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Original Article

Detailed pathologic evaluation on endomyocardial biopsy provides long-term prognostic information in patients with acute myocarditis

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ABSTRACT

Background: The long-term prognosis of biopsy-proven myocarditis is not well known. We hypothesized that a detailed pathological examination of an endomyocardial biopsy (EMB) would reveal prognostic information in patients with acute myocarditis.
Methods: Fifty-four patients were diagnosed with acute myocarditis based on an EMB. Pathological diagnosis was categorized into lymphocytic dominant (29.6%), eosinophilic dominant (22.2%), and borderline myocarditis (48.1%). Masson's trichrome staining and further immunohistochemical staining for CD3, CD20, CD68, HLA-DR, TLR4, TLR8, enteroviral VP1, and caspase-3 expression were performed. The clinical outcomes were defined as all-cause and cardiovascular (CV) death.
Results: During the median 10.4 years of follow up (9.7±5.7 years), the overall all-cause mortality was 20.4% and the CV mortality was 14.8% in patients with acute myocarditis. Lymphocytic dominant myocarditis patients showed a poor clinical outcome when compared with eosinophilic dominant myocarditis patients for both all-cause (37.5% vs. 0%, $p=0.015$) and CV (31.2% vs. 0%, $p=0.029$) mortality. Among borderline myocarditis patients, the presence of fibrosis was linked with poor clinical outcomes in both all-cause (75.0% vs. 21.4%, $p=0.045$) and CV (100.0% vs. 25.0%, $p=0.034$) mortality. No significant associations between clinical outcome and all other immunohistochemical staining targets were observed.
Conclusions: Detailed pathological evaluation on an EMB provides prognostic information in patients with acute myocarditis. EMB evaluation should be considered in patients with suspected myocarditis.

1. Introduction

Myocarditis is an inflammatory disease of the myocardium with a wide range of clinical and histological manifestations [1–3]. The Dallas criteria for defining myocarditis require that an inflammatory cellular infiltrate with or without associated myocyte necrosis should be present on conventionally stained endomyocardial biopsy (EMB) specimens [4]. Even though histological evaluation is the gold standard for the diagnosis of myocarditis, not all patients undergo EMB due to the lack of specificity, risk of complications, and/or a limited ability to perform the procedure. However, EMB should be considered when the incremental diagnostic and prognostic information gained from the biopsy outweigh the risks and cost associated with the procedure. An AHA/ACCF/ESC scientific statement recommended that EMB should be done in patients with new onset heart failure who also had (1) a normal sized or dilated left ventricle, less than 2 weeks of symptoms, and hemodynamic compromise; or (2) a dilated ventricle, 2 weeks to 3 months of symptoms, new ventricular arrhythmias, second- or third-degree heart block, or failure to respond to usual care within 1 to 2 weeks [5].

Recent studies demonstrated not only the diagnostic role but also the prognostic value of EMB in patients with suspected myocarditis [6–8]. Therefore, we hypothesized that the pathological evaluation of an EMB would provide long-term prognostic information in patients with biopsy-proven acute myocarditis.

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2. Methods

2.1. Study population

We retrospectively identified 138 clinically suspected acute myocarditis patients who underwent EMB between January 1990 and December 2010. Patients with symptoms lasting more than 3 months and those with no evidence of inflammatory cell infiltration on EMB were excluded. The final analysis included 54 biopsy-proven acute myocarditis patients. Clinical characteristics, laboratory and echocardiographic findings, treatment method, and clinical outcomes were evaluated. Primary outcomes were defined as all-cause and cardiovascular (CV) death. The institutional review board of Yonsei University College of Medicine approved the study protocol.

2.2. Histopathological analysis

For all samples, histopathological examination was done by two pathologists (H.S.S and J.S.L) on a 4-μm-thick tissue section from a paraffin-embedded EMB stained with hematoxylin and eosin. Histological analysis followed the Dallas criteria, which are considered as the gold standard for the evaluation of suspected myocarditis. According to this classification, acute myocarditis is defined by inflammatory cell infiltrates in association with myocyte necrosis. We categorized the biopsy-proven myocarditis into three subgroups based on types of major dominant inflammatory cell infiltration; lymphocytic dominant, eosinophilic dominant, and borderline myocarditis. Borderline myocarditis was characterized by the presence of inflammatory infiltrates without definite microscopic signs of myocyte injury.

2.3. Immunohistochemical and Masson's trichrome staining

Formalin-fixed and paraffin-embedded tissues were sectioned at a thickness of 4 μm and stained using the Ventana automated immunostainer, Discovery XT (Ventana Medical Systems, Tucson, AZ, USA). The slides were dried at 60°C for 1 hour and deparaffinized using EZ Prep (Ventana Medical Systems) at 75°C for 8 minutes. Cell conditioning was performed using CC1 solution (Ventana Medical Systems) at 100°C for 48 minutes. Signals were detected using the DAB Map Detection Kit (Ventana Medical Systems). Counterstaining was performed with hematoxylin (Ventana Medical Systems) for 4 minutes at room temperature. The following monoclonal antibodies were applied for identification, localization, and characterization of mononuclear cell infiltrates: CD3 for T cells (Clone F7.2.38; 1:50; Dako, Glostrup, Denmark), PGM1 (CD68) for macrophages and natural killer cells (Clone PG-M1; 1:100; Dako), CD20 for B cells (Clone L26; 1:400; Dako), and HLA-DR (TAL.1B5; 1:20; Dako) to assess HLA class II expression in professional antigen-presenting immune cells. A mouse monoclonal antibody against human TLR4 (Clone HTA125; 1:10; Santa Cruz Biotechnology, Santa Cruz, CA), human TLR8 (Clone 44C143; 1:10; Imgenex, San Diego, CA), enteroviral capsid protein VP1 (Clone 5-D8/1; 1:10; Dako) and a rabbit monoclonal antibody against human activated cleaved caspase-3 (Clone 5A1E; 1:10; Cell Signaling Technology, Beverly, Massachusetts) were used as primary antibodies as well. To quantify the inflammatory cell infiltration, we examined sections using a high-power objective and counted the number of positive cell numbers found in the microscope field within three hot spots, each centered around intact myocytes, and the average cell number was analyzed. Masson's trichrome staining was also used to identify collagenous fibrosis. Minimal fibrosis without myocyte damage was considered as negative, while the presence of interstitial or replacing fibrosis was considered as positive. To classify HLA-DR, TLR4, TLR8, enteroviral VP1, and caspase-3 expression, positivity was considered as the cytoplasmic expression of these targets in over 50% of the myocytes evaluated.

2.4. Statistical analysis

Continuous variables are presented as a mean±standard deviation (SD). Categorical variables are expressed as a percentage of the group total. To compare variables amongst histopathological classifications, we used ANOVA for continuous variables and the chi-square test for categorical variables. The cumulative incidence of mortality was estimated using the Kaplan-Meier method and log-rank test was used to compare groups. All analyses were conducted using SPSS Statistics (version 18.0.0, IBM Corp., Armonk, NY, USA). A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Histopathological classification of biopsy proven myocarditis patients

The baseline clinical characteristics and laboratory findings of the study population according to histopathological classification of biopsy-proven myocarditis are shown in Table 1. Representative pathological examinations are presented in Fig. 1.

Patients with eosinophilic dominant myocarditis showed statistically significant increases in total white blood cell count both at

Table 1
Clinical characteristics, laboratory, and immunohistochemical findings of biopsy-proven myocarditis patients

	Lymphocytic (n=16, 29.6%)	Eosinophilic (n=12, 22.2%)	Borderline (n=26, 48.1%)	*p* value
Age (years)	34±14	31±8	37±14	0.338
Male	10 (62.5%)	7 (58.3%)	18 (69.2%)	0.786
Initial WBC (cells/μL)	9758±5989	13333±3277	8302±2941	0.004
Initial neutrophil (%)	73±13	73±9	62±16	0.030
Initial eosinophil (%)	3±4	7±4	4±11	0.488
Initial lymphocyte (%)	16±10	16±6	27±11	0.001
Time from admission to biopsy (days)	4±4	5±4	7±13	0.477
WBC at biopsy (cells/μL)	7225±3327	11525±3477	7898±2836	0.005
Neutrophil (%) at biopsy	74±12	67±15	60±16	0.065
Eosinophil (%) at biopsy	3±3	10±9	6±11	0.138
Lymphocyte (%) at biopsy	14±6	17±9	26±11	0.005
ESR	38±41	22±24	30±34	0.694
LVEF (%)	36±14	48±18	37±17	0.115
Steroid use	6 (42.9%)	10 (83.3%)	2 (8.0%)	<0.001
Follow up duration (yrs)	6±6	10±4	12±5	0.009
All-cause mortality	6 (37.5%)	0 (0%)	5 (19.2%)	0.019
CV mortality	5 (31.2%)	0 (0%)	3 (11.5%)	0.033

Values are mean±SD or number (%). WBC, white blood cell; ESR, erythrocyte sedimentation rate; LVEF, left ventricular ejection fraction; CV, cardiovascular.