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Letters

TO THE EDITOR

In Situ Immune Profiling Identifies Immune Players Involved in Allograft Rejection

A Call for Precision Medicine

We read with interest about the use of quantitative multiplex immunofluorescence (QmIF) methodology (1), which found significantly increased programmed death-ligand 1 (PD-L1)-positive, forkhead box P3 (FoxP3)-positive, and cluster of differentiation 68 (CD68)-positive cells suppressed in clinically evident rejections, whereas PD-L1-positive, FoxP3-positive, and cluster of differentiation 68-positive cell proportions were significantly higher in "never-rejection" than in "future-rejection." Peyster et al. (1) suggest that in situ immune players regulate the severity of cardiac allograft rejection (CAR). Conventional study of CAR detection involves the identification and quantification of basophilic immune cells on hematoxylin and eosin-stained slides by using endomyocardial biopsy (EMB), which provides few pathogenic insights into both immune cells and individual mechanisms of rejection. In 2005, expert panels required a more detailed characterization of the inflammatory infiltrate in order to have a clinically relevant framework useful for discovering patients with progressive CAR (2). To date, there has been limited application of tissue-level immune phenotyping in transplanted heart tissues. The authors performed useful in situ identification and quantification of CD3, CD8, CD68, FoxP3, and PD-L1, which were selected from a rejection panel of animal models and renal transplantation due to limited research in transplanted heart tissues. Only 33 EMB samples completed the study analysis: 22 had low International Society for Heart and Lung Transplantation (ISHLT) grades of 1R and 0R, and 11 had high ISHLT grades of 2R and 3R (2). First, data must be considered in the setting of the limited sample size. Second, it is well known that discordantly high ISHLT grade designations are uncertain because it is standard practice for all high ISHLT grade biopsy events to receive some form of altered immunosuppression regardless of the presence of altered clinical data. Third, the most interesting findings support the existence of different "immunobiologies" in comparison to concordantly high ISHLT grade cases. This concept is not entirely new because the presence of distinct phenotypes with distinct fates was proposed in 2003 (3). On the other hand, the relatively high rate of a technical failure of QmIF analysis (26% of EMB) is a real concern in common clinical practice, but as the authors recognized, "it may reflect our dependence on residual material following routine clinical processing and the 6- to 12-year interval between EMB sampling and QmIF analysis" (1). Because of intrinsic limitations of the study and future reorganization of costs for the health care system due to the emergent coronavirus 2019 (COVID-19) pandemic, Peyster et al. (1) performed a valuable study of pathogenesis of clinical interest in the surveillance of CAR. Additionally, it would be interesting to analyze their data also in correlation to human leukocyte antigen-DR isotype (HLA-DR) matching at the time of transplantation, because this index may influence outcomes (4). Nevertheless, cost rationing is an inevitable occurrence where the potential demand for effective high-cost techniques will exceed supply. Despite this rather harmful consideration, the future need is to investigate prospectively whether integration of EMB with tissue (1) and liquid biopsy (5) could act synergistically to form a novel precision medicine paradigm (5) leading to the optimized management of patients undergoing heart transplantation.

*Claudio Napoli, MD, PhD
Francesco Donatelli, MD
Ciro Maiello, MD

*Clinical Department of Internal Medicine and Specialistic Units, and University Department of Advanced Medical and Surgical Sciences
University of Campania "Luigi Vanvitelli"
Piazza Miraglia 1
Naples 80138
Italy
E-mail: claudio.napoli@unicampania.it

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REPLY: In Situ Immune Profiling Identifies Immune Players Involved in Allograft Rejection

A Call for Precision Medicine

We appreciate the interest shown by Dr. Napoli and colleagues in our recent publication in *JACC: Basic to Translational Science*. We also appreciate their clear recognition of the need for improved biological characterization of transplanted endomyocardial biopsy tissues, as well as their appreciation of the in situ methodology we used in our work. Their letter raises several important points which we address in this response.

In our publication, approximately one-fourth of cases failed technical quality control. There were several reasons for this, some of which are mentioned in our paper (e.g., sample age), but other more technical factors are likely to improve with further experience and optimization. For example, the automated quantitative multiplex immunofluorescence (QmIF) staining workflow had not previously been applied to samples of heart tissue, and the default temperature of a heating step used for most tissues caused coverslips to become loose in heart tissues and created artifacts. Issues like this are relatively easily addressed once identified, and we expect a higher quality control pass rate moving forward.

The second point by Dr. Napoli and colleagues refers to the difficulty in determining if a high-grade asymptomatic case is truly "discordant" or the development of overt graft dysfunction is avoided by early treatment. It is true that the widespread convention of treating high-grade endomyocardial biopsy tissue with augmented immunosuppression based on histology alone makes it difficult to control for this potential confounder in retrospective investigations. Nevertheless, the clear differences in in situ immune profiles between high-grade endomyocardial biopsies with and without evidence of graft dysfunction and much higher expression of the anti-inflammatory mediators PD-L1 and FoxP3 in the clinically silent cases suggests that there are real biological differences between these groups. Although this cannot be proven conclusively until a prospective investigation is performed, it is compelling circumstantial evidence.

Finally, Dr. Napoli and colleagues discuss the issue of cost containment in the context of advanced diagnostic approaches such as QmIF. Although cost considerations may influence adoption of new technologies, if QmIF substantially improves the accuracy of rejection diagnosis and aids in risk stratification, then initial assay costs may be offset by reduced complications and improved patient outcomes. In low-risk populations, minimizing low-yield procedures saves money, especially if associated with reductions in excess immunosuppression which can predispose patients to iatrogenic injury. In high-risk populations, more aggressive surveillance, prevention, and treatment strategies can reduce hospitalizations and major complications and potentially reduce cost. Whether this potential will be realized will, of course, require further investigation.

Eliot G. Peyster, MD, MSc
Michael D. Feldman, MD, PhD
***Kenneth B. Margulies, MD**

*Perelman School of Medicine
University of Pennsylvania
Translational Research Center, Room 11-101
3400 Civic Center Boulevard, Building 421
Philadelphia, Pennsylvania 19104
E-mail: kenneth.margulies@pennmedicine.upenn.edu

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