Validating the INTERHEART algorithms for molecular diagnosis of rejection in 558 new endomyocardial biopsies

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INTRODUCTION

We previously reported a Molecular Microscope system for diagnosis of heart transplant rejection (MMDx-Heart) in 331 endomyocardial biopsies (EMB) (*J Heart Lung Transplant* 36 (11):1192-1200, 2017). In the present study we validated these locked algorithms in 558 new EMBs and re-derived the diagnostic algorithm in the combined set of 889 EMBs to make use of all available data.

METHODS

In addition to the previously published cohort of 331 EMBs, we obtained 558 new single EMB bites from 273 recipients at eight centers in Canada, USA, Australia and Europe. We analyzed their gene expression using Affymetrix PrimeView™ microarrays. We normalized expression across all biopsies and assessed rejection using unsupervised principal component analysis (PCA) and archetypal analysis (AA) based on kidney-derived rejection-associated transcripts (RATs). PCA and AA models were first derived in cohort 331, and then applied to 558 for comparison. Algorithms were re-derived in the combined set of 889 EMBs for reporting the molecular phenotype in new biopsies with MMDx-Heart. To compare molecular to histology classes, we designated ABMR, TCMR, possible ABMR, and possible TCMR based on ISHLT grades and molecular AA score cut-offs. An overview of our approach is shown in Figure 1.

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RESULTS

The 558 new EMBs were similar in case mix to the previous 331 EMBs (Table 1) except histologic ABMR was less frequent (6% vs. 18%). The archetype cluster assignment (imposed on PCA) separated ABMR and TCMR, and possible ABMR and TCMR by molecules (Figure 2 A&C) and histology (Figure 2 B&D). In cohort 1 (N=331) and cohort 2 (N=558), the TCMR scores ($S2_{TCMR}$) were significantly associated with histologic TCMR, and the ABMR scores ($S3_{ABMR}$) were significantly associated with histologic ABMR and DSA (Table 3). The mean archetype scores for the respective type of rejection ABMR increased from histologic no rejection to possible rejection to full rejection (Table 2). However, there were many disparities between molecular and histology classes, as expected given the limited reproducibility (kappa values) of histology diagnoses. The same results were achieved when the diagnostic algorithms were re-derived the combined set of 889 biopsies, and a new injury dimension ($S4_{Injury}$) was identified by expanding the archetypal analysis to capture 4 archetypes instead of the 3 archetypes originally captured in 331 (Table 4). The 889-based diagnostic algorithms form the basis of the MMDx-Heart reporting system for new biopsies (Figure 3).

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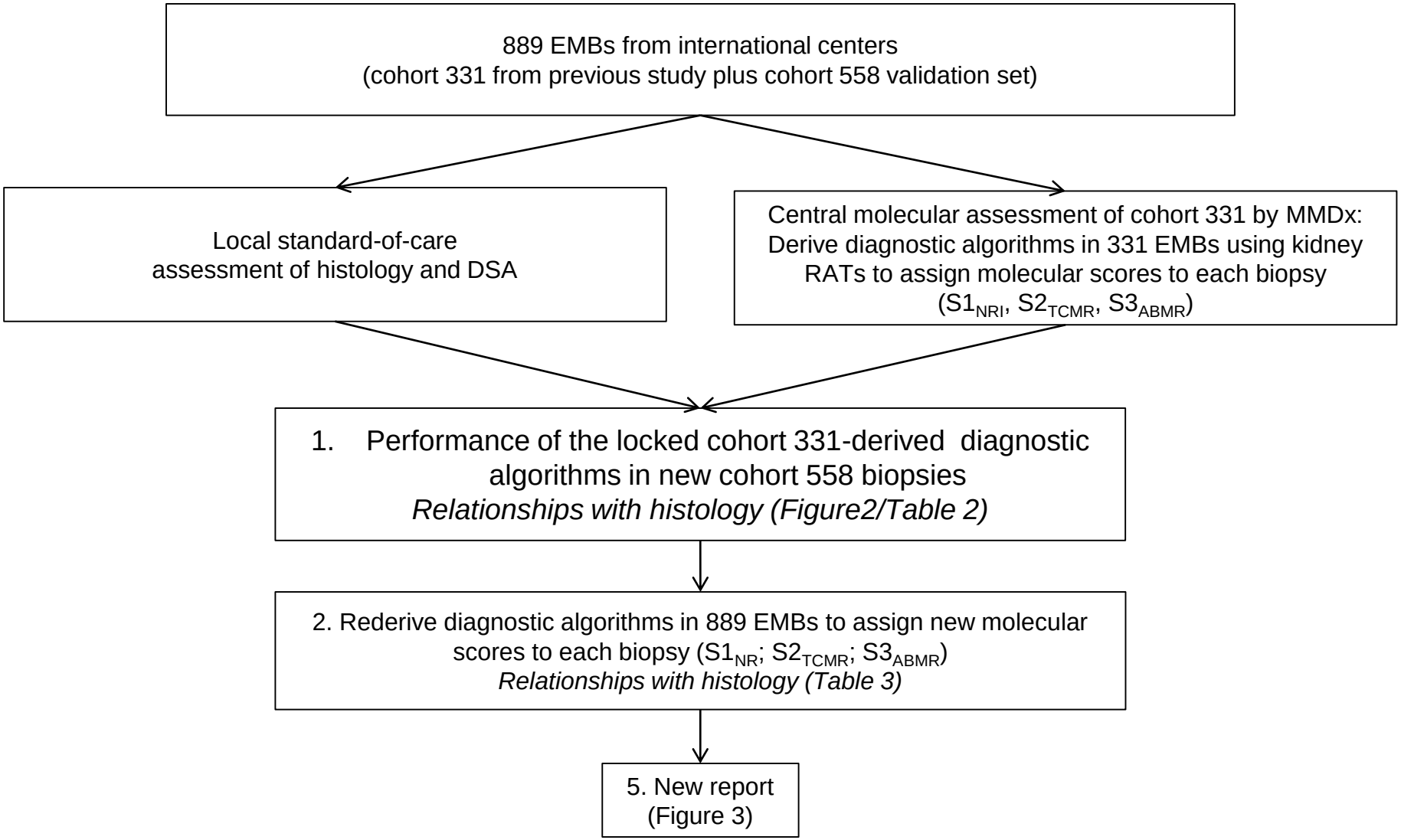

Figure 1 – Overview of the work plan implemented in this investigation.

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Table 1. Histology summary available in 889 EMBs

Histology diagnoses* (% of known diagnoses)		Cohort 1 (331 biopsies)	Cohort 2 (558 biopsies)
No Rejection		89 (27%)	245 (44%)
ABMR Related	**ABMR**	33 (10%)	18 (3%)
	pABMR	44 (13%)	19 (3%)
TCMR Related	**TCMR**	36 (11%)	48 (9%)
	pTCMR	90 (27%)	183 (33%)
Other	**ABMR/TCMR (Mixed)**	5 (2%)	4 (1%)
	pABMR/pTCMR	34 (10%)	37 (7%)
Missing		0	4
DSA Status		**Cohort 1†** (210 patients)	**Cohort 2†** (273 patients)
DSA at any time‡			
Positive		92 (44%)	108 (39%)
Negative or unknown		118 (56%)	165 (61%)
DSA at most recent biopsy			
Positive		61 (29%)	84 (31%)
Negative		111 (53%)	152 (56%)
Unknown		38 (18%)	37 (14%)

* Biopsies were classified as follows according to ISHLT rejection grades:

pAMR1, pAMR1I+, pAMR1H+……..Possible ABMR (pABMR);
pAMR2, pAMR3……..ABMR;
TCMR1……Possible TCMR (pTCMR);
TCMR2, TCMR3……TCMR

† Some patients were part of both cohorts if biopsies were taken in both legs of the INTERHEART study.
‡ The most recent DSA status at time of most recent biopsy was used, if known. DSA statuses dated more than 14 days after the biopsy were not considered. If the most recent DSA status at time of biopsy was not known, but the patient was most recently PRA negative, the DSA status was presumed negative.

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Table 2. Unsupervised algorithms for rejection derived in cohort 331 predict histologic classes in 331 and 558

Cohort 1 (N=331 biopsies)				Cohort 2 (N=558 biopsies)			
Histology in 331	Molecular mean 331-derived scores in 331			Histology in 558	Molecular mean 331-derived scores in 558		
	$S1_{NRI}$	$S2_{TCMR}$	$S3_{ABMR}$		$S1_{NRI}$	$S2_{TCMR}$	$S3_{ABMR}$
No rejection (NR)	0.82	0.07	0.11	*No rejection (NR)*	0.81	0.06	0.13
pTCMR	0.66	0.15	0.18	*pTCMR*	0.63	0.12	0.24
TCMR	0.45	0.36	0.18	*TCMR*	0.50	0.24	0.26
No rejection (NR)	0.82	0.07	0.11	*No rejection (NR)*	0.81	0.06	0.13
pABMR	0.33	0.26	0.40	*pABMR*	0.65	0.11	0.24
ABMR	0.36	0.12	0.51	*ABMR*	0.53	0.11	0.36
Statistical analysis							
Histology in 331	Mann-Whitney U Test p Value			Histology in 558	Mann-Whitney U Test p Value		
	$S1_{NR}$	$S2_{TCMR}$	$S3_{ABMR}$		$S1_{NR}$	$S2_{TCMR}$	$S3_{ABMR}$
NR vs. TCMR	2.90E-08	1.80E-07	9.50E-03	*NR vs. TCMR*	1.70E-10	1.30E-10	2.80E-06
pTCMR vs. NR	9.70E-06	1.60E-05	2.20E-03	*pTCMR vs. NR*	8.10E-13	7.90E-07	2.30E-10
TCMR vs. pTCMR	7.10E-04	1.20E-03	4.90E-01	*TCMR vs. pTCMR*	7.00E-03	1.40E-04	2.90E-01
NR vs. ABMR	2.90E-11	4.70E-02	1.30E-12	*NR vs. ABMR*	5.70E-06	6.00E-02	9.50E-06
pABMR vs. NR	4.10E-13	3.30E-07	3.80E-10	*pABMR vs. NR*	1.00E-02	8.20E-01	1.30E-02
ABMR vs. pABMR	6.00E-01	1.00E+00	3.60E-02	*ABMR vs. pABMR*	4.90E-02	8.50E-02	3.30E-02

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Table 3. DSA status for biopsies clustered according to their molecular rejection class (grouped by highest score)

Most recent known DSA status at biopsy*	**Number of biopsies assigned to each rejection class (cluster)**		
	No Rejection ($A1_{NRI}$; N=634)	*TCMR ($A2_{TCMR}$; N=77)*	*ABMR ($A3_{ABMR}$; N=178)*
Positive (N=262)	139	28	95
Negative (N=483)	394	32	57
Missing/not done (N=144)	101	17	26
p-value†:	$<1.0\times10^{-15}$		

* The most recent DSA status at time of biopsy was used, if known. DSA/PRA statuses dated more than 14 days after the biopsy were not considered. If the most recent DSA status at time of biopsy was not known, but the patient was most recently PRA negative, the DSA status was presumed negative.

† Pearson's Chi-squared test on observations where DSA status was known.

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Table 4. Unsupervised algorithms for rejection derived in cohort 889 predict histologic classes in 889

Cohort 1 + 2 (N=889 biopsies)							
Histology in 889	**Molecular mean 889-derived scores in 889 (3 Archetype Model/Model 1)**			**Molecular mean 889-derived scores in 889 (4 Archetype Model/Model 2)**			
	S1NRI	*S2TCMR*	*S3ABMR*	*S1NRI*	*S2TCMR*	*S3ABMR*	*S4Injury*
No rejection or injury (NRI)	0.75	0.06	0.16	0.76	0.04	0.13	0.05
pTCMR	0.58	0.12	0.27	0.59	0.08	0.21	0.09
TCMR	0.43	0.28	0.27	0.44	0.18	0.18	0.17
No rejection or injury (NRI)	0.75	0.06	0.16	0.76	0.04	0.13	0.05
pABMR	0.38	0.19	0.4	0.39	0.12	0.33	0.13
ABMR	0.37	0.1	0.50	0.39	0.06	0.43	0.1
Statistical analysis							
Histology in 889	**Mann-Whitney U Test p Value**						
	S1NR	*S2TCMR*	*S3ABMR*	*S1NRI*	*S2TCMR*	*S3ABMR*	*S4Injury*
NRI vs. TCMR	3.9E-17	8.8E-16	8.5E-07	3.5E-17	2.0E-10	1.2E-02	6.0E-07
pTCMR vs. NRI	4.6E-17	7.8E-09	4.2E-12	4.4E-17	1.1E-06	2.9E-10	3.8E-06
TCMR vs. pTCMR	6.5E-05	6.6E-07	4.6E-01	4.4E-05	3.9E-04	9.6E-01	8.9E-03
NRI vs. ABMR	3.4E-16	3.1E-02	1.7E-17	4.4E-16	4.5E-02	3.8E-14	2.4E-02
pABMR vs. NRI	3.6E-15	1.7E-04	3.0E-12	6.4E-15	3.1E-04	3.1E-10	2.2E-05
ABMR vs. pABMR	4.7E-01	9.3E-01	3.0E-02	4.9E-01	9.2E-01	3.0E-02	9.0E-01

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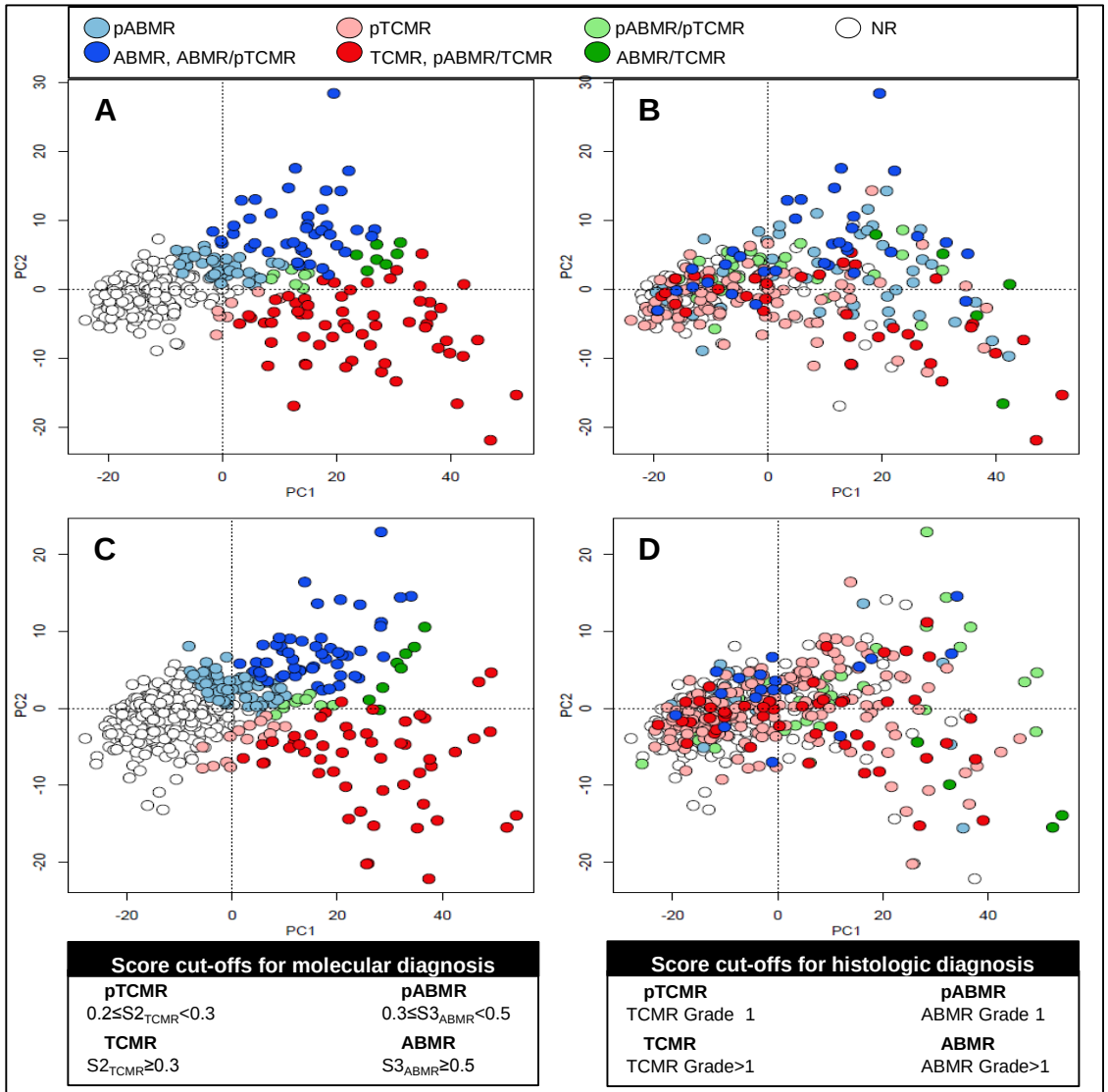

Figure 2. Comparing the cohort 331(A & B) and cohort 558 biopsies (C & D) annotated by the molecular algorithms derived in cohort 1 (n=331), projected in the principal component analysis (PCA) space based on rejection associated transcript (RAT) expression in cohort 1 (N=331). As per the key at the bottom, the left panels (A and C) are colored by molecular diagnoses assigned by archetype score cut-offs, and the right panels (B and D) are colored by histology diagnoses.

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ATAGC

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Molecular Diagnostic Report

General			
LIMS ID		Name	
Sample Name		Patient Age at Bx	
Date Reported (Y-M-D)		Biopsy Indication	
Date Received (Y-M-D)		Estimated LVEF	
Date of Transplant (Y-M-D)		DSA	
		Clinical Diagnosis	
Date of Biopsy (Y-M-D)		Histologic Diagnosis	
Time of Biopsy Post-Tx			

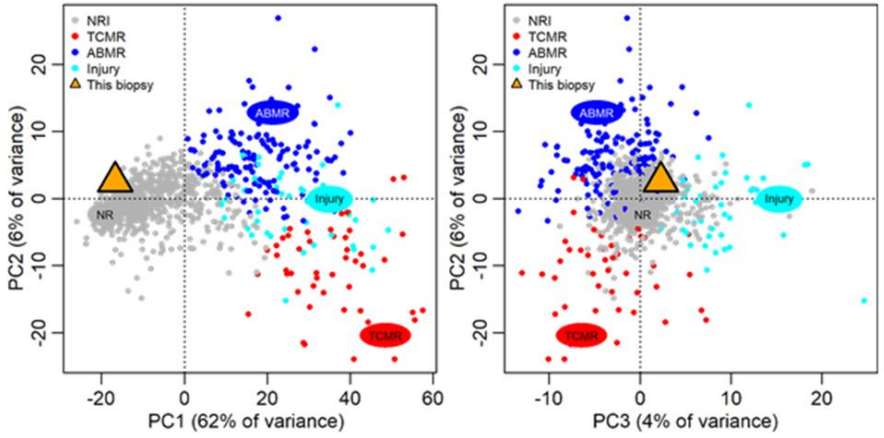
Pure molecular interpretation

Relatively healthy cardiac transplant. No ABMR. No TCMR. Well differentiated parenchymal tissue (HT1s in normal range) and minimal parenchymal injury (S4, IRRAT, QCMAT scores in normal range).

Signed out by Dr. P.F. Halloran

Proportion Rejection and Injury*	Model 1	NRI	0.90	TCMR/Injury	0.00	ABMR/Injury	0.10		
	Model 2	NRI	0.91	TCMR	0.00	ABMR	0.09	Injury	0.00
Probable Rejection Diagnosis*	Using Model 1	NRI	1.00	TCMR/Injury	0.00	ABMR/Injury	0.00		
	Using Model 2	NRI	1.00	TCMR	0.00	ABMR	0.00		

NRI = No Rejection or Injury. *Based on new algorithms accepted for presentation at the 2018 ISHLT meeting, April 11-14, Nice, France.



Legend: NA = not available, ABMR = antibody-mediated rejection, TCMR = T cell-mediated rejection

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Molecular Diagnostic Report

Molecular Phenotype				
PBT/Gene		Biopsy Score	Normal limit	Interpretation
ABMR-related	DSA-selective (DSAST)	-0.00	< 0.08	normal
	Endothelial DSA-selective (eDSAST)	0.19	< 0.16	slightly abnormal
	NK cell burden (NKB)	-0.07	< 0.12	normal
	ROBO4	9.25	< 9.49	normal
TCMR-related	Cytotoxic T cell transcripts (QCAT)	-0.53	< 0.15	normal
	T cell burden (TCB)	-0.62	< 0.30	normal
	Enzyme (ADAMDEC1)	2.32	< 2.96	normal
	Cytokine (CXCL13)	4.60	<5.20	normal
	Interferon gamma (IFNG)	3.75	<4.13	normal
	Checkpoint (CTLA4)	2.98	<3.66	normal
All rejection and injury-related	IFNG inducible (GRIT)	-0.13	< 0.11	normal
Injury-related	Heart transcripts (HT1)	0.00	> -0.05	normal
	Injury transcripts (IRRAT)	0.17	< 0.24	normal
	Injury cluster (S4)	0.00	< 0.10	normal
	Macrophage transcripts (QCMAT)	-0.25	< 0.14	normal

Other Clinical Information

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Figure 3. Example of an MMDx-Heart report. This sample has no molecular rejection. On page 1 of the report (left panel), the biopsy's position in PCA is illustrated alongside its molecular rejection scores, which inform the pure molecular interpretation of the biopsy's disease state. Page 1 also provides clinical information about the sample. On page 2, additional information about the molecular phenotype is reported, along with addition clinical information (if available)

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CONCLUSION

1. Despite differences in case mix, the MMDx-Heart system algorithms created using 3-archetype clustering (3AA) in 331 biopsies produced diagnoses in 558 new biopsies that significantly correlated with the histologic diagnoses and DSA. The 3AA model assigns algorithm scores for no rejection or injury ($S1_{NRI}$), TCMR ($S2_{TCMR}$), and ABMR ($S3_{ABMR}$).
2. Both 3AA and four archetype (4AA) models (incorporating parenchymal injury $S4_{injury}$ score) correlated with histologic ABMR and TCMR in the combined set, and the $S3_{ABMR}$scores was associated with donor-specific antibody (DSA).
3. This allows creation of an MMDx report format incorporating the 3AA and 4AA scores. ClinicalTrials.gov # NCT02670408

DISCLOSURE

Dr Jeff Reeve has no relevant disclosures
Dr Phil Halloran has shares in TSI, University of Alberta research company