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Lymphocytic myocarditis occurs with myocardial infarction and coincides with increased inflammation, hemorrhage and instability in coronary artery atherosclerotic plaques☆

Linde Woudstra [a,b,*], P. Stefan Biesbroek [b,c,d], Reindert W. Emmens [b], Stephane Heymans [e], Lynda J. Juffermans [b,c], Albert C. van Rossum [b,c], Hans W.M. Niessen [a,b,f], Paul A.J. Krijnen [a,b]

[a] Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands
[b] ICaR-VU, Institute for Cardiovascular Research, VU University Medical Center, The Netherlands
[c] Department of Cardiology, VU University Medical Center, The Netherlands
[d] ICIN, Inter-university Cardiology Institute of the Netherlands, Utrecht, The Netherlands
[e] Center for Heart Failure Research, Cardiovascular Research Institute Maastricht (CARIM), University Hospital Maastricht, Maastricht, The Netherlands
[f] Department of Cardiothoracic Surgery, VU University Medical Center, The Netherlands

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ABSTRACT

Objective: Although lymphocytic myocarditis (LM) clinically can mimic myocardial infarction (MI), they are regarded as distinct clinical entities. However, we observed a high prevalence (32%) of recent MI in patients diagnosed post-mortem with LM. To investigate if LM changes coronary atherosclerotic plaque, we analyzed in autopsied hearts the inflammatory infiltrate and stability in coronary atherosclerotic lesions in patients with LM and/or MI.

Methods: The three main coronary arteries were isolated at autopsy of patients with LM, with MI of 3–6 h old, with LM and MI of 3–6 h old (LM + MI) and controls. In tissue sections of atherosclerotic plaque-containing coronary segments inflammatory infiltration, plaque stability, intraplaque hemorrhage and thrombi were determined via (immuno)histological criteria.

Results: In tissue sections of those coronary segments the inflammatory infiltrate was found to be significantly increased in patients with LM, LM + MI and MI compared with controls. This inflammatory infiltrate consisted predominantly of macrophages and neutrophils in patients with only LM or MI, of lymphocytes in LM + MI and MI patients and of mast cells in LM + MI patients. Moreover, in LM + MI and MI patients this coincided with an increase of unstable plaques and thrombi. Finally, LM and especially MI and LM + MI patients showed significantly increased intraplaque hemorrhage.

Conclusions: This study demonstrates prevalent co-occurrence of LM with a very recent MI at autopsy. Moreover, LM was associated with remodeling and inflammation of atherosclerotic plaques indicative of plaque destabilization pointing to coronary spasm, suggesting that preexistent LM, or its causes, may facilitate the development of MI.

1. Introduction

Lymphocytic myocarditis (LM) is an inflammatory disease of the heart, predominantly characterized by diffuse focal aggregates of inflammatory infiltrate in the myocardium that in majority is associated with viral infection [1]. The prevalence of LM in the general population is uncertain as it often may have a clinically silent course. The clinical presentation of LM is very diverse and varies from mild flu-like symptoms to acute heart failure and sometimes sudden death [1]. In addition, patients with LM can present with a variety of clinical symptoms suggestive of myocardial infarction (MI), such as chest pain, electrocardiographic ST-segment elevation, wall motion abnormalities and increased blood levels of cardiac enzymes [2–10]. Moreover, coronary artery spasms have been reported to occur in patients with LM [11–13]. In fact coronary vasospasm was demonstrated in 70.9% of patients with proven LM on endomyocardial biopsy (EMB) who presented with chest pain [11].

☆ Each author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation

* Corresponding author at: VU University Medical Center, Department of Pathology, Room 0[E]46, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands.
E-mail address: l.woudstra@vumc.nl (L. Woudstra).

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In clinical practice, LM is only considered as potential underlying cause of infarct-like complaints when MI is ruled out, based on the absence of coronary narrowing or obstruction as detected by coronary angiography. In case of normal or non-obstructed coronary arteries, cardiac magnetic resonance (CMR) imaging is often employed as a complementary imaging tool to further differentiate between MI and LM, wherein myocardial injury is mainly located in the subendocardium with MI as opposed to a more (sub)epicardial location with LM [14–16].

The general consensus in the literature is that although LM and MI can be similar in clinical presentation they are distinct clinical entities. However, there is accumulating evidence suggesting an interrelatedness between LM and MI. For instance, recent respiratory tract infections of the cardiotropic influenza virus, which is commonly associated with LM, are significantly associated with MI also [17]. In addition, markers of infection of another group of cardiotropic viruses, i.e. enteroviruses, were detected in the hearts of 40% of patients who died of sudden MI versus only 4% of matched subjects without cardiac disease [18]. In line herewith, as we will show below, we have observed in autopsied cases of patients diagnosed post-mortem with LM a high prevalence of very recent MI, demonstrating that LM and MI can be present simultaneously. Even more, these data may suggest that LM or its cause can facilitate the development of MI.

MI most often is the result of coronary plaque complication, either through rupture or erosion of atherosclerotic plaques. Inflammation has been found to be an important mediator of atherosclerotic plaque destabilization that renders them more vulnerable for complication [19]. For instance, in autopsied cases MI was found to be associated with increased inflammatory cell infiltration in the coronary arteries, including macrophages, T-lymphocytes [20,21] as well as mast cells [22]. Interestingly, via the secretion of vasoactive factors such as histamine, chymase and tryptase, mast cells have been associated with coronary vasospasm also [23,24].

However, knowledge regarding coronary artery changes associated with LM is scarce. In EMB-proven myocarditis, impairment of endothelium-dependent vasodilation of the epicardial coronary arteries correlated with the number of T-lymphocytes in the myocardium [25], indicative of endothelial dysfunction in the coronary arteries. Conversely, in autopsied hearts of patients LM did not coincide with increased infiltrate of T-lymphocytes in the coronary arteries [26]. However, infiltration of other inflammatory cell types was not analyzed.

Therefore, the aim of this study was to analyze in autopsied hearts the inflammatory infiltrate, plaque bleeding and plaque stability in coronary atherosclerotic lesions in patients with LM coinciding with very recent MI, in patients with only LM or very recent MI and control patients without heart disease.

2. Materials and methods

2.1. Patient material

A total of 38 autopsied cases were retrospectively selected and divided into four groups:

1) A group with lymphocytic myocarditis (LM; $n = 10$): In these patients LM was diagnosed based on immunohistochemical analysis of multiple heart slides of both the left ventricle (the septum, the anterior, lateral and posterior wall) and right ventricle (anterior wall). LM was diagnosed based on the presence of multiple aggregates of extravascular lymphocytes (CD45+ cells) with or without cardiomyocyte damage (based on presence of complement activation product C3d). MI was excluded in these patients based on absence of localized nitro blue tetrazolium (NBT) decoloration and the absence of thrombi in the epicardial coronary arteries.
2) A group with a MI of 3–6 h old ($n = 10$): In these patients a MI was diagnosed of 3–6 h old based on reduced localized nitro blue tetrazolium (NBT) staining that appears after three hours after infarction on a mid-ventricular macroscopic cross-section of the heart and/or a thrombus in the epicardial coronary artery, in the absence of neutrophil infiltration in the NBT staining-identified infarction area. MI-induced neutrophil infiltration in the infarcted myocardium was shown previously to start after 6 h after onset of MI (19). In addition, in four cases a thrombus was present in the epicardial coronary artery.
3) A group with lymphocytic myocarditis and a MI of 3–6 h old ($n = 13$): In these patients in addition to LM, a MI of 3–6 h old was diagnosed based on reduced localized NBT staining of the heart. Moreover, in four cases a thrombus in the epicardial coronary artery was found. In one patient no NBT decoloration was found, but a thrombus was found in the epicardial coronary artery indicative of a MI of <3 h old.
4) A control group ($n = 5$): Control patients were selected whose death was not related to cardiac disease. Patients had no NBT decoloration of the heart and patients with diseases that theoretically could coincide with cardiac inflammation were excluded.

In all groups, patients that used prednisolone were excluded. The patient characteristics are shown summarized in Table 1 and in detail in the Supplementary Table.

The infarct area in patients with MI was determined on a mid-ventricular macroscopic cross-section stained with NBT. The percentage of the infarct area was calculated by the dividing the infarct area with the total area of the left ventricle.

From all patients, the three main epicardial coronary arteries were dissected from the heart (left anterior descending, left circumflex and right coronary artery). Segments with macroscopically the most profound stenosis were microscopically analyzed. The number of segments per coronary artery varied between 1 and 6. In total 307 segments were fixed in formalin and embedded in paraffin: 85 from LM, 98 from LM + MI, 86 from MI, 38 of control patients. The coronary segments of the two patients groups with MI were further subdivided in the infarct- and non-infarct-related coronary arteries. The infarct-related coronary arteries were first identified based on the occurrence of a thrombus. In the absence of a thrombus, the infarct related coronary arteries were defined related to location of the NBT staining-identified infarct area.

This study was approved by and performed according to the guidelines of the ethics committee of the VU University Medical Center, Amsterdam, and conforms to the principles of the Declaration of Helsinki. Use of the leftover material after the pathological examination has been completed is part of the patient contract in our hospital.

2.2. Immunohistochemistry

Paraffin tissue sections (4 μm) of the coronary segments were stained with antibodies detecting CD45 (lymphocytes), CD68 (macrophages), MPO (neutrophils), tryptase (mast cells) and Glut-1 (erythrocytes). Sections were first deparaffinized, rehydrated and blocked for endogenous peroxidases by incubation in H_2O_2 (0.3%) diluted in methanol for 30 min. As an antigen retrieval step the slides were heated in 10 mM citrate buffer (pH 6.0) for 10 min to boiling and then cooled for 20 min for slides to be stained for CD68, MPO and tryptase or were heated in a Tris/EDTA buffer (pH 9.0) for 10 min to boiling and then cooled for 20 min for slides to be stained for Glut-1. Slides to be stained for CD45 required no antigen retrieval step. The sections were subsequently incubated with either mouse-anti-human CD45 (1:100, Dako M0701), rabbit-anti-human CD68 (1:400, Dako M0814), mouse-anti-human MPO (1:500, Dako A0398), mouse-anti-human Tryptase (1:500, Dako RB9052-P) or rabbit-anti-human Glut-1 (1:100, Thermo Scientific RB-9052-P) for 60 min. The primary antibodies were diluted in normal antibody diluent (ImmunoLogic ABB500). The slides were then incubated with anti-mouse/rabbit Envision (Dako, K5007) for 30 min. The staining was visualized via incubation in 3,3′-diaminobenzidine (0.1 mg/ml, Dako K3468) for 10 min. Subsequently, the slides were counterstained with haematoxylin, dehydrated and covered. With each staining slides were included incubated with antibody diluent without a primary antibody as a negative control and all these controls showed no staining (not shown).

2.3. Quantification of immune cells and morphometric analysis

In all individual segments of each coronary artery the numbers of extravascular inflammatory cells were quantified separately in the intima, media, adventitia. The total surface area of the intima, media and adventitia were determined on the scanned slides using the Panoramic Desk scanner and analyzed with Pannoramic Viewer 1.15.2 software

Table 1
General patient characteristics.

	Control ($n = 5$)	LM ($n = 10$)	LM + MI ($n = 13$)	MI ($n = 10$)	p value
Age (years), median (IR)	66.0 (55.0–71.5)	63.5 (52.5–77.3)	68.0 (44.5–69.0)	63.0 (53.25–90.0)	0.582
Gender, male/female	4/1	6/4	11/2	8/2	0.556
MI 3–6 h old	0/5	0/10	13/13	10/10	NA
LM	0/5	10/10	13/13	0/10	NA
Clinical history and medication §:					
Diabetes Mellitus type 2	0/5	0/9	2/12	1/10	0.503
COPD	0/5	2/9	3/12	2/10	0.689
Beta blockers	0/5	3/9	5/12	1/8	0.219
Diuretics	0/5	2/9	4/12	0/8	0.120
Anti-coagulation	0/5	2/9	2/12	3/8	0.418
Ace inhibitors	0/5	3/9	2/12	1/8	0.214

LM = lymphocytic myocarditis; MI = myocardial infarction; NA = not applicable; § = data included if available; COPD = chronic obstructive pulmonary disease.

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