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Biventricular endomyocardial biopsy in patients with suspected myocarditis: Feasibility, complication rate and additional diagnostic value

Thomas Stiermaier [a,b,1], Felix Föhrenbach [c,1], Karin Klingel [d,1], Reinhard Kandolf [d,1], Enno Boudriot [c,1], Marcus Sandri [c,1], Axel Linke [c,1], Karl-Philipp Rommel [c,1], Steffen Desch [a,b,1], Gerhard Schuler [c,1], Holger Thiele [a,b,1,2], Philipp Lurz [c,*,1,2]

[a] University Heart Center Luebeck, Medical Clinic II (Cardiology/Angiology/Intensive Care Medicine), Luebeck, Germany
[b] German Center for Cardiovascular Research (DZHK), partner site Hamburg/Kiel/Luebeck, Luebeck, Germany
[c] University of Leipzig–Heart Center, Department of Internal Medicine–Cardiology, Leipzig, Germany
[d] University Hospital Tuebingen, Department of Molecular Pathology, Tuebingen, Germany

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ABSTRACT

Background: Previous retrospective analyses have suggested that biventricular (BV) endomyocardial biopsy (EMB) is superior compared to selective left ventricular (LV) or right ventricular (RV) EMB. This study prospectively assessed the feasibility, safety and diagnostic performance of implementing a routine BV-EMB approach in patients with suspected myocarditis.

Methods: Consecutive patients with clinically suspected myocarditis underwent EMB ($n = 136$). Myocarditis was defined as ≥14 infiltrating leukocytes/mm^2 in addition to enhanced human leukocyte antigen class II expression in professional antigen-presenting immune cells. The presence of viral genomes was assessed by nested (reverse transcriptase–) polymerase chain reaction.

Results: BV-EMB was attempted in 132 patients (LV thrombus, $n = 3$; complication during RV-EMB, $n = 1$) and resulted in sufficient samples from both ventricles in 127 patients (96.2%). One major complication (pericardial tamponade requiring surgical revision) was observed during the 136 RV-EMB (0.7%). No severe complications occurred during the 132 LV procedures. Of the 127 patients with BV-EMB, myocarditis was diagnosed in 89 patients (70.1%). While 67 patients (75.3%) fulfilled the diagnostic criteria in both ventricles, the diagnosis of myocarditis was based on the results of LV-EMB only in 16 patients (18%) and of RV-EMB only in 6 patients (6.7%). Viral genomes were found in 45 of the 127 patients (35.4%) with evidence of virus genome only in the left ventricle in 10 patients (22.2%) and only in the right ventricle in 3 patients (6.7%).

Conclusions: Implementing a routine BV-EMB approach is feasible and safe. In patients with suspected myocarditis, BV-EMB yields superior diagnostic performance compared to selective RV- or LV-EMB.

1. Introduction

Endomyocardial biopsy (EMB) is still considered the reference standard for the diagnosis of myocarditis despite advances in imaging techniques such as cardiovascular magnetic resonance (CMR) [1–5]. A joint scientific statement from the American Heart Association, the American College of Cardiology and the European Society of Cardiology identified certain clinical scenarios in which the diagnostic benefit of EMB outweighs the risk of complications [6]. Furthermore, clinical trials have demonstrated the therapeutic and prognostic value of histopathological findings in patients with myocarditis [7,8]. However, the preferable ventricular site of EMB is still a matter of debate and varies between cardiovascular centers. Recently, 2 retrospective studies analyzed patients undergoing EMB for suspected myocarditis or non-ischemic cardiomyopathy and suggested that a biventricular (BV) approach results in a significantly higher number of diagnostic samples compared to selective left ventricular (LV) or right ventricular (RV) EMB, whereby LV-EMB was diagnostically more contributive [9,10]. Consistently, morphological changes were found to be more reliably determined in LV-EMB in a small prospective study in patients with suspected myocarditis [11]. However, taking samples from both ventricles might increase the risk

* Corresponding author at: University of Leipzig–Heart Center, Department of Internal Medicine/Cardiology, Struempellstrasse 39, 04289 Leipzig, Germany.
E-mail address: philipp.lurz@gmx.de (P. Lurz).
[1] This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.
[2] The last two authors should both be considered as senior authors.

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of complications, which is already a widespread concern. Most previous investigations have reported a low major complication rate < 1% for both LV- and RV-EMB with some authors suggesting a slightly superior safety profile of LV-EMB [9,10,12,13].

The retrospective data regarding the safety and efficacy of routine BV-EMB have not been validated in prospective trials, yet. Therefore, the aim of the present study was to prospectively evaluate the feasibility, safety and additional diagnostic value of implementing a routine BV-EMB approach in consecutive patients with suspect myocarditis at an institution that primarily performed selective LV-EMB beforehand.

2. Methods

2.1. Study population

This prospective study included 136 consecutive patients presenting to the University of Leipzig–Heart Center with signs and symptoms suggestive of myocarditis between August 2012 and May 2015. Myocarditis was clinically suspected in patients who fulfilled all of the following criteria: (a) symptoms suggestive of myocarditis such as dyspnea, chest pain, palpitations, exercise intolerance and/or abnormal fatigue; (b) evidence of recent or ongoing myocardial damage (regional or global wall motion abnormalities leading to left ventricular dysfunction, ECG-abnormalities or elevated troponin); (c) history of systemic viral disease; and (d) exclusion of relevant coronary artery disease on selective angiography with a culprit lesion explaining clinical presentation.

Patients of either sex between 18 and 80 years of age were included in the trial if they provided informed consent for LV- and RV-EMB. Exclusion criteria were pregnancy, participation in another study and evidence of acute coronary syndrome (culprit lesion) on coronary angiography.

The present analysis was a predefined sub-study of the Comprehensive Cardiac Magnetic Resonance Imaging in Patients with Suspected Myocarditis (MyoRacer) trial (ClinicalTrials.gov number NCT02177630) [5]. The study protocol was approved by the local ethics committee and complied with the principles of the Helsinki Declaration. All patients provided written informed consent for study participation.

2.2. Clinical evaluation and endomyocardial biopsy

All patients underwent physical examination, laboratory testing including cardiac enzymes, 12-lead ECG and 2-dimensional echocardiography at admission. Invasive procedures were performed after obtaining written informed consent. A right or left femoral access was used to place guiding sheaths in the femoral vein and artery. Coronary angiography was performed in every patient before the EMB procedure. Significant obstructive coronary artery disease was defined as ≥ 50% diameter stenosis. Consequently, EMB sampling was performed using a myocardial biopsy forceps (Teleflex Medical Tuttlingen GmbH, Tuttlingen, Germany). For LV-EMB, an 8F JR4 guiding catheter was passed retrogradely through the aortic valve and positioned into the LV cavity. The biopsy forceps was then advanced through the JR4 guiding catheter and 5–7 specimens were taken from different locations. For RV-EMB, an 8F Mullins sheath was positioned in the right ventricle using a pigtail catheter on an anterior–posterior view. After confirmation of the correct position of the sheath pointing towards the RV septum on left-anterior–oblique projection by contrast injection, the biopsy forceps was advanced out of the sheath into the RV septum and 5–7 specimens were taken. After closure of the forceps, the bioptome was removed through the sheath. One experienced operator performed > 90% of EMB procedures. New or progressive pericardial effusion was excluded after EMB on echocardiography. Major and minor complications, including death, pericardial tamponade, atrioventricular block, arrhythmias, myocardial infarction, stroke or transient ischemic attack, were recorded during and after EMB.

2.3. Histopathological analysis

All histological, immunohistological and molecular pathological analyses were performed at the Department of Molecular Pathology, University Hospital of Tuebingen (Tuebingen, Germany) as described previously [10,14,15]. Diagnoses based on EMB results were established according to the World Health Organization/International Society and Federation of Cardiology Task Force Report [16]. Myocardial inflammation was defined as the detection of ≥ 14 infiltrating immune cells/mm^2 (CD3 + T-lymphocytes and/or CD68 + macrophages) in addition to enhanced human leukocyte antigen class II expression in professional antigen-presenting immune cells.

Acute myocarditis revealing myocyte injury/necrosis was differentiated from chronic myocarditis, which was defined by the following criteria: absence of myocyte necrosis, but detection of interstitial fibrosis, the presence of ≥ 14 infiltrating immune cells/mm^2 (CD3 + T-lymphocytes and/or CD68 + macrophages) and degeneration of neighboring myocytes—being morphologically consistent with the formerly called "borderline" myocarditis.

For the detection of viral genomes, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) were extracted using proteinase-K digestion followed by extraction with phenol/chloroform. Nested (reverse transcriptase–)polymerase chain reaction (PCR) was performed to detect enteroviruses (including coxsackieviruses of groups A and B and echoviruses), parvovirus B19 (PVB19), adenoviruses, human cytomegalovirus, Epstein–Barr virus (EBV) and human herpesvirus type 6 (HHV6) in the cardiac tissue samples. Oligonucleotide sequences were chosen from the glyceraldehyde-3-phosphate-dehydrogenase gene as a control for successful extraction of DNA and RNA. Negative and positive controls were included in each PCR reaction. Automatic DNA sequencing was used to confirm specificity of all viral amplification products [15,17].

Evidence of viral genomes in the absence of inflammation was considered as latent virus persistence. Healed myocarditis required multifocal fibrosis/scarring without inflammation. Masson trichrome staining allowed the visualization of fibrosis. For the diagnosis of hypertrophic cardiomyopathy [18,19] and amyloidosis [20], additionally electron microscopy and Congo red staining, respectively, were performed. The diagnosis of dilated cardiomyopathy was primarily made in association with additional angiographic and CMR data, especially when histopathological data were ambiguous for dilated cardiomyopathy.

2.4. Statistical analysis

Categorical variables are presented as number and percentage of patients and compared with the chi-square test or the Fisher exact test. Continuous data are expressed as mean ± standard deviation for normally distributed variables or median and interquartile range for non-normally distributed variables. Comparisons between groups were performed with the Student *t*-test in case of normal distribution or the Mann–Whitney *U* test for non-normally distributed values. Correlations were analyzed with the Pearson method. Baseline characteristics were compared between patients exhibiting myocarditis in EMB and patients with other diagnoses.

All statistical analyses were performed with SPSS (version 17.0; SPSS Inc.; Chicago, IL). A two-sided probability value ≤ 0.05 was considered statistically significant.

3. Results

3.1. Baseline patient characteristics

A total of 136 consecutive patients with suspected myocarditis underwent EMB. The cardiovascular risk profile, leading clinical symptoms and electrocardiographic findings, did not differ significantly between patients with the confirmed histopathological diagnosis of myocarditis and patients with other final diagnoses (Table 1). However,

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