International Journal of Cardiology 177 (2014) 76–78

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Letter to the Editor

Analysis of endomyocardial biopsies in suspected myocarditis—Diagnostic value of left versus right ventricular biopsy

F. Escher [a,b,*], D. Lassner [b], U. Kühl [a,b], U. Gross [b], D. Westermann [c], W. Poller [a], C. Skurk [a], K. Weitmann [d], W. Hoffmann [d], C. Tschöpe [a,e,1], H.-P. Schultheiss [a,1]

[a] Department of Cardiology and Pneumology Charité, University Medicine Berlin, Campus Benjamin Franklin, Berlin, Germany
[b] Institut für kardiale Diagnostik und Therapie, IKDT, Berlin Germany
[c] University Heart Center Hamburg, Hamburg, Germany
[d] Institute for Community Medicine, University Medicine Greifswald, Greifswald, Germany
[e] Berlin-Brandenburg Center for Regenerative Therapies, Berlin, Germany

ARTICLE INFO

Article history:
Received 29 August 2014
Accepted 17 September 2014
Available online 28 September 2014

Keywords:
Diagnostical methods
Endomyocardial biopsy
Inflammation
Matricellular remodeling

Endomyocardial biopsies (EMBs) are the gold standard for the identification of causative factors in structural heart disease [1–3]. Besides detection of inflammation and viral persistence, histopathological findings in EMB samples, such as variation in myocyte size and apparent interstitial fibrosis are morphologic features which can add important diagnostic and prognostic information influencing therapeutic decisions in patients with cardiomyopathies [4–6]. However, the practice and number of EMB procedures differ substantially, even among experienced cardiovascular centers. In regard to detecting inflammation, virus persistence, and morphological changes, no clear recommendations of a diagnostic value for left ventricular (LV-) versus right ventricular (RV-) EMB have yet been established, although the safety of both procedure has been validated [7,8].

The aim of our study was to determine the diagnostic value of LV- versus RV-EMB specimens in patients with suspected myocarditis.

In this prospective study, 65 patients (47 males, 18 females) with clinically suspected myocarditis were included. Mean age was 42.3 ± 15.7 years and mean LV ejection fraction (LVEF) was 49.8 ± 18.3%. Coronary artery disease and other possible causes of myocardial dysfunction had been excluded by angiography prior to EMB in all patients. Patients indicating symptoms or signs of acute myocarditis (e.g. documented electrocardiographic or laboratory signs of acute myocardial injury) were excluded.

Up to 7 EMBs taken simultaneously from both the RV and LV ventricle were obtained using a flexible biotome (Fa. Westmed, Germany) via the femoral vein approach (RV), and a stiff biotome (Fa. Medwork, Germany) using the femoral artery approach (LV).

Quantitative immunohistochemical analysis of EMB specimens [9] showed no significant difference in cardiac immune cell infiltration and cell adhesion molecules (CAMs) between LV- and RV-EMBs: 11.4 ± 9.3/mm^2 versus 12.0 ± 9.9/mm^2 LFA-1$^+$ lymphocytes, $P = 0.23$; 5.0 ± 4.4/mm^2 versus 5.0 ± 4.4/mm^2 CD3$^+$ T-lymphocytes, $P = 0.61$; 13.3 ± 12.4/mm^2 versus 15.1 ± 14.0/mm^2 CD45R0$^+$ T memory cells, $P = 0.43$; 2.0 ± 1.1/Area Fraction (AF) versus 2.0 ± 1.1/AF ICAM-1, $P = 0.089$; 6.4 ± 2.3/AF versus 6.5 ± 2.6/AF HLA-1, $P = 0.19$; 0.06 ± 0.05/AF versus 0.06 ± 0.05/AF VCAM-1, $P = 0.83$, LV- versus RV-EMBs (Fig. 1).

Due to low detection rates of Human Herpes Virus 6 (HHV6) genomes (4.6%) in EMBs, only data of erythrovirus by Polymearse-chain reaction (PCR)-analyses are reported. The erythrovirus prevalence of this data set was 89.2%. Presence of erythrovirus genome within the myocardium was solely diagnosed in LV-EMBs in 3.5% (n = 2), solely in RV-EMBs in 5.2% (n = 3) and concomitantly in LV- and RV-EMBs in the remaining 91.4% of the patients. Based on the simulation data set generated from our data, a saturation model was fitted. The limit of the saturation model was 0.8947 (=89.47%). This value corresponds to the "true prevalence" (89.23%) of erythroviruses in the raw data. No virus genome was found in 9.2% (n = 6) of the patients studied. All or some of these patients might be free of virus, or virus genome might be present only in certain locations within the heart where no EMB was obtained (i.e., sampling error) [10]. Therefore, the regression curve does not reach the best probability (1.0). When 4 EMBs were studied, in our sample the "true" diagnosis was found in 100% of the

Abbreviations: AF, area fraction; CAMs, cell adhesion molecules; EF, ejection fraction; EMB, endomyocardial biopsy; HLA-1, human leukocyte antigen-1; ICAM-1, intercellular cell adhesion molecule-1; LFA-1, leukocyte function antigen-1; LV, left ventricular; PCR, polymerase chain reaction; RV, right ventricular; α-SMA, α-smooth muscle actin; VCAM-1, vascular cell adhesion molecule-1.

* Corresponding author at: Department of Cardiology and Pneumology, Campus *Benjamin Franklin* Charité-Universitätsmedizin Berlin, Hindenburgdamm 30, 12200 Berlin, Germany. Tel.: +49 30 8445 2349.
E-mail address: felicitas.escher@charite.de (F. Escher).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.ijcard.2014.09.071
0167-5273/

patients. To assess whether the erythroviral load differed between LV- and RV-EMBs, a quantification of erythroviral genomes was performed. Mean viral load in both LV (237.5 copies/μg DNA, range 10.0–644.4) and RV (374.7/mg DNA, range 7.2–776.6) cardiac tissue did not differ significantly but it did show a great variability among different samples taken from the same ventricle ($P = 0.6$). Higher erythrovirus loads were not associated with increased intramyocardial inflammation ($P = 0.3$). Moreover, quantification of erythrovirus copy numbers did not reliably improve the probability of a positive diagnostic result.

Taken together, the erythrovirus prevalence of this data set was any difference in the detection of cardiac erythrovirus genomes in LV- and RV-EMBs (LV 84.6%, RV 86.2% $P = 0.65$). In this sample a number of 4 EMBs was mandatory to compensate for the procedural sampling error of EMB in order to reliably identify an erythrovirus-positive patient.

Determination of interstitial fibrosis severity based on the pathologic score was enhanced in LV- compared to RV-EMBs. The results of histopathological findings are demonstrated in Fig. 2. Pronounced or severe fibrosis was determined in LV-EMBs of 27.6% (n = 18) of the patients. In contrast, RV-EMB samples of only 4.6% (n = 3) of the patients were characterized by enhanced fibrotic tissue (Fig. 2A). As illustrated in Fig. 2B, immunohistological quantification of cardiac collagen type I protein expression associated with increasing myocardial stiffness was significantly augmented in LV- compared to RV-EMBs (0.8/AF versus 0.4/AF for LV- and RV-EMBs, respectively, $P = 0.03$). Moreover, mRNA abundance of collagen I subtype was increased in LV- EMB in contrast to RV-EMB ($P = 0.03$) as shown in Fig. 2C. Furthermore and in accordance with these results, transdifferentation from fibroblasts to myofibroblasts as suggested by α-SMA mRNA expression (Fig. 2D) was significantly enhanced in LV- versus RV-EMBs ($P = 0.04$).

Besides accumulation of fibrotic tissue, cardiac myocyte hypertrophy is a determinant of clinical outcome in cardiomyopathy. Cardiac myocyte diameter was significantly increased in LV- in contrast to RV-EMBs (mean 23.0 μm versus 18.0 μm, $P < 0.0001$) indicating higher mechanical stress. In fact, omitting LV-EMBs in these patients would have resulted in missing diagnostic information of hypertrophy in 26.1% (n = 17) of the cases whereas RV-EMBs did not have any additional value in characterizing myocyte hypertrophy (Fig. 2E).

Taken together, LV tissue has a significant diagnostic advantage when interstitial fibrosis, cardiac remodeling, and hypertrophy are to be investigated. Collagen I subtype, representing a stiff fibrillar protein providing tensile strength, as well as α-SMA expression, was associated with the course of disease in LV-EMBs. Thus, for the detection of clinically relevant changes in matricellular remodeling in the myocardium, it is the analysis of LV-EMBs which is advantageous.

In conclusion, we prospectively assessed the diagnostic yield of left versus right ventricular (LV versus RV) biopsies with respect to inflammation, detection of virus genomes, and morphological changes in the myocardium. Both LV- and RV-EMBs can be used to quantify cardiac inflammatory infiltration, and to detect viral genomes, provided that sufficient numbers of cardiac tissue samples have been taken. Morphological changes were found to be more reliably determined in LV-EMBs.

This work was supported by the German Research Foundation (DFG)— Transregional Collaborative Research Centre “Inflammatory Cardiomyopathy — Molecular Pathogenesis and Therapy” (SFB/TRR19, Z1).

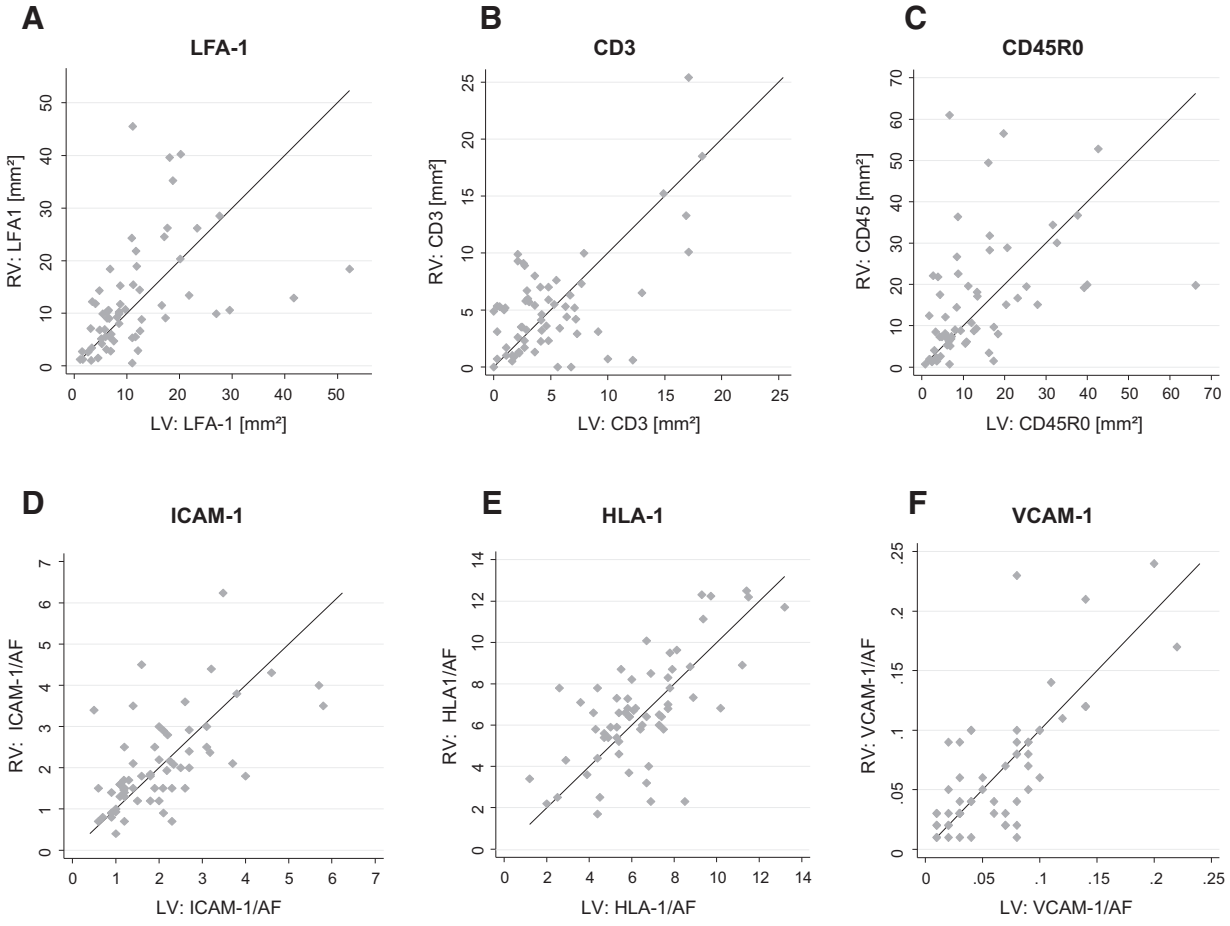

Fig. 1. Cardiac immune cell accumulation and cell adhesion molecules expression in cardiomyopathy patients. Protein expression of $CD45R0^+$-cells, $CD3^+$-cells and $LFA\text{-}1^+$-cells. Cell adhesion molecules HLA-1, ICAM-1 and VCAM-1 were quantified in LV- and RV-EMBs by digital image analysis. Data are expressed per area of heart tissue (cells per millimeter squared, CAMs per area fraction percentage).

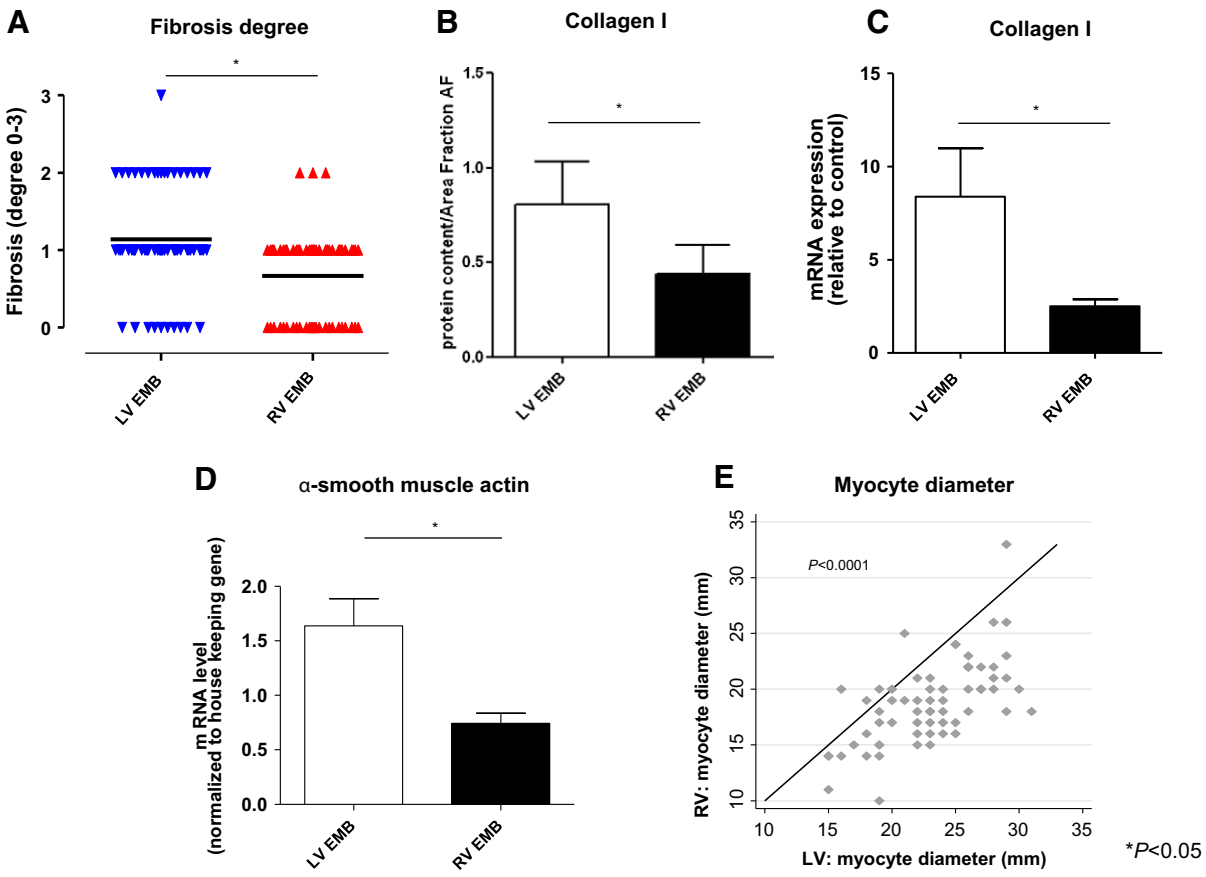

Fig. 2. Characterization of fibrotic tissue within the myocardium of LV and RV. A. Pathological score of interstitial fibrosis severity assessed by azan staining in LV- and RV-EMBs. A semi quantitative scale with severity scores of 0 to 3 was used to quantify overall interstitial fibrosis and scarring (score: 0, no fibrosis; 1, modest enhanced fibrosis; 2, pronounced increased fibrosis; 3, severe increased fibrosis), $P < 0.0001$. B. Quantification of collagen I per area of heart tissue (area fraction, percentage) in LV- and RV-EMB by digital image analyses. Data are expressed as mean ± SD. $^*P < 0.05$. C. Relative mRNA expression of collagen I in heart tissue in LV- and RV-EMBs. Data expressed as mean ± SD. $^*P < 0.05$. D. Relative mRNA expression of α-smooth muscle actin in heart tissue in LV- and RV-EMBs. Data expressed as mean ± SD. $^*P < 0.05$. E. Quantification of myocyte diameter (in μm) in LV- and RV-EMBs. Data are expressed as mean ± SD.

Conflict of interest

The authors have no relationship with any industries, no financial associations and no conflicts of interest.

References

[1] Sagar S, Liu PP, Cooper Jr LT. Myocarditis. Lancet 2012;379:738–47.
[2] Schultheiss HP, Kühl U, Cooper LT. The management of myocarditis. Eur Heart J 2011;32:2616–25.
[3] Mahrholdt H, Wagner A, Deluigi CC, Kispert E, Hager S, Meinhardt G, et al. Presentation, patterns of myocardial damage, and clinical course of viral myocarditis. Circulation 2006;114:1581–90.
[4] Cooper LT, Baughman KL, Feldman AM, Frustaci A, Jessup M, Kuhl U, et al. American Heart Association; American College of Cardiology; European Society of Cardiology. The role of endomyocardial biopsy in the management of cardiovascular disease: a scientific statement from the American Heart Association, the American College of Cardiology, and the European Society of Cardiology. Circulation 2007;116:2216–33.
[5] Gulati A, Jabbour A, Ismail TF, Guha K, Khwaja J, Raza S, et al. Association of fibrosis with mortality and sudden cardiac death in patients with nonischemic dilated cardiomyopathy. JAMA 2013;309:896–908 [21].
[6] Yilmaz A, Kindermann I, Kindermann M, Mahfoud F, Ukena C, Athanasiadis A, et al. Comparative evaluation of left and right ventricular endomyocardial biopsy: differences in complication rate and diagnostic performance. Circulation 2010;122:900–9.
[7] Chimenti C, Frustaci A. Contribution and risks of left ventricular endomyocardial biopsy in patients with cardiomyopathies: a retrospective study over a 28-year period. Circulation 2013;128(14):1531–41.
[8] Caforio AL, Pankuweit S, Arbustini E, Basso C, Gimeno-Blanes J, Felix SB, et al. Current state of knowledge on aetiology, diagnosis, management, and therapy of myocarditis: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. Eur Heart J Sep. 2013;34:2636–48 [2648a-2648d].
[9] Escher F, Westermann D, Gaub R, Pronk J, Bock T, Al-Saadi N, et al. Development of diastolic heart failure in a 6-year follow-up study in patients after acute myocarditis. Heart 2011;97:709–14.
[10] Kühl U, Pauschinger M, Seeberg B, Lassner D, Noutsias M, Poller W, et al. Viral persistence in the myocardium is associated with progressive cardiac dysfunction. Circulation 2005;112:1965–70.