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CASE REPORT

Enrico Silingardi,[1] *M.D.; Francesco Rivasi,*[2] *M.D.; Anna L. Santunione,*[1] *M.D.; and Lorella Garagnani,*[2] *Ph.D.*

Sudden Death from Tubercular Myocarditis

ABSTRACT: Tuberculous myocarditis is a rare finding. We present the case of a 33-year-old woman who was in good health and who died suddenly at home. Autopsy and histopathologic examinations revealed granulamatous lesions in the myocardium, lungs, lymph nodes, liver, and spleen. No fast acid bacilli were demonstrated on histological examination. The presence of a Mycobacterium tuberculosis DNA complex was identified using a polymerase chain reaction (PCR) on formalin-fixed paraffin-embedded histological samples. An HIV test carried out on the blood obtained during the autopsy was negative according to the DNA amplification technique (PCR) and enzyme-linked immunosorbent assay serological test. We hypothesize that the mechanism of death was severe ventricular arrhythmia due to granulomatous proliferation in the structures of the interventricular septum.

KEYWORDS: forensic science, forensic pathology, tubercular myocarditis, sudden death, autopsy, polymerase chain reaction

Tuberculosis (TB) is still a serious problem for public health, even in highly developed countries. In Italy, the annual incidence was 7.3 per 100,000 inhabitants in 2003, with 3.2 new cases/year. In the same year, mortality from TB was 0.8 per 100,000 inhabitants (1).

The specific finding of TB in the myocardium (MTB) is rarely seen and coexists with localization in other organs. For the most part, it affects subjects in their youth and it is generally asymptomatic. For this reason, the diagnosis is usually obtained only postmortem.

The MTB average incidence on the total number of autopsies is $<0.3\%$. A systematic study relative to 13,658 autopsies reported a frequency of 0.14%, mainly in males (2). Unusual cases of sudden death from MTB have been reported in the literature (3–5).

Case Report

A 33-year-old Italian woman, married, a teacher and in good health died suddenly at home. Cardiopulmonary resuscitation carried out by the first emergency unit was unsuccessful. In accordance with the Italian law, autopsy was performed by a forensic pathologist.

Her familial and personal history were completely negative. No use of medication was reported. There was no warning symptom preceding the death. External examination revealed a good physical state and the absence of traumatic lesions. At autopsy, the heart weighed 370 g. The surface of the incision of the left ventricular myocardium showed nodules, which were pale in color and poorly delimited (Fig. 1). These nodules localized throughout the antero-septal and posterior wall and they extended longitudinally for 50 mm from the atrio-ventricular groove toward the apex. Pale infiltrations of lesser intensity were present on the anterior and the lateral walls of the left ventricle. Nothing was found in the endocardium, the other cardiac sections, the valves, the coronary tree, and the pericardium.

The lymph nodes and the spleen (300 g) were also increased in volume and infiltrated by nodular formations. Pale nodules of small dimensions were observed on the surface of the liver. Examination of the remaining organs and systems was negative.

Histological sections colored according to the hematoxylin & eosin (Fig. 2), periodic acid-Schiff, Grocott, and Ziehl–Neelsen methods were prepared on fragments of the myocardium, lungs, lymph nodes, liver, and spleen. The histological exams of the myocardium showed large cell granulomas, epithelial cells, and lymphocytes immersed in a wide context of dense fibrous replacement tissue. The nodules at the hepato-splenic and lymphnodal level showed similar aspects. Some microscopic granulomas were

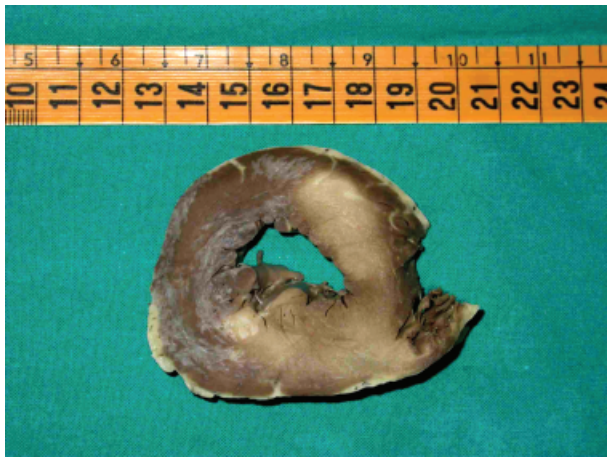

FIG. 1—*Transverse section of the left ventricle showing large nodules, pale in color and poorly delimited.*

[1]Institute of Legal Medicine, University of Modena and Reggio Emilia, Modena, Italy.
[2]Institute of Pathological Anatomy, University of Modena and Reggio Emilia, Modena, Italy.

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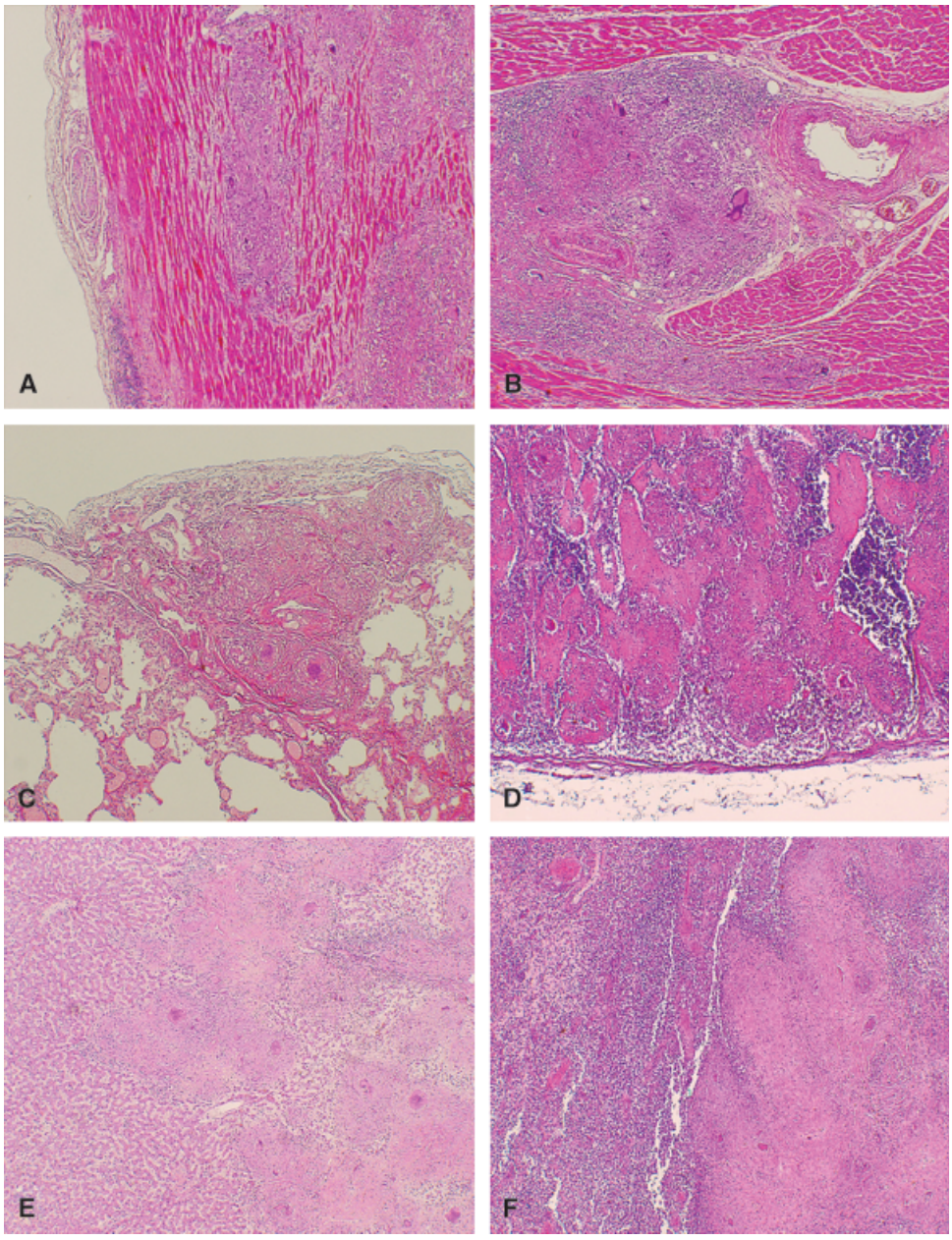

FIG. 2—*Photomicrographs showing myocardial large cell granulomas (A, B), microscopic granulomas in the lung (C), large cell granulomas in a lymph node (D), in the liver (E), and in the spleen (F) (hematoxylin & eosin × 40).*

observed in the lungs. Examination of the remaining organs was negative. The test for alcohol-acid resistant bacilli was negative.

Given the probable tubercular nature of the granulomatous inflammation, the mycobacterial DNA was examined using the nested polymerase chain reaction (PCR) method. DNA was extracted from all formalin-fixed, paraffin-embedded tissues (6). Three 5-µm sections from each block were cut with a microtome. In order to prevent carryover of contaminating DNA, a fresh blade was used for each sample and the microtome overlay was covered with a piece of adhesive tape that was changed for each sample. As a negative extraction control, three serial 5-µm-thick sections were cut from formalin-fixed, paraffin-embedded tissue samples having a histopathological diagnosis other than mycobacterial infection and were then processed in the same manner as the test sample. As a positive extraction control, three serial 5-µm-thick sections were cut from formalin-fixed, paraffin-embedded tissue samples having a clinical history of mycobacterial infection with microbiological confirmation by a positive smear for acid-fast bacilli (by Ziehl–Neelsen staining). The sections were collected in 1.5-mL microfuge tubes and were deparafinized twice. Subsequently, the tissue pellets were vacuum dried, resuspended in 200 µL of digestion buffer made up of 50 mM Tris (pH 8.5), 1 mM ethylenediamine tetraacetic acid, 0.5% Tween 20, and 200 µg of proteinase K per mL, and the mixture was incubated for 3 h at 56°C. Proteinase K was inactivated by incubating the samples at 95°C for 10 min.

Nested PCR carried out with specific oligonucleotides was positive for *Mycobacterium avium*, identifying the ISI110 insertion sequence (7) in all the samples examined (left ventricle, liver, spleen, right lung, and kidneys). Nested PCR with specific oligonucleotides was positive for the tubercular complex (*M. tuberculosis*, *M. bovis*, and *M. africanum*), identifying the IS6110 insertion sequence (8) in the samples examined: left ventricle, liver, spleen, and kidneys (Fig. 3).

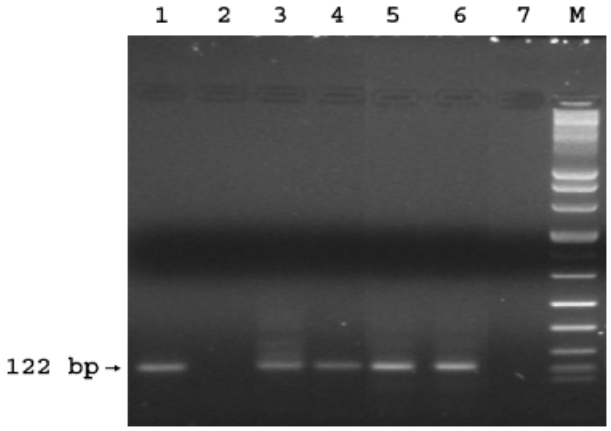

FIG. 3—*Electrophoresis of 2% agarose gel of nested polymerase chain reaction product of IS6110 M. tuberculosis complex DNA. Dominant bands about 122 bp indicated the presence of mycobacterial DNA in samples. Lanes 1–5, DNA extracted from spleen, lung, liver, kidneys, heart; lane 6, positive control of reaction; lane 7, negative control of reaction; M, molecular weight marker.*

HIV tests were performed on the blood obtained during the autopsy and which had been conserved under suitable conditions; they were performed according to the DNA amplification technique (PCR) and the ELISA serological test and both were negative.

Clinical and laboratory research carried out on family members of the patient and in the school community where she worked excluded other cases of infection.

Discussion

We reported a case of sudden death from previously undiagnosed MTB in a subject in apparently good health and without known historical or environmental risk factors. Cases of sudden death from undiagnosed MTB have rarely been reported in the literature and they refer exclusively to male subjects. MTB can present in a miliary or a nodular form, occasionally with aneurysms. The infection can reach the myocardium through the blood or the lymphatic system, or as a result of contiguity with mediastinic lymph nodes (2).

MTB is hardly ever diagnosed while the person is alive. In addition, research on alcohol-acid resistant bacilli is rarely positive. Therefore, the differential diagnosis from the other forms of granulomatous inflammations including sarcoidosis and large cell idiopathic granulomatous myocarditis is challenging (9). This emphasizes the role and the possibility of applying molecular biology techniques to diagnose MTB. In particular, DNA amplification (PCR) on formalin-fixed paraffin-embedded histological samples is currently considered a valid method.

The considerable discrepancy between the extension of myocardial lesions and the absence of symptoms is not surprising. Indeed, in several cases, the myocardial involvement is not associated with cardiac dysfunction. In contrast, extended pericardial localization causes symptoms due to the development of effusion and adhesions. In spite of the negative HIV tests, we believe that a compromised immune system was probably at the origin of the infection.

The identification of the mechanism of death is of specific interest. The absence of coronary and valvular pathologies, the typical circumstances of death without preceding or accompanying symptoms, suggests a sudden and severe ventricular arrhythmia. The presence of granulomatous proliferation in the structures of the interventricular septum supports our hypothesis.

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Additional information and reprint requests:
Enrico Silingardi, M.D.
Institute of Legal Medicine
University of Modena e Reggio Emilia
Policlinico L.go del Pozzo, 71
41100 Modena
Italy
E-mail: silingardi.enrico@unimo.it