Journal of Critical Care 32 (2016) 196–200

Contents lists available at ScienceDirect

Journal of Critical Care

journal homepage: www.jccjournal.org

Myocarditis in patients with subarachnoid hemorrhage: A histopathologic study

Ivo A.C. van der Bilt, MD [a,*], Jean-Paul Vendeville, MD [b], Tim P. van de Hoef, MD, PhD [a], Mark P.V. Begieneman, MSc [c,d], Wim K. Lagrand, MD, PhD [e], Johan M. Kros, MD, PhD [f], Arthur A.M. Wilde, MD, PhD [a], Gabriel J.E. Rinkel, MD, PhD [g,h], Hans W.M. Niessen, MD, PhD [c,d]

[a] Heart Center, Department of Clinical and Experimental Cardiology, Academic Medical Center, Amsterdam, The Netherlands
[b] Department of Cardiology, VU University Medical Center, Amsterdam, The Netherlands
[c] Department of Pathology, ICaR-VU, VU University Medical Center, Amsterdam, The Netherlands
[d] Department of Cardiac Surgery, ICaR-VU, VU University Medical Center, Amsterdam, The Netherlands
[e] Department of Intensive Care, Academic Medical Center, Amsterdam, The Netherlands
[f] Department of Pathology, Erasmus Medical Center, Rotterdam, The Netherlands
[g] Department of Neurology, Brain Center Rudolf Magnus, University Medical Center, Utrecht, The Netherlands
[h] Department of Neurosurgery, Brain Center Rudolf Magnus, University Medical Center, Utrecht, The Netherlands

ARTICLE INFO

Keywords:
Subarachnoid hemorrhage
Myocarditis
Inflammation
Takotsubo
Cardiac abnormalities
Outcome

ABSTRACT

Cardiac abnormalities after subarachnoid hemorrhage (SAH) such as electrocardiographic changes, echocardiographic wall motion abnormalities, and elevated troponin levels are independently associated with a poor prognosis. They are caused by catecholaminergic stress coinciding with influx of inflammatory cells into the heart. These abnormalities could be a sign of a myocarditis, potentially giving insight in pathophysiology and treatment options. These inflammatory cells are insufficiently characterized, and it is unknown whether myocarditis is associated with SAH. Myocardium of 25 patients who died of SAH and 18 controls was stained with antibodies identifying macrophages (CD68), lymphocytes (CD45), and neutrophil granulocytes (myeloperoxidase). Myocytolysis was visualized using complement staining (C3d). CD31 was used to identify putative thrombi. We used Mann-Whitney *U* testing for analysis.

In the myocardium of SAH patients, the amount of myeloperoxidase-positive ($P < .005$), CD45-positive ($P < .0005$), and CD68-positive ($P < .0005$) cells was significantly higher compared to controls. Thrombi in intramyocardial arteries were found in 22 SAH patients and 1 control. Myocytolysis was found in 6 SAH patients but not in controls.

Myocarditis, consisting of an influx of neutrophil granulocytes, lymphocytes, and macrophages, coinciding with myocytolysis and thrombi in intramyocardial arteries, occurs in patients with SAH but not in controls. These findings might explain the cardiac abnormalities after SAH and may have implications for treatment.

1. Introduction

Cardiac dysfunction after subarachnoid hemorrhage (SAH), such as electrocardiographic changes, wall motion abnormalities, and troponin release, occurs frequently and is associated with poor prognosis [1]. The massive sympathetic activation after the SAH leads to a catecholamine release in the myocardium, which is thought to cause these cardiac abnormalities [2]. On a cellular level, catecholamine release has been associated with myocardial damage [2-6]. Literature is limited, and early pathology studies that reported on myocardial cellular infiltration and myocytolysis after SAH, using immunohistochemical staining [7], did not classify the inflammatory response nor did they specify the types of inflammatory cells in the heart. According to the Dallas criteria, myocardial infiltration of inflammatory cells and myocytolysis qualifies as a myocarditis [8]. Myocarditis after SAH has never been established but is a plausible explanation in the pathway from catecholamine release; it may explain the cardiac abnormalities and may have important clinical implications for treatment and prognosis. Therefore, the objectives of the present study were to characterize the infiltration of inflammatory cells in the heart after SAH compared to controls, to investigate whether this cellular infiltration meets criteria for the diagnosis myocarditis and to search for other myocarditis stigmata such as myocytolysis and intra-arterial thrombi.

2. Materials and methods

2.1. Patients' selection

Myocardium of patients who died of SAH between 1994 and 2004 was obtained from the departments of pathology of the VU University

* Corresponding author at: Haga Teaching Hospital, Heartcenter, Leyweg 275, 2545 CH, The Hague, The Netherlands. Tel.: +31 70 2100000.
E-mail address: vanderbilt@cardionetworks.org (I.A.C. van der Bilt).

http://dx.doi.org/10.1016/j.jcrc.2015.12.005
0883-9441/

Medical Center Amsterdam, the Netherlands, and the Erasmus Medical Center Rotterdam, the Netherlands. The pathology databases were searched for cases in which SAH was documented as cause of death, as indicated by the clinician requesting autopsy, in concomitance with the presence of subarachnoid blood documented in the autopsy report. As a control group, myocardium of oncologic patients without cardiac involvement of the disease who did not receive cardiotoxic chemotherapy or radiotherapy in the cardiac region was obtained. Control patients who died of a neurologic disease or potentially had an underlying disease associated with cardiac inflammation, for example, sepsis, were excluded. The study was approved by and performed according to the guidelines of the local medical ethics committee and in accordance with the Declaration of Helsinki. Use of material for research after completion of a pathological examination is part of the patient contract in the participating hospitals.

2.2. Immunohistochemistry

The heart tissue samples of both the SAH patients and the control subjects were fixed in 4% buffered formaldehyde solution and embedded in paraffin for the preparation of 4-μm sections. Sections were then dewaxed and dehydrated, and antigen retrieval was performed by boiling in 10 mmol/L sodium citrate buffer, pH 6.0, for 10 minutes in a microwave oven. Sections were preincubated with normal serum for 10 minutes. Rabbit serum was used for monoclonal antibodies; swine serum was for polyclonal antibodies. Preincubation was followed by incubation with primary antibodies for 1 hour.

The primary antibodies that we used are myeloperoxidase (MPO), mouse anti-human CD68, mouse anti-human CD45, rabbit anti-human complement C3d, and mouse anti-human CD31, all from Dako Cytomation, Denmark.

Sections were subsequently rinsed in phosphate-buffered saline (PBS) and incubated with a biotin-labeled secondary antibody (rat–anti-mouse–biotin or swine–anti-rabbit–biotin) for 30 minutes. After washing in PBS, sections were incubated with streptavidin-biotin complex/Horseradish Peroxidase Complex for 1 hour. After the streptavidin-biotin complex/Horseradish Peroxidase Complex incubation, sections were rinsed with PBS, followed by visualization with 3,3′-diaminobenzidine (0.1 mg/mL, 0.02% H_2O_2). Sections were subsequently counterstained with hematoxylin, dehydrated, and covered. As a control, the same staining procedure was used, but instead of primary monoclonal or polyclonal antibody, PBS was used.

2.3. Morphometric analysis

Two observers (IB and WL) scored the number of extravascular neutrophil granulocytes (MPO positive), lymphocytes (CD45 positive), and macrophages (CD68 positive). This was done by counting the number of positive cells within a fixed grid drawn on the microscopic slide with the specimen. To minimize interobserver variability, some of the slides were scored on a 2-person multiviewing microscope, some were done separately. In addition, a third observer, an experienced pathologist, checked samples at random. Both observers were blinded with respect to the origin of the slides (SAH vs controls). In case of significant differences in scoring results between the 2 observers, both observers examined the same myocardial slides simultaneously. After this, consensus was achieved by the 2 observers. The number of extravascular inflammatory cells per 100 mm^2 was then calculated. *Myocytolysis* was defined as complement (C3d) positivity of cardiomyocytes. Finally, the number of putative thrombi (CD31 positive) in intramyocardial arteries was scored and calculated per 100 mm^2. Myocarditis was classified according the Dallas criteria as "myocarditis": an aggregation of inflammatory cells in the myocardium coincided with areas of myocytolysis, or "borderline myocarditis": when aggregation of inflammatory cells in the myocardium was documented without myocytolysis.

2.4. Statistical analysis

Distribution of data was checked using Kolmogorov-Smirnov analyses. After that, nonparametric testing using Mann-Whitney *U* test was used for differences between groups. A sensitivity analysis was performed for patients with a proven aneurysm on autopsy and the patients with SAH without an aneurysm. $P < .05$ was considered statistically significant.

3. Results

Myocardial tissue samples of 25 patients were retrieved from the pathology databases. Baseline characteristics could be retrieved for 23 patients. Mean age was 59 ($\pm$SD 15) years of age, and 11 patients (44%) were female. In 16 patients (64%), a culprit aneurysm was reported on autopsy. Duration from hospital admission to death ranged from 2 hours to 21 days. As depicted in Fig. 1, there was a large spread in the number of cells per individual patient. Myocardial tissue of 18 control patients was used as a control group. Fig. 2 shows the mean number of cells in SAH patients compared to controls. Compared to the control group, the amount of MPO-, CD45-, and CD68-positive cells was significantly higher in SAH patients ($P < .005$ for all). In 6 patients with SAH (24%), spots with C3d-positive cells, indicating myocytolysis, were documented, and these patients consequently fulfilled the Dallas criteria for myocarditis. In contrast, no C3d-positive cells were documented in the controls. Intramyocardial thrombi were found in 22 SAH patients (88%) and in only 1 control patient. Sensitivity analyses showed no difference in the number of inflammatory cells or myocytolysis in patients with or without proven aneurysm.

4. Discussion

In the present autopsy study, we documented an influx of neutrophil granulocytes, lymphocytes, and macrophages into the myocardium of patients who died after SAH. In some, this coincided with myocytolysis and thrombi in intramyocardial arteries. According to the Dallas criteria, this finding suggests that patients with SAH have a borderline myocarditis and some have a myocarditis.

Although other studies have reported on myocardial cellular infiltration and myocytolysis after SAH, classification of the inflammatory cells is not described before, and we could not find previous studies establishing myocarditis after SAH. Most studies focused on the myocardial cell damage after SAH; only a few studies used immunohistochemical staining methods which were not specific for the type of cell.

There is overwhelming evidence from clinical and experimental studies that catecholaminergic stress after acute cerebral lesions causes myocardial cell damage [3,9-16]. However, influx of inflammatory cells in the myocardium was not investigated.

Intramyocardial catecholamine release by sympathetic nerve endings has been suggested as the primary source of the catecholamines because experimental SAH studies showed no myocardial damage after SAH in sympathectomized baboons, whereas extensive myocardial damage was documented in adrenalectomized dogs [17]. This hypothesis is supported by the finding that cardiomyocytolysis after SAH is more prevalent in the direct surrounding of the sympathetic nerve terminals [18].

We found evidence of thrombi in the intramyocardial arteries. It is known that myocarditis may cause vasospasm, which may cause the formation of thrombi. Thrombi might cause obstruction of the microarteries, thus causing myocardial infarction [19,20]. Several other studies reported patchy subendocardial infarction after SAH, suggesting that thrombi and likely myocarditis were present in these cases as well [6,12,21].

The clinical relevance of our study is that treatment of cardiac abnormalities after SAH may improve outcome as they are associated with poor outcome independent of other clinical parameters [22]. Our