Mapping the injury phenotypes of heart transplants

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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 and Natera

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Backgrounds and Methods

- **Purpose.** In previous studies (see references below), we used microarray analysis to characterize the rejection phenotypes of heart transplant endomyocardial biopsies, based on rejection-associated transcripts (RATs). Although these phenotypes were associated with graft survival, gene-based analyses indicated that survival was more strongly associated with injury- than with rejection-related genes. We therefore built a second model using injury gene sets, analogous to our earlier rejection model, in order to have an independent classification system more concordant with outcomes.
- **Goal: new understanding by combining injury and rejection analysis.**
- **Methods.** We used microarrays to analyze gene expression of previously annotated injury-associated transcript sets in 1320 biopsies (645 patients) from 13 centers in the INTERHEART study. Injury categories were defined using unsupervised archetypal analysis. These categories and those from the rejection analysis were used to predict low LVEF (≤50), and 3-year graft survival.

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Loupy A, Duong Van Huyen JP, Hidalgo LG, Reeve J, Racape M, Venner J, et al. Gene Expression Profiling for the Identification and Classification of Antibody-Mediated Heart Rejection. Circulation. 2017;135(10):917-35.
Parkes MD, Aliabadi AZ, Cadeiras M, Crespo-Leiro MG, Deng M, Depasquale EC, et al. An integrated molecular diagnostic system for rejection and injury in heart transplant biopsies. Journal of Heart and Lung Transplantation. 2019;38(6):636-46.

Main Histologic diagnoses: 9% TCMR, 5% ABMR, 39% no rejection, and 31% possible TCMR

DSA status: 37% of those tested were +ve

Histologic diagnoses and DSA status in 1320 endomyocardial biopsies

Histology diagnoses[A]	N (% of total)
No Rejection	519 (39%)
TCMR	113 (9%)
ABMR	71 (5%)
ABMR/TCMR (Mixed)	14 (1%)
pTCMR	411 (31%)
pABMR	69 (5%)
pABMR/pTCMR	81 (6%)
Missing	42 (3%)
DSA status at biopsy	N (% of known)
Positive	307 (37%)
Negative	517 (63%)
Not tested	496

[A] Biopsy labels were converted as follows:

pAMR0	No ABMR;
pAMR1, pAMR1I+, pAMR1H+	Possible ABMR (pABMR);
pAMR2, pAMR3	ABMR;
TCMR0R	No TCMR;
TCMR1R	Possible TCMR (pTCMR);
TCMR2R, TCMR3R	TCMR

New rejection model

Rejection (based on rejection associated transcripts (RATs):

$R1_{\text{No-rejection}}$ $R2_{\text{TCMR}}$ $R3_{\text{ABMR}}$ R4 Injury-no rejection R5 Minor

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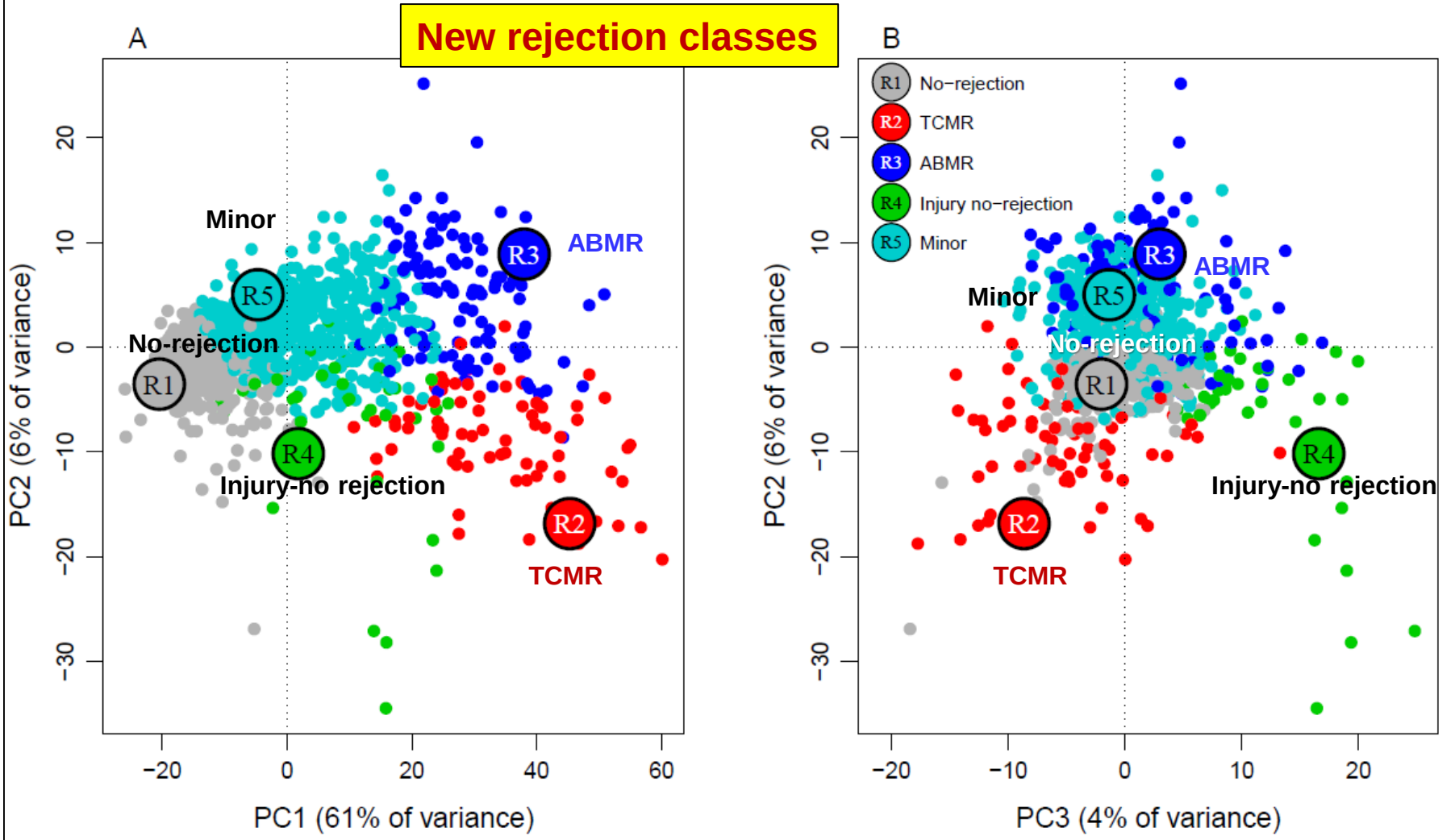
New rejection classes
A
B
Minor
ABMR
No-rejection
Injury-no rejection
TCMR
R1
R2
R3
R4
R5
PC1 (61% of variance)
PC2 (6% of variance)
PC3 (4% of variance)
No−rejection
TCMR
ABMR
Injury no−rejection
Minor

Injury PCA and AA of input variables

analysis of the variation in injury-induced gene sets in the biopsy population

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Injury was measured by 10 input variables: injury related transcript sets characterized in experiemnetal models and clinical transplant biopsies

The ten injury-related pathogenesis-based transcript sets[A,B] (PBTs) used for the injury-based principal component (PCA) and archetypal (AA) analyses

Biological processes	PBTs	Description	Detail
Expressed in macrophages	QCMAT	Quantitative Constitutive Macrophage-Associated Transcripts	Transcripts with high expression in human primary macrophages, not inducible by IFNG, and highly correlated with levels of macrophage RNA in a sample (1)
	AMAT	Alternative Macrophage Associated Transcripts	Alternative activation of macrophages in mouse model of ischemic acute kidney injury (1)
Increased in injury	IRRAT	Injury-repair response associated transcripts	Transcript set estimating kidney transplant injury, developed in early transplants (2)
	cIRIT	Cardiac injury and repair induced transcripts	Injury and repair induced transcripts derived from mouse cardiac isografts
	IRITD3	Injury and rejection induced transcripts – intermediate time post-transplant	Human orthologs of mouse genes induced by non-immune kidney injury in isografts, peaking around day 3 post-transplant in mouse kidney transplants (3)
	IRITD5	Injury and rejection induced transcripts – late time post-transplant	Human orthologs of mouse genes induced by non-immune kidney injury in isografts, peaking around day 5 post-transplant in mouse kidney transplants (3)
	DAMP	Damage-associated molecular pattern transcripts	Literature-based damage-associated molecular pattern (DAMP) transcripts annotated as markers of cellular stress (4, 5)
Highly expressed in normal heart	HT1	Heart transcripts - Set 1	Human orthologues of genes with high expression in normal mouse heart (6)
	HT2	Heart transcripts - Set 2	Human orthologs of solute carrier genes showing high expression in normal mouse heart (6)
Increased in atrophy-fibrosis	IGT	Immunoglobulin transcripts	Time-dependent increase in injured tissue that reflects plasma cell infiltrate (7)

[A] https://www.ualberta.ca/medicine/institutes-centres-groups/atagc/research/gene-lists

[B] The gene sets were empirically derived in human cell lines, human transplants, and mouse models. They reflect biological processes relevant to rejection and injury.

Abbreviations: AMAT - alternative macrophage associated transcripts; cIRIT – cardiac injury-repair induced transcripts; DAMP – damage-associated molecular pattern transcripts; HT1 – heart transcripts set 1; HT2 – heart transcripts 2; IGT – immunoglobulin transcripts; IRITD3 - injury-repair induced transcripts day 3; IRITD5 - injury-repair induced transcripts day 5; IRRAT – AKI transcripts; QCMAT - quantitative constitutive macrophage-associated transcripts

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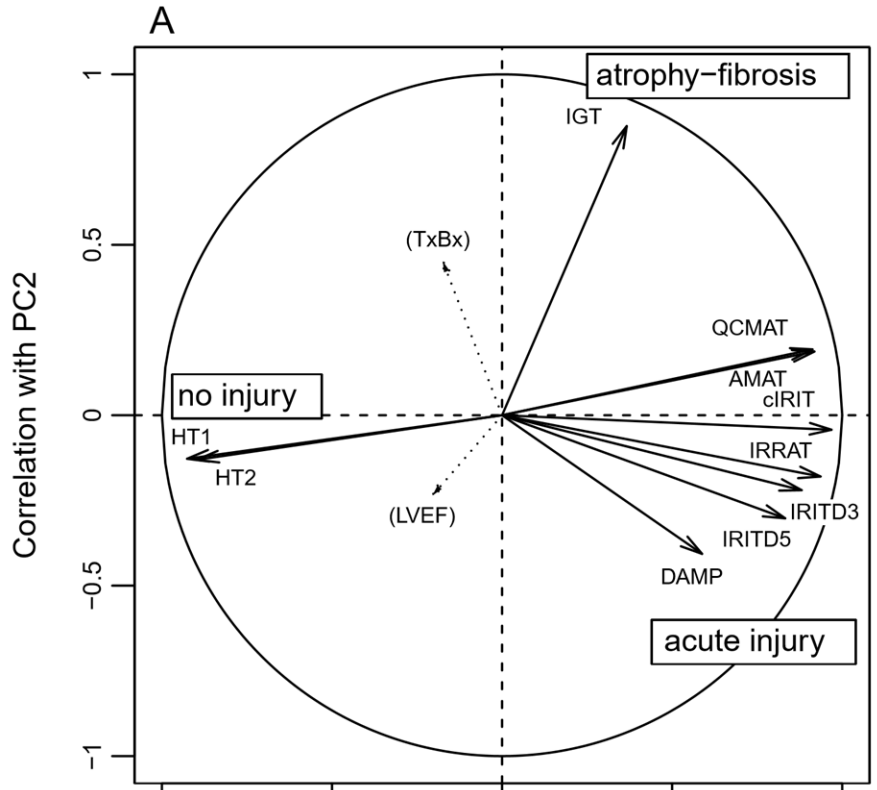
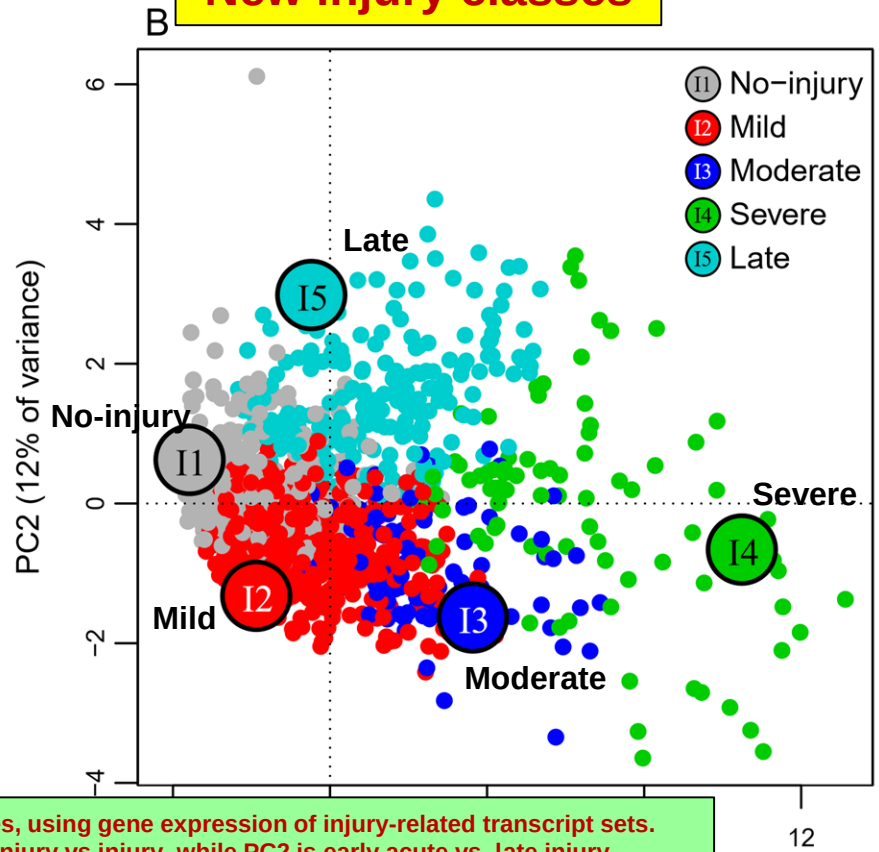
7. Einecke G, Reeve J, Mengel M, Sis B, Bunnag S, Mueller TF, et al. Expression of B cell and immunoglobulin transcripts is a feature of inflammation in late allografts. *American Journal of Transplantation*. 2008;8(7):1434-43.

New injury classes





We performed principal component analysis (PCA) in 1320 heart transplant biopsies, using gene expression of injury-related transcript sets.

A) Correlation between input (transcript set scores) and PCs. PC1 represents no injury vs injury, while PC2 is early acute vs. late injury (atrophy-fibrosis).

B) PCA plot of the 1320 biopsies colored by archetypal analysis clusters. Biopsy location reflects their correlation with inputs in A).
The five archetypes were $I1_{No\text{-}injury}$ $I2_{Early\text{-}mild}$ $I3_{Late}$ $I4_{Severe}$ $I5_{Early\text{-}moderate.}$

I5.Late has the atrophy-fibrosis associated transcript set changes

I4.severe has high expression of macrophage transcripts and DAMPs

Table 4. Mean of pathogenesis-based transcript set (PBT) scores and clinical variables, in biopsies belonging to the five Injury archetype clusters

		Injury archetype groups				
Biological processes	**PBT**	**I1$_{No\text{-}injury}$ (N=376)**	**I2$_{Mild}$ (N=526)**	**I3$_{Moderate}$ (N=110)**	**I4$_{Severe}$ (N=87)**	**I5$_{Late}$ (N=221)**
Expressed in macrophages	QCMAT [A]	1.05	1.17	1.45	2.80	1.54
	AMAT [A]	1.08	1.24	1.67	3.28	1.78
Increased in injury	IRRAT [A]	0.99	1.15	1.61	2.16	1.26
	cIRIT [A]	1.00	1.05	1.22	1.47	1.15
	IRITD3 [A]	0.99	1.04	1.19	1.26	1.08
	IRITD5 [A]	0.99	1.07	1.35	1.40	1.10
	DAMP [A]	0.92	1.13	1.02	1.41	1.03
Highly expressed in normal heart	HT1 [A]	0.98	0.98	0.86	0.68	0.88
	HT2 [A]	0.97	0.99	0.79	0.54	0.83
Increased in atrophy-fibrosis	IGT [A]	1.03	0.99	1.03	1.79	3.19
Mean days post-transplant (median)		1065 (329)	408 (126)	218 (65)	548 (85)	1430 (712)
LVEF		62	64	62	54	55
Probability of failure at 3 years post-biopsy [B]		0.15	0.09	0.00	**0.30**	**0.21**
Fraction DSA+		0.31	0.27	0.51	0.52	0.55

[A] These were the 10 transcript sets used in the principal component and archetypal analyses.
[B] Based on a Kaplan-Meier estimate using one randomly selected biopsy per transplant

Heart injury classes (Archetype names)

Injury (based on injury-related transcript sets (injury PBTs)

$I1_{\text{No-injury}}$ $I2_{\text{mild}}$ $I3_{\text{moderate}}$ $I4_{\text{Severe}}$ $I5_{\text{Late}}$

Rejection classes for comparison

Rejection (based on rejection associated transcripts (RATs):

$R1_{\text{No-rejection}}$ $R2_{\text{TCMR}}$ $R3_{\text{ABMR}}$ $R4_{\text{Injury-no rejection}}$ $R5_{\text{Minor}}$

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Injury-rejection relationships

Injury is often present in biopsies with no rejection

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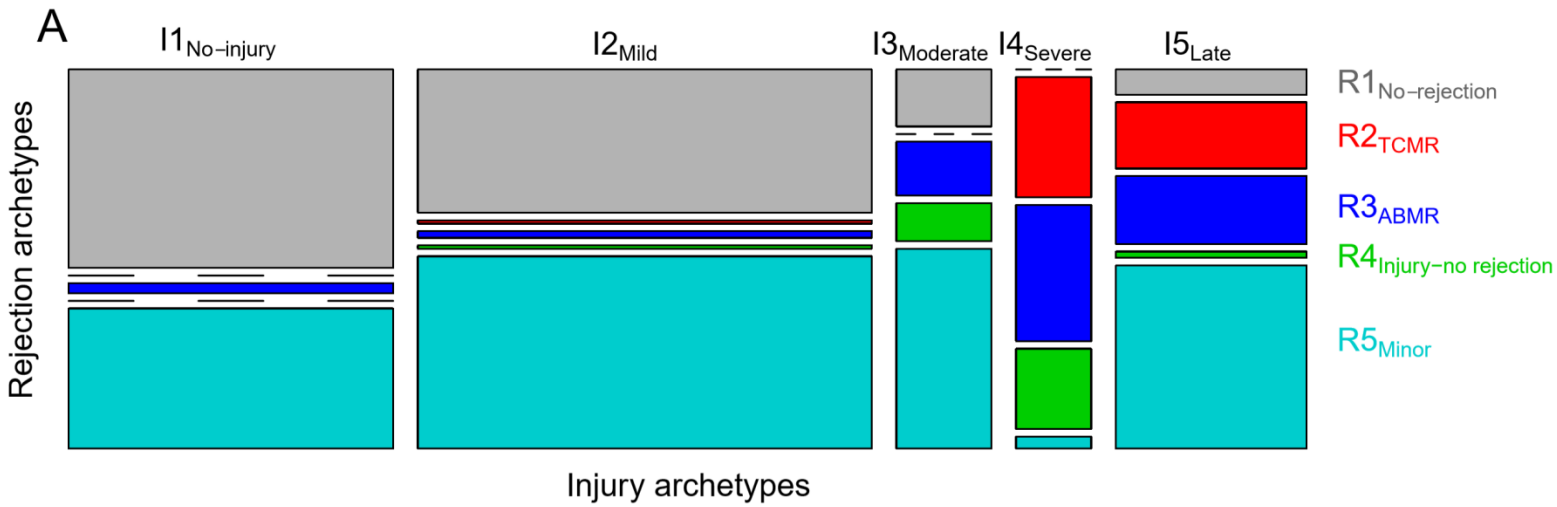
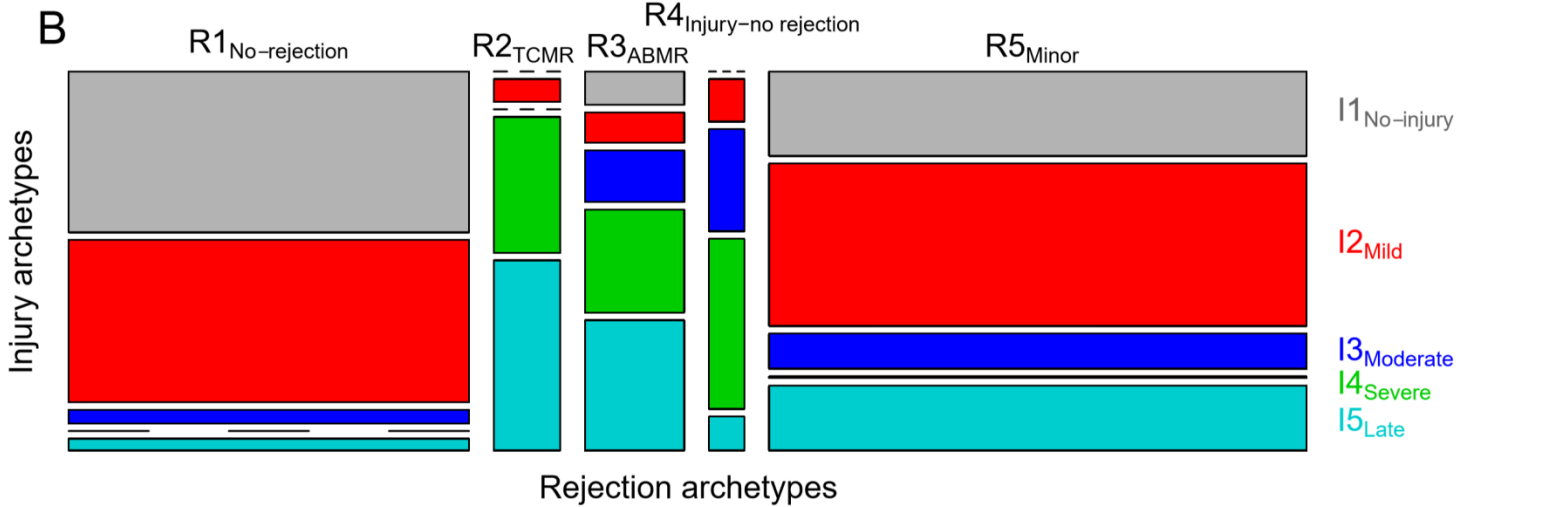
Fig 2

The I5Late and I4severe injury groups have much of the TCMR (red) and ABMR (blue) cases, but I5Late is often not rejection (R5minor – cyan) .



R2TCMR and R3ABMR groups usually have I5Late (cyan) or I4severe (green) injury phenotypes.



Injury-rejection time course

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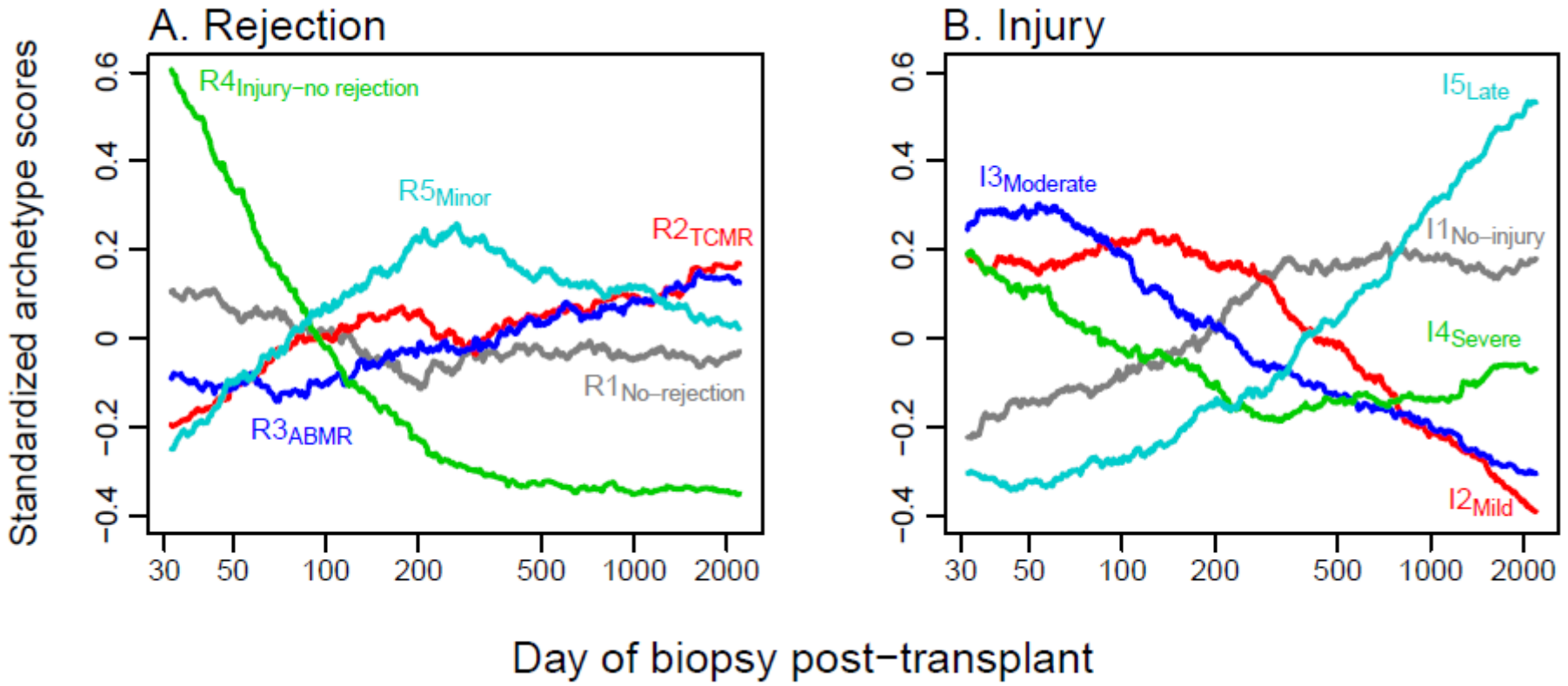
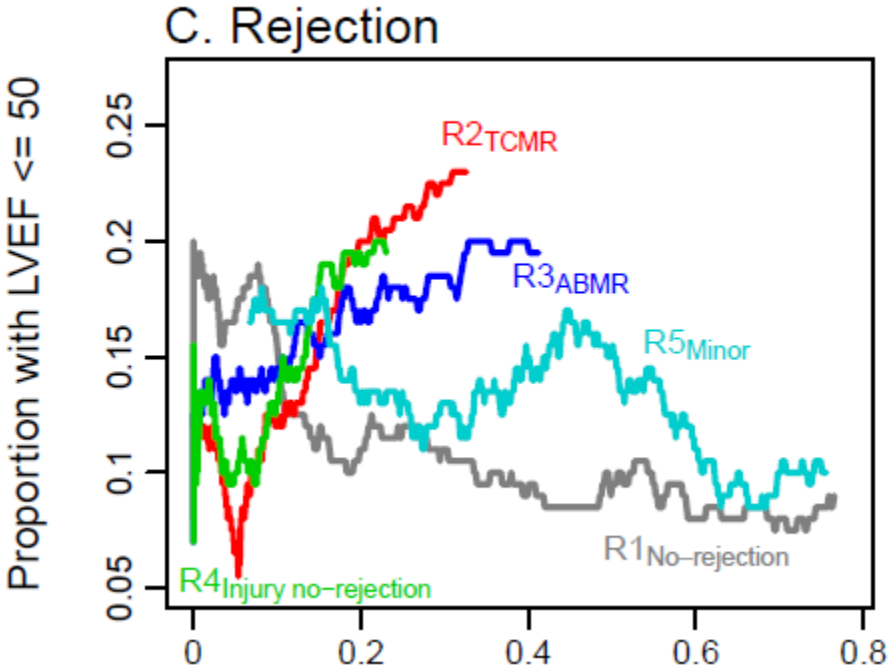
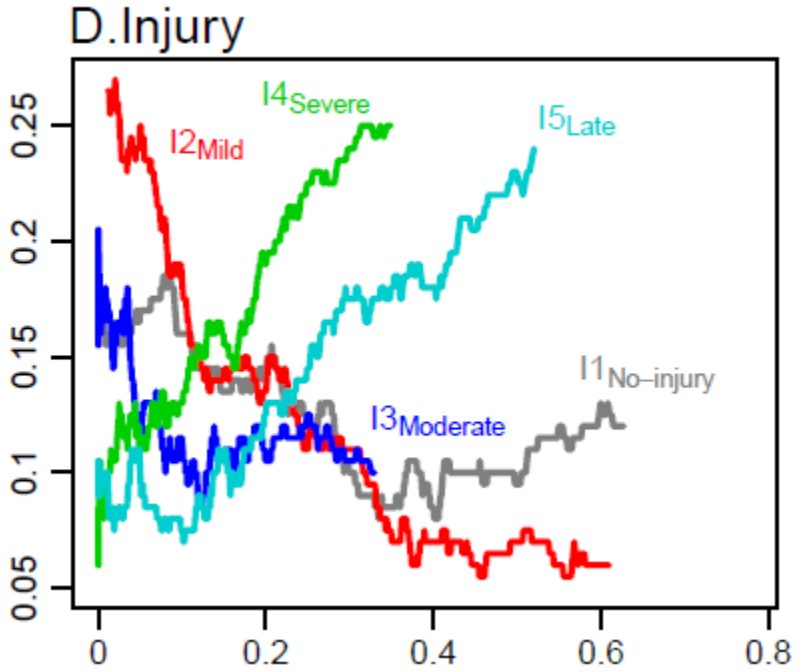
Comparing the time course of the rejection scores (A) to the injury scores (B)



Association with LVEF<50 of rejection scores (C) and injury (D) scores





Injury and rejection together give the best model for survival

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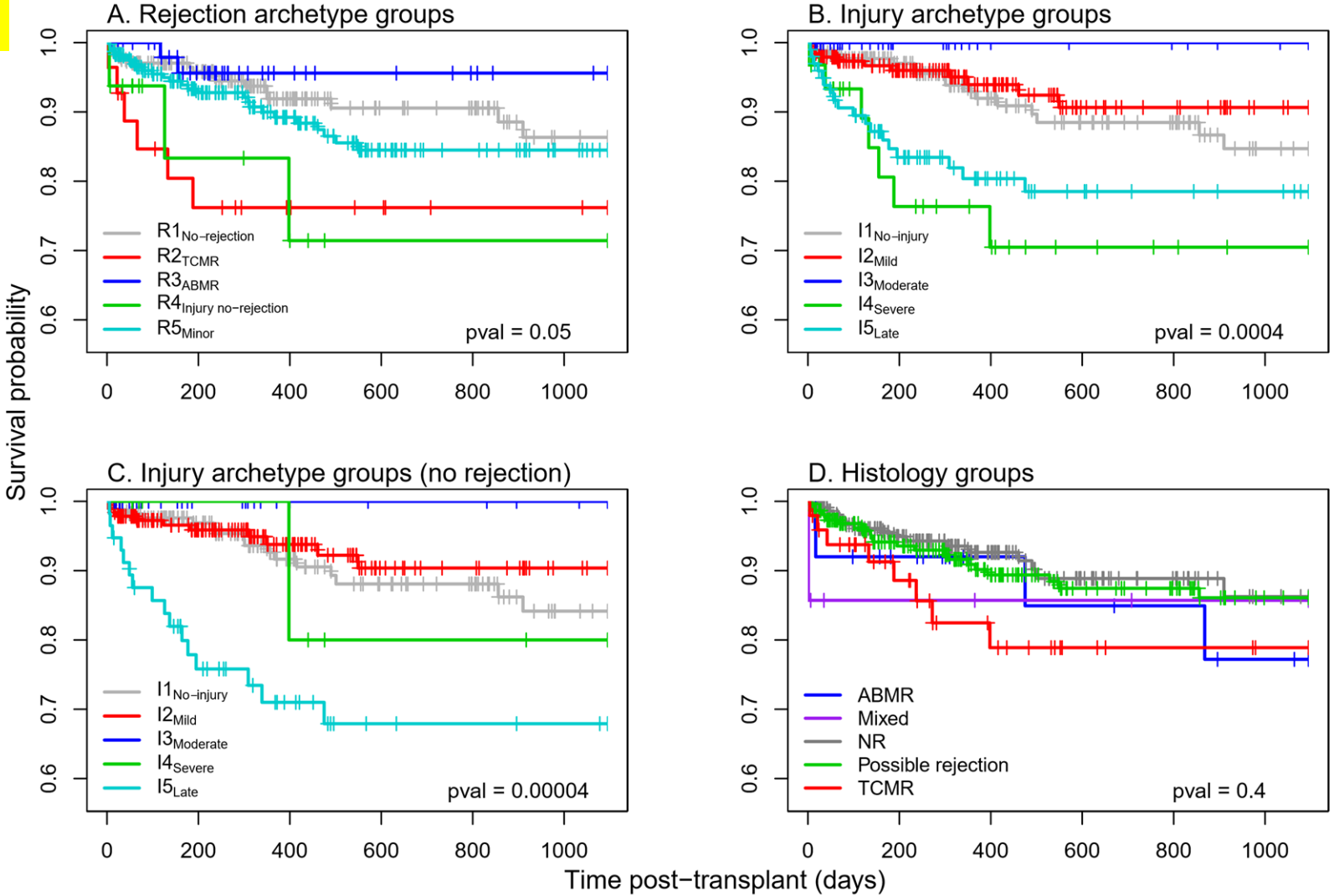
Figure 6


A. Rejection archetype groups
R1 No−rejection
R2 TCMR
R3 ABMR
R4 Injury no−rejection
R5 Minor
pval = 0.05
B. Injury archetype groups
I1 No−injury
I2 Mild
I3 Moderate
I4 Severe
I5 Late
pval = 0.0004
C. Injury archetype groups (no rejection)
I1 No−injury
I2 Mild
I3 Moderate
I4 Severe
I5 Late
pval = 0.00004
D. Histology groups
ABMR
Mixed
NR
Possible rejection
TCMR
pval = 0.4
Survival probability
Time post−transplant (days)

Injury-rejection relationships to 3 year graft survival

We combined the I and R scores in a multiple Cox regression model to predict three-year post-biopsy survival. Inputs remaining after backward elimination were $I5_{Late}$, $I4_{Severe}$, and $R3_{ABMR,}$ the last being "protective" i.e. associated with relatively low risk.

Adding I scores to a model with only R scores improves the model (NRI=0.24, p-value=0.046). Adding R scores to I scores alone also improves the model (NRI=0.31, p-value=0.004).

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Conclusion

- Heart transplant parenchymal injury can be mapped by analysis using injury-related transcript sets.
- The injury phenotypes are sometimes associated with active rejection but often not.
- Injury phenotypes are the top predictors of impaired function and important predictor of risk of graft loss. Rejection acts by inducing injury.
- Added to the molecular rejection phenotype, the molecular injury phenotype adds new understanding of the state of heart transplants.
- **Note the emergence of the new I4 Late biopsy group, 62% of which have no rejection, which have reduced LVEF and increased failure**
  - **Relationship to CAV?**