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Quantitative diagnosis of lymphocytic myocarditis in forensic medicine

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ABSTRACT

The aim of this study was to establish quantitative diagnostic criteria for lymphocytic myocarditis on autopsy samples by using a stereological cell profile counting method. We quantified and compared the presence of lymphocytes and macrophages in myocardial autopsy specimens from 112 deceased individuals who had been diagnosed with myocarditis according to the Dallas criteria and 86 control subjects with morphologically normal hearts.

We found the mean number to be 52.7 lymphocyte profiles/mm^2 (range 3.7–946; standard deviation 131) in the myocarditis group and 9.7 (range 2.1–25.9; standard deviation 4.6) in the control group. The cut-off value for the diagnosis of myocarditis was determined by calculating sensitivity plus specificity, which reached the highest combination at 13 lymphocyte profiles/mm^2 (sensitivity 68%; specificity 83%). A considerable proportion of subjects in both the myocarditis and control groups had lymphocyte profile counts below 30/mm^2, representing a diagnostic challenge due to the increased risk of creating false negative or false positive results. We found it practically impossible to obtain a reliable macrophage count.

The present data add new important information on lymphocyte counts in inflamed and non-inflamed myocardium. We suggest a cut-off value in the range of 11–16 lymphocyte profiles/mm^2 for a reliable diagnosis of lymphocytic myocarditis from autopsy samples. To evaluate small inflammatory changes at low lymphocyte counts, a multidisciplinary approach should be implemented, in which diagnostic tools are used ancillary to histological examination. We advise against semi-quantification of macrophages based on cell profile counting.

1. Introduction

Myocarditis is an inflammatory disease of the cardiac muscle caused by myocardial infiltration of immunocompetent cells. Myocarditis constitutes one of the most important unsolved challenges in the practice of cardiovascular medicine and is a major cause of sudden unexpected death in infants and young adults [1–3]. The incidence of myocarditis at autopsy varies considerably in the published literature, ranging from 0.5 to 20% [4–6], mainly due to difficulties in establishing the diagnosis. Despite improvements in non-invasive diagnostic tests, histological examination remains the gold standard for the diagnosis of myocarditis, as this enables characterization and quantification of the inflammatory changes [7–10]. In 1987, The Dallas Classification System was developed to establish uniform criteria for the diagnosis of myocarditis on endomyocardial biopsies (EMB) [11]. However, this classification had important limitations regarding interobserver variability and the risk of sampling error [12,13]. Due to these limitations, alternative pathological diagnostic criteria including semi-quantitative histopathological evaluations have

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been proposed [14–16]. Latest, inflammation in the myocardium was defined by Maisch et al. as the presence of >14 lymphocytes and macrophages/mm^2 in immunohistochemical stained sections [17]. However, a description of which cell counting method to employ, including selection of areas for counting and identification and separation of positive cell profiles, was not included in their recommendations. To the best of our knowledge, quantification of lymphocytes and macrophages in healthy and inflamed myocardium has not been assessed by using a stereological counting method with systematic sampling of fields of view. This method will reduce the subjective component that is common to conventional histopathological evaluations, increasing the reproducibility of the data [18].

The primary aim of this study was to quantitatively assess and compare the presence of lymphocytes and macrophages in non-inflamed and inflamed myocardium using a 2-dimensional stereological cell profile counting method, to establish quantitative diagnostic criteria for lymphocytic myocarditis on autopsy samples.

2. Materials and methods

2.1. Study subjects

All post-mortem cases diagnosed with myocarditis were identified in an electronic database at the Institute of Forensic Medicine, Aarhus University, Denmark. A total number of 150 cases were identified from 1992 to 2010. As the diagnoses were made by different investigators, H&E-stained heart sections from all cases were re-evaluated for the presence of inflammation and myocyte necrosis. Only cases with active or borderline myocarditis according to the Dallas criteria were included in this study, n = 112 (78 men and 34 women; median age 35.8 years, age range 3 weeks to 77 years). According to the Dallas criteria, a diagnosis of myocarditis is based on the presence of inflammatory infiltrates with/without myocyte necrosis. For this study, an infiltrate was defined as a cluster of ≥10 inflammatory cells profiles. Myocyte necrosis was defined by fraying cell borders, pyknotic nuclei and cytoplasmatic vacuolization.

A control group of 84 suicide cases was selected (51 men and 33 women; median age 36.9 years, age range 10–71 years) from 1992 to 2010. Cases with proven clinical or pathological heart diseases (myocarditis, atherosclerosis AHA IV-VIII, valvular diseases and cardiomyopathies) or severe traumatic cardiac injury following suicide were not included.

2.2. Sampling of material

For each deceased individual four myocardial tissue samples obtained at autopsy were included: one from the anterior wall of the left ventricle; one from the posterior wall of the left ventricle, one from the interventricular septum and one from the right ventricle, according to the department-specific protocol for routine histologic examination of the heart. The specimens were initially fixed in 4% buffered formalin for 48 h and subsequently embedded in paraffin.

2.3. Immunohistochemical staining and stereological quantification

Histopathological examinations were performed on 3 μm tissue sections cut from all available myocardial tissue samples. As the study was retrospective covering a period of 19 years, some tissue samples were missing for unknown reasons, and as a consequence less than four tissue specimens were available for examination in a few cases (1–4; mean: 3.5). For the diagnosis of lymphocytic myocarditis, sections were stained for T-lymphocytes with CD3 (Novocastra PS1) and for macrophages with CD 68 (DAKO PG-M1) using monoclonal mouse anti-human antibodies. Staining was performed according to manufacturer's protocol. All sections were blinded for group (project vs. control) and for clinical information before examination.

The sections were analyzed using systematic sampling of fields of view and a stereological cell profile counting method. A microscope (Nikon ECLIPSE 80i) modified for stereology with a motorized stage and digital camera connected to a PC with newCAST 3.4.3.0 software (Visiopharm, Hørsholm, Denmark) was used. All sections were analyzed by the same investigator.

A 2-dimensional quantification of positive cell profiles was performed using a 2-dimensional unbiased counting-frame. Approximately 30 counting frames were placed systematically, evenly distributed and non-overlapping, over each section by the software, see Fig. 1 for illustration. Each counting frame covered an area of 383 × 10^3 μm^2. Counting was performed using a 20× lens. For a detailed description of the counting rules employed, see Figs. 2 and 3.

The total number of positive cell profiles/mm^2 was calculated by the following formula:

$$Q_A(\mathrm{CD3/Myo}) = \frac{\Sigma Q(\mathrm{CD3})}{(a/p)\,\Sigma P(\mathrm{Myo})}$$

where Q_A(CD3/Myo) was the total number of positive cell profiles/mm^2 myocardium; ΣQ(CD3) was the sum of CD3 positive cell profiles counted per section (e.g., sum of cell profiles in all counting frames); (a/p) was the area of the counting frame divided by the number of test points (=4); and ΣP(Myo) was the sum of the counting frame corners hitting the myocardium.

The study was approved by the Danish National Ethical Committee protocol no. 1209317.

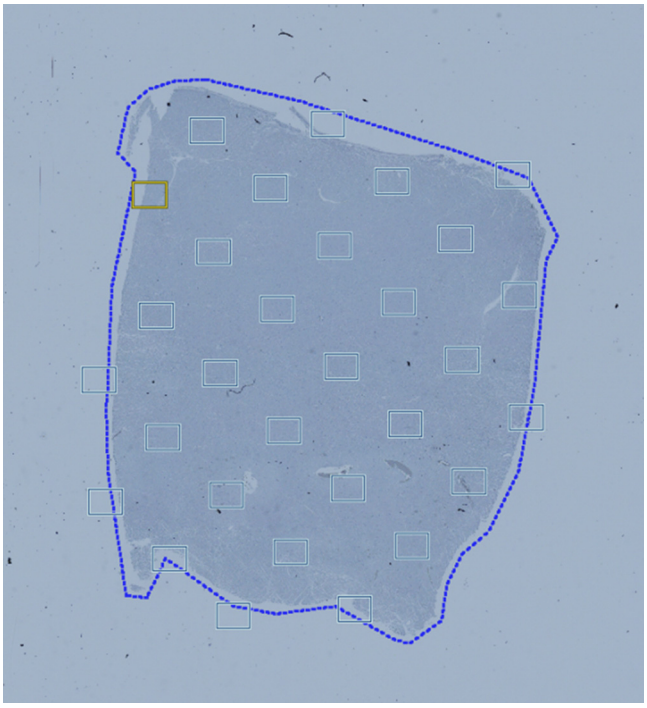

Fig. 1. Light microscopic image of a heart section stained for T-lymphocytes (CD3). Applied on the photo are 30 counting frames. The figure illustrates the systematic and non-overlapping placement of the counting frames made by the software. The blue line indicates the area in which the counting frames are placed within. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)