Molecular diagnosis of rejection in liver transplant biopsies: the INTERLIVER study

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ABSTRACT

Diagnosing rejection in liver transplant biopsies is challenging given the limitations of histology e.g. low kappa values among pathologists. A Molecular Microscope Diagnostic system (MMDx) analogous to that for kidney and heart transplants could offer improved precision and accuracy.

METHODS

We used gene expression microarrays to study 235 liver transplant biopsies (73% for indications) from 10 international centers (INTERLIVER ClinicalTrials.gov NCT03193151). Archetypal analysis (AA) and principal component analysis (PCA) were used to assign molecular classes based on expression of 417 rejection-associated transcripts (RATs) derived in renal transplants (**Fig1A**).

RESULTS

Antibody-mediated rejection (ABMR) transcripts separated from T cell-mediated rejection (TCMR) transcripts in PC2 in kidney and heart biopsy populations (**Fig1B-C**), but did not separate in liver (**Fig1D**), indicating that no ABMR phenotype can be detected in liver using approaches that readily detect ABMR in kidney and heart transplants.

AA assigned four distinct groups in the population: $R1_{normal}$, $R2_{TCMR}$, $R3_{early\ injury}$, and $R4_{late}$ (**Fig1E-F**), differing in time post-transplant e.g. median $R3_{injury}$ 99 vs. $R4_{late}$ 3117 days.

Groups were characterized by unique features. $R1_{normal}$ biopsies were relatively normal; $R2_{TCMR}$ biopsies expressed IFNG-induced TCMR-related transcripts (e.g. CXCL11); injury transcripts increased in $R3_{injury}$ (e.g. hypoxia inducible factor EGLN1); and $R4_{late}$ biopsies showed associations with atrophy-fibrosis (e.g. immunoglobulin transcripts) and injury transcripts. Groups R2-R4 were biochemically abnormal. AA scores associated with TCMR and acute injury decreased over time, while scores associated with 'normalness' and atrophy-fibrosis increased (**Fig2**).

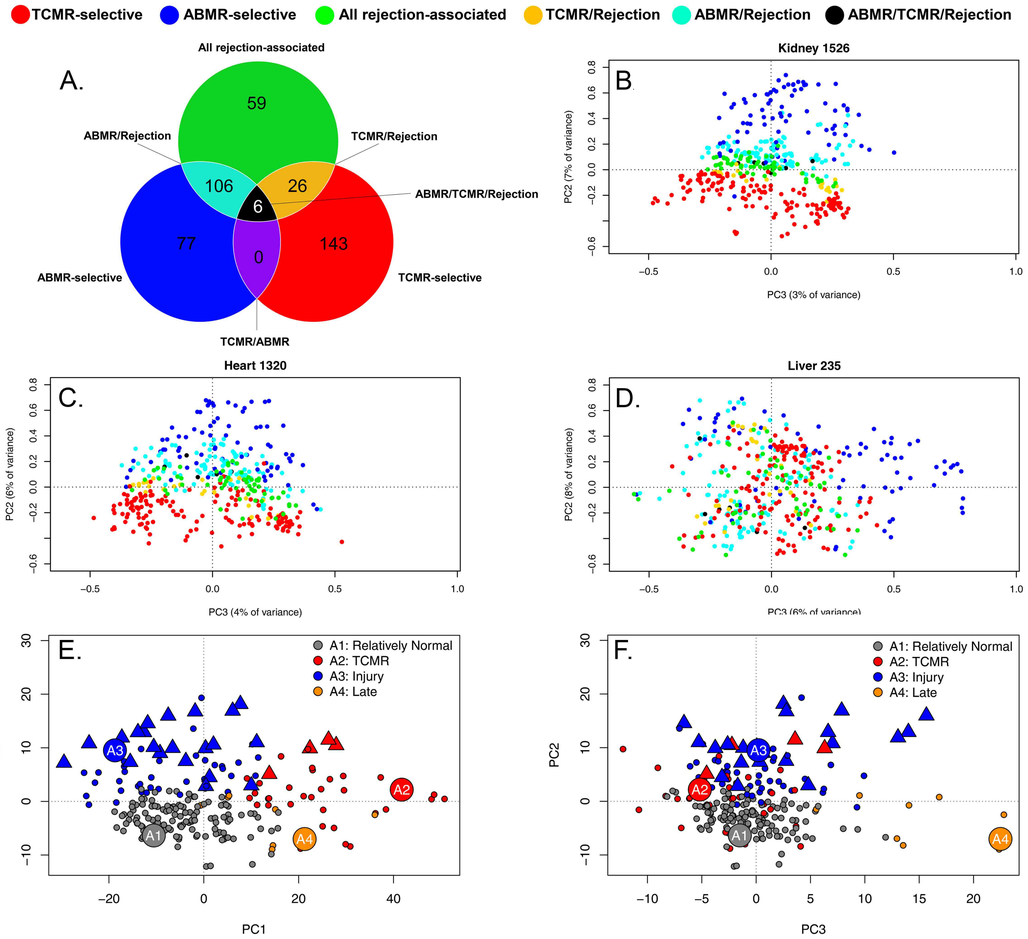

Figure 1. Venn diagram showing (A) the rejection-associated transcripts (RATs) used as input in the rejection analyses, (B-D) the results of the PCA showing a lack of ABMR/TCMR separation in livers compared to heart and lung transplant populations, (E-F) the results of the archetypal analysis showing the four unique groups assigned: $R1_{relatively\ normal}$, $R2_{rejection}$, $R3_{injury}$, and $R4_{late}$, and (G) characteristics of the official MMDx rejection sign-outs (which combine all molecular information) vs. various cut-offs defining histologic rejection.

Table 1. Characterizing the relationship between histologic and molecular diagnoses in the liver biopsy population (N=235)

Crosstab of individual histology features of acute rejection and overall histologic rejection vs. molecular rejection archetype clusters.

		MMDx-Liver sign-outs (% of column)		*Row totals*
		No rejection	TCMR	
Overall Histologic acute rejection >0[a]	**No rejection**	51 (33%)	7 (11%)	*58*
	Rejection	104 (67%)	58 (89%)	*162*
	Column totals	*155*	*65*	***220***
Overall Histologic acute rejection >1[b]	**No rejection**	102 (66%)	23 (35%)	*125*
	Rejection	53 (34%)	42 (65%)	*95*
	Column totals	*155*	*65*	***220***
Overall Histologic acute rejection >2[c]	**No rejection**	124 (80%)	39 (60%)	*163*
	Rejection	31 (20%)	26 (40%)	*57*
	Column totals	*155*	*65*	***220***

Confusion matrix statistics for MMDx Diagnoses predicting the histologic diagnosis in liver transplant acute rejection

Reference Standard	Diagnostic Test	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Accuracy	Balanced Accuracy
Histologic rejection lesion score >0[a]	MMDx Diagnosis[d]	0.36	0.89	0.89	0.33	0.50	0.62
Histologic rejection lesion score >1[b]	MMDx Diagnosis[d]	0.44	0.82	0.65	0.66	0.65	0.63
Histologic rejection lesion score >2[c]	MMDx Diagnosis[d]	0.46	0.76	0.40	0.80	0.68	0.60

Samples with missing information were excluded from this analysis (N=15) , except where those samples were missing a single score and already clearly met the threshold for histologic rejection sum >0 (N=2).
[a] Based on our algorithm interpreting the acute rejection scores/features, where the presence of any score >0 in portal, bile duct, or venous inflammation classified the biopsy as acute rejection.
[b] Based on our algorithm interpreting the acute rejection scores/features, where the presence of any score >1 in portal, bile duct, or venous inflammation classified the biopsy as acute rejection.
[c] Based on our algorithm interpreting the acute rejection scores/features, where the presence of any score >2 in portal, bile duct, or venous inflammation classified the biopsy as acute rejection.
[d] Based on the diagnosis of acute rejection (TCMR) or no rejection by an expert signing out the official MMDx report. Diagnoses were based on position of the biopsy in the report figure, archetypal data, and PBT information provided on the MMDx report page 2.

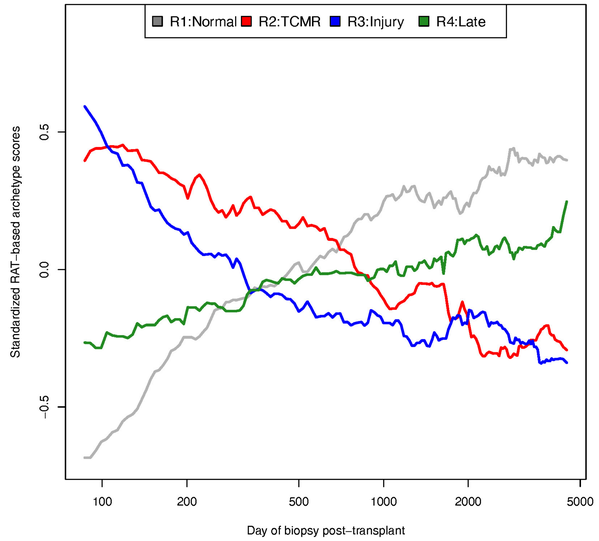

Figure 2. Moving average plots showing the relationships between rejection archetype scores, PC scores, and time post-transplant. A) High R2 and R3 scores were more common in early biopsies, consistent with the predominance of early rejection in liver transplant populations and post-implantation injury. High R1 and R4 scores were common as time increased post-transplant, showing that the liver biopsy population becomes more normal over time.

TCMR-related transcripts increased in biopsies with TCMR histology lesions (p=0.027). Biopsies called TCMR by MMDx had moderate agreement with histology, but with considerable disagreement similar to that in heart transplants (**Table 1**).

The $R2_{TCMR}$ score predicted histologic TCMR with an AUC of 0.70.

CONCLUSIONS

- **By MMDx-Liver, early acute rejection (TCMR) is common in liver transplants, and becomes rare over time compatible with tolerogenic properties of the liver.**
- **MMDx TCMR correlates with histologic TCMR lesions, but no distinct ABMR phenotype was identified.**
- **MMDx-Liver phenotyping shows promise for improving accuracy and precision of rejection diagnoses and for guiding immunosuppressive management.**