VOL. 70, NO. 23, 2017
ISSN 0735-1097/$36.00

Letters

Immunological Serum Protein Profiles for Noninvasive Detection of Acute Cellular Rejection After Heart Transplantation

Acute cellular rejection (ACR), a T-cell-mediated form of organ rejection, remains a common problem after transplantation that compromises long-term allograft survival. Surveillance for ACR has been based on endomyocardial biopsy since the 1970s, which is burdensome due to its invasiveness, risk of complications, significant cost, interobserver variability, and risk of false negative findings. Circulating biomarkers reflecting inflammation or myocardial injury, such as C-reactive protein or troponins, are not recommended for rejection surveillance due to poor specificity (1). Recent developments in affinity-based technologies have made systematic discovery of more informative biomarkers possible by allowing robust, simultaneous quantification of large sets of low-abundance proteins in blood (2). We aimed to comprehensively explore immunological protein profiles in heart transplant recipients with ACR.

We applied a proximity extension assay (2) to measure 92 immune-related proteins, selected to provide broad coverage of immune pathways (Inflammation panel, Olink Proteomics, Uppsala, Sweden), in 2 heart transplant cohorts. Protein abundance is quantified from real-time polymerase chain reaction quantification cycle values, and normalized protein expression units, expressed on the log2 scale. The discovery cohort consisted of recipients of heart transplants between February 2009 and September 2013 at 6 Canadian centers, including 22 biopsy-confirmed ACR cases (91% grade 2R, 9% 1R, median 23 days after transplantation [interquartile range: 13 to 98 days]) and 38 controls without evidence of rejection in routine surveillance biopsies, all graded by 3 blinded expert pathologists (3). In this cohort, 10 of the 92 inflammatory proteins were significantly higher in patients with ACR (Table 1). Lasso regression analysis retained 5 proteins as independently informative, which were strongly correlated with the excluded markers (Pearson pairwise $r = 0.50$ to 0.86), particularly the chemokines ($r = 0.86$ for CXCL10 and CXCL9). These 5 proteins were combined into a multimarker protein score based on normalized protein expression sums weighted by beta-coefficients, which allowed significant separation between ACR cases and controls ($p < 0.0001$) and discrimination of ACR (area under the curve [AUC]: 0.82). A maximally informative score cutoff point was 0.359 (maximum Youden J statistic), with 82% sensitivity and 76% specificity, and 100% sensitivity was obtained with a cutoff of 0.102.

An increase during ACR was confirmed for 4 proteins using linear mixed models (Table 1) in a validation cohort consisting of serial serum samples (before, during, and after ACR) from 10 heart transplant recipients (Skåne University Hospital in Lund, Sweden, 1997 to 2010) with biopsy-proven ACR (78% grade 3A, 11% 3B, and 11% 2, median 356 days after transplantation [interquartile range: 225 to 731]) (4). The multimarker score provided similar diagnostic accuracy as in the discovery cohort (AUC: 0.80). The score cutoffs from the discovery cohort for maximum information content and 100% sensitivity provided a sensitivity of 67% and 100%, respectively, and a specificity of 67% and 27%.

The pathway most enriched among the 5 biomarkers was related to lymphocyte signaling (canonical pathway "Altered T Cell and B Cell Signaling in Rheumatoid Arthritis" from Ingenuity Pathway Analysis Software build 430520M, Qiagen, Hilden, Germany). Four of the proteins (SLAMF1, LTA, IL-12B, and CSF-1) were included in this pathway as compared with 17 of the 92 proteins in the full assay (p for enrichment $= 0.004$). Ex vivo activation of human CD4+ T cells ($n = 6$) with CD3/CD28 beads (Dynabeads Human T-Activator CD3/CD28, Life Technologies, Carlsbad, California) resulted in increased expression of mRNA transcripts for all 5 biomarkers ($p < 0.01$).

In summary, circulating proteins associated with T-cell activation were increased in heart transplant

TABLE 1 Immunological Protein Biomarkers Associated With ACR

	Discovery Cohort				Validation Cohort					
Marker	ACR	NR	FDR	AUC	BR	DR	AR	p Value 1	p Value 2	AUC
CCL19	9.01 ± 1.37	8.59 ± 1.07	0.047	0.59	–	–	–	–	–	–
CD244	6.04 ± 0.39	5.75 ± 0.43	0.024	0.70	–	–	–	–	–	–
CSF1	8.57 ± 0.26	8.43 ± 0.25	0.030	0.64	8.50 ± 0.19	8.50 ± 0.10	8.43 ± 0.20	0.97	0.37	–
CXCL9	8.56 ± 1.20	7.63 ± 1.25	0.008	0.72	–	–	–	–	–	–
CXCL10	10.24 ± 1.24	9.20 ± 1.15	0.004	0.74	9.33 ± 1.19	10.13 ± 1.10	9.38 ± 1.44	<0.001	<0.001	–
CXCL11	8.16 ± 1.34	7.30 ± 1.27	0.017	0.68	–	–	–	–	–	–
IL-6	6.04 ± 1.49	5.42 ± 1.61	0.049	0.63	–	–	–	–	–	–
IL-12B	3.35 ± 1.11	2.57 ± 1.18	0.024	0.71	3.29 ± 0.72	3.76 ± 1.30	3.00 ± 1.05	<0.001	0.01	–
LTA	3.03 ± 0.49	2.67 ± 0.49	0.022	0.69	2.88 ± 0.47	3.07 ± 0.77	2.74 ± 0.69	0.006	<0.001	–
SLAMF1	3.56 ± 0.68	3.06 ± 0.59	0.004	0.72	2.80 ± 0.62	3.30 ± 0.66	2.77 ± 0.50	<0.001	<0.001	–
Multimarker model	0.54 ± 0.24	0.25 ± 0.19	–	0.82	0.26 ± 0.21	0.53 ± 0.26	0.28 ± 0.20	–	–	0.80

Values are mean ± SD. Biomarkers with significantly higher expression in acute cellular rejection (ACR) cases (n = 22) as compared to control patients (NR) (n = 38) in the discovery cohort (false discovery rate [FDR] <0.05 based on p values from Student's *t*-tests comparing ACR and NR patients). In the validation cohort, repeat measurements of protein expression were performed in serum samples from 10 heart transplant patients before rejection (BR) (n = 8), during rejection (DR) (n = 9), and after rejection (AR) (n = 9). Measurements were compared using logistic regression analysis and linear mixed models, with p value 1 referring to AR versus DR, and p value 2 referring to DR versus AR.

AUC = area under the receiver-operating characteristic curve.

recipients with ACR. A multimarker model including 5 such biomarkers provided strong and reproducible discrimination of ACR. The only noninvasive modality currently recommended for ACR surveillance is gene expression profiling of 20 mRNA transcripts in peripheral blood (AlloMap, CareDx, Brisbane, California) (1), used at many U.S. centers at a cost similar to endomyocardial biopsy. We note that our score achieves substantially higher AUC than reported for AlloMap in the CARGO II (Cardiac Allograft Rejection Gene Expression Observational II) trial (0.80 vs. 0.69) and substantially higher specificity at the 100% sensitivity cutoff (27% vs. 2%), but recognize that no direct comparison has been performed (5). The encouraging results from this study motivate a large, prospective study to determine whether plasma biomarkers reflecting T-cell activation can reduce the burden of endomyocardial biopsy in heart transplant recipients.

Ihdina Sukma Dewi, MD, PhD
Olof Gidlöf, PhD
Zsuzsanna Hollander, PhD
Karen K. Lam, PhD
Mark D. Benson, MD, PhD
Oscar O. Braun, MD, PhD
Johan Nilsson, MD, PhD
Scott J. Tebbutt, PhD
Raymond T. Ng, PhD
Jenny Öhman, MD, PhD
Bruce M. McManus, MD, PhD
***J. Gustav Smith, MD, PhD**

*Department of Cardiology, Clinical Sciences
Faculty of Medicine
Lund University
Skåne University Hospital
SE-221 85, Lund
Sweden
E-mail: gustav.smith@med.lu.se

https://doi.org/10.1016/j.jacc.2017.10.012

Please note: The authors thank Siv Svensson and Rebecca Rylance (Department of Cardiology, Clinical Sciences, Lund University, Lund, Sweden), the Prevention of Organ Failure (PROOF) Centre of Excellence team (Vancouver, British Columbia, Canada) including Janet Wilson-McManus, Paul Keown, and Robert McMaster, the Science for Life Laboratory and Olink Proteomics teams (Uppsala, Sweden) for technical assistance, and all heart transplant recipients who contributed to the study through the Biomarkers in Transplantation (BiT) consortium and at Skåne University Hospital in Lund. This work was supported by the European Research Council, the Swedish Heart-Lung Foundation, the Wallenberg Center for Molecular Medicine in Lund, the Swedish Research Council, the Crafoord Foundation, governmental funding of clinical research within the Swedish National Health Service, Skåne University Hospital in Lund, and the Scania county (to Dr. Smith). The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

REFERENCES

1. Costanzo MR, Dipchand A, Starling R, et al. The International Society of Heart and Lung Transplantation Guidelines for the care of heart transplant recipients. J Heart Lung Transplant 2010;29:914–56.

2. Smith JG, Gerszten RE. Emerging affinity-based proteomic technologies for large-scale plasma profiling in cardiovascular disease. Circulation 2017;135:1651–64.

3. Lin D, Hollander Z, Ng RT, et al. Whole blood genomic biomarkers of acute cardiac allograft rejection. J Heart Lung Transplant 2009;28:927–35.

4. Sukma Dewi I, Torngren K, Gidlof O, et al. Altered serum miRNA profiles during acute rejection after heart transplantation: potential for non-invasive allograft surveillance. J Heart Lung Transplant 2013;32:463–6.

5. Crespo-Leiro MG, Stypmann J, Schulz U, et al. Clinical usefulness of gene-expression profile to rule out acute rejection after heart transplantation: CARGO II. Eur Heart J 2016;37:2591–601.