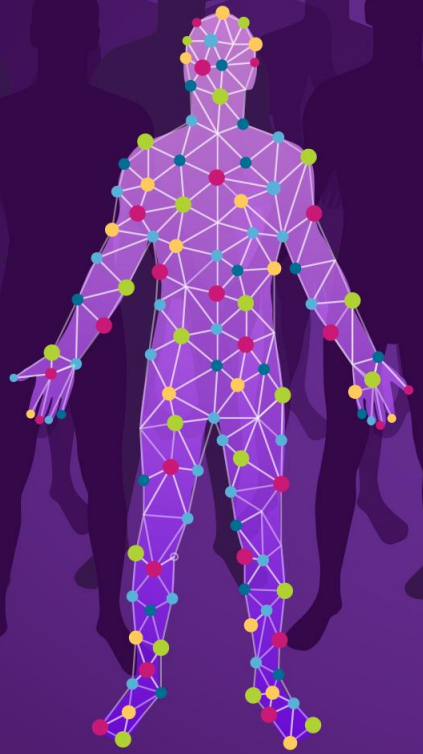
AST | AMERICAN SOCIETY OF TRANSPLANTATION

Molecular Signals of Intragraft Rejection: Is INTERHEART true NORTH?



Phil Halloran, MD PhD

Alberta Transplant Applied Genomics Center (ATAGC)
University of Alberta
and
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Edmonton, AB

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Relevant Financial Relationship Disclosure Statement

The Molecular Microscope® Diagnostic System

Presenter: Phil Halloran

Our studies are supported in part by a licensing agreement with One Lambda/Thermo Fisher

- Phil Halloran
  - Has shares in Transcriptome Sciences Inc (TSI), a University of Alberta research company with an interest in molecular diagnostics
  - Has been a speaker in symposia for One Lambda/Thermo Fisher
  - Is a consultant to CSL-Behring

http://atagc.med.ualberta.ca/Services/MolecularMicroscopeSystem/

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Learning Objectives: The INTERHEART study Clinicaltrials.gov NCT02670408

To understand:

1. The unmet need in heart transplant diagnostics
2. The principles of microarray analysis
3. Unsupervised and supervised analysis of high dimensionality data
4. The relationship of the MMDx diagnoses to histology diagnoses
5. The role of myocardial injury in heart transplant outcomes

Supplementary Table 1. Participating centers

Center	Principal investigators	Number in 889 cohort
A Coruña, Spain	Dra. Maria G. Crespo-Leiro	92
Bologna, Italy	Dr. Luciano Potena	201
Edmonton, Canada	Dr. Daniel Kim	113
France	Drs. Alex Loupy, P. Bruneval, and Xavier Jouven	
Centre Hospitalier Universitaire de Bordeaux		1
Centre Hospitalier Universitaire de Rouen		9
Hôpital Européen Georges-Pompidou		203
Centre Hospitalier Universitaire de Nantes		11
Hôpital Necker		7
Hôpital de la Pitié		24
Los Angeles, USA		
Cedars-Sinai Medical Center	Dr. Jon Kobashigawa	51
University of California Los Angeles	Drs. Mario Deng, Martin Cadeiras, and Eugene C. Depasquale	7
Sydney, Australia	Dr. Peter Macdonald	92
Vienna, Austria	Drs. Andreas Zuckermann, Arezu Aliabadi, and Johannes Goekler	76
Virginia, USA*	Dr. Keyur B. Shah	2
TOTAL		**889**

** Two biopsies from Virginia Commonwealth University were not formally part of the INTERHEART study but we included them on request of the center, with patient consent.*

MMDx-Heart

INTERHEART
Clinicaltrials.gov NCT02670408

The problem: unreliable (“imprecise”) histology diagnoses
We cannot train strong supervised classifiers on unreliable diagnoses

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Gene Expression Profiling for the Identification and Classification of Antibody-Mediated Heart Rejection

Editorial, see p 936

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Jean Paul Duong Van Huyen, MD, PhD*
Luis Hidalgo, PhD
Jeff Reeve, PhD
Maud Racapé, PhD
Olivier Aubert, MD
Jeffery M. Venner, MSc
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Sabine Pattier, MD
Claire Toquet, MD
Arnaud Gay, MD
Philippe Rouvier, MD, PhD
Shaida Varnous, MD
Pascal Leprince, MD, PhD
Jean Philippe Empana, MD, PhD
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*Drs Loupy, Duong Van Huyen, Jouven, and Halloran contributed equally.

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Sources of Funding, see page 933

Key Words: genetics
- microarray analysis
- transplantation

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BACKGROUND: Antibody-mediated rejection (AMR) contributes to heart allograft loss. However, an important knowledge gap remains in terms of the pathophysiology of AMR and how detection of immune activity, injury degree, and stage could be improved by intragraft gene expression profiling.

METHODS: We prospectively monitored 617 heart transplant recipients referred from 4 French transplant centers (January 1, 2006–January 1, 2011) for AMR. We compared patients with AMR (n=55) with a matched control group of 55 patients without AMR. We characterized all patients using histopathology (ISHLT [International Society for Heart and Lung Transplantation] 2013 grades), immunostaining, and circulating anti-HLA donor-specific antibodies at the time of biopsy, together with systematic gene expression assessments of the allograft tissue, using microarrays. Effector cells were evaluated with in vitro human cell cultures. We studied a validation cohort of 98 heart recipients transplanted in Edmonton, AB, Canada, including 27 cases of AMR and 71 controls.

RESULTS: A total of 240 heart transplant endomyocardial biopsies were assessed. AMR showed a distinct pattern of injury characterized by endothelial activation with microcirculatory inflammation by monocytes/macrophages and natural killer (NK) cells. We also observed selective changes in endothelial/angiogenesis and NK cell transcripts, including CD16A signaling and interferon-γ–inducible genes. The AMR-selective gene sets accurately discriminated patients with AMR from those without and included NK transcripts (area under the curve=0.87), endothelial activation transcripts (area under the curve=0.80), macrophage transcripts (area under the curve=0.86), and interferon-γ transcripts (area under the curve=0.84; $P<0.0001$ for all comparisons). These 4 gene sets showed increased expression with increasing pathological AMR (pAMR) International Society for Heart and Lung Transplantation grade ($P<0.001$) and association with donor-specific antibody levels. The unsupervised principal components analysis demonstrated a high proportion of molecularly inactive pAMR1(I+), and there was significant molecular overlap between pAMR1(H^+) and full-blown pAMR2/3 cases. Endothelial activation transcripts, interferon-γ, and NK transcripts showed association with chronic allograft vasculopathy. The molecular architecture and selective AMR transcripts, together with gene set discrimination capacity for AMR identified in the discovery set, were reproduced in the validation cohort.

CONCLUSIONS: Tissue-based measurements of specific pathogenesis-based transcripts reflecting NK burden, endothelial activation, macrophage burden, and interferon-γ effects accurately classify AMR and correlate with degree of injury and disease activity. This study illustrates the clinical potential of a tissue-based analysis of gene transcripts to refine diagnosis of heart transplant rejection.

Circulation. 2017;135:917–935. DOI: 10.1161/CIRCULATIONAHA.116.022907

Molecular phenotype of rejection in heart transplants is very similar to kidney rejection: permits kidney phenotype to guide development of MMDx-heart

A. Loupy, J. P. Duong Van Huyen, L. G. Hidalgo, J. Reeve, M. Racape, J. Venner, K. Famulski, M. C. Bories, T. Beuscart, R. Guillemain, A. Francois, S. Pattier, C. Toquet, A. Gay, P. Rouvier, S. Varnous, P. Leprince, J. P. Empana, C. Lefaucheur, P. Bruneval, X. Jouven, and P. F. Halloran. Gene Expression Profiling for the Identification and Classification of Antibody-Mediated Heart Rejection. Circulation 135 (10):917-935, 2017.

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JCI insight

CLINICAL MEDICINE

Exploring the cardiac response to injury in heart transplant biopsies

Philip F. Halloran,[1,2] Jeff Reeve,[1,2] Arezu Z. Aliabadi,[3] Martin Cadeiras,[4] Marisa G. Crespo-Leiro,[4] Mario Deng,[4] Eugene C. Depasquale,[5] Johannes Goekler,[6] Xavier Jouven,[7] Daniel H. Kim,[3] Jon Kobashigawa,[8] Alexandre Loupy,[9] Peter Macdonald,[10] Luciano Potena,[11] Andreas Zuckermann,[6] and Michael D. Parkes[1]

[1]Alberta Transplant Applied Genomics Centre, Edmonton, Alberta, Canada. [2]Department of Medicine, University of Alberta, Edmonton, Alberta, Canada. [3]Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada. [4]Medical University of Vienna, Vienna, Austria. [5]Ronald Reagan UCLA Medical Center, Los Angeles, California, USA. [6]Complexo Hospitalario Universitario A Coruña, A Coruña, Spain. [7]Paris Transplant Group, Paris, France. [8]Cedars-Sinai Medical Center, Los Angeles, California, USA. [9]Hôpital Necker, Paris, France. [10]The Victor Chang Cardiac Research Institute, Sydney, Australia. [11]Cardiovascular Department, University of Bologna, Bologna, Italy.

BACKGROUND. Because injury is universal in organ transplantation, heart transplant endomyocardial biopsies present an opportunity to explore response to injury in heart parenchyma. Histology has limited ability to assess injury, potentially confusing it with rejection, whereas molecular changes have potential to distinguish injury from rejection. Building on previous studies of transcripts associated with T cell–mediated rejection (TCMR) and antibody-mediated rejection (ABMR), we explored transcripts reflecting injury.

METHODS. Microarray data from 889 prospectively collected endomyocardial biopsies from 454 transplant recipients at 14 centers were subjected to unsupervised principal component analysis and archetypal analysis to detect variation not explained by rejection. The resulting principal component and archetype scores were then examined for their transcript, transcript set, and pathway associations and compared to the histology diagnoses and left ventricular function.

RESULTS. Rejection was reflected by principal components PC1 and PC2, and by archetype scores $S2_{TCMR}$ and $S3_{ABMR}$, with $S1_{normal}$ indicating normalness. PC3 and a new archetype score, $S4_{injury}$, identified unexplained variation correlating with expression of transcripts inducible in injury models, many expressed in macrophages and associated with inflammation in pathway analysis. $S4_{injury}$ scores were high in recent transplants, reflecting donation-implantation injury, and both $S4_{injury}$ and $S2_{TCMR}$ were associated with reduced left ventricular ejection fraction.

CONCLUSION. Assessment of injury is necessary for accurate estimates of rejection and for understanding heart transplant phenotypes. Biopsies with molecular injury but no molecular rejection were often misdiagnosed rejection by histology.

TRAIL REGISTRATION. ClinicalTrials.gov NCT02570408

FUNDING. Roche Organ Transplant Research Foundation, the University of Alberta Hospital Foundation, and Alberta Health Services.

Introduction

Heart transplant endomyocardial biopsies (EMBs) provide an opportunity to characterize pathogenic processes unique to transplants such as rejection but also offer potential insight into states that are of general interest in cardiology such as the parenchymal response to injury. Currently, EMBs are studied by histology to diagnose rejection, following the guidelines of the International Society for Heart and Lung Transplantation (ISHLT) (1–4). The features of T cell–mediated rejection (TCMR) include interstitial inflammation and myocyte damage, and the features of antibody-mediated rejection (ABMR) include microvascular inflammation, complement factor C4d deposition (5–9), and a positive test for circulating donor-specific anti-HLA antibodies (DSAs) (10–13). However, DSA is present in many patients with no

Conflict of interest: PF Halloran holds shares in Transcriptome Sciences Inc., a University of Alberta research company with an interest in molecular diagnostics.

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JCI Insight. 2018;3(20):e123674.
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Distinguishing a heart injury phenotype from rejection

P. F. Halloran, A. Z. Aliabadi, M. Cadeiras, M. G. Crespo-Leiro, M. Deng, E. C. Depasquale, J. Goekler, X. Jouven, D. H. Kim, J. Kobashigawa, A. Loupy, P. Macdonald, L. Potena, A. Zuckermann, and M. D. Parkes. Exploring the cardiac response-to-injury in heart transplant biopsies. *JCI Insight 3 (20):e123674, 2018.*

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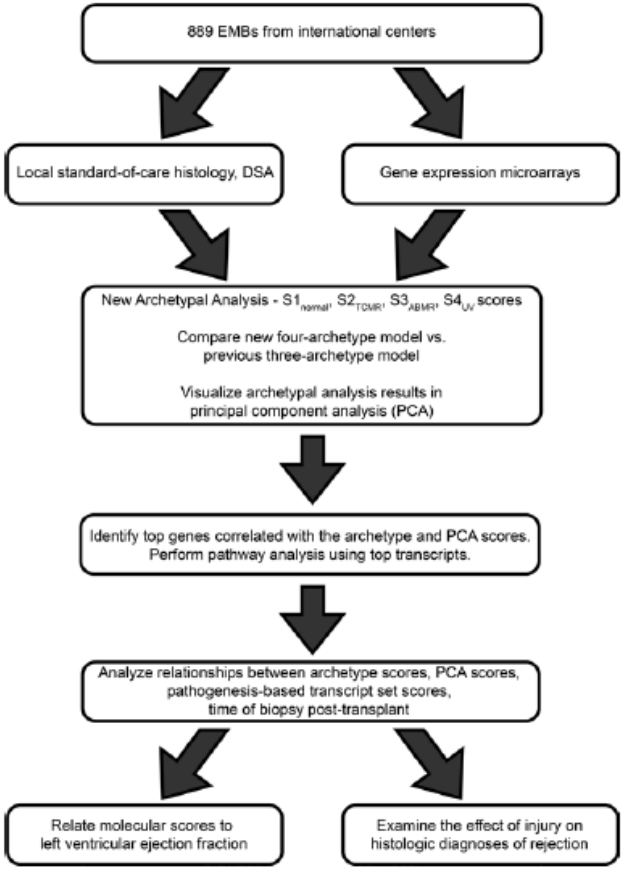

Figure 1. Overview of the work plan implemented in this investigation.

Table 2. Histology summary available in 889 EMBs

Histology diagnoses[A] (% of known diagnoses)		All (889 biopsies)
No Rejection		334 (38%)
TCMR Related	TCMR	84 (9%)
	pTCMR	273 (31%)
ABMR Related	ABMR	51 (6%)
	pABMR	63 (7%)
Other	ABMR/TCMR (Mixed)	9 (1%)
	pABMR/pTCMR	71 (8%)
Missing		4 (0%)
DSA Status		**All (454 patients)**
Last known DSA status at most recent biopsy[B]		
Positive		158 (37%)
Negative		267 (63%)
Not tested		29 (6%)

[A]Biopsies in the 889 cohort were labeled as follows:

pAMR ..... No ABMR
pAMR1, pAMR1I+, pAMR1H+ ..... Possible ABMR (pABMR)
pAMR2, pAMR3 ..... BMR
TCMR0R ..... No TCMR
TCMR1R ..... Possible TCMR (pTCMR)
TCMR2R, TCMR3R ..... TCMR

Biopsies in the 331 cohort were reclassified using the above criteria.

[B]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. PRA statuses dated more than 14 days after the biopsy were not considered.

P. F. Halloran, A. Z. Aliabadi, M. Cadeiras, M. G. Crespo-Leiro, M. Deng, E. C. Depasquale, J. Goekler, X. Jouven, D. H. Kim, J. Kobashigawa, A. Loupy, P. Macdonald, L. Potena, A. Zuckermann, and M. D. Parkes. Exploring the cardiac response-to-injury in heart transplant biopsies. *JCI Insight 3 (20):e123674, 2018.*

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Developing the Molecular Microscope® system for EMBs (MMDx-Heart)

Rejection-associated transcripts

New four-state (4 archetype) model:

- $S1_{normal}$ = no rejection or injury
- $S2_{TCMR}$ = TCMR
- $S3_{ABMR}$ = ABMR
- $S4_{injury}$ = recent heart injury

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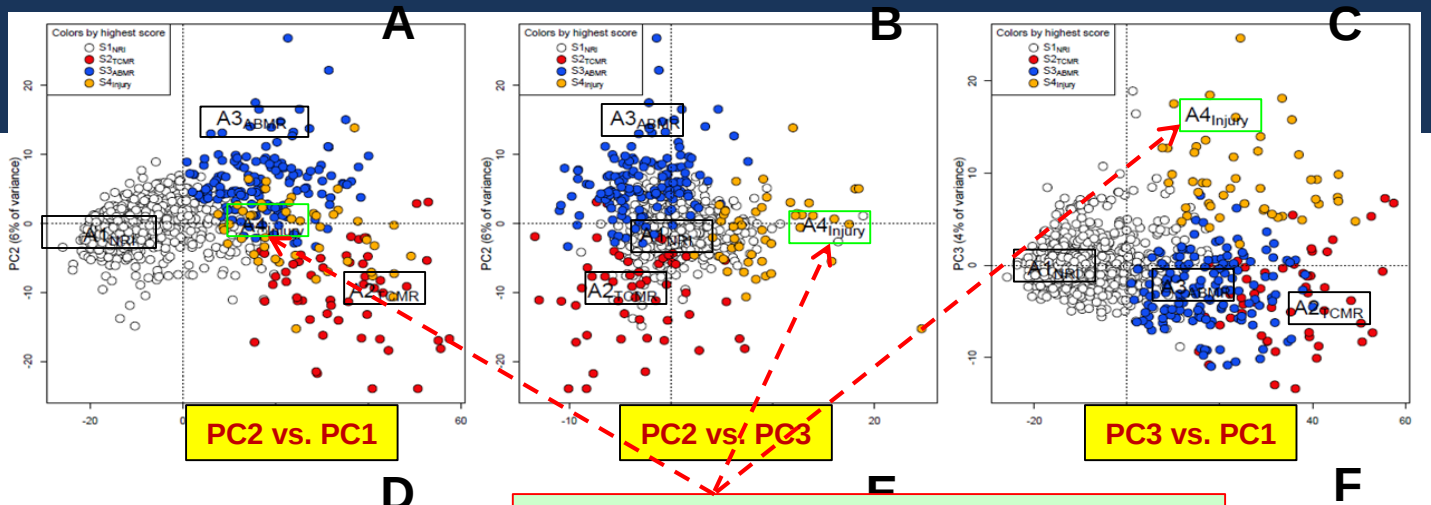
Molecular classes of heart biopsies

P. F. Halloran, A. Z. Aliabadi, M. Cadeiras, M. G. Crespo-Leiro, M. Deng, E. C. Depasquale, J. Goekler, X. Jouven, D. H. Kim, J. Kobashigawa, A. Loupy, P. Macdonald, L. Potena, A. Zuckermann, and M. D. Parkes. Exploring the cardiac response-to-injury in heart transplant biopsies. *JCI Insight 3 (20):e123674, 2018.*



S4 - Injury archetype: driver of PC3

Distributed in PCA based on RAT expression
Colored by MMDx diagnoses
Adding the fourth archetype

Principal component analysis of 889 heart transplant biopsies based on their expression of rejection associated transcripts (RATs). Samples in A-C are colored according to their highest archetype score (white = $S1_{normal}$, red = $S2_{TCMR}$, blue = $S3_{ABMR}$, orange = $S4_{Injury}$) in the four-archetype model trained on RAT expression. The large A1-A4 text labels superimposed on plots A-C mark the position of the theoretical archetypes to which each sample is compared. Panels D-F are colored by ISHLT rejection grade cut-offs according to the key at the bottom of the figure.

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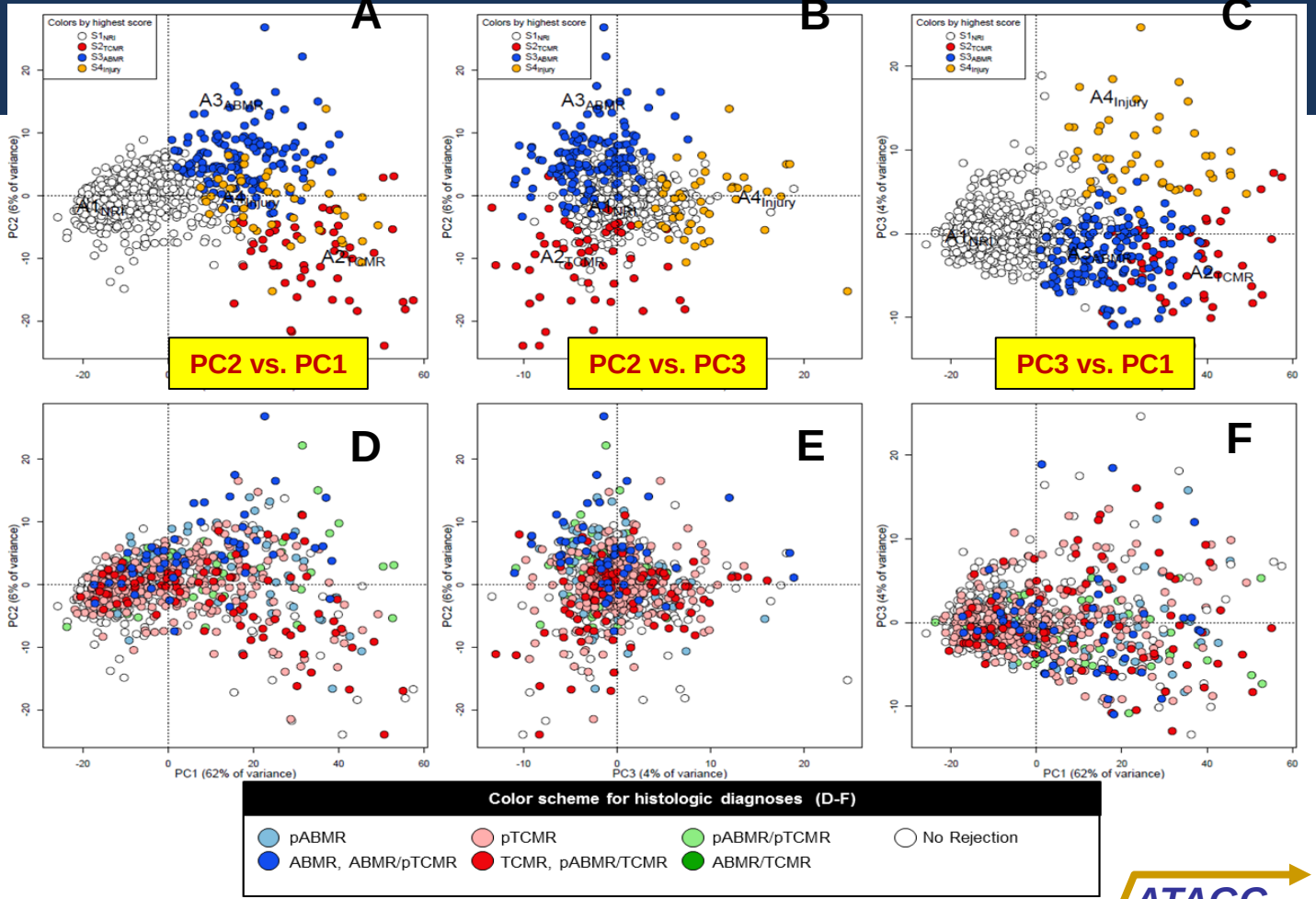
Histology diagnoses: extensive disagreement, e.g. in "normal" and in "injury"



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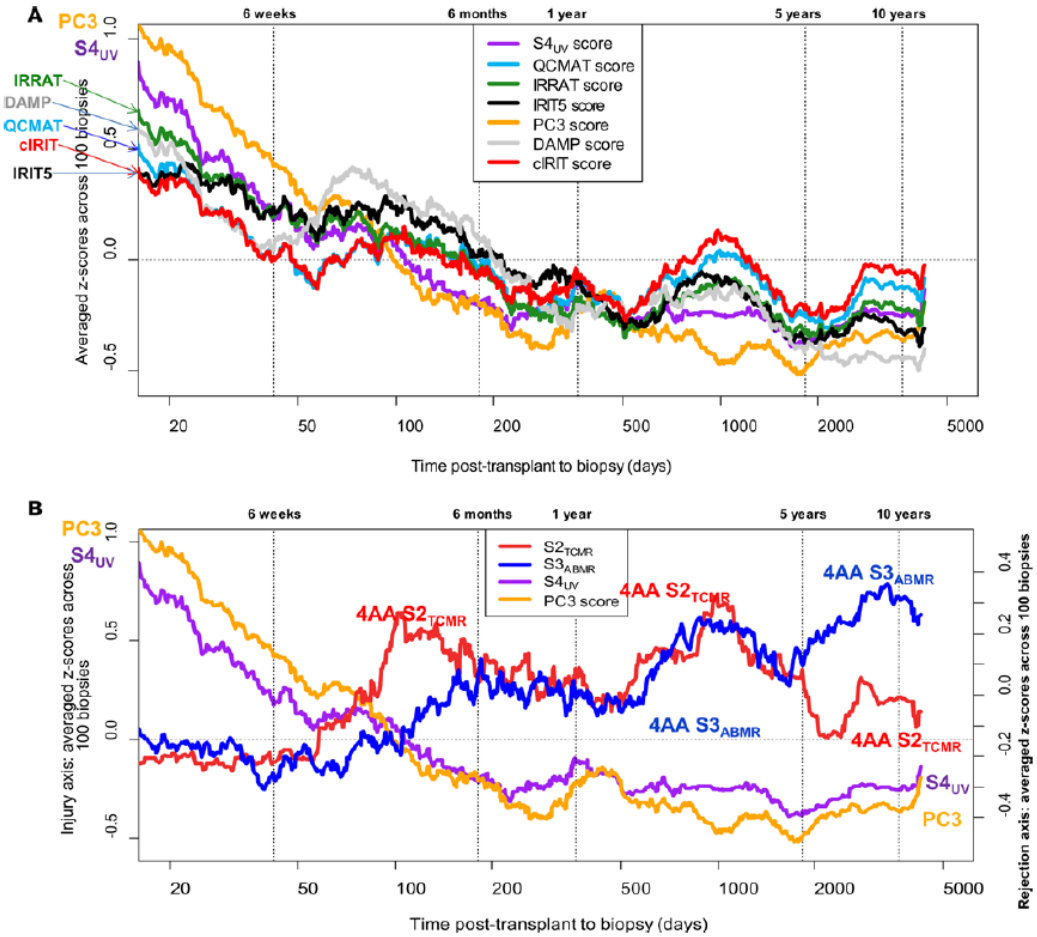

Figure 3. Moving average of standardized pathogenesis-based transcript (PBT) scores and archetype scores in biopsies ordered by increasing time after transplant (period = 100 biopsies). The $S4_{injury}$ score is compared to injury PBT scores (**A**) and rejection-related scores (**B**). Time after transplant is given in days, reported on a logarithmic scale. The $S2_{TCMR}$, $S3_{ABMR}$, and $S4_{UV}$ scores are all taken from the 4-archetype model (4AA). PC3, principal component 3 from principal component analysis of 889 EMBs based on rejection-associated transcript expression; IRIT5, 5-day injury-and-repair-induced kidney transcripts; cIRIT, cardiac injury-and-repair-induced transcripts; IRRATs, kidney injury-and-repair-associated transcripts; DAMP, damage-associated molecular pattern transcripts; QCMATs, macrophage-associated transcripts; QCATs, effector T cell–associated transcripts.

The response to injury dominates the first weeks after heart transplant and is sometimes confused with rejection

P. F. Halloran, A. Z. Aliabadi, M. Cadeiras, M. G. Crespo-Leiro, M. Deng, E. C. Depasquale, J. Goekler, X. Jouven, D. H. Kim, J. Kobashigawa, A. Loupy, P. Macdonald, L. Potena, A. Zuckermann, and M. D. Parkes. Exploring the cardiac response-to-injury in heart transplant biopsies. *JCI Insight 3 (20):e123674, 2018.*

Molecular Microscope® system for EMBs: correlations with depressed heart function (left ventricular ejection fraction, LVEF)

(Rejection/Injury Archetype) scores and LVEF:

- $S4_{injury}$ and $S2_{TCMR}$: low LVEF
- $S1_{normal}$: high LVEF
- $S3_{ABMR}$: little effect

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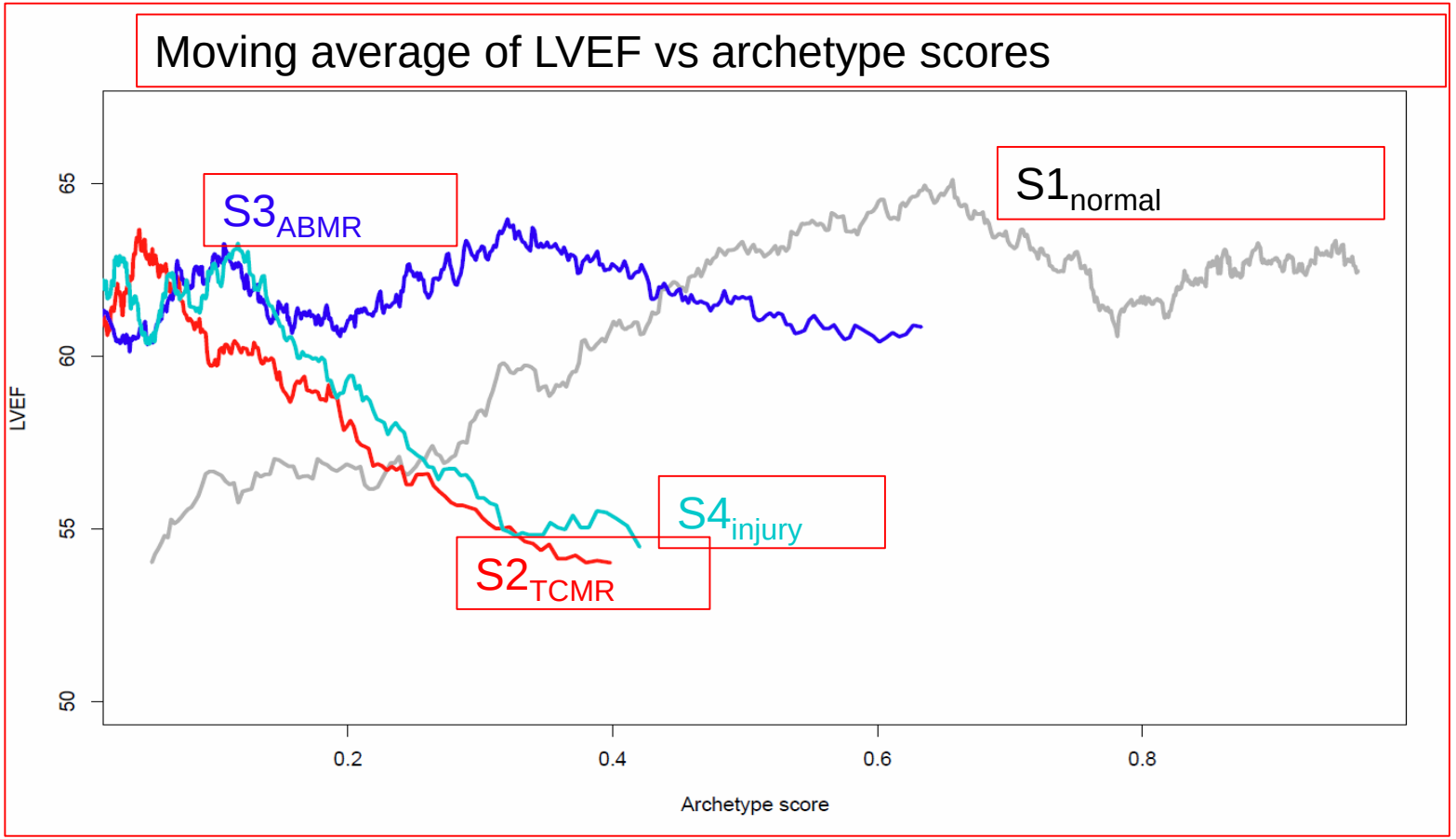

Figure 4. Running average of LVEF vs. archetype scores. For each of the four archetype scores, the 606 biopsies with available LVEF data were sorted by the archetype score being plotted. Then a sliding window of size N=85 biopsies was used to plot the mean LVEF vs. mean archetype score. I.e., the first data point on the left on the A1 line corresponds to the mean LVEF and mean S1 of the 1st through 85th biopsies (sorted in ascending order of the 606 S1 scores), the second point to the 2nd through 86th biopsies, etc. The lines have different x-axis ranges because, e.g., the highest 85 S2 scores is ~0.4, while the highest 85 scores for each of S1, S3, and S4 are larger.

ELSEVIER

The Journal of Heart and Lung Transplantation
http://www.jhltonline.org

ORIGINAL CLINICAL SCIENCE

An integrated molecular diagnostic report for heart transplant biopsies using an ensemble of diagnostic algorithms

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From the [a]Alberta Transplant Applied Genomics Centre, Edmonton, Alberta, Canada; [b]Medical University of Vienna, Vienna, Austria; [c]Ronald Reagan UCLA Medical Center, Los Angeles, California, USA; [d]Complexo Hospitalario Universitario A Coruña, A Coruña, Spain; [e]Department of Medicine, University of Alberta, Edmonton, Alberta, Canada; [f]Cedars-Sinai Medical Center, Beverly Hills, California, USA; [g]Hôpital Necker, Paris, France; [h]The Victor Chang Cardiac Research Institute, Sydney, New South Wales, Australia; and the [i]Cardiovascular Department, University of Bologna, Bologna, Italy.

KEYWORDS:
antibody-mediated rejection;
T-cell–mediated rejection;
injury;
heart transplant

BACKGROUND: We previously reported a microarray-based diagnostic system for heart transplant endomyocardial biopsies (EMBs), using either 3-archetype (3AA) or 4-archetype (4AA) unsupervised algorithms to estimate rejection. In the present study we examined the stability of machine-learning algorithms in new biopsies, compared 3AA vs 4AA algorithms, assessed supervised binary classifiers trained on histologic or molecular diagnoses, created a report combining many scores into an ensemble of estimates, and examined possible automated sign-outs.

METHODS: We studied 889 EMBs from 454 transplant recipients at 8 centers: the initial cohort ($N = 331$) and a new cohort ($N = 558$). Published 3AA algorithms derived in Cohort 331 were tested in Cohort 558, the 3AA and 4AA models were compared, and supervised binary classifiers were created.

RESULTS: Algorithms derived in Cohort 331 performed similarly in new biopsies despite differences in case mix. In the combined cohort, the 4AA model, including a parenchymal injury score, retained correlations with histologic rejection and DSA similar to the 3AA model. Supervised molecular classifiers predicted molecular rejection (areas under the curve [AUCs] >0.87) better than histologic rejection (AUCs <0.78), even when trained on histology diagnoses. A report incorporating many AA and binary classifier scores interpreted by 1 expert showed highly significant agreement with histology ($p < 0.001$), but with many discrepancies, as expected from the known noise in histology. An automated random forest score closely predicted expert diagnoses, confirming potential for automated signouts.

CONCLUSIONS: Molecular algorithms are stable in new populations and can be assembled into an ensemble that combines many supervised and unsupervised estimates of the molecular disease states.

J Heart Lung Transplant ▮▮▮▮;▮:▮▮▮–▮▮▮

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Reprint requests: Philip F. Halloran, MD, PhD, Alberta Transplant Applied Genomics Centre, 250 Heritage Medical Research Centre, University of Alberta, Edmonton, AB T6G 2S2, Canada. Telephone: 780-492-6160. Fax: 780-407-7450.

An integrated molecular diagnostic system for rejection and injury in heart transplant biopsies. JHLT in press 2019.

M. D. Parkes, A. Z. Aliabadi, P. Bruneval, M. Cadeiras, M. G. Crespo-Leiro, M. Deng, E. C. Depasquale, J. Goekler, X. Jouven, D. H. Kim, J. Kobashigawa, A. Loupy, P. Macdonald, L. Potena, A. Zuckermann, and P. F. Halloran.

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Table 3. Ability of binary molecular classifiers trained in histology or molecular diagnoses to predict histologic or molecular diagnoses

4AA Scores	Prediction tested	Areas under the receiver-operator characteristic curve (AUCs) for classifiers predicting the diagnosis of:		
		All Rejection*,† (ABMR, TCMR, Mixed)	ABMR* (ABMR, Mixed)	TCMR* (TCMR, Mixed)
$S1_{normal}$, $S2_{TCMR}$, and				0.67
Classifiers traine				**TCMR* (TCMR, Mixed)**
Histologic diagnos				0.74
Molecular diagnos				0.70
Histologic diagnos				0.92
Molecular diagnos				1.00

We trained (supervised) molecular classifiers using either molecular diagnoses or histology diagnoses.
Even the histology trained molecular classifiers agreed much better with MMDX diagnoses than histology diagnoses

** All rejection = ABMR, TCMR, Mixed (ABMR/TCMR), ABMR/pTCMR, and pABMR/TCMR vs all other biopsies. ABMR = ABMR, Mixed, and ABMR/pTCMR vs all other biopsies. TCMR = TCMR, Mixed, and pABMR/TCMR vs all other biopsies.*

† Molecular scores derived from the RAT-based four-archetype model of rejection. For classification purposes, we used cut-offs of $S2_{TCMR} \geq 0.3$ for TCMR, $S3_{ABMR} \geq 0.5$ for ABMR, and either cut-off for all rejections.

‡ Genes used: top 20 transcripts associated with histologic diagnoses. TCMR included biopsies with histologic TCMR grades > 1R, ABMR included biopsies with histologic ABMR grades > 1, and either cut-off for all rejections.

§ Genes used: top 20 transcripts associated with molecular diagnoses based on the four-archetype model of rejection using cut-offs of $S2_{TCMR} \geq 0.3$ for TCMR, $S3_{ABMR} \geq 0.5$ for ABMR, and either cut-off for all rejections.

M. D. Parkes, A. Z. Aliabadi, P. Bruneval, M. Cadeiras, M. G. Crespo-Leiro, M. Deng, E. C. Depasquale, J. Goekler, X. Jouven, D. H. Kim, J. Kobashigawa, A. Loupy, P. Macdonald, L. Potena, A. Zuckermann, and P. F. Halloran. An integrated molecular diagnostic system for rejection and injury in heart transplant biopsies. *JHLT in press 2019.*

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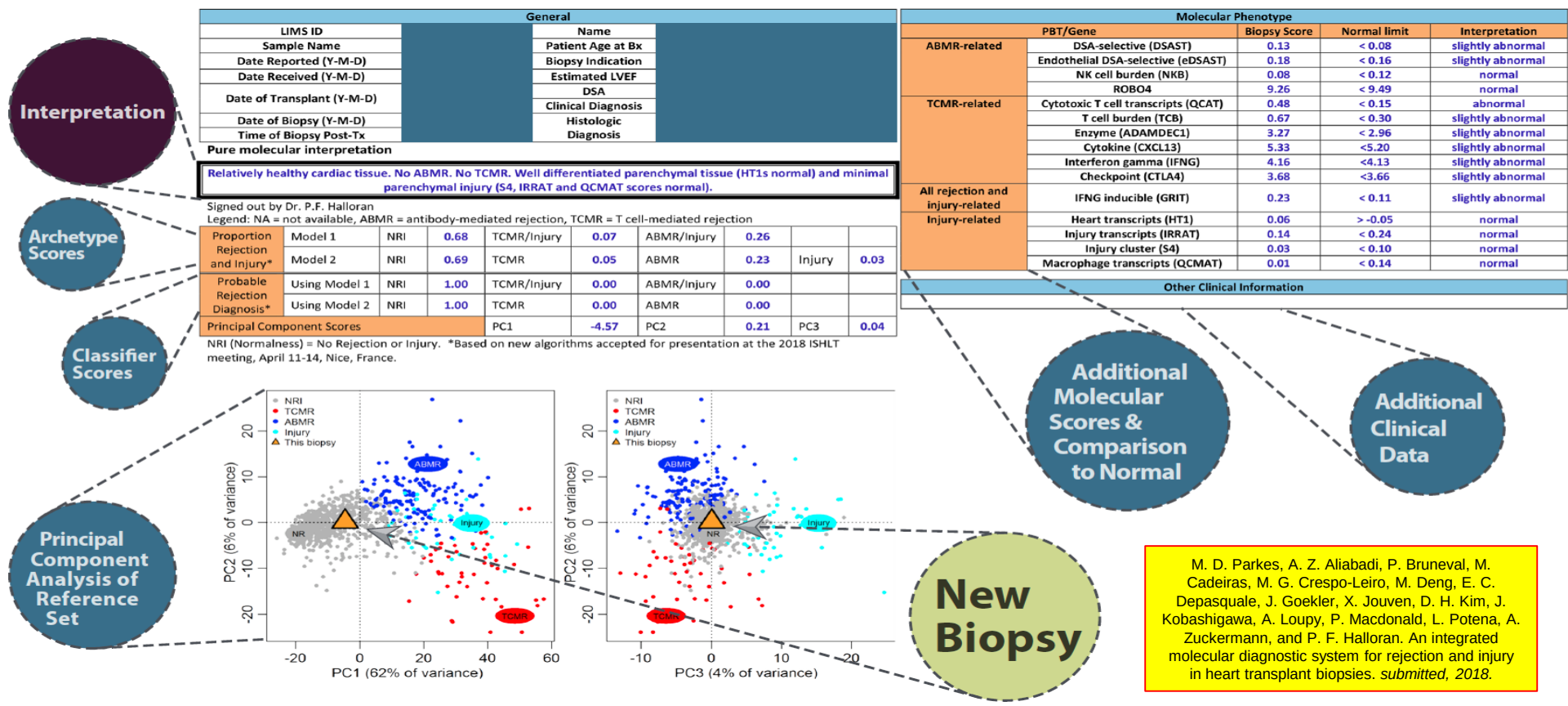
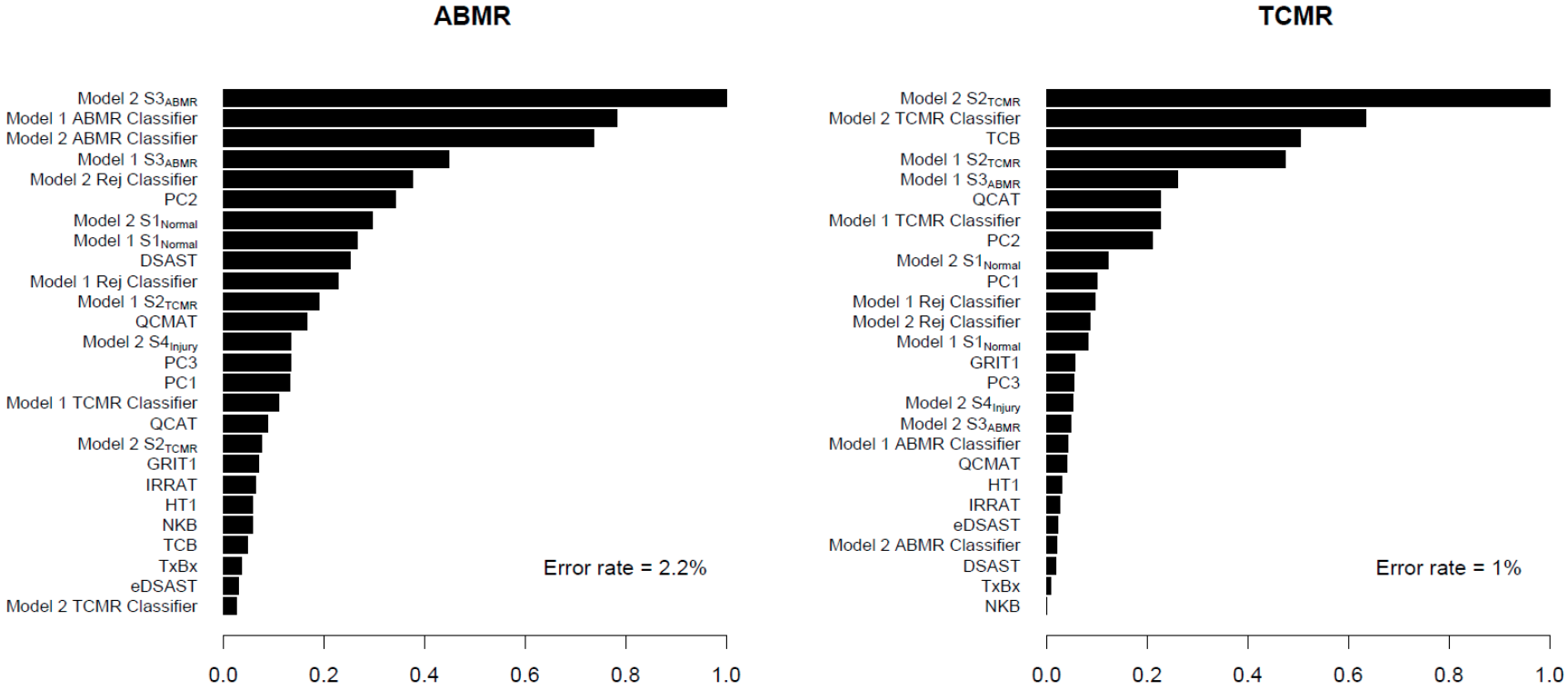


M. D. Parkes, A. Z. Aliabadi, P. Bruneval, M. Cadeiras, M. G. Crespo-Leiro, M. Deng, E. C. Depasquale, J. Goekler, X. Jouven, D. H. Kim, J. Kobashigawa, A. Loupy, P. Macdonald, L. Potena, A. Zuckermann, and P. F. Halloran. An integrated molecular diagnostic system for rejection and injury in heart transplant biopsies. *submitted, 2018.*

Figure 3. Molecular Microscope® Report for heart transplant biopsies (MMDx-Heart). The new biopsy is compared to the reference set of 889 endomyocardial biopsies and given a series of molecular scores culminating in the assignment of a molecular interpretation. This new biopsy was relatively normal with molecular features typical of well-differentiated parenchymal tissue with minimal injury or rejection. Patient information in the first table has been redacted. Archetype scores $S1_{Normal}$ (NRI), $S2_{TCMR}$, $S3_{ABMR}$, and $S4_{Injury}$ from the 3-archetype model (3AA/model 1) or 4-archetype model(4AA/model 2) are given for the new biopsy in addition to corresponding binary classifier scores predicting the probability of molecular non-rejection, TCMR, and ABMR. The report provides a visualization of the new biopsy (yellow triangle) projected into the rejection-associated transcript-based principal component analysis of the 889 reference set biopsies. Biopsies in the reference set are colored according to their highest of four archetype scores in the 4AA model. Grey indicates that $S1_{Normal}$ was the highest score, red corresponds to $S2_{TCMR}$, blue to $S3_{ABMR}$, and cyan to $S4_{Injury}$. The right hand side of the report provides a table of addition molecular data including pathogenesis-based transcript (PBT) set scores and singular transcript expression scores relating to all rejections, ABMR, TCMR, and injury. Score are represented as the log fold change in the new biopsy vs. normal biopsies (i.e. reference set biopsies with $S1_{Normal}$>0.7). For each score a normal limit is given, defined as the 95th percentile score in the normal biopsies. Scores in the 95th-99th percentile are labeled “slightly abnormal” and scores in the 99th percentile are labeled “abnormal.” The report also has space for additional clinical information if provided.

Automated ensemble sign-out for heart biopsies



M. D. Parkes, A. Z. Aliabadi, P. Bruneval, M. Cadeiras, M. G. Crespo-Leiro, M. Deng, E. C. Depasquale, J. Goekler, X. Jouven, D. H. Kim, J. Kobashigawa, A. Loupy, P. Macdonald, L. Potena, A. Zuckermann, and P. F. Halloran. An integrated molecular diagnostic system for rejection and injury in heart transplant biopsies. *JHLT in press 2019.*

ATAGC

A Molecular Analysis of Graft Survival in the INTERHEART Study: The importance of parenchymal injury

ATAGC

ATC1394. A Molecular Analysis of Graft Survival in the INTERHEART Study; The importance of parenchymal injury

Reeve, J1; and Halloran, PF1 .and the INTERHEART Investigators

Purpose: Rejection is a major cause of graft loss in heart and kidney transplants. The principal diagnosis associated with risk in kidneys is antibody-mediated rejection 'ABMR' (JCI Insight 2(12), 201710.1172/jci.insight.94197), and molecular rejection predicts graft failure better than histology (JASN 26(7):1711-1720, 2015). Similar comparisons in a heart transplant endomyocardial biopsy (EMB) population have not been performed.

Methods: The INTERHEART study population contains 1265 indication and protocol EMB single bite biopsies from 18 centers in Canada, the USA, Australia and Europe. Affymetrix microarrays analyzed gene expression. 948 biopsies from 483 transplants (478 patients) had follow-up time and graft status. We selected 1 random biopsy per transplant and analyzed 3-year post-biopsy survival. Median follow-up time in this subset was 394 days, and 51/483 hearts failed by 3 years post-biopsy. We analyzed rejection by unsupervised archetype analysis using kidney-derived rejection-associated transcripts 'RATs' (JHLT 36:1192-1200, 2017) and by our interpretation of ISHLT histologic diagnosis.

Results: Four clusters of biopsies were found by archetypal analysis: 1) Non-rejection (N=686), 2) TCMR (129), 3) ABMR (437), and 4) Injury (13), used for the Kaplan-Meier plot of survival analysis in 483 transplants (Figure 1). Because only 4 hearts from cluster 4 remained (too few to analyze as a group), and 1 failed, each was incorporated into the next most closely associated archetype (3 moved to non-rejection, 1 of which failed, and 1 to TCMR). TCMR was a greater hazard than ABMR, both by molecules and histology (Figure 1). The results using histologic diagnoses were similar to those from molecular archetypes, except that the separation between ABMR and non-rejection was not as distinct using histology. Eleven biopsies, including one that failed, lacked a histologic diagnosis and were not included in the histologic survival analysis.

In addition to belonging to a dominant archetype, each biopsy also has a score for each of the 4 archetypes, which permit the degree of molecular injury to be considered. These were used for multivariable Cox regression (Table 1). The only significant predictor of survival is degree of injury.

Conclusion: In our study population, graft loss within the first years after EMB is more highly associated with TCMR than with ABMR. However, the best predictor of graft loss, as in kidneys, is the extent of injury regardless of its cause. Presumably TCMR (and ABMR) produce graft loss via molecular injury to the parenchyma. ClinicalTrials.gov # NCT02670408

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Table 1. Hazard ratios (HR) for 3-year death-censored survival after biopsy

Variable	HR	Lower/Upper limit		p-val
Molecular archetype scores:				
Non rejection score	Reference			
ABMR score	0.85	0.26	2.8	0.80
TCMR score	2.32	0.77	7.0	0.14
Injury score	11.03	2.1	58.5	0.005

Molecular Microscope Determinants of Graft Survival in the INTERHEART Study

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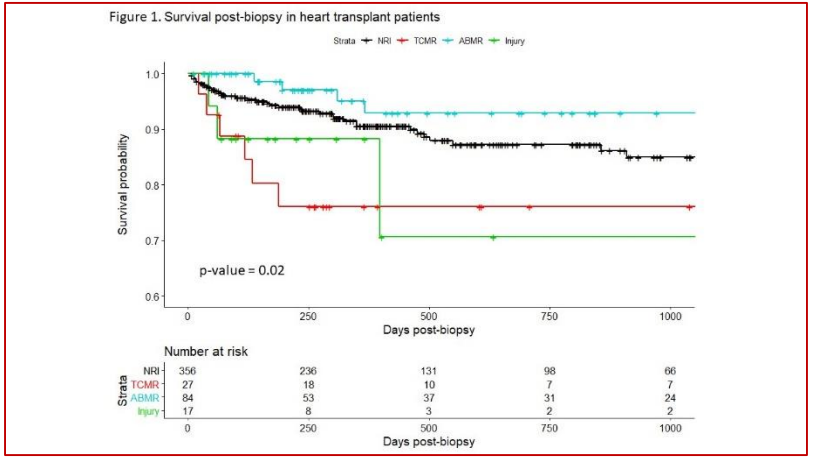
Unlike kidneys, short term graft loss (particularly within one year) after EMB is highly associated with TCMR but not ABMR. TCMR may reflect failure of immunosuppression or non-adherence. This difference between the heart and renal transplant populations raises the possibility that TCMR is relatively more destructive, and ABMR less destructive, in heart than in kidney transplants. ClinicalTrials.gov # NCT02670408



ATAGC

MMDx-Heart in EMBs

- Defined rejection in **unsupervise**d analysis
- Defined parenchymal injury as a fourth archetype
- Added **supervised** analyses of TCMR and ABMR
- Showed that LVEF is depressed by TCMR and injury, not ABMR
- Found the early losses after biopsy often are related to TCMR and injury
- Continuing studies:
  - More bites, more events (survival)
  - Define CAV and fibrosis
  - Define effects of treatment

New INTERHEART extension (INTERHEARTEX) focus on Rx, late phenotypes, events

- Define fibrosis
  - MRI T1
  - Histology grades in EMB
- Define CAV suspected CAV
- Define survival events (death retx):
  - What did clinician suspect/ attribution? Classify deaths
    - Rejection
    - Non-adherence
    - Completely unexpected previously well
- Known or suspected dysfunction/rejection
  - HFLEF – heart failure low ejection fraction
  - New phenotype: HFPEF - heart failure/symptoms/dysfunction preserved ejection fraction
- Post Rx bx is standard of care
- Question: do MTORIs inhibit fibrosis/hypertrophy? If so do they preserve/improve LVEF? (Peter MacDonald)

Potential of molecular measurements to change care

Mechanisms (not just “biomarkers”)

Reclassify the disease states

New tests

International standard

Recalibrate conventional tests

Guide and monitor response to therapy

Empower clinical trials: new treatments

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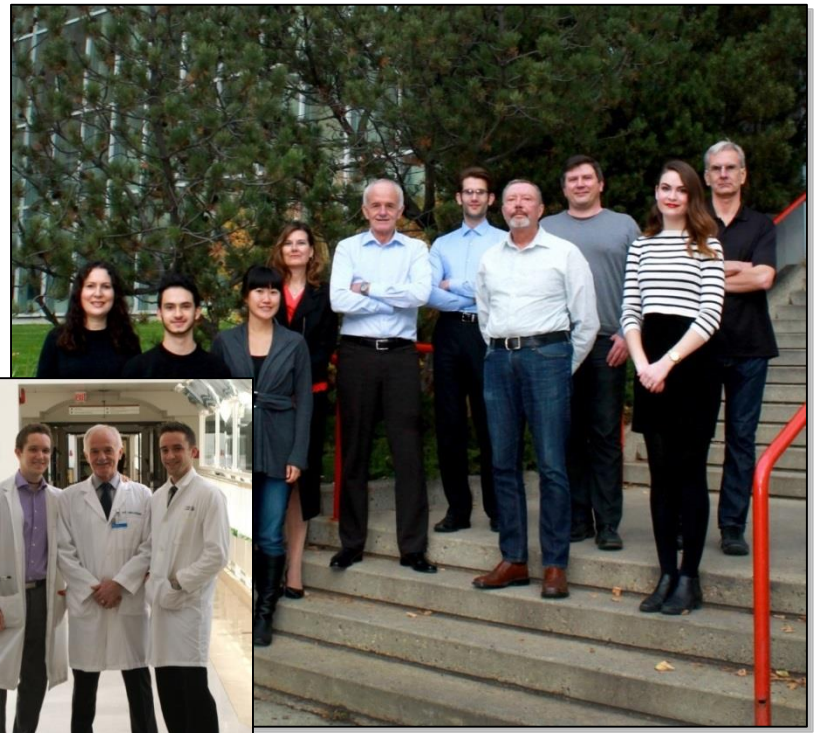

Jeffery Venner (not shown)
Luis Hidalgo (not shown)
Kieran Halloran
Brendan Halloran

Mendez NATIONAL INSTITUTE of TRANSPLANTATION FOUNDATION

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Thank you

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