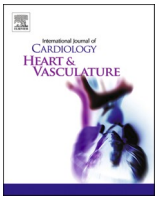
IJC Heart & Vasculature 53 (2024) 101468

Contents lists available at ScienceDirect

IJC Heart & Vasculature

journal homepage: www.sciencedirect.com/journal/ijc-heart-and-vasculature



Recent highlights from the *International Journal of Cardiology Heart and Vasculature*: Spatiotemporal and functional immunoprofiling and its theranostic potential

Cardiovascular diseases like coronary artery disease (CAD) are attributed to an interplay of numerous modifiable and non-modifiable risk factors that culminate in multiple critical pathological processes. These include the coronary microvascular dysfunction, the development of lipid-laden plaques that progressively occlude vessels and potentially rupture to trigger an acute thromboembolism, as well as a chronic low-grade inflammatory state [1,2]. Inflammatory signaling is closely linked with an inappropriately regulated immune response, in which a constellation of immune cells orchestrates systemic and local pro- and anti-inflammatory responses throughout all stages of CAD development and progression. This provides a landscape for candidate therapeutic approaches to blunt aberrant immune cell functions while boosting pathways of resolution. For clinical translation, however, a spatiotemporal and functional map of the immune response during disease initiation, progression and maintenance is critically required.

The recently published study by Cortenbach et al. [3] details a novel imaging approach for *ex vivo* immunoprofiling and scoring during development of human CAD. The authors applied multiplex immunohistochemistry (mIHC) with a comprehensive profiling panel to map the cellular subsets of the innate and adaptive immune systems in coronary artery biopsies. The 4 definitive stages of atherosclerotic vessel disease were represented: (i) eccentric, intimal thickening, (ii) pathological intimal thickening, (iii) fibroatheroma and (iv) fibrous plaque. The net finding was that immune cells in diseased vessels are more numerous, but show no immediately apparent qualitative differences throughout disease progression. Potentially, a deeper subset analysis might unmask subtle changes. What this finding means for targeted immune cell-based therapies in the CAD context remains to be clarified. *En face*, this seems a disappointingly negative result, yet the value of the report goes way beyond the stated finding. The accompanying editorial by Bruno et al. [4] praises the seminal nature of the study by Cortenbach et al. [3], pointing to the increasing attention paid to the holistic immune landscape in various pathological contexts. In the cancer setting, as the editorial authors emphasize, the incorporation of an *in situ* immunoprofiling ("Immunoscore") has revolutionized tumor classification and provides a therapeutic framework to exploit features of the host immune system to eliminate cancers. The authors of the study [3] and of the editorial [4] critically reflect on the small sample size, yet provide a convincing case to take this pilot study as the first step towards translation of immunoscoring into cardiovascular research and practice. A tantalizing idea would be to correlate the plaque immune landscape with the circulating profile, ultimately enabling insight into dynamic plaque characteristics with a liquid biopsy. Bruno et al. [4] conclude with the perspective of applying immune cell maps to guide next generation immunotherapy or even repurpose traditional drugs in a new context. However, this enterprise will certainly require further extensive studies not only with a larger numbers of biopsies and patients, but also with a significantly deeper profiling of immune cell composition. Novel techniques such as single-cell RNA-sequencing, in-situ proteomics or spatial transciptomics could be employed to more precisely identify rare and disease-state specific subsets of immune cells.

Standardized immunoprofiling may also find application beyond degenerative and progressive CVD, particularly in combination with other novel diagnostic tools. A comprehensive review by Almas et al. [5] highlights emergent nanotechnologies as powerful adjuncts to contemporary diagnostic and interventional modalities in cardiology. The authors discuss how nanotechnology improves sensitive biomarker detection and the characterization of atherosclerotic vessel disease by optical coherence tomography (OCT), infrared luminescence (IR) and gadolinium-based visualization. Teaming liquid biopsy immune profiling with these techniques, or with other innovative multimodal diagnostics such as cardiac magnetic resonance imaging or 3D speckle-tracking strain-imaging echocardiography [6,7] might provide a means to classify patients according to their symptom severity and improve therapeutic management. One disease setting that would benefit greatly from a multimodal stratification is the post- or long-COVID syndrome [8]. Since the peak of the COVID-19 pandemic, survivors with all levels of COVID-19 disease severity have been afflicted by a wide-ranging and often diffuse spectrum of post-discharge symptoms. Typical manifestations encompass dyspnea and fatigue, persistent chest pain, palpitations and sensory disturbances [8]. Currently, the cardiovascular symptoms seen in COVID survivors are still too diffuse and inadequately linked with inflammatory and other biomarkers or invasive read-outs to allow for stratification and directed therapies [9,10], but this may change with the implementation of immunoprofiling teamed with non-invasive nano- and imaging technologies. Once established, such a tandem approach might also shed light on the causal and bi-directional interplay between inflammation and the elusive Takotsubo syndrome [11,12]. As the case-study by Rroku et al. [13] exemplifies, inflammation is not easily assigned to an acute myocarditis or to Takotsubo syndrome, and its role as cause, consequence or bystander in the disease remains unclear. Monitoring of immune cell subsets, correlated with functional parameters, might provide valuable insight into the temporal and mechanistic nature of immune cell-driven inflammation in Takotsubo syndrome [12,14] and myocarditis [15], and identify improved treatment paradigms.

Alongside the powerful theranostic potential of comprehensive immunoprofiling and scoring, the distinct functional profiles of the

https://doi.org/10.1016/j.ijcha.2024.101468

Available online 17 July 2024

individual cellular subsets should be considered. While the study by Cortenbach et al. [3] may show that the immune cell composition of the developing atherosclerotic plaque do not overtly change, this is no longer the case in the aftermath of plaque rupture. In this scenario, lipid and cholesterol cargo are directly exposed to the blood flow and trigger thromboembolism [16,17]. In alignment with the kinetics of classical circulating cardiac biomarkers such as troponin and creatine kinase after myocardial infraction (MI) [18], the *cardiac* immune cell landscape will show dramatic dynamics in terms of composition and of function. Haj-Yehia et al. [19] for example provide new insight into the phagocytic role of neutrophils post-MI and identify the phagocytic regulator CD47 as a novel target to improve cardiac healing. The clearance of dead cardiomyocytes and debris is typically seen as the key role of macrophages. These recognize CD47 on the surface of resident cardiac cells such as endothelial cells as a "don't eat me" signal and instead target cells with low CD47 expression, such as dead and dying cardiomyocytes. Accordingly, a CD47-targeting antibody enhances macrophage-mediated phagocytosis and improves MI injury repair. In their study, Haj-Yehia et al. [19] show that neutrophils, the earliest immune cell population to arrive within the reperfused myocardium, also contribute critically to early post-MI phagocytosis and thereby reduce the extent of injury. Thus targeting CD47 during specific time-windows after MI, when different immune cells subsets dynamically infiltrate the myocardium, could increase debris clearance and accelerate healing, without disturbing other critical reconstitutional components of the inflammatory phase after MI.

In line with this, Moratal et al. [20] more specifically examined the capacity for phenotypic macrophage transition after MI in the context of type 2 diabetes. Although the clear distinction of the different macrophage-phenotypes is challeging, pro-inflammatory M1-like macrophages are critical in the early stages post-MI by virtue of their ability to phagocytose and digest damaged cells, tissue and debris. Subsequent transition to M2-like anti-inflammatory macrophages is considered causal for inflammation-resolution and tissue repair. The authors hypothesized that diabetes may compromise timely macrophage polarization and functional switching, and thereby worsen outcome. To test this, circulating monocytes were isolated from peripheral blood taken from male patients with and without diabetes at 1, 3 and 5 days after MI and directed toward pro- or anti-inflammatory macrophage phenotypes by standard *in vitro* protocols. Neither the phenotype of freshly isolated monocytes, nor the kinetics of their polarization in culture, differed between the two groups of patients. Whether this also holds true for macrophages – or neutrophils – *in situ* in the post-MI myocardium requires systematic study to eventually identify potential therapeutic windows.

Majmundar et al. [21] focussed particularly on lymphocytes in patients with heart failure, and established an association between low absolute lymphocyte count (ALC) and increased mortality. Although limited by its retrospective design, the cohort study by Majmundar et al. [21] yet considered convincingly large cohorts of 1,029 patients for the matched analyses and 766 patients in the propensity-score matched analyses. In the matched analyses, comparing ALS $\leq$ 1,500 cells/μl and ALS $>$ 1,500 cells/μl, the lower ALS count was associated with a significantly higher risk of death. While this study does not provide a mechanistic basis for the association, this finding does suggest that immunoprofiling might also be of value to stratify risk in patients with established heart failure. In a broader perspective, continuous monitoring the circulating immune cell composition may also provide feedback regarding the success of pharmacological and non-pharmacological interventions, such as exercise training. The meta-analysis by Malandish & Gulati [22] provides insight into the dynamics of the classical inflammatory markers in patients with heart failure and various degrees of adiposity who underwent exercise training. Aerobic, resistance and concurrent training paradigms were considered and read-outs were the cytokines tumor necrosis factor-alpha (TNF-α), IL-1β, IL-8, and high sensitivity C-reactive protein (hs-CRP). Robust drops in the levels of circulating TNF-α, IL-6 and hs-CRP were noted across a range of ages, heart failure types, exercise intensities and follow-up durations. Whether profiling immune cell and/or subset provides additional insight into disease severity, progression or regression in response to treatment warrants further study.

Considering the current studies, it becomes clear that we are only at the very beginning of a comprehensive understanding of the immune labyrinth in cardiovascular disease. Since abnormal immune system and inflammatory signaling appear a common theme in almost all cardiovascular conditions including arrhythmias [23,24], each incremental step towards in-depth spatio-temporal characterization of immune cell subpopulations will raise critical questions opening novel therapeutic avenues. The coming era of computer-assisted deep immunoprofiling will likely provide novel insights into hitherto underrecognized aspects of immunocardiology.

Sources of funding

Grants from the Deutsche Forschungsgemeinschaft (FE 1365/4-1 to A.F.; Research Training Group 2989, project 517043330 to D.D.), National Institutes of Health (R01HL136389, R01HL163277, R01HL131517, R01HL160992, R01HL165704, and R01HL164838 to D. D.), and the European Union (large-scale network project MAESTRIA No. 965286 to D.D.).

Disclosures

None.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] A.R. Bhandiwad, I. Valenta, S. Jain, T.H. Schindler, Pet-determined prevalence of coronary microvascular dysfunction and different types in a cardio-metabolic risk population, Int. J. Cardiol. Heart Vasc. 46 (2023) 101206.
[2] J.C. Sæther, E.K. Vesterbekkmo, B. Gigante, G.F. Giskeødegård, T.F. Bathen, T. Follestad, R. Wiseth, E. Madssen, A. Bye, The association between circulating lipoprotein subfractions and lipid content in coronary atheromatous plaques assessed by near-infrared spectroscopy, Int. J. Cardiol. Heart Vasc. 46 (2023) 101215.
[3] K.R.G. Cortenbach, D. Morales Cano, J. Meek, M.A.J. Gorris, A.H.J. Staal, M. Srinivas, M.d.V.I. Jolanda, J. Fog Bentzon, R.R.J. van Kimmenade, Topography of immune cell infiltration in different stages of coronary atherosclerosis revealed by multiplex immunohistochemistry, Int. J. Cardiol. Heart Vasc. 44 (2023) 101111.
[4] A. Bruno, M. Teresa Palano, M. Cucchiara, G. Ambrosio, Immunoprofiling and immunoscoring in cor onary artery disease: From vascular research to the bedside? Int. J. Cardiol. Heart Vasc. 44 (2023) 101140.
[5] Almas T, Haider R, Malik J, Mehmood A, Alvi A, Naz H, Satti DI, Zaidi SMJ, AlSubai AK, AlNajdi S, Alsufyani R, Ramtohul RK, Almesri A, Alsufyani M, A HA-B, Alghamdi HAS, Sattar Y, Alraies MC, Raina S, Nanotechnology in interventional cardiology: A state-of-the-art review, Int. J. Cardiol. Heart Vasc. 43 (2022) 101149.
[6] F. Bruns, D. Dobrev, Assessment of atrial function by echocardiography: Can this be used to assess the arrhythmogenic atrial substrate? Int. J. Cardiol. 362 (2022) 190–191.
[7] P.R. Goody, S. Zimmer, C. Öztürk, A. Zimmer, J. Kreuz, M.U. Becher, A. Isaak, J. Luetkens, A. Sugiura, F. Jansen, G. Nickenig, C. Hammerstingl, V. Tiyerili, 3d-speckle-tracking echocardiography correlates with cardiovascular magnetic resonance imaging diagnosis of acute myocarditis - an observational study, Int. J. Cardiol. Heart Vasc. 41 (2022) 101081.
[8] S.A. Elseidy, A.K. Awad, M. Vorla, A. Fatima, M.A. Elbadawy, D. Mandal, T. Mohamad, Cardiovascular complications in the post-acute covid-19 syndrome (pacs), Int. J. Cardiol. Heart Vasc. 40 (2022) 101012.
[9] O. Ogungbe, B. Kumbe, O.A. Fadodun, T. Latha, D. Meyer, A.F. Asala, P. M. Davidson, C.R. Dennison Himmelfarb, W.S. Post, Y. Commodore-Mensah, Subclinical myocardial injury, coagulopathy, and inflammation in covid-19: A meta-analysis of 41,013 hospitalized patients, Int. J. Cardiol. Heart Vasc. 40 (2022) 100950.
[10] J.A. Keefe, V. Avadhanula, E.G. Nicholson, S. Devaraj, P.A. Piedra, B. Bozkurt, X.H. T. Wehrens, Abnormalities in cardiac and inflammatory biomarkers in ambulatory subjects after covid-19 infection, Int. J. Cardiol. Heart Vasc. 43 (2022) 101144.

[11] S. Khalid Ahmed, M. Gamal Mohamed, R. Abdulrahman Essa, E. Abdelaziz Ahmed Rashad Dabou, S. Omar Abdulqadir, R. Muhammad Omar, Global reports of takotsubo (stress) cardiomyopathy following covid-19 vaccination: A systematic review and meta-analysis, Int. J. Cardiol. Heart Vasc. 43 (2022) 101108.
[12] A. Tedeschi, E. Ammirati, N. Contri, D. Dobrev, Recent highlights on acute myocardial infarction and takotsubo syndrome from the international journal of cardiology: Heart & vasculature, Int. J. Cardiol. Heart Vasc. 43 (2022) 101155.
[13] A. Rroku, S. Grahl, U. Landmesser, B. Heidecker, A case report of myocardial inflammation in takotsubo syndrome. A chicken-or-the-egg phenomenon, Int. J. Cardiol. Heart Vasc. 39 (2022) 100958.
[14] A.V. Bairashevskaia, S.Y. Belogubova, M.R. Kondratiuk, D.S. Rudnova, S. S. Sologova, O.I. Tereshkina, E.I. Avakyan, Update of takotsubo cardiomyopathy: Present experience and outlook for the future, Int. J. Cardiol. Heart Vasc. 39 (2022) 100990.
[15] S. Talukder, A. Ghose, T. Chakraborty, A. Olsson-Brown, S. Ramalingam, S. D. Rosen, K. Young, A.R. Lyon, A.K. Ghosh, Evolving cardiac biomarkers for immune checkpoint inhibitor related myocarditis in cancer patients, Int. J. Cardiol. Heart Vasc. 49 (2023) 101278.
[16] A. Kato, D. Kinoshita, T. Nagata, K. Asakura, M. Katamine, A. Katsura, T. Hashimoto, Y. Minami, J. Ako, Lipoprotein (a) levels and vulnerable characteristics in nonculprit plaque in patients with acute coronary syndrome, Int. J. Cardiol. Heart Vasc. 43 (2022) 101120.
[17] Y. Katayama, A. Taruya, M. Kashiwagi, Y. Ozaki, Y. Shiono, T. Tanimoto, T. Yoshikawa, T. Kondo, A. Tanaka, No-reflow phenomenon and in vivo cholesterol crystals combined with lipid core in acute myocardial infarction, Int. J. Cardiol. Heart Vasc. 38 (2022) 100953.
[18] J. Henrik Kristensen, C. Amalie Wistisen Koczulab, E. Anton Frandsen, R. Bo Hasselbalch, N. Strandkjær, N. Jørgensen, M. Østergaard, P. Hasse Møller-Sørensen, J. Christian Nilsson, S. Afzal, P. Rørbæk Kamstrup, M. Dahl, M.V. Bor, R. Frikke-Schmidt, N. Rye Jørgensen, L. Rode, L. Holmvang, J. Kjærgaard, L. Evi Bang, J. Forman, K. Dalhoff, H. Bundgaard, K. Karmark Iversen, Kinetics of cardiac troponin and other biomarkers in patients with st elevation myocardial infarction, Int. J. Cardiol. Heart Vasc. 48 (2023) 101250.
[19] E. Haj-Yehia, S. Korste, R. Jochem, A. Lusha, A. Roth, N. Dietzel, J. Niroomand, P. Stock, A.M. Westendorf, J. Buer, U.B. Hendgen-Cotta, T. Rassaf, M. Totzeck, Cd47 blockade enhances phagocytosis of cardiac cell debris by neutrophils, Int. J. Cardiol. Heart Vasc. 48 (2023) 101269.
[20] C. Moratal, J. Murdaca, C. Cruzel, A. Zamiti-Smondel, N. Heme, F. Asarisi, J. G. Neels, E. Ferrari, G. Chinetti, An exploratory human study investigating the influence of type 2 diabetes on macrophage phenotype after myocardial infarction, Int. J. Cardiol. Heart Vasc. 49 (2023) 101309.
[21] M. Majmundar, T. Kansara, H. Park, G. Ibarra, J. Marta Lenik, P. Shah, A. Kumar, R. Doshi, H. Zala, S. Chaudhari, A. Kalra, Absolute lymphocyte count as a predictor of mortality and readmission in heart failure hospitalization, Int. J. Cardiol. Heart Vasc. 39 (2022) 100981.
[22] A. Malandish, M. Gulati, The impacts of exercise interventions on inflammaging markers in overweight/obesity patients with heart failure: A systematic review and meta-analysis of randomized controlled trials, Int. J. Cardiol. Heart Vasc. 47 (2023) 101234.
[23] D. Dobrev, J. Heijman, R. Hiram, N. Li, S. Nattel, Inflammatory signalling in atrial cardiomyocytes: A novel unifying principle in atrial fibrillation pathophysiology, Nat. Rev. Cardiol. 20 (3) (2023) 145–167.
[24] L. Scott Jr., A.C. Fender, A. Saljic, L. Li, X. Chen, X. Wang, D. Linz, J. Lang, M. Hohl, D. Twomey, T.T. Pham, R. Diaz-Lankenau, M.G. Chelu, M. Kamler, M.L. Entman, G. E. Taffet, P. Sanders, D. Dobrev, N. Li, Nlrp3 inflammasome is a key driver of obesity-induced atrial arrhythmias, Cardiovasc. Res. 117 (7) (2021) 1746–1759.

Florian Bruns
Institute of Pharmacology, West German Heart and Vascular Center, University Duisburg-Essen, Germany

Dobromir Dobrev
Institute of Pharmacology, West German Heart and Vascular Center, University Duisburg-Essen, Germany
Department of Medicine and Research Center, Montreal Heart Institute and Université de Montréal, Montréal, Canada
Department of Integrative Physiology, Baylor College of Medicine, Houston, TX, USA

Anke C. Fender*
Institute of Pharmacology, West German Heart and Vascular Center, University Duisburg-Essen, Germany

* Corresponding author at: Institute of Pharmacology, Medical Faculty, University Duisburg-Essen, Hufelandstr. 55, 45122 Essen, Germany.
E-mail address: anke.fender@uk-essen.de (A.C. Fender).