Forensic Science International 208 (2011) e1–e5

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Case report

Immunohistochemical characterisation and TNF-α expression of the granulomatous infiltration of the brainstem in a case of sudden death due to neurosarcoidosis

S. D'Errico, S. Bello, S. Cantatore, M. Neri, I. Riezzo, E. Turillazzi, V. Fineschi *

Department of Forensic Pathology, University of Foggia, Ospedale Colonnello D'Avanzo, Viale degli Aviatori 1, 71100 Foggia, Italy

ARTICLE INFO

Article history:
Received 3 May 2010
Received in revised form 28 November 2010
Accepted 14 January 2011
Available online 24 February 2011

Keywords:
Neurosarcoidosis
Granulomas
TNF-α
Brainstem
Sudden death

ABSTRACT

Neurosarcoidosis carries a mortality of 10%, over twice that of sarcoidosis overall, although it has been rarely reported as a cause of sudden death. The current evidence suggests that sarcoidosis results from an enhanced immune reaction to a variety of antigens, non-self or self which causes CD4 (helper–inducer) T-cell accumulation with a ratio of helper–inducer T cells to suppressor-cytotoxic T cells usually high in affected organs, activation and release of inflammatory cytokines, and formation of granulomatous lesions. Numerous cytokines and other mediators are produced by both activated macrophages and T lymphocytes bearing the CD4-helper phenotype during the granuloma responses. A number of data suggest that interferon-gamma (IFN-gamma) and cytokines such as TNF-α, IL-2, and IL-18 play a critical role in the formation of granulomas. In this article, we describe the clinical and pathological characteristics of a patient who suddenly died due to acute respiratory failure. Neurosarcoidosis with massive and extensive involvement of the brainstem was established as the cause of death. Western blot analysis in the patient demonstrated the TNF-α presence as a 51-kDa protein in the brain tissue. The immunohistochemical analysis showed a poor positiveness for CD4 in all samples around the granulomas, as well as moderate positiveness for CD8, CD15, and CD20; CD45 and CD68 showed a strong positiveness in all the brain samples. Histological findings, immunohistochemical analysis, and proteomic studies addressed the diagnosis of neurosarcoidosis with involvement of the nucleus of the solitary tract in the brainstem and central hypoventilation as the cause of death.

Among the central nervous system diseases that are most likely to lead acutely and unexpectedly to death, neurosarcoidosis is considered to be relatively rare. Sarcoidosis is a multisystemic disorder of unknown cause, characterised by the presence of noncaseating epithelioid cell granulomas in involved organs. It is a ubiquitous disease with incidence (varying according to age, sex, race and geographic origin) estimated at around 16.5/100,000 in men and 19/100,000 in women [1,2]. Clinical manifestations and prognosis are dependent on the location and extent of granulomatous infiltrates and although the respiratory tract and the lymphatic system are most often affected, the disease may affect almost any organ. The incidence of clinical involvement of the nervous system in a sarcoidosis population is estimated to be 5–15%, however, the incidence of subclinical neurosarcoidosis may be much higher [1,3–12]; autopsy results suggest a rate of more than 25% [13]. Necropsy studies also suggest that ante mortem diagnosis is made in only 50% of patients with nervous system involvement [14]. There is a female preponderance and most commonly, it presents in young adults between the ages of 20 and 40 years [12].

Neurosarcoidosis carries a mortality of 10%, over twice that of sarcoidosis overall [15], although it has been rarely reported as a cause of sudden death. Arrhythmias from infiltration of autonomic centres, epilepsy, and obstructive hydrocephalus from brainstem involvement have been suggested as the mechanism of sudden death [16–19], as well as respiratory failure from central hypoventilation [20–22]. Here, we report autopsy findings of a sudden death in a patient with neurosarcoidosis infiltrating the brainstem. Expression of CD4 and TNFα in granuloma and intergranulomatous areas at himmunoistochemical investigation was also confirmed at confocal laser scanning microscopy and proteomic analysis with retained activity, suggesting for an early phase of the neurological disease.

1. Case report

1.1. Clinical history

A 42-year-old man was admitted to the local hospital complaining of a low-grade remittent fever not responding to

* Corresponding author. Tel.: +39 0881733193; fax: +39 0881736903.
E-mail address: vfinesc@tin.it (V. Fineschi).

0379-0738/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.forsciint.2011.01.021

antibiotic therapy and headache from the past 2 months. In the case history, he was shown to be affected from severe obesity, diabetes mellitus, and hypertension. Both physical and neurological examinations were unremarkable, except for mild tachycardia (heart rate (HR) 118 beats/min) and low-grade fever (body temperature (BT) 37.4°). Lab tests showed mild leukocytosis and increasing of erythrocyte sedimentation rate (ESR) (21 s). Microbiological and immunological investigations as well as ECGs and X-ray scan of chest and abdomen were negative. Cranial computed tomography (CT) and nuclear magnetic resonance (NMR) were not performed. A few days later, the man suddenly complained of recurrent episodes of sudden stomach ache, tachycardia (HR 147 beats/min), and dyspnea; hypotensive crisis (90/n.a.) and low oxygen saturation of blood (70%) were also recorded, providing oxygen support in ventimask. The day after the respiratory crisis, the man was found dead lying on his bed.

1.2. Autopsy findings

A complete postmortem examination was performed 24 h after death.

A complete neuropathological examination was performed, including gross brain examination and microscopic examination of paraffin-embedded sections from the gray and white matter of all lobes of the brain, central nuclei (caudate, putamen, pallidum and thalamus), cerebellum, and brainstem. Gross examination of the brain revealed normal gyral pattern, unremarkable dura, and leptomeninges. There was no evidence of uncal or tonsillar herniation. Coronal sections (10 mm) demonstrated a normal cortical ribbon, a well-demarcated gray/white matter, and a normal ventricular system. Brainstem, cerebellum and spinal cord showed no abnormalities on gross examination. Lungs were heavy with white foam on the main bronchi. Heart was normal in size and weight with conical shape; coronary arteries were unremarkable.

1.3. Histological study

A routine microscopic histopathological study was performed on all samples collected during autopsy using haematoxylin–eosin (H&E), trichromic stains (Masson, Mallory, phosphotungstic acid hematoxylin (PTAH), Azan, and Van Gieson), and combined Luxol–periodic acid–Schiff (PAS) stains. In addition, an immunohistochemical investigation of brain samples was performed utilising antibodies anti-CD45, CD3, CD4, CD8, CD15, CD20, CD68 and anti-TNF-α (Santa Cruz, CA, USA). For this study, we used 4-μm-thick paraffin sections mounted on slides covered with 3-aminopropyl-triethoxysilane (Fluka, Buchs, Switzerland). Pretreatment was necessary to facilitate antigen retrieval and to increase membrane permeability to antibodies anti-CD45, CD3, CD4, CD8, CD15, CD20 boiling in 0.25 M ethylendiaminetetracetic acid (EDTA) buffer, to antibody anti-CD 68 for 15 min in proteolytic enzyme (Dako, Copenhagen, Denmark) at 20 °C, and to antibodies anti-TNF-α boiling in 0.1 M citric acid buffer. The primary antibody was applied in 1:600 ratio for CD45 and TNFα, in 1:100 ratio for CD3 and CD8, in 1:50 ratio for CD4 and CD15, in 1:200 ratio for CD20 and CD68 and incubated for 120 min at 20 °C. The detection system utilised was the LSAB+ kit (Dako, Copenhagen, Denmark), a refined avidin–biotin technique in which a biotinylated secondary antibody reacts with several peroxidase-conjugated streptavidin molecules. The sections were counterstained with Mayer's hematoxylin, dehydrated, cover slipped, and observed in a Leica DM4000B optical microscope (Leica, Cambridge, UK). The samples were also examined under a confocal laser scanning microscope (CLSM) (True Confocal Scanner, Leica TCS SPE) to obtain images with an optimal spatial resolution.

1.4. Western blot study

Approximately, 100 mg of frozen brain tissue was dissected and immediately transferred to radioimmunoprecipitation assay (RIPA) buffer (Sigma, Eugene, OR, USA) with protease inhibitor cocktail (Sigma) and homogenised on ice utilising homogeniser SilentCrusher (Sigma–Aldrich, St. Louis, MO, USA). The homogenate was centrifuged (12,000 rpm for 10 min at 4 °C). The supernatant was collected, estimated by Bradford method [23], and it was boiled for 5 min, at 95 °C. Brain total protein extracts (~40 μg/lane) were run on 12% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) at 130 V for about 2.5 h. For Western blot, proteins from SDS gels were electrophoretically transferred to nitrocellulose membranes in mini trans blot apparatus (Bio-Rad laboratories, Hercules, CA, USA) (1 h at 250 mA). Non-specific binding was blocked by incubating membranes in Western blocker solution (Sigma) for 1 h at room temperature. The membranes were incubated with primary antibodies CD4 (Santa Cruz, CA, USA) and TNFα (Santa Cruz, CA, USA) diluted in Western blocker solution, in 1:800 ratio CD4 and in 1:1000 ratio TNFα, overnight at 4 °C. Blots were washed with phosphate-buffered saline (PBS)/Tween-20 and then incubated for 1 h at room temperature with horseradish peroxidase (HRP)-conjugated secondary antibodies diluted in Western blocker solution, in 1:2000 ratio. Membranes were washed with PBS/Tween-20 and the immune reaction was developed in IMMUNOSTAR Kit Western C (Bio Rad., Hemel Hempstead, UK) and then visualised by chemiluminescent detection methods. The light is then detected by a photographic film. The image was then analyzed by Versadoc (Bio-Rad laboratories) to detect protein staining.

1.5. Histological findings

Histological examination revealed mild cerebral oedema. A diffuse, perivascular cuffing lymphocyte infiltrate was also observed on brain samples as well as the presence of multiple compact and well-circumscribed noncaseating granulomas with multinucleated giant cells observed in the meninges and brainstem (Fig. 1A). The external arcuate nucleus and the nucleus of the solitary tract were characterised by the presence of multiple granulomas. Granulomas were detected in the periacqueductal gray sections. Noncaseating granulomas were mainly composed of an aggregate of epithelioid cells and multinucleated giant cells of Langhans and foreign body type, without necrosis in the centre surrounded by lymphocytes, plasma cells, and mastcells (Fig. 1B and C). The giant cells sometimes showed asteroid bodies and Schaumann bodies (Fig. 2A). The absence of fibrotic and sclerotic changes in the granulomas and intergranulomatous areas indicates an early phase of the granulomas. The same granulomas were also detected in the brainstem as well as in the lungs where massive pulmonary oedema was detected. Intensity of immunopositive infiltrates were assessed semiquantitatively in the scale 0–4 as follows: 0 = no immunoreactivity, 1 = mild immunopositivity in scattered cells, 2 = immunopositivity in up to a third of cells, 3 = immunopositivity in up to a half of cells, and 4 = strong immunopositivity in the majority or all cells. Strong immunopositivity was revealed for CD68 in all the brain samples (Fig. 2B and C). The immunohistochemical analysis showed a poor positiveness (+) for CD4 in all the samples around the granulomas and a moderate positiveness (++) for CD8 in all the samples (Fig. 3B). We found a moderate positiveness (++) for CD15 around the granulomas, a moderate positiveness (++) for CD20 (Fig. 3D), and CD45 showed a strong positiveness (++++). Immunohistochemical reaction for TNFα was positive (+++) in all the brain samples. Mycobacterium tuberculosis and fungal infections were