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Quantification of leucocytes, T-lymphocytes and macrophages in autoptical endomyocardial tissue from 56 normal human hearts during the first year of life

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ABSTRACT

This study evaluated the normal number of inflammatory cells in the heart in the first year of life using two methods to compare their ability to quantitate physiological myocardial infiltration. Eight endomyocardial samples from both ventricles were obtained at autopsy from 56 structurally normal hearts during the first year of life. In each sample the numbers of leucocytes, T-lymphocytes and macrophages were counted once in 20 randomly chosen high-power fields (400×) as well as in a 10 mm^2 area of randomly chosen myocardial tissue (100×) by two independent investigators. Compared to the literature a greater representative proportion of myocardial tissue was analyzed. The results of the enumeration in mm^2 were converted into high-power-fields to compare both methods. The mean numbers and standard deviations for leucocytes, T-lymphocytes and macrophages were calculated. Both counting methods showed similar results with low inflammatory cell counts per single heart and staining. A greater understanding of the physiological myocardial infiltration by leucocytes, T-lymphocytes and macrophages is important for postmortem forensic cases, and for the interpretation of endomyocardial biopsies in infants.

1. Introduction

The use of endomyocardial biopsies is absolutely necessary to determine an accurate diagnosis of myocarditis in clinical cardiology. The investigation of cardiac tissue is based on histopathological criteria such as myocytolysis, lymphomonocytic infiltrates and necrosis [1,2], as well staining by immunohistochemical methods. Using these procedures, the diagnostic sensitivity and specificity have been increased and the inter-observer variability between a correct positive and false negative diagnosis of myocarditis have been reduced [3–13].

According to Foley and Edwards [14], quantitative evidence of interstitial lymphocyte infiltrate associated with myocyte injury, is currently the best available hallmark for myocarditis in biopsy specimens. Determining the normal physiological number of inflammatory cells in the heart may help to minimize false positive interpretations of myocarditis in biopsy specimens of endomyocardial tissue as well as in tissues obtained at autopsy. Studies on the normal amount of inflammatory cells in adult myocardium, reported up to 5 lymphocytes per high power field (HPF) were regarded as normal [1,2,14,15]. Currently, >14 leucocytes per mm^2 tissue are the lower limit for the diagnosis of myocarditis [9]. However, these limits were developed for endomyocardial biopsies of living adults.

Nevertheless, knowledge regarding the physiological infiltration of inflammatory cells in infantile myocardium is poor. Currently, the cut-off limits for the diagnosis of myocarditis or suspicion of myocarditis in the first year of life are ≤15 leucocytes resp. ≤10–15 lymphocytes per HPF are regarded as normal [16–18]. However, despite the recommended cut-off levels for the diagnosis of myocarditis, investigations on the normal distribution of inflammatory cells in this age group have not yet been performed.

The random selection of cut-off limits without determining the physiological number of inflammatory cells in normal infantile myocardium has been criticized [19,20]. This arbitrarily chosen cut-off value has led to a high rate of false positive myocarditis cases among sudden infant death syndrome (SIDS) samples [6,18]. These results are in contrast to results from other recent investigations [19–25].

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Therefore, we have quantified, for the first time, the number of interstitial leucocytes, T-lymphocytes and macrophages in autoptical endomyocardial tissues from structurally normal human hearts during the first year of life.

2. Material and methods

Structurally normal myocardium samples from 56 infants under the age of 1 year were evaluated. The study group contains forty-four SIDS cases (category IB San-Diego definition) [26] with no signs of infection prior to death, structurally normal hearts and without histological evidence of inflammation, oedema, cellular infiltration, necrosis or myocytolysis, and 12 cases of unnatural but non-infectious causation of death (brain death caused by violence or trauma, smoke inhalation, drug intoxication, strangulation, and drowning). The heart weights were always within the reference frame for age, weight and height [27,28].

Post-mortem myocardial tissue was collected at eight standard locations within a time period of 2 days after death at the latest (Fig. 1). Then conventional haematoxylin–eosin staining and immunohistochemical stainings for LCA (monoclonal antibody CD45, Abcam, MEM-28), CD68 (monoclonal anti-human CD68, Dako, PG-M1) and CD45-R0 (monoclonal antibody anti-human CD45-R0, Dako, UCHL1) were performed.

After a thorough review by two different investigators confirming no histological signs of inflammation using conventional stainings, the quantification of the inflammatory cells was carried out by two independent investigators as follows:

- *Method 1*: By using a purpose-built grid, inflammatory cells were counted at 100× magnification. The area of the grid was exactly 1 mm^2 (Fig. 2). At each of the eight sections, 10 loci per 1 mm^2 were counted. This equals to 80 mm^2 enumerated myocardial tissue per case and staining.
- *Method 2*: Twenty HPF areas per sample were investigated. HPF was defined as the microscopically visible area at 400× magnification. The size of each HPF equalled 0.2375 mm^2. A total area of 4.75 mm^2 myocardial tissue per sample (38 mm^2 per case) was investigated.

With both methods the investigated area was considerably increased in comparison with former studies [6,17–19] or endomyocardial biopsies [29–31] (Fig. 3).

After enumeration, the results from both investigators were analyzed individually, and then the two groups of results were compared. For statistical significance the Wilcoxon signed-rank test for related samples was used. The significance level was defined as 5% ($p \leq 0.05$). To compare the results of both methods the cell counts from method 1 (mm^2) were converted into HPF (1 mm^2 = 4.21 HPF). All hearts were evaluated by haematoxylin–eosin staining to exclude inflammation, cellular infiltration, necrosis or myocytolysis. Counting was only performed in immunostained tissues.

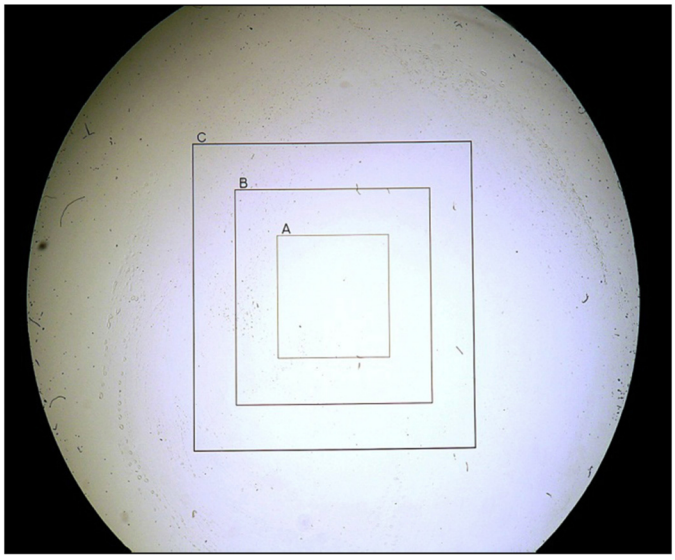
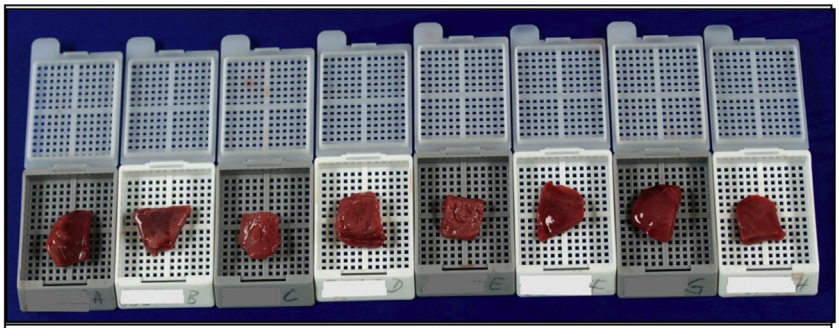


Fig. 2. Microscopy image with overlying grid. The area exactly refers to 1 mm^2 (divided into four squares A–C).

3. Results

The cell counts for leucocytes, macrophages and T-lymphocytes in immunohistochemical stained tissues were low and were similar between the methods evaluated (Fig. 4); thus both procedures were considered valid counting methods. There was no statistically significant difference between the methods used (Table 1).

It became evident, that in 98.2% (method 1) resp. in 100% (method 2) of all cases the mean infiltration was <5 lymphocytes per HPF. None of the cases showed more than 3 macrophages per HPF in the investigated area, and 92.8% (method 1) resp. 85.7% (method 2) of all cases contained no macrophages. However, leucocytes were commonly observed in all cases. Especially for



A	Right ventricle, basis	E	Anterior wall, left ventricle, basis
B	Right ventricle, apex	F	Anterior wall, left ventricle, apex
C	Interventricular septum, basis	G	Posterior wall, left ventricle, basis
D	Interventricular septum, apex	H	Posterior wall, left ventricle, apex

Fig. 1. Tissue samples from a sudden infant death syndrome case in 2009. Standardized locations A–H.