review boards and national regulatory agencies (when required) approved the study protocol at each clinical centre.

The study was divided in three phases. In the discovery phase, blood and biopsy samples were used for genome-wide expression analysis. We selected samples based on availability and histological criteria of concomitant renal allograft biopsies (excluding cases with diagnosis of glomerulonephritis or polyomavirus nephropathy, and cases with unclear diagnosis). Based on local biopsy readings, a first selection was made, which was then further refined by judgment of the clinical courses and final confirmation by central pathology, independent from the original centre (see the Supplementary Appendix). The same study design was used for the second derivation phase, for targeted

validation of the results obtained in the discovery set, and derivation of the multigene marker. In the validation cohort, all samples with concomitant adequate renal allograft biopsy histology, prospectively collected according to the BIOMARGIN protocol between June 24, 2014 and July 2, 2015 were serially included. In this cohort, no selection was made on histology, demographics, time or any other factor than sample availability. In this validation cohort, the analyses were entirely based on central pathology scores.

2.2. Primary and secondary end points

The primary end point was the diagnostic accuracy of a multigene marker for antibody-mediated rejection in the validation cohort in relation to the current gold standard of tissue pathology, based on the area under the receiver operating characteristic curve (ROC AUC). Secondary endpoints were the diagnostic accuracy in specific clinical situations (at time of graft dysfunction leading clinicians to perform an indication biopsy versus at time of stable graft function, early (<1 year) versus later (>1 year) after transplantation), and net benefit for clinical decision-making.

2.3. Sample collection and biopsy scoring

Peripheral blood samples were collected at time of the renal allograft biopsies, directly in PAXgene Blood RNA tubes® (PreAnalytiX GmbH, a Qiagen/ BD Company, Switzerland). Two needle cores were taken at each kidney allograft biopsy. One was used for histology, at least half of the other one was immediately stored in Allprotect Tissue Reagent® (Qiagen Benelux BV, Venlo, The Netherlands) for RNA expression analysis (in the discovery set). All biopsies were rescored semiquantitatively according to the updated Banff 2017 classification [4]. Index test results were not available to assessors of the histopathological scoring (reference standard), whereas reference standard results and clinical information were available to the readers of the index test.

In the discovery cohort, RNA extracted from blood and biopsies was hybridized onto Affymetrix GeneChip Human Genome U133 Plus 2.0 Arrays (Affymetrix Inc., High Wycombe HP10 0HH, UK). In the derivation and validation cohorts, RNA expression analysis of mRNA extracted from blood samples was evaluated by real-time polymerase chain reaction (RT-PCR) using OpenArray® technology on the Quantstudio™ 12 K Flex Real-Time PCR System (Life Technologies Europe BV, Ghent, Belgium) with ACTB, GAPDH and SDHA as endogenous controls. Details on blood and biopsy sample collection and gene expression analysis are provided in the Supplementary Appendix.

2.4. Statistical analysis

In the discovery phase, robust-multiarray average-normalized mRNA expression data of the 117 peripheral blood samples and 95 biopsies were analyzed in a statistical pipeline developed under the R framework in an extension of the *biosigner* R package as developed for this study [20], with addition of Elastic-Net and Shrunken Centroids multivariate methods to the Sparse Partial Least Squares (SPLS), Random Forrest and Support Vector Machines-Recursive Feature Elimination (SVM-RFE) multivariate methods already available in the biosigner package. More information on the constructed statistical pipeline and determination of a multivariate score for antibody-mediated rejection (ABMR score) and T-cell mediated rejection (TCMR score) is given in the Supplementary Appendix. A multivariate score $> 0{\cdot}25$ was considered as specific for antibody-mediated and/or T-cell mediated rejection. Ingenuity Pathway Analysis (IPA, Build: 478438 M Content version: 44691306) was used for canonical pathway enrichment analysis.

In the derivation phase, we identified the multivariate combination of transcripts that lead to the best model performance, based on the extended list of transcripts obtained in the discovery phase. This identification of the multigene signature was done by ranking a combination of genes according to the C-statistic of logistic regression models trained on this combination and estimated under a 3-folds cross validation. Instead of identifying the best combination as the final multigene signature, we integrated the combinations obtained by the top K models (see Supplementary Appendix). The best multigene signature was then used to build a multivariable logistic regression model in a nested-cross validation approach on the derivation cohort. The ensuing logistic regression model (intercept and estimations) was then locked and represented the final multigene assay.

The diagnostic accuracy of the locked multigene signature and logistic regression model calculated in the derivation phase was then evaluated on the validation cohort. We used receiver operating characteristic (ROC) curves to evaluate the C-statistic (area under the curve, AUC) of the multigene assay. The optimal marker threshold from the derivation phase, at the highest combination of sensitivity and specificity (which are independent of disease prevalence), was then evaluated in this validation cohort. In addition, arbitrarily defined low and high thresholds with respectively high negative and positive predictive values (which are dependent on actual disease prevalence) were evaluated in the validation cohort.

Finally, post-hoc sensitivity analyses were performed to evaluate the accuracy of the marker in specific clinical situations. The net benefit of the 8-gene marker for clinical decision-making was evaluated using decision curve analysis [21]. To allow for comparison and assess added value of the 8-gene assay compared with clinical parameters alone, we built a clinical model using the 8 clinical parameters that were differently prevalent in the ABMR vs. no AMBR group. This model was built and cross-validated in the discovery and derivation phases combined and then assessed in the independent validation phase. The diagnostic performances of the clinical model, the 8-gene assay and an integrated model (adding the 8-gene assay to the clinical model) were assessed and compared using ROC CONTRAST and random forest out-of-bag error rates (using 500 trees). For variance analysis of continuous clinical variables in different groups, non-parametric Wilcoxon-Mann-Whitney U, non-parametric ANOVA and parametric one-way ANOVA were used. Dichotomous variables were compared using the chi-square test. R [22], SAS (version 9.4; SAS institute, Cary, NC) and GraphPad Prism (version 7; GraphPad Software, San Diego, CA) were used for data presentation. Normalized signal intensities and. CEL files of the transcriptomic data were deposited at the NIH Gene Expression Omnibus http://www.ncbi.nlm.nih.gov/geo under the series accession number GSE 129166) and the microarray data were handled in accordance with the MIAME (Minimum Information About a Microarray Experiment) guidelines. The BIOMARGIN study is registered with ClinicalTrials.gov, number NCT02832661. The checklist according the STARD guidelines for diagnostic accuracy studies was completed and is included in the Supplementary Appendix (Table S9).

2.5. Study approval

All patients provided informed written consent.

3. Results

3.1. Baseline characteristics

We included 687 peripheral blood samples from 630 patients obtained at the time of a renal allograft biopsy, 120 with antibody-mediated rejection and 567 without (Fig. 1). In the discovery phase, 117 blood samples and 95 biopsy samples were used for genome-wide expression analysis from 117 patients, in a case-control study design. In the derivation phase, we included 183 samples from 183 individual patients, again in a case-control setting. In the independent validation cohort, 387 consecutively collected and unselected samples from 365 patients were included in the analyses; 56 samples were

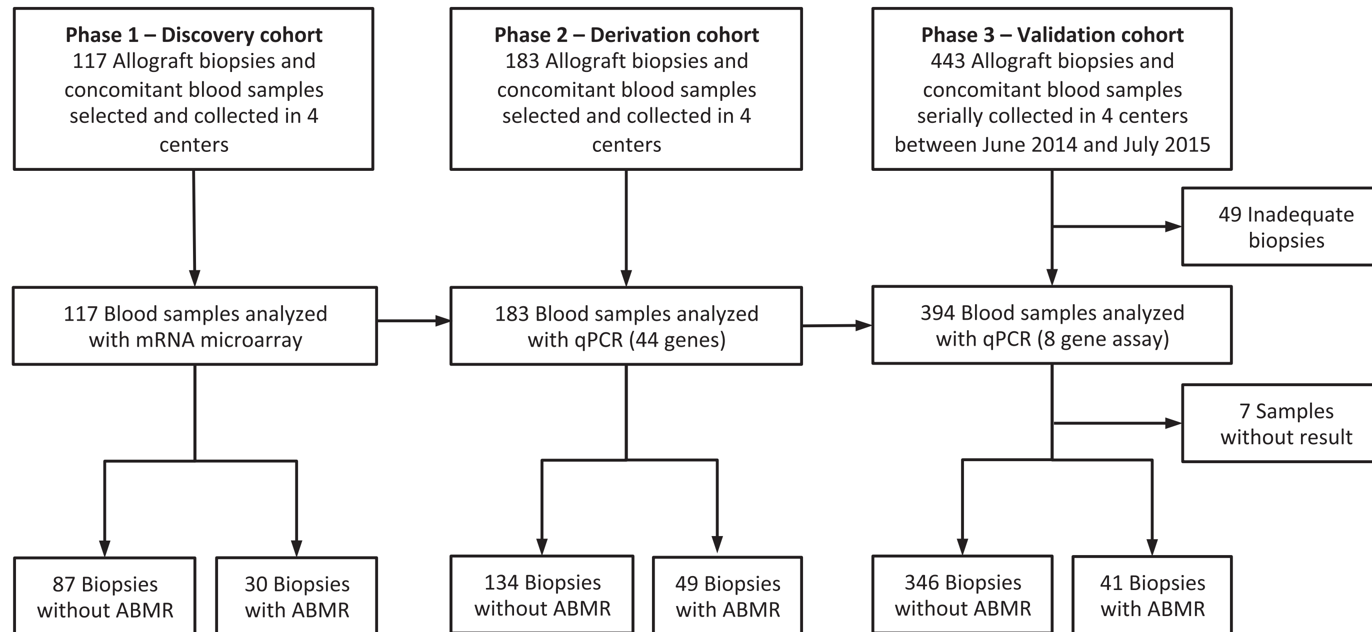


Fig. 1. Study design. Peripheral blood samples were obtained at the time of a renal allograft biopsy in four European transplant centres. In the discovery and derivation cohort, samples were selected based on availability and histological criteria of concomitant renal allograft biopsies (excluding cases with diagnosis of glomerulonephritis or polyomavirus nephropathy, and cases with unclear diagnosis), while graft function was not taken into account. In the validation cohort, all samples with concomitant adequate renal allograft biopsy histology, prospectively collected between June 24, 2014 and July 2, 2015, were serially included without selection on histology, demographics or time. The gene expression profile was not complete in seven of these samples, leading to a total of 387 cases in the validation phase. ABMR = antibody-mediated rejection.

Table 1
Characteristics of the patients and biopsies included in the validation cohort ($N = 365$ patients, 387 biopsies).

Variable	Mean (median) ± standard deviation (min – max) or no. (%)
Transplant characteristics (N = 365 patients)	
Recipient age at transplantation (years)	50·2 (52·2) ± 15·3 (2·7–78·5)
Recipient age at time of biopsy (years)	52·4 (54·1) ± 14·4 (19·0–79·6)
Recipient sex (male/female)	224/141 (61·4%/38·6%)
Repeat transplantation	64 (17·5%)
Recipient ethnicity (European/ Asian/ African/ Other)	318/3/6/35* (87·8%/0·8%/1·7%/9·7%)
Donor age (years)	50·6 (52·0) ± 15·5 * (5·0–91·0)
Donor sex (male/female)	177/180* (50·4%/49·6%)
Deceased/Living donor	278/83* (77·0%/23·0%)
Heart-beating/ Non-heart-beating donor	250/28* (89·9%/10·1%)
Cold ischemia time (hours)	12·1* (12·6) ± 7·8 (0·27–35·8)
Biopsy characteristics ($N = 387$ biopsies)	
Indication/protocol biopsy	134/253 (34·6%/65·4%)
Time after transplantation (days)	908 (359) ± 1733 (6–12,564)
Biopsy time after transplantation	
< 1 year	207 (53·5%)
> 1 year	180 (46·5%)
MDRD eGFR, (ml/min/1·73 m^2)	43·0 (41·8) ± 17·5 (5·8–96·2)
Proteinuria (g/g creatinine)	0·4* (0·1) ± 1·0 (0·0–8·0)
Immunosuppression at time of biopsy	
Cyclosporine	40 (10·3%)
Tacrolimus	331 (85·5%)
Mycophenolate	320 (82·7%)
Azathioprine	9 (2·3%)
mTOR inhibitor	49 (12·7%)
Corticosteroids	352 (91·0%)
Histological diagnosis	
No rejection	330 (85·3%)
T-cell mediated rejection	
Borderline changes	15 (3·9%)
Grade 1 or 2	4 (1·0%)
Antibody-mediated rejection	41 (10·6%)
Mixed rejection^	3 (0·8%)
Interstitial fibrosis/tubular atrophy	
Grade 0	182 (47·0%)
Grade 1	93 (24·0%)
Grade 2	64 (16·5%)
Grade 3	48 (12·4%)
Polyomavirus-associated nephropathy	14 (3·6%)
De novo/recurrent glomerulonephritis	26 (6·7%)

^Mixed rejection cases are defined by co-occurrence of antibody-mediated rejection and T-cell mediated rejection.
* Missing data on donor age, donor gender, donor type, cold ischemia time, proteinuria, recipient ethnicity and induction therapy.

excluded, 49 because of inadequate biopsy histology and seven because of incompleteness of the gene expression profile. Patients' demographics and clinical characteristics of the three independent peripheral blood sample sets are provided in Table 1 and Table S1 in the Supplementary Appendix. There were significant differences between patients with versus without antibody-mediated rejection in some baseline characteristics, reflecting the background risk for development of antibody-mediated rejection as shown in Table 2. Details on the clinical characteristics of the biopsy samples used for micro-array gene expression ($N = 95$) were provided separately [18]. Histological characteristics of the biopsies in the validation set are provided in Table S2.

3.2. Discovery of transcripts specific for antibody-mediated rejection

In peripheral blood and biopsy samples, respectively 970 and 783 probesets (730 and 576 individual genes) had an ABMR score > 0·25. Pathway enrichment analysis of the biopsy signature was previously published [18]. Based on ABMR scores in peripheral blood and biopsies, 2 genelists were determined (Fig. S1 in the Supplementary Appendix). In both genelists, there was significant enrichment in natural killer cell signaling, crosstalk between dendritic and natural killer cells, communication between innate and adaptive immune cells and antigen presentation pathways. Predicted upstream factor analysis identified interferons and interferon regulatory factors as the most likely upstream regulators (Table S3 in the Supplementary Appendix).

3.3. Gene signature identification and model development in the derivation cohort

From the genelists obtained in the discovery phase, we selected 44 transcripts for RT-PCR analysis in the derivation cohort, based on combinations of ABMR and TCMR scores in blood and allograft biopsies, robustness of the results with different probesets of the same gene and by their involvement in relevant canonical pathways (Table S4 in the Supplementary Appendix). First, 26 genes with ABMR score > 0·25 and TCMR score < 0·20 in blood were selected (of these selected transcripts, nine also had a high ABMR score > 0·25 in kidney biopsies). We additionally selected 17 genes with an ABMR score > 0.25 in biopsies and ABMR score > 0.20 in peripheral blood. Finally, given the biological homology of *CXCL11* with *CXCL10* and an ABMR score of *CXCL11* of 0·49 in biopsy samples (but only 0·08 in blood), we added *CXCL11* to the gene panel in the derivation phase. The univariate associations of the expression of these 44 genes with rejection phenotypes are shown in Fig. S2 in the Supplementary Appendix. From these 44 genes, a gene signature specific for antibody-mediated rejection was identified on the samples of the derivation cohort ($N = 183$), and included the following eight genes: *CXCL10, FCGR1A, FCGR1B, GBP1, GBP4, IL15, KLRC1, TIMP1*. More information on the role of these eight genes in ABMR is provided in Table S5. Subsequently, this 8-gene signature was used to build a logistic regression model with nested loop internal cross-validation for discrimination between cases with and without antibody-mediated rejection in the derivation cohort. Applied to the samples of the derivation cohort, this gene signature and logistic regression model yielded a ROC AUC of 78·1% (95% confidence interval [CI], 70·7 to 85·6; $p < 0·0001$)(Fig. 2).

3.4. Diagnostic accuracy of the 8-gene assay in the validation cohort

The 8-gene signature and logistic regression model built and locked on the derivation cohort were evaluated on the 387 samples collected in the validation cohort, which contained 41 cases with antibody-mediated rejection (10·6%), representing the natural prevalence of this phenotype in the cohort of biopsies performed at the participating centres. The 8-gene assay reached a ROC AUC of 79·9% (95% CI, 72·6 to 87·2; $p < 0·0001$)(Fig. S3 in the Supplementary Appendix). When we evaluated the diagnostic accuracy for discrimination between pure antibody-mediated rejection ($N = 38$) and pure borderline changes and T-cell mediated rejection ($N = 13$ and 3 respectively), the 8-gene assay reached a ROC AUC of 82·2% (95% CI 70·7 to 93·8, $p = 0·001$), and 79·3% (95% CI, 71·6 to 86·9; $p < 0·0001$) for discrimination between pure antibody-mediated rejection (N = 38) and absence of rejection ($N = 330$). This 8-gene assay was not diagnostic for the group of T-cell mediated rejections (containing mostly borderline changes) (Fig. S4 in the Supplementary Appendix).

We next validated the cut-off value of the 8-gene assay that was determined in the derivation phase (Fig. S5 in the Supplementary Appendix). The optimal cut-off from the derivation phase for the 8-gene biomarker (−1·08) had a sensitivity of 73·2%, specificity of 75·7%, Youden index of 0·49, positive predictive value of 26·3% and negative predictive value of 96·0% in the validation cohort (Table 3 and Fig. S6 in the Supplementary Appendix).

3.5. Sensitivity analysis

The 8-gene assay retained its accuracy for antibody-mediated rejection in patients with stable graft function and at time of graft

Table 2
Comparison between cases with antibody-mediated rejection vs. without antibody-mediated rejection in the validation cohort ($N = 387$). For variance analysis of continuous variables *t*-test was used; dichotomous variables were compared with the Chi-square test.

Variable	Antibody-mediated rejection (N = 41)	No antibody-mediated rejection (N = 346)	*P* value
	Mean ± SD or No. (%)	*Mean ± SD or No. (%)*	
Recipient age at transplantation (years)	42·9 ± 17·3	50·8 ± 14·8	0·002
Recipient age at time of biopsy (years)	50·5 ± 14·4	52·7 ± 14·4	0·37
Recipient sex (male/female)	16/25 (39·0%/61·0%)	224/122 (64·7%/35·3%)	0·001
Recipient ethnicity* (European/Asian/African/Other)	38/0/0/3 (92·7%/0%/0%/7·3%)	300/3/6/34 (86·7%/0·9%/1·7%/9·8%)	0·77
Donor age (years) *	41·2 ± 17·5	51·3 ± 14·9	<0·0001
Donor sex* (male/female)	24/14 (63·2%/36·8%)	168/173 (49·3%/50·7%)	0·10
Deceased/Living donor *	34/5 (87·2%/12·8%)	261/83 (75·9%/24·1%)	0·11
Heart-beating/ Non-heart-beating donor*	32/2 (94·1%/5·9%)	231/30 (88·5%/11·5%)	0·32
Cold ischemia time (hours)*	13·3 ± 6·9	12·0 ± 7·9	0·36
Repeat transplantation (yes/no)	60/286 (17·3%/82·7%)	9/32 (22·0%/78·0%)	0·92
Indication/protocol biopsy	30/11 (73·2%/26·8%)	104/242 (30·1%/69·9%)	<0·0001
Time after transplantation (days)	2806·0 ± 3233·7	683·5 ± 1293·5	0·0002
MDRD eGFR, (ml/min/1.73 m^2)	34·7 ± 21·4	43·9 ± 16·7	0·01
Proteinuria (g/g creatinine)*	1·4 ± 1·9	0·3 ± 0·7	0·0006
Presence of DSA	18 (43·9%)	46 (13·3%)	<0·0001

* missing data on recipient ethnicity, donor age, donor sex, deceased/living donor, heart-beating/non-heart-beating donor, cold ischemia time and proteinuria.

dysfunction, within the first year and also later after transplantation (Table 3, Fig. 3, Table S6 in the Supplementary Appendix). In all scenarios, the 8-gene assay allowed to rule out ongoing antibody-mediated rejection with high negative predictive values. However, the power was low in these sub-analyses, and these accuracy parameters should be interpreted cautiously. The 8-gene assay reached universal high specificities for antibody-mediated rejection, but the positive predictive value was lower due to the low prevalence of this disease in the validation cohort, especially in protocol-specified biopsies. From this, the positive predictive value was higher at time of graft dysfunction than in stable graft function. If restricted to cases with donor-specific antibodies ($N = 64$), the positive predictive value of the 8-gene assay was even higher: at the optimal threshold (−1·08), positive predictive value was 57·1%, with sensitivity of 66·7%, specificity of 80·4%, Youden index of 0·47 and negative predictive value of 86·0%.

3.6. Correlation of the 8-gene assay with histological and clinical variables

The 8-gene assay correlated with graft functional parameters like eGFR and proteinuria, and with histological lesions diagnostic for antibody-mediated rejection like glomerulitis, peritubular capillaritis, microvascular inflammation, and transplant glomerulopathy in the validation cohort (Table S7 in the Supplementary Appendix). Distribution of the 8-gene assay score per histological lesion grade is shown in Fig. 4. There was no correlation with histological lesions of T-cell mediated rejection. The 8-gene biomarker did not associate with diagnosis of glomerulonephritis, polyomavirus associated nephropathy or interstitial fibrosis with tubular atrophy. A weak association with recipient gender ($p = 0·04$) was found. No other donor-recipient demographics associated with the 8-gene assay.

3.7. Comparison with traditional biomarkers and added clinical value of the 8-gene assay

The 8-gene assay associated with diagnosis of antibody-mediated rejection, independent of traditional factors associating with antibody-mediated rejection (female gender, recipient age, time after transplantation, presence of donor-specific antibodies and proteinuria) (Table S8 in the Supplementary Appendix). We next built and internally cross-validated a clinical model on the data from the discovery and derivation phase combined, which consisted of the 8 clinical parameters that differed between ABMR vs. no ABMR cases (donor-specific

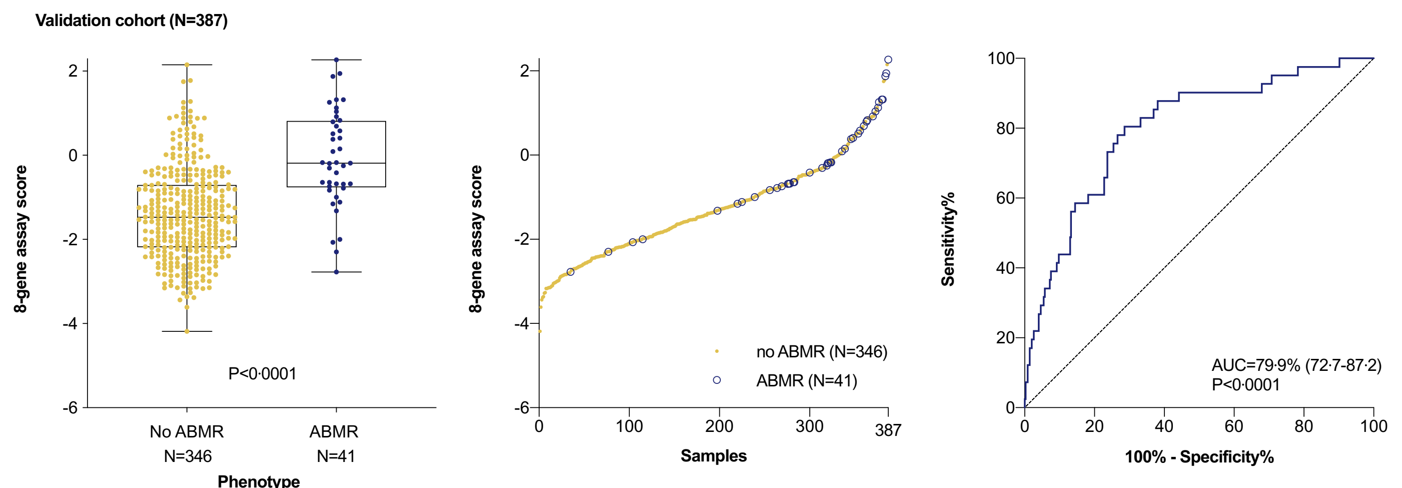


Fig. 2. Diagnostic accuracy of the 8-gene assay for non-invasive diagnosis of antibody-mediated rejection in the validation cohort ($N = 387$). The left panel shows the 8-gene assay score in cases with versus without antibody-mediated rejection. The middle panel shows the distribution of cases with antibody-mediated rejection across all scores of the 8-gene assay. The right panel shows the ROC curves for samples with versus without antibody-mediated rejection, with presentation of the area under the receiver operating characteristic curve (ROC AUC) and the 95% confidence interval.

Table 3
Diagnostic accuracy of the 8-gene marker for non-invasive diagnosis of antibody-mediated rejection in the independent validation cohort ($N = 387$).

	Diagnostic Accuracy (ROC AUC)	Accuracy (Total correct/total)	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Population (N = ABMR/total)	% (95% CI)	%	%	%	%	%
Validation cohort						
All biopsies (N = 41/387)	79·9% (72·7–87·2)					
Low threshold[§] (−2·50)	$p < 0·0001$	35·1%	95·1%	28·0%	13·5%	98·0%
Optimal threshold* (−1·08)		75·5%	73·2%	75·7%	26·3%	96·0%
High threshold[§] (0·50)		89·1%	22·0%	97·1%	47·4%	91·3%
Post-hoc sensitivity analyses						
Early biopsies <1 year ($N = 10/207$)	90·9% (85·3–96·4)					
Low threshold (−2·50)	p < 0·0001	33·8%	100%	30·5%	6·8%	100%
Optimal threshold (−1·08)		79·2%	90·0%	78·7%	17·6%	99·4%
High threshold (0·50)		93·2%	20·0%	97·0%	25·0%	96·0%
Late biopsies >1 year ($N = 31/180$)	73·5% (63·6–83·4)					
Low threshold (−2·50)	p < 0·0001	36·7%	93·5%	24·8%	20·6%	94·9%
Optimal threshold (−1·08)		71·1%	67·7%	71·8%	33·3%	91·5%
High threshold (0·50)		84·4%	22·6%	97·3%	63·6%	85·8%
Biopsies at time of graft dysfunction ($N = 30/134$)	75·3% (64·9–85·8)					
Low threshold (−2·50)	p < 0·0001	36·6%	93·3%	20·2%	25·2%	91·3%
Optimal threshold (−1·08)		71·6%	73·3%	71·1%	42·3%	90·2%
High threshold (0·50)		79·1%	26·7%	94·2%	57·1%	81·7%
Biopsies at time of stable graft function (N = 11/253)	83·4% (75·4–91·3)					
Low threshold (−2·50)	p < 0·0001	34·4%	100%	31·4%	6·2%	100%
Optimal threshold (−1·08)		77·5%	72·7%	77·7%	12·9%	98·4%
High threshold (0·50)		94·5%	9·1%	98·3%	20·0%	96·0%

*The optimal threshold for the ROC curve was chosen in the derivation phase at maximum sensitivity and specificity. [§]Low and high thresholds were arbitrarily selected in the independent validation cohort. ROC AUC = area under the receiver operating characteristic curve.

antibodies, proteinuria, eGFR, time after transplantation, recipient age at time of transplantation, donor age, recipient sex and protocol vs. indication biopsy). In the validation set, this clinical model reached an AUC of 77·3% (95% CI 68·5 to 86·0, $p < 0·0001$). Adding the result of the 8-gene assay to the clinical model increased the diagnostic accuracy for ABMR to 88·0% (95% CI, 82·7 to 93·3; p < 0·0001) (Fig. S7 in the Supplementary Appendix). Random forest out-of-bag error rates in the validation set were comparable for the 8-gene assay and the clinical model (14·99% and 14·73%, respectively). When adding the 8-gene assay to the clinical model the error rate dropped to 11·37%. In protocol biopsies (at time of stable graft function), the clinical model was not contributive (AUC of 55·7%, 95% CI, 37·2–74·2). Both in indication and protocol biopsies the 8-gene assay had added diagnostic value on top of the clinical model (Fig. S7 in the Supplementary Appendix). Decision curve analysis confirmed the net benefit of using the 8-gene assay for diagnosis of antibody-mediated rejection across the range of probability thresholds between 5% and 35% (Fig. S8 in the Supplementary Appendix).

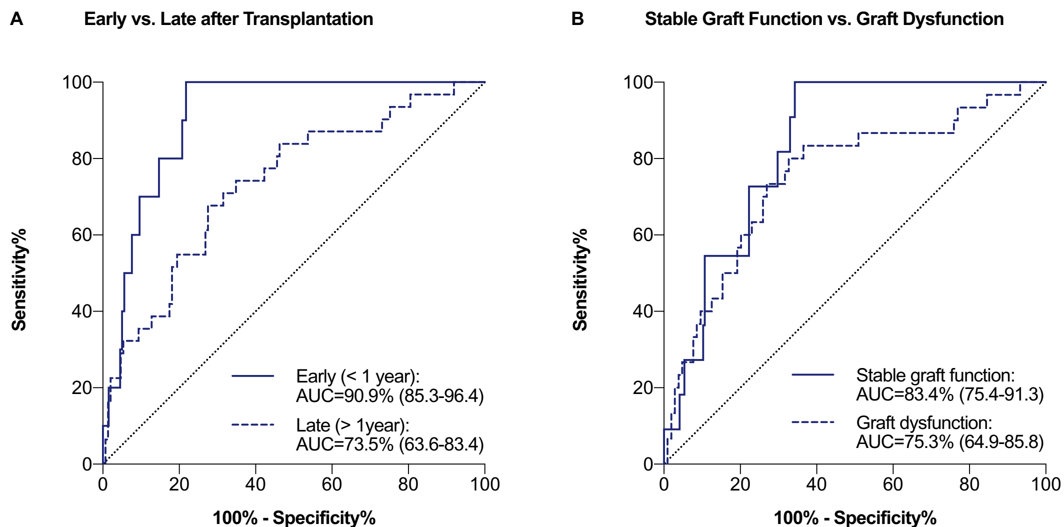
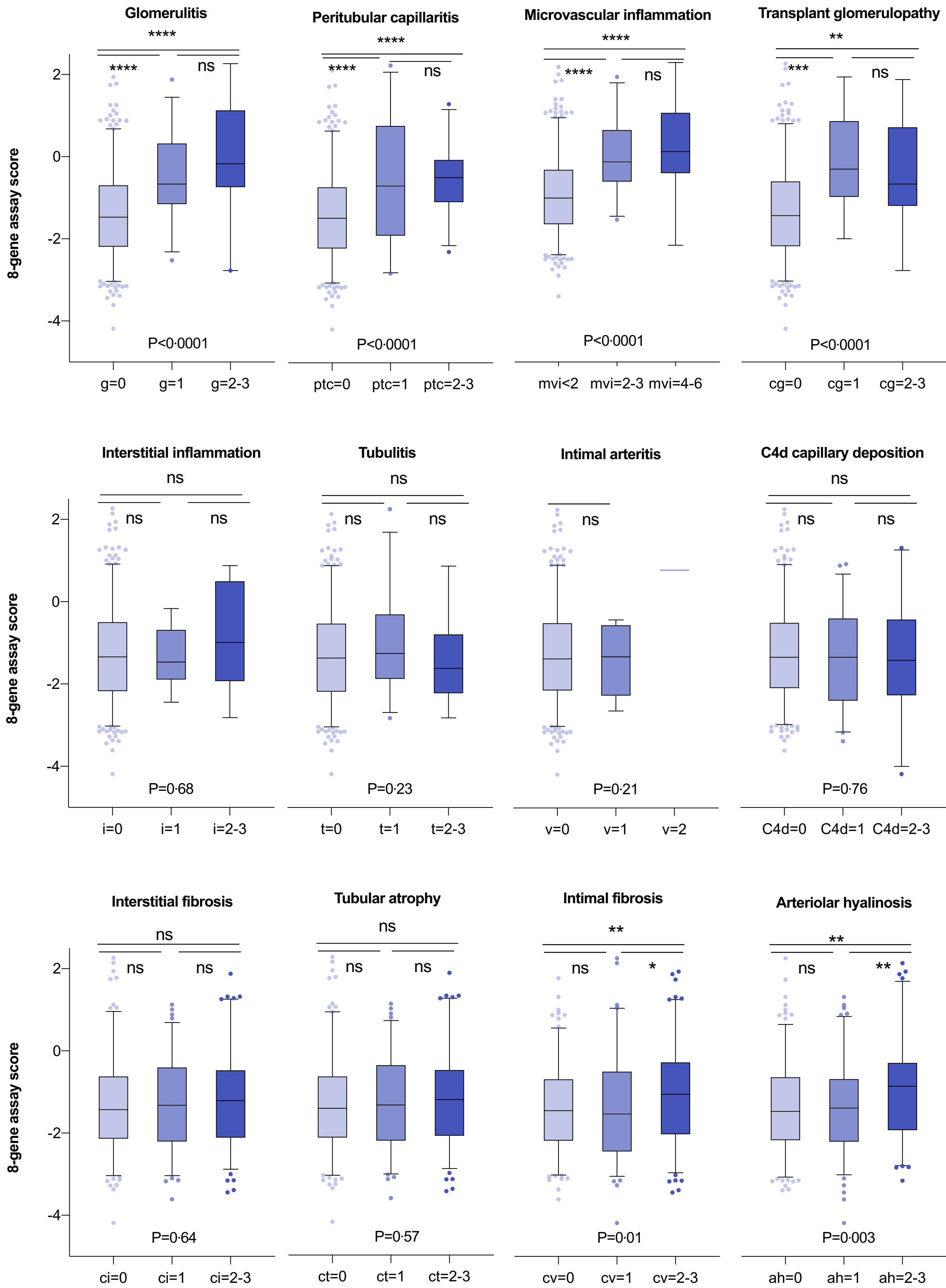


Fig. 3. Diagnostic accuracy of the 8-gene assay for antibody-mediated rejection in specific subgroups in the validation set ($N = 387$). Post-hoc sensitivity analysis of the 8-gene marker according to time after transplantation is shown in panel A and according to stable graft function vs. graft dysfunction in panel B.


Glomerulitis
****
****
ns
P<0·0001
g=0
g=1
g=2-3
Peritubular capillaritis
****
****
ns
P<0·0001
ptc=0
ptc=1
ptc=2-3
Microvascular inflammation
****
****
ns
P<0·0001
mvi<2
mvi=2-3
mvi=4-6
Transplant glomerulopathy
**
***
ns
P<0·0001
cg=0
cg=1
cg=2-3
Interstitial inflammation
ns
ns
ns
P=0·68
i=0
i=1
i=2-3
Tubulitis
ns
ns
ns
P=0·23
t=0
t=1
t=2-3
Intimal arteritis
ns
P=0·21
v=0
v=1
v=2
C4d capillary deposition
ns
ns
ns
P=0·76
C4d=0
C4d=1
C4d=2-3
Interstitial fibrosis
ns
ns
ns
P=0·64
ci=0
ci=1
ci=2-3
Tubular atrophy
ns
ns
ns
P=0·57
ct=0
ct=1
ct=2-3
Intimal fibrosis
**
ns
*
P=0·01
cv=0
cv=1
cv=2-3
Arteriolar hyalinosis
**
ns
**
P=0·003
ah=0
ah=1
ah=2-3
8-gene assay score
2
0
-2
-4

4. Discussion

In this multicentre, prospective, multiphase study we identified and validated an 8-gene expression assay in peripheral blood samples with good diagnostic accuracy for non-invasive diagnosis of antibody-mediated rejection. The 8-gene assay retained this accuracy both at time of stable graft function and at time of graft dysfunction, within the first year and also later after transplantation. The diagnostic accuracy of the 8-gene assay for antibody-mediated rejection was superior to that of clinical indicators, and the assay offered benefit in clinical decisions to perform or not perform a biopsy for diagnosis of antibody-mediated rejection.

This study is a landmark in the field of biomarker discovery and development in renal transplantation in several aspects. First, its multiphase study design with independent discovery, derivation and validation sets allowed for robust biomarker development and validation [15,16]. After nested loop internal cross-validation in the derivation set, we performed independent external validation of the locked and fully specified model in a representative population with natural prevalence of antibody-mediated rejection, using the same technology platform. Second, stringent phenotypic selection with central re-assessment was applied, minimizing the interobserver variability in the current gold standard for diagnosis of rejection and reference standard for performance of the biomarker. Third, the comparison with an internally cross-validated and externally validated clinical model, and assessment of the net benefit of using this 8-gene assay indicate the clinical usefulness of this marker on top of routinely available clinical markers.

The unbiased transcriptomic analysis on the discovery set illustrated that the molecular changes that occur in renal allograft biopsies with antibody-mediated rejection, primarily related to immune regulation, are also reflected in transcriptional differences in peripheral blood. The selected gene transcripts in our study come from biologically plausible molecular pathways, based on gene pathway enrichment analyses. Especially natural killer cells and their interplay with dendritic cells are known to be notorious players in humoral rejection [23]. The significant enrichment of these pathways in our gene transcripts corroborates the specificity of our marker for antibody-mediated rejection. Furthermore, the eight individual genes have relevant pathophysiological functions and most have references to rejection as found from proposed gene transcript lists [4,17].

In addition, we assessed the accuracy of this biomarker in different clinical scenarios. The clinical value of a biomarker in renal transplantation depends on the setting in which biopsies are performed, as we also observed in our post-hoc sensitivity analyses. The better diagnostic accuracy of our marker in the first year after transplantation is relevant, as therapeutic implications will be greatest when antibody-mediated rejection is detected early, before chronic damage has developed and the disease becomes irreversible [3,24]. The high negative predictive value and high sensitivity of our 8-gene assay in all settings indicates that the assay can be used to rule out antibody-mediated rejection, both at time of graft dysfunction and at time of stable graft function.

Another strength of our study is the comparison with readily available clinical information, where we illustrated the added value of the 8-gene assay both at time of stable graft function and at time of graft dysfunction. As protocol biopsies are not guided by clinical information, the clinical model is of little use in this scenario, and was outperformed by the 8-gene assay. Part of the relevance of this biomarker indeed lies in its accuracy independent of graft functional parameters (estimated glomerular filtration rate and proteinuria) as subclinical histological changes of antibody-mediated rejection often remain undetected but are nevertheless associated with an increased risk of graft failure [5,6]. In centres that are currently not performing protocol-specified biopsies to detect subclinical rejection, it could be considered to include this biomarker in the follow-up of patients at increased risk of antibody-mediated rejection (e.g. patients with donor-specific antibodies) and restrict performing protocol-specified biopsies only to patients with a higher value of the 8-gene assay, when antibody-mediated rejection is not excluded. In centres that routinely perform protocol-specified biopsies, a low value of the 8-gene assay could be used to avoid performing the biopsy in such case. This indicates the usefulness of the biomarker and possibility to adapt cutoff values of the biomarker according to centre preference, as was illustrated using arbitrarily chosen low and high cutoffs. For further clinical implementation, validation of these cutoffs in the same way the optimal cutoff was validated, is still warranted.

Finally, the high specificity for antibody-mediated rejection at all instances is clinically meaningful, although the positive predictive value is lower, primarily related to the low prevalence of antibody-mediated rejection in this cohort. Yet, the decision curve analysis of our 8-gene assay confirmed that the test offers benefit in clinical decision making over the range of clinically reasonable thresholds for performing a biopsy. It can be anticipated that the positive predictive value of our test would be even higher in higher-risk cohorts, as was illustrated in our subanalysis of cases with donor-specific antibodies. In further clinical validation of this biomarker, the diagnostic performance in specific risk groups, at different time points and in different clinical situations will need to be addressed in larger populations.

Our study has several limitations. Definition of antibody-mediated rejection is a topic of active discussion, which makes our reference standard of histology imperfect [4]. This may have affected the final diagnostic accuracy of our 8-gene assay and re-evaluation of the diagnostic performance of our assay in updated versions of the Banff diagnostic classification will be needed. Moreover, given the inherent difficulties with histological diagnosis of antibody-mediated rejection as gold standard for diagnosis of antibody-mediated rejection (reproducibility, sampling error), better diagnostic accuracy of any test is not expected. In addition, the study design of the validation cohort did not allow evaluating the prognostic performance of the assay for future antibody-mediated rejection, or for prediction of outcome of patients with antibody-mediated rejection. Also, in clinical practice, the combination of our 8-gene assay with non-invasive mRNA markers for T-cell mediated rejection and chronic tubulo-interstitial injury, as were previously proposed [25–29], would be interesting to assess different graft injury processes simultaneously. Correlations of the marker with lesions like transplant glomerulopathy are rather weak, leaving biopsies in some cases indispensable for determining the degree of injury and the utility of treatment. As the vast majority of our population was treated with a calcineurin inhibitor-based immunosuppressive regimen, further studies are necessary to assess whether the 8-gene assay is also valuable in patients on other immunosuppressive regimens. The same remark applies for the fact that the majority of our population was of Caucasian ethnicity, requiring further studies to assess whether the 8-gene assay is also valuable in patients of other ethnical groups. Also, our validation cohort with real-life disease prevalence had very low incidence of TCMR, with a majority of the TCMR samples meeting only criteria for borderline changes. Although this reflects the natural disease prevalence in our clinical centres, this could differ from other clinical centres

Fig. 4. Distribution of the 8-gene assay score per histological lesion grade in the validation cohort (N = 387). The 8-gene assay score was significantly associated with lesions of antibody-mediated rejection. Significance was assessed with nonparametric one-way ANOVA and pairwise comparisons with *t*-test. Significance was apparent for higher severity grades of lesions associated with antibody-mediated rejection (glomerulitis, peritubular capillaritis, microvascular inflammation score, transplant glomerulopathy). No significant association was present with lesions of T-cell mediated rejection (tubulitis, interstitial inflammation) or non-specific chronic damage (interstitial fibrosis, tubular atrophy). g = glomerulitis; ptc = peritubular capillaritis; mvi = microvascular inflammation; cg = transplant glomerulopathy; i interstitial inflammation; t = tubulitis; v = intimal arteritis; C4d = C4d deposition in peritubular capillaries; ci = interstitial fibrosis; ct = tubular atrophy; cv = intimal fibrosis; ah = arteriolar hyalinosis. ns = not significant, $^{*}p < 0{\cdot}05$, $^{**}p < 0{\cdot}01$, $^{***}p < 0{\cdot}001$, $^{****}p < 0{\cdot}0001$.

with different clinical practice, and perhaps overestimate the discriminative performance of our marker for ABMR versus TCMR.

In conclusion, we present a novel 8-gene biomarker with robust accuracy for non-invasive diagnosis of antibody-mediated rejection after kidney transplantation. Further evaluation and validation is warranted in larger prospective studies.

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Data sharing statement

All of the individual de-identified participant data that underlie the results reported in this article (text, tables, figures, and appendices) can be made available on a collaborative basis following institutional review board approval, immediately following publication, without end date and for any purpose. Proposals should be directed at maarten.naesens@kuleuven.be.

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Declaration of interests

This manuscript is related to a European patent application (Title: mRNA-based biomarkers for antibody-mediated transplant rejection; Application Number EP19152365.3), which has been filed on January 17, 2019).

Author contributions

MN, PM, DA, WG and ME conceived and designed the study.

EL, HdL, L-HL, BS, DK, ME, WG, DG and MN collected the clinical data and samples.

SY, HdL, LVL, FS and MN performed the gene expression analyses.

EVL, SG, SY, MC, LT and MN did the statistical analyses and interpreted the data.

EVL, SY and MN wrote the draft of the report.

All authors revised the report.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2019.07.028.

References

[1] Nankivell BJ, Kuypers DR. Diagnosis and prevention of chronic kidney allograft loss. Lancet 2011;378(9800):1428–37.
[2] Wekerle T, Segev D, Lechler R, Oberbauer R. Strategies for long-term preservation of kidney graft function. Lancet 2017;389(10084):2152–62.
[3] Loupy A, Lefaucheur C. Antibody-mediated rejection of solid-organ allografts. N Engl J Med 2018;379(12):1150–60.
[4] Haas M, Loupy A, Lefaucheur C, et al. The Banff 2017 kidney meeting report: revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. Am J Transplant 2018;18(2):293–307.
[5] Loupy A, Vernerey D, Tinel C, et al. Subclinical rejection phenotypes at 1 year post-transplant and outcome of kidney allografts. J Am Soc Nephrol 2015;26(7):1721–31.
[6] Schinstock CA, Cosio F, Cheungpasitporn W, et al. The value of protocol biopsies to identify patients with De novo donor-specific antibody at high risk for allograft loss. Am J Transplant 2017;17(6):1574–84.
[7] Naesens M, Lerut E, Emonds MP, et al. Proteinuria as a noninvasive marker for renal allograft histology and failure: an observational cohort study. J Am Soc Nephrol 2016;27(1):281–92.
[8] Blydt-Hansen TD, Sharma A, Gibson IW, et al. Urinary metabolomics for noninvasive detection of antibody-mediated rejection in children after kidney transplantation. Transplantation 2017;101(10):2553–61.
[9] Rabant M, Amrouche L, Lebreton X, et al. Urinary C-X-C motif chemokine 10 independently improves the noninvasive diagnosis of antibody-mediated kidney allograft rejection. J Am Soc Nephrol 2015;26(11):2840–51.
[10] Matignon M, Ding R, Dadhania DM, et al. Urinary cell mRNA profiles and differential diagnosis of acute kidney graft dysfunction. J Am Soc Nephrol 2014;25(7):1586–97.
[11] Veale JL, Liang LW, Zhang Q, et al. Noninvasive diagnosis of cellular and antibody-mediated rejection by perforin and granzyme B in renal allografts. Hum Immunol 2006;67(10):777–86.
[12] Ashton-Chess J, Giral M, Mengel M, et al. Tribbles-1 as a novel biomarker of chronic antibody-mediated rejection. J Am Soc Nephrol 2008;19(6):1116–27.
[13] Iwasaki K, Yamamoto T, Inanaga Y, et al. MiR-142-5p and miR-486-5p as biomarkers for early detection of chronic antibody-mediated rejection in kidney transplantation. Biomarkers 2017;22(1):45–54.
[14] Anglicheau D, Naesens M, Essig M, Gwinner W, Marquet P. Establishing biomarkers in transplant medicine: a critical review of current approaches. Transplantation 2016;100(10):2024–38.
[15] Menon MC, Murphy B, Heeger PS. Moving biomarkers toward clinical implementation in kidney transplantation. J Am Soc Nephrol 2017;28(3):735–47.
[16] Kurian SM, Whisenant T, Mas V, et al. Biomarker guidelines for high-dimensional genomic studies in transplantation: adding method to the madness. Transplantation 2017;101(3):457–63.
[17] Halloran PF, Venner JM, Madill-Thomsen KS, et al. Review: the transcripts associated with organ allograft rejection. Am J Transplant 2018;18(4):785–95.
[18] Yazdani S, Callemeyn J, Gazut S, et al. Natural killer cell infiltration is discriminative for antibody-mediated rejection and predicts outcome after kidney transplantation. Kidney Int 2019;95(1):188–98.
[19] Weinberger J, Jimenez-Heredia R, Schaller S, et al. Immune repertoire profiling reveals that clonally expanded B and T cells infiltrating diseased human kidneys can also be tracked in blood. PLoS One 2015;10(11):e0143125.
[20] Rinaudo P, Boudah S, Junot C, Thevenot EA. Biosigner: a new method for the discovery of significant molecular signatures from Omics data. Front Mol Biosci 2016;3:26.
[21] Vickers AJ, Van Calster B, Steyerberg EW. Net benefit approaches to the evaluation of prediction models, molecular markers, and diagnostic tests. BMJ 2016;352:i6.
[22] R Core Team. R: A language and environment for statistical computing. Vienna, Austria; 2014.
[23] Resch T, Fabritius C, Ebner S, Ritschl P, Kotsch K. The role of natural killer cells in Humoral rejection. Transplantation 2015;99(7):1335–40.
[24] Fehr T, Gaspert A. Antibody-mediated kidney allograft rejection: therapeutic options and their experimental rationale. Transpl Int 2012;25(6):623–32.
[25] Roedder S, Sigdel T, Salomonis N, et al. The kSORT assay to detect renal transplant patients at high risk for acute rejection: results of the multicenter AART study. PLoS Med 2014;11(11):e1001759.
[26] Kurian SM, Williams AN, Gelbart T, et al. Molecular classifiers for acute kidney transplant rejection in peripheral blood by whole genome gene expression profiling. Am J Transplant 2014;14(5):1164–72.
[27] O'Connell PJ, Zhang W, Menon MC, et al. Biopsy transcriptome expression profiling to identify kidney transplants at risk of chronic injury: a multicentre, prospective study. Lancet 2016;388(10048):983–93.
[28] Friedewald JJ, Kurian SM, Heilman RL, et al. Development and clinical validity of a novel blood-based molecular biomarker for subclinical acute rejection following kidney transplant. Am J Transplant 2019;19(1):98–109.
[29] Christakoudi S, Runglall M, Mobillo P, et al. Development of a multivariable gene-expression signature targeting T-cell-mediated rejection in peripheral blood of kidney transplant recipients validated in cross-sectional and longitudinal samples. EBioMedicine 2019;41:571–83.