Molecular profiles of injury in heart transplant allograft: Relationship to rejection, ejection fraction, and survival.

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3. INTERHEART ClinicalTrials.gov NCT02670408

ATAGC

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- 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

MMDx-Heart (N=1320)

MMDx-Heart Participating Centers in N=1320 biopsies		
Name	**Center**	**Location**
Daniel Kim	University of Alberta	Edmonton, AB, Canada
Jon Kobashigawa	Cedars-Sinai Medical Center	Los Angeles, CA, USA
Josef Stehlik	University of Utah	Salt Lake City, UT, USA
Mario Deng	University of California Los Angeles	Los Angeles, CA, USA
Martin Cadeiras	University of California Los Angeles	Los Angeles, CA, USA
Eugene Depasquale	University of California Los Angeles	Los Angeles, CA, USA
Keyur Shah	Virginia Commonwealth University	Richmond, VA, USA
Luciano Potena	University of Bologna	Bologna, Italy
Marisa Crespo-Leiro	A Coruña University Hospital	A Coruña, Spain
Alexandre Loupy	Necker Hospital	Paris, France
Andreas Zuckermann	Medical University of Vienna	Vienna, Austria
Arezu Zuckermann-Aliabadi	Medical University of Vienna	Vienna, Austria
Johannes Gökler	Medical University of Vienna	Vienna, Austria
Peter Macdonald	St. Vincent's Hospital	Sydney, Australia

INTERHEART ClinicalTrials.gov NCT02670408

Backgrounds and Methods

- **Purpose.** In previous studies[1,2,3,4] we used microarrays to characterize the rejection phenotypes of heart transplant endomyocardial biopsies (EMBs), based on rejection-associated transcripts (RATs). We found that survival was strongly associated with rejection but also with a class of biopsies with early injury-no rejection. In the present study, we built a second model using injury genes rather than rejection genes.
- **Goal: new understanding of each biopsy scoring injury and rejection independently.**
- **Methods.** We used microarrays to analyze gene expression of previously annotated injury-associated transcript sets in 1320 biopsies (645 patients) from 10 centers in the INTERHEART study. Injury classes were defined using unsupervised principal component (PCA) and archetypal analysis (AA). These injury and rejection scores were used to predict dysfunction (LVEF (≤50) and 3-year graft survival.

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

DSA status: 37% of those tested as per standard-of-care 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:

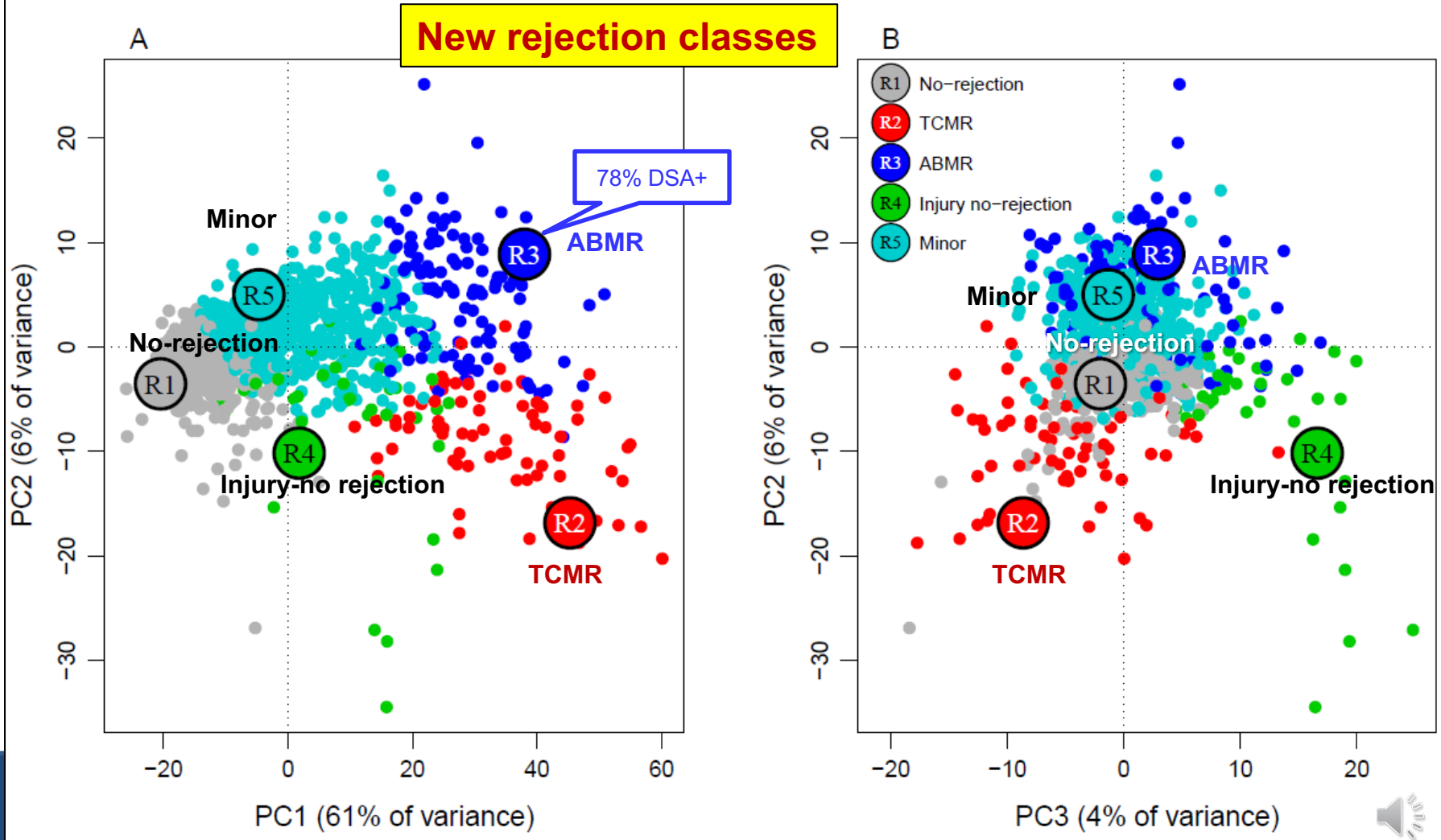
pAMR0	No ABMR;
pAMR1, pAMR1I+, pAMR1H+	Possible ABMR (pABMR);
pAMR2, pAMR3	ABMR;
TCMR0R	No TCMR;
TCMR1R	Possible TCMR (pTCMR);
TCMR2R, TCMR3R	TCMR

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**

New rejection classes



New injury model

Analysis of the variation in injury-induced gene sets in the biopsies using PCA and AA

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

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

Biological processes	PBTs	Description	Detail
Expressed in macrophages	QCMAT	Quantitative Constitutive Macrophage-Associated Transcripts	high expression in human primary macrophages, not inducible by IFNG (1)
	AMAT	Alternative Macrophage Associated Transcripts	Alternative activation of macrophages in mouse model(1)
Increased in recent injury	IRRAT	Injury-repair response associated transcripts	Human kidney transplant acute injury (2)
	cIRIT	Cardiac injury and repair induced transcripts	Injury in mouse cardiac isografts
	IRITD3	Injury-induced transcripts day 3	Acute kidney injury in mouse isografts at day 3 (3)
	IRITD5	Injury-induced transcripts day 5	Acute kidney injury in mouse isografts at day 5 (3)
	DAMP	Damage-associated molecular pattern transcripts	Literature-based damage-associated molecular pattern (DAMP) transcripts (4, 5)
Highly expressed in normal heart	HT1	Heart transcripts - Set 1	High expression in normal mouse heart (6)
	HT2	Heart transcripts - Set 2	Solute carriers with high expression in normal heart (6)
Increased in atrophy-fibrosis	**IGT**	**Immunoglobulin transcripts**	**Plasma cell infiltrate in atrophy-fibrosis (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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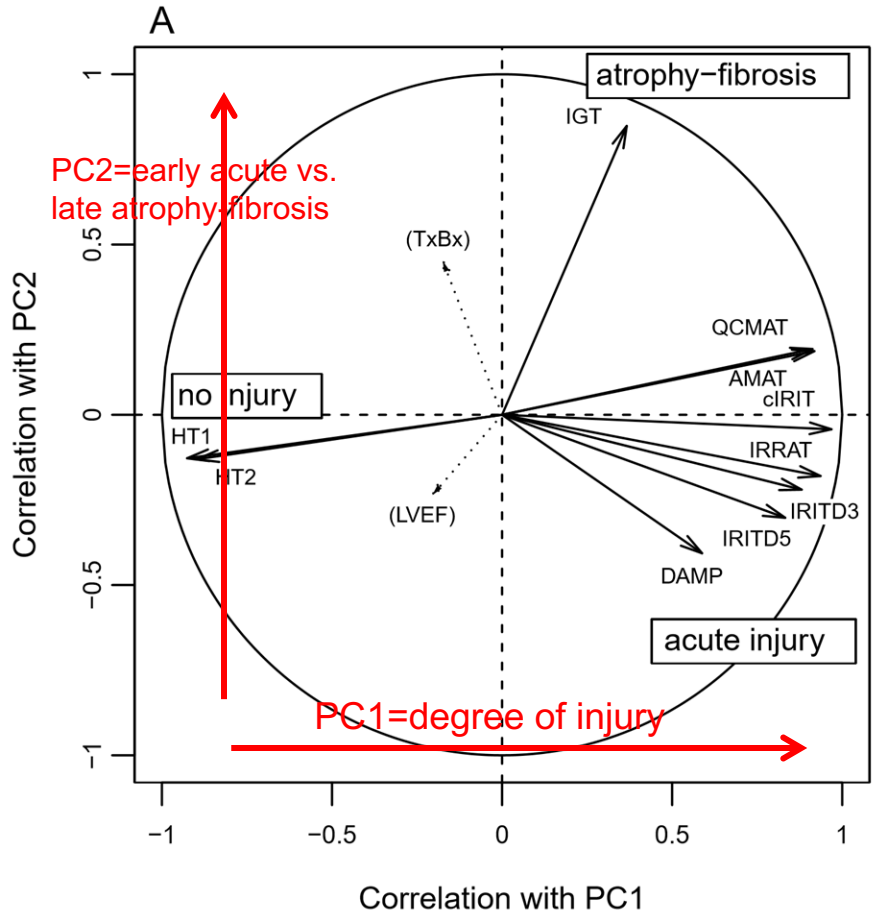


We performed principal component analysis (PCA) in 1320 heart transplant biopsies, using gene expression of injury-related transcript sets.

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).

Every biopsy can be assesed independently for injury and rejection

- Heart injury classes (Archetype names);
  - $I1_{\text{No-injury}}$ $I2_{\text{mild}}$ $I3_{\text{moderate}}$ $I4_{\text{Severe}}$ $I5_{\text{Late}}$

- Heart rejection classes (Archetype names);
  - $R1_{\text{No-rejection}}$ $R2_{\text{TCMR}}$ $R3_{\text{ABMR}}$ $R4_{\text{Injury-no rejection}}$ $R5_{\text{Minor}}$

Special interest:

$I5_{Late}$ has the atrophy-fibrosis associated transcript set changes

$I4_{severe}$ has high expression of macrophage transcripts, injury transcripts, and DAMPs

Pathogenesis-based transcript set (PBT) scores and clinical variables in each Injury group

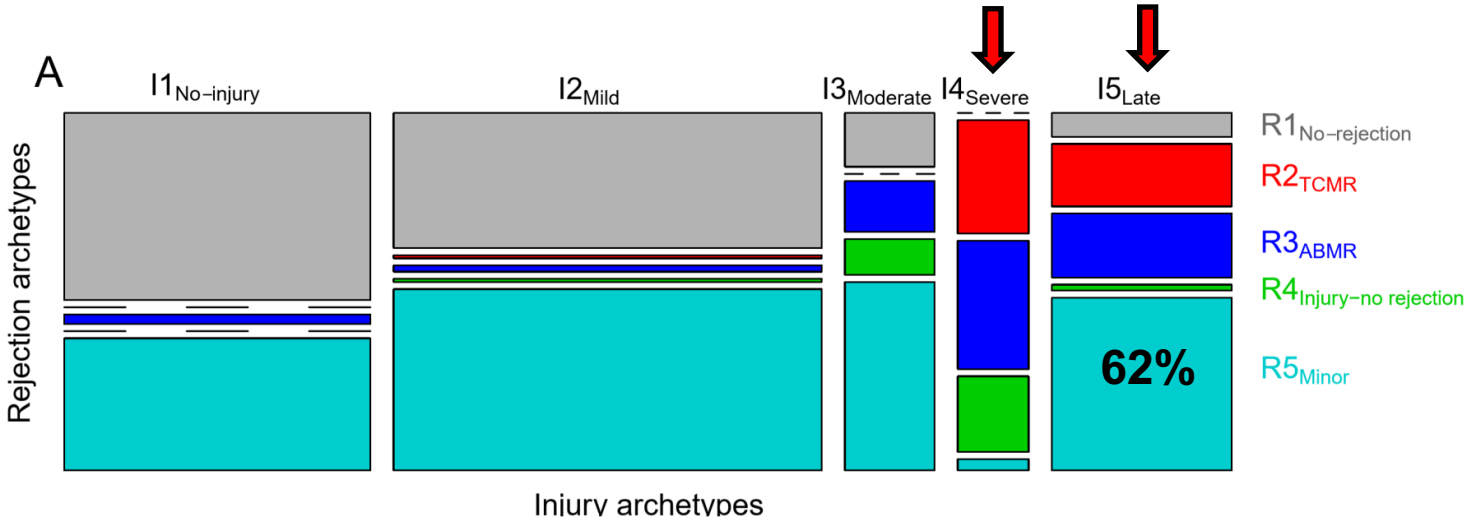
Molecular injury-related changes		Injury archetype groups				
Biological processes	PBT	$I1_{No\text{-}injury}$ (N=376)	$I2_{Mild}$ (N=526)	$I3_{Moderate}$ (N=110)	$I4_{Severe}$ (N=87)	$I5_{Late}$ (N=221)
Expressed in macrophages	QCMAT [A]	1.05	1.17	1.45	2.80	1.54
	AMAT [A]	1.08	1.24	1.67	3.28	1.78
Increased 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
ghly 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

Injury-rejection relationships

Injury is often present in biopsies with no active rejection

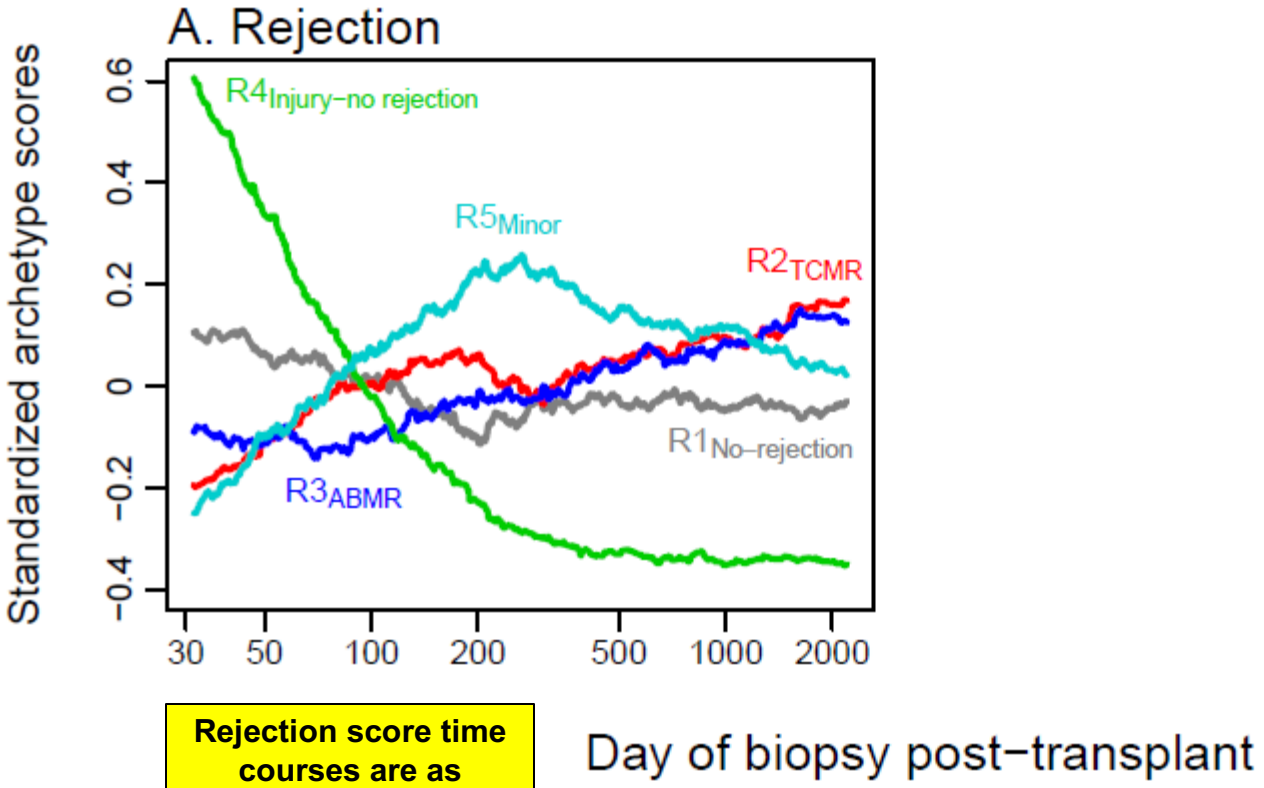
Fig 2

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



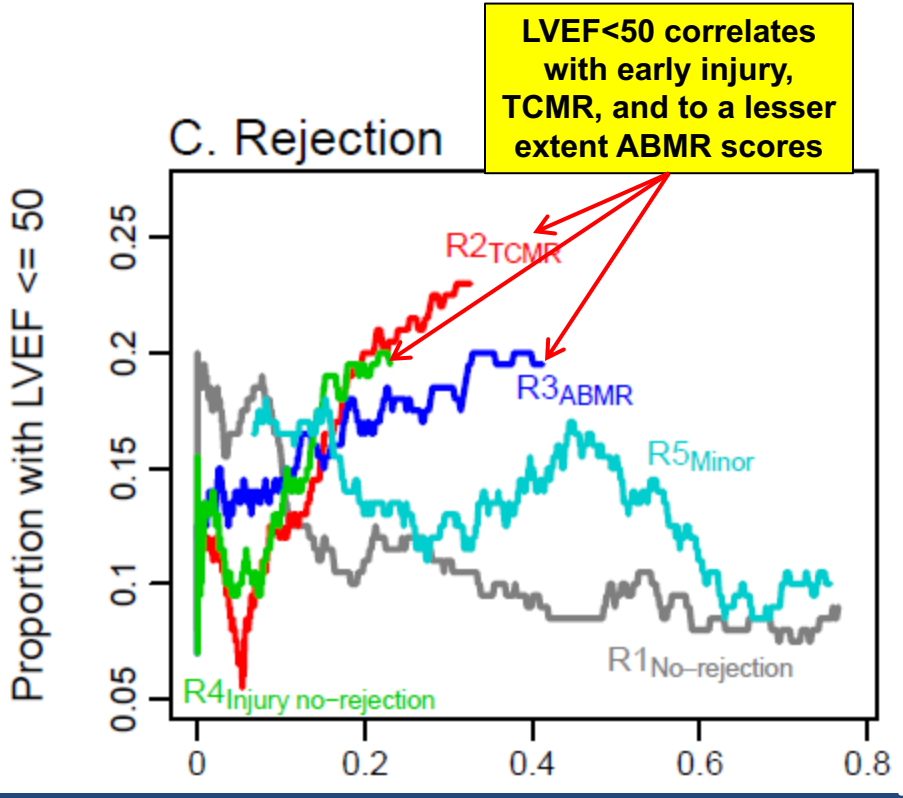
Injury-rejection time course

Time course of rolling average rejection and injury scores

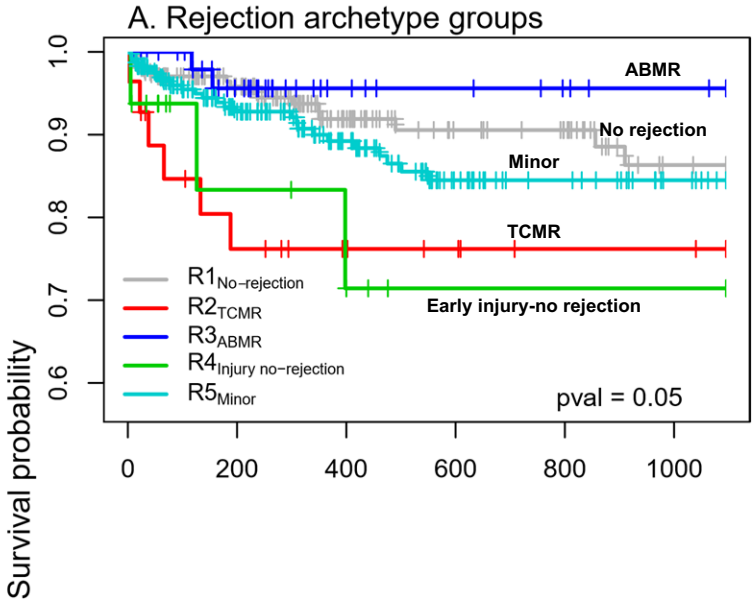


Rejection score time courses are as previously published

Association with LVEF<50 with rejection and injury scores



Injury and rejection together give the best model for survival


A. Rejection archetype groups
Survival probability
1.0
0.9
0.8
0.7
0.6
0
200
400
600
800
1000
ABMR
No rejection
Minor
TCMR
Early injury-no rejection
R1No−rejection
R2TCMR
R3ABMR
R4Injury no−rejection
R5Minor
pval = 0.05
Time post−transplant (days)

Injury-rejection relationships in backward elimination provide best estimates of 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).

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. Rejection acts on function by inducing injury.
- Added to the molecular rejection phenotype, the molecular injury phenotype improves predictions of survival.
- **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?**

Thank you

Molecular profiles of injury in heart transplant allograft: Relationship to rejection, ejection fraction, and survival.

- **Background.** In previous studies we used microarray analysis of gene expression to identify rejection in heart transplant endomyocardial biopsies, and found that this also revealed an acute injury phenotype. The present study aimed for comprehensive study of early and late parenchymal injury compared to the rejection phenotypes, and to define how parenchymal injury relates to function and outcomes.
- **Methods.** We studied injury-related changes in gene expression in 1320 biopsies (645 patients) from 13 centers in the INTERHEART study using microarrays. Biopsies were classified based on expression of previously annotated injury-associated transcript sets, analyzed by unsupervised principal component and archetypal analysis.
- **Results.** Analysis of injury transcript expression identified four injury phenotypes (fig. 1A): I1severe; I2late (atrophy-fibrosis); I3mild; and I4no-injury. Injury scores varied over time, indicating an early injury group (figure 1B), a relatively normal group, a late injury group, and a severe injury group. Comparison with rejection diagnoses showed that severe and late injury were often, but not always, associated with rejection. The late changes included high expression of B cell and plasma cell transcripts, which in other organs indicate atrophy-fibrosis. TCMR was almost always associated with injury, but ABMR was sometimes not. Injury classes (severe and late – fig. 2), like rejection classes reported previously, were associated with depressed LVEF. The severe and late injury groups were associated with reduced 3-year survival. When the relative impact of injury vs. rejection were compared in random forests, the strongest predictors of impaired LVEF and reduced 3-year survival were the injury archetype scores, not the rejection classes.
- **Conclusions.** Molecular changes of parenchymal injury in heart transplant biopsies reveal severe and late changes associated with reduced LVEF and 3-year survival. Injury is often, but not always, associated with (presumably caused by) rejection, particularly TCMR, and is the principal determinant of function and survival. ClinicalTrials.gov # NCT02670408.

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