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Exosomal profiling in cardiac allograft rejection: Best basic science article in 2018.

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Identifying patients who are at risk for complications after thoracic transplantation assists in better defining outcomes and promoting discovery of new mechanisms that can be targeted to mitigate these complications. The gold-standard for diagnosing rejection, a major cause of morbidity and mortality after transplantation, is biopsy, which (1) is not risk-free, (2) may not be practical in critically ill patients and (3) may result in false-negative findings.¹ Exosomes had been described as far back as in 1981 as membrane fragments from reticulocytes detected in body fluids.² Over the last three decades, they have been appreciated as having key roles in cellular homeostasis (for example, by removing obsolete or harmful cargo such as cytoplasmic DNA), transferring genetic information (such as miRNAs that can facilitate adaptation to environmental changes) and immunomodulation (such as carrying MHC Class I, II and costimulatory molecules that can activate T cells).³ However, exosomes have also gained notoriety by facilitating dysregulated intercellular communication and expressing receptors and enzymes that can facilitate tumor growth, infiltration and metastasis. While these properties have formed the basis of many fundamental discoveries in immunology and cell biology, they have prompted the consideration of exosomes as biomarkers for cancer and cellular injury.^{2,3} This is logical and inherently attractive, because pathogenic cells have been found to extrude and/or release their components into the extracellular space in multiple body fluids, and components of

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these exosomes can provide potential insights into the source, tissue/organ specificity and dysregulated mechanisms of immune evasion and/or injury.³

Based on this foundation, Kennel et al sought to develop serum exosomal profiling as a novel tool to detect and monitor rejection in heart transplantation in comparison with endomyocardial biopsy (EMB), the current gold standard.¹ They isolated exosomes from the first available blood sample on admission (for graft rejection) in heart transplant (HTx) recipients and used liquid-tandem mass spectrometry to compare the exosomal profiles in these patients versus healthy subjects, heart failure (HF) patients who had not received a transplant and HTx recipients without rejection. The median time from transplant to rejection was approximately 6 months. Principal component analysis identified 17 differentially expressed proteins that helped discriminate those with HF or HTx without rejection versus controls; 15 genes discriminated those who developed either acute cellular rejection (ACR) or acute antibody-mediated rejection (AMR) from those who were not diagnosed with any rejection (Table 1). Only 3 of the differentially expressed proteins were common between the two comparisons (Figure 1) - KV302 (representing Ig κ chain V-III region SIE, an immunoglobulin structural component), FIBB (fibrinogen beta chain) and FIBG (fibrinogen gamma chain). A majority of the differentially expressed exosomes were comprised of immune proteins (such as complement and immunoglobulins) and proteins involved in coagulation (fibrinogen, thrombospondin-1) that have also been implicated in other forms of tissue injury and repair. Interestingly, a majority of the differentially expressed proteins were decreased in both ACR and AMR relative to non-rejection HTx samples. Additionally, while the levels in ACR and/or AMR were different compared to non-rejection HTx samples, they were similar to non-transplanted controls in most instances. This observation suggests that the transplant procedure alters the exosomal profile in patients with end-stage HF, and this “new profile” subsequently gets dysregulated in the setting of rejection. The authors interpret some of these changes to suggest that an episode of rejection can deplete certain circulating exosomal proteins due to their utilization and deposition in the graft, which could indicate that these biomarkers provide mechanistic insights into the underlying disease process; however, understanding the profiles of those who do not develop rejection may also provide some insights into mechanisms facilitating tolerance. It is tempting to comment on differences in profiles of recipients developing ACR and AMR; however, the sample size was too small to make an accurate and meaningful discrimination, and subsequent studies are necessary to delineate these groups.

What implications does this study have for the thoracic transplant community? Firstly, we interpret the findings of this initial study to suggest that serum exosomal profiling is a promising, non-invasive biomarker to identify those developing acute rejection post-HTx, but it needs further investigation and validation in independent cohorts. In addition to discriminating these patients, it carries the potential to provide insights into the underlying mechanisms driving acute rejection, which can subsequently provide novel candidates for further investigation to halt the disease process. To that accord, similar studies conducted in lung transplant recipients have suggested that exosomes expressing donor HLA, self-antigens (K α 1 tubulin, collagen V) and immunoregulatory miRNAs can be detected in both serum and bronchoalveolar lavage of those undergoing acute rejection and bronchiolitis-obliterans syndrome.⁴ These investigators are now conducting *in vivo* studies that suggest

mice immunized with exosomes of patients with bronchiolitis-obliterans syndrome develop elevated circulating pro-inflammatory cytokines (such as IL-2 and TNF- α) and a decrease in anti-inflammatory cytokines (such as IL-10), as well as an increase in cells producing IL-17 and IFN- γ , and a decrease in those producing IL-10.⁴ Thus, we hope that these initial single-center studies, despite a limited sample size, will encourage the thoracic transplant community to conduct multi-center analyses by collecting and processing samples appropriately,⁵ validating these biomarkers and utilizing the insights gained from these profiles to better understand mechanisms underlying rejection, with the goal of improving long-term outcomes from both heart and lung transplantation.

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Figure 1: Differentially regulated exosomes.

Figure showing differentially regulated exosome proteins ($q < 0.05$) in patients who had a heart transplant (HTx) but did not develop rejection versus those who had heart failure, or healthy controls (blue circle). Similarly, the group on the right are differentially regulated exosome proteins in HTx recipients who developed acute cellular rejection (ACR) or acute antibody-mediated rejection (AMR) versus those who have not been diagnosed with rejection. The proteins in the middle represent those that were differentially regulated in both comparisons. * denotes those with $q < 0.0001$. Adapted from Kennel et al.¹

TABLE 1:

Exosomal profile comparison

Protein	Control vs. HF vs. HTx				HTx vs. ACR vs. AMR			
	Control (n=10)	HF (n=10)	HTx (n=10)	q	HTx (n=10)	ACR (n=10)	AMR (n=8)	q
IGJ	24.70 ± 6.41	0.10 ± 0.32	0.00 ± 0.00	< 0.0001				
KV302	45.30 ± 12.93	56.20 ± 24.40	0.00 ± 0.00	< 0.0001	0.00 ± 0.00	60.50 ± 24.54	60.50 ± 22.96	< 0.0001
KV303	11.40 ± 4.81	8.70 ± 3.95	2.50 ± 3.98	0,0125				
KV1A1	0.00 ± 0.00	5.40 ± 3.66	0.00 ± 0.00	0,0001				
LV101	3.30 ± 3.09	0.00 ± 0.00	0.00 ± 0.00	0,0001				
LV102	0.00 ± 0.00	0.00 ± 0.00	3.60 ± 3.57	0,0341				
CO5	212.90 ± 39.58	212.30 ± 39.04	299.20 ± 41.31	0,0119				
C1RL	11.30 ± 8.07	8.60 ± 8.51	0.00 ± 0.00	0,0128				
ITIH2	143.60 ± 29.52	142.40 ± 59.22	0.00 ± 0.00	< 0.0001				
PON1	18.20 ± 11.91	21.40 ± 19.01	0.00 ± 0.00	0,0002				
A2AP	4.40 ± 2.80	0.00 ± 0.00	4.00 ± 2.87	0,016				
PLMN	81.30 ± 7.92	76.10 ± 12.24	54.80 ± 12.15	0,0034				
FIBB	3.1 ± 4.012	3.5 ± 6.04	47.6 ± 39.94	0,0067	47.60 ± 39.94	5.00 ± 8.27	1.63 ± 3.85	0,0051
FIBG	3.00 ± 3.23	3.30 ± 5.33	37.60 ± 33.38	0,0115	37.60 ± 33.38	3.10 ± 5.63	0.75 ± 1.49	0,0014
STK36	5.00 ± 2.98	0.90 ± 1.91	0.90 ± 1.37	0,0128				
L1CAM	2.70 ± 1.64	0.30 ± 0.48	0.60 ± 0.84	0,0397				
C1QA					130.60 ± 52.59	64.90 ± 15.65	57.75 ± 20.77	0,015
C1R					120.10 ± 41.62	60.30 ± 18.34	55.50 ± 13.67	0,0079
HV304					0.00 ± 0.00	15.00 ± 8.26	13.88 ± 9.31	< 0.0001
HV315					13.70 ± 4.64	0.00 ± 0.00	0.00 ± 0.00	< 0.0001
ITIH1					76.00 ± 16.26	99.00 ± 23.03	0.00 ± 0.00	< 0.0001
APOL1					25.70 ± 10.30	0.00 ± 0.00	0.00 ± 0.00	< 0.0001
F13A					26.10 ± 23.23	0.80 ± 1.14	0.38 ± 1.06	0,015
FIBA					56.40 ± 36.93	15.70 ± 8.00	10.00 ± 7.31	0,0058
FINC					974.50 ± 226.05	560.90 ± 151.49	477.38 ± 133.86	0,0009
TSP1					148.70 ± 69.99	60.30 ± 23.67	42.00 ± 18.97	0,0005
FRMPD1					0.00 ± 0.00	3.10 ± 2.28	0.00 ± 0.00	0,0014
ACTB					4.20 ± 2.53	0.00 ± 0.00	0.00 ± 0.00	0,0001

q: adjusted p-values obtained using an optimized false-discovery rate (FDR) approach. In this case, the FDR threshold was 5%. HF: heart failure, HTx: heart transplant; ACR: acute cellular rejection, AMR: acute antibody-mediated rejection. Adapted from Kennel et al.¹