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The role of serum amyloid A staining of granulomatous tissues for the diagnosis of sarcoidosis

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ABSTRACT

Background: Previous studies demonstrated that SAA staining of sarcoidosis granulomas was qualitatively and quantitatively different from other granulomatous diseases. These data suggest that positive SAA staining of granulomatous tissue may have adequate specificity to establish a diagnosis of sarcoidosis. Our objective was to determine the diagnostic specificity of SAA staining for sarcoidosis relative to other granulomatous disorders.

Methods: Pathological specimens demonstrating granulomatous inflammation were retrospectively identified at one institution, plus 4 specimens were obtained from New York City firefighters with biopsy-confirmed World Trade Center "sarcoidosis-like" pulmonary disease. Specimens were analyzed if specific diagnoses related to the granulomatous inflammation were confirmed through medical record review. SAA staining was performed using previously developed methods. Two pathologists, blinded to each other and the diagnoses, determined if the stained material was SAA positive or negative. Discordant results were adjudicated by the two pathologists.

Measurements and main results: 106 specimens were analyzed from 100 patients, with 36 biopsies (34%) from sarcoidosis tissues and 70 (66%) from other granulomatous disorders. The Cohen Kappa correlation between the two pathologists for SAA staining positivity was excellent (0.85, 0.73–0.98). The overall specificity of SAA staining for the diagnosis of sarcoidosis was 84% (59/70). The sensitivity was 44% (16/36).

Conclusions: Although SAA staining of various granulomatous tissues was fairly specific for the diagnosis of sarcoidosis, the specificity was inadequate for SAA staining to be used as a diagnostic test for sarcoidosis in isolation. These data suggest that SAA production may not be a universal mechanism in the development of sarcoidosis.

1. Introduction

Sarcoidosis is a multisystem granulomatous disease of unknown cause. Currently, there is no reliable test that is adequately specific to ensure a diagnosis of sarcoidosis. At present, the diagnosis of sarcoidosis is established on the basis of clinical-radiographic findings that are supported by histological evidence of non-caseating epithelioid cell granulomas [1] in absence of alternative causes of granulomatous inflammation [1–3].

Serum amyloid A (SAA) is an amyloid precursor protein that may

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amplify ongoing helper T cell type 1 (Th1) granulomatous reactions to mycobacterial antigens [4]. Chen and colleagues demonstrated SAA was expressed much more intensely and with a characteristic distribution pattern in tissues involved with sarcoidosis compared to many non-sarcoidosis granulomatous tissues [4]. These authors concluded that their findings suggested that SAA plays a central, perhaps defining, role in the pathobiology of sarcoidosis.

The investigations of Chen and coworkers prompt consideration of SAA staining as a test with adequate specificity to be diagnostic of sarcoidosis. We therefore retrospectively obtained histological specimens from our institution that revealed granulomatous inflammation from sarcoidosis and known alternative causes. We also obtained histological specimens from the Fire Department of New York City (FDNY) firefighters who were exposed to World Trade Center (WTC) dust from World Trade Center disaster on September 11, 2001 and who subsequently developed sarcoidosis. The primary purpose of our study was to determine the specificity of SAA staining for the diagnosis of sarcoidosis relative to other granulomatous disorders. To that end, we attempted to modify the SAA staining technique to enhance its specificity.

2. Methods

This study was approved by the Institutional Review Board of Albany Medical College (study# 3731). Pathological specimens that demonstrated granulomatous inflammation between January 1, 2004 and December 31, 2013 were retrospectively identified. The medical records of these patients were reviewed for confirmation of a specific diagnosis related to the granulomatous inflammation. In the case of granulomatous infections, confirmation included documentation of a positive culture and/or stain consistent with the granulomatous diagnosis. In the case of sarcoidosis and other non-infectious granulomatous disorders, confirmation included a review of medical records, serologic studies, radiographic and/or additional clinical data to ensure that a specific diagnosis could be determined. Cases where there was significant doubt concerning a specific granulomatous diagnosis, were dropped from analysis prior to performing SAA staining. We also obtained pathological specimens from FDNY firefighters who had accumulated a minimum of 1-month exposure to WTC dust from the WTC disaster of September 11, 2001 who subsequently developed WTC “sarcoidosis-like” pulmonary disease [5].

As only biopsies demonstrating granulomatous inflammation were analyzed in this study, all patients with sarcoidosis had to have biopsy-proven granulomatous inflammation with no evidence of an alternative cause of granulomatous inflammation. In addition, they were required to satisfy at least one additional criterion for “at least probable” sarcoidosis on the basis of the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) organ assessment instrument [6]. All biopsy specimens were stained at least 18 months after the biopsy was performed in order to ensure that the diagnosis of sarcoidosis had not been changed to an alternative cause of granulomatous inflammation over this time period.

Staining for SAA was performed using modifications of methods previously developed by Chen and coworkers [4]. The modifications of the previous staining method were made to increase the specificity of the stain for the diagnosis of sarcoidosis at the potential expense of lowering the sensitivity. This was gauged by repeatedly staining 10 known sarcoidosis specimens as well as 10 known granulomatous specimens with a specific diagnosis other than sarcoidosis. The details of the staining technique are described in e-Appendix 1. This SAA staining technique was confirmed to stain amyloidosis AA using slides of renal tissue involved with amyloidosis AA (e-Fig. 1). This positive renal amyloidosis AA control was verified by liquid chromatography tandem mass spectrometryMayo Clinic Laboratories, Rochester MN, on peptides extracted from Congo red positive microdissected areas of the paraffin embedded tissue block.

The two pathologist investigators (LF, TJ) independently reviewed the Haematoxylin and Eosin (H&E) and SAA stained slides on all cases. Immunostaining was semi-quantitatively scored based on the stain intensity and distribution. The slides were assessed for the abundancy of granulomas and the following characteristics of the granulomas: the degree of inflammatory cells, the abundancy of hyaline fibrosis, and the degree of necrosis (Table 1). SAA stain intensity and distribution was also graded (Table 2).

Slides were assessed by each pathologist for a global SAA result of negative, equivocal, or positive. The slides were determined to be negative for SAA if there was no staining. In addition, slides were determined to be negative if the pathologist viewed the combined intensity and distribution to be very low (faint staining that was identified in sparse cells). SAA was determined to be positive if slides demonstrated moderate to high intensity staining and the distribution of staining was in areas of granulomatous inflammation. All discrepancies in global SAA staining results between the two pathologists were re-reviewed by both pathologists and a consensus was reached that was either globally positive or negative for SAA staining. When analyses of the individual global assessment by individual pathologists were performed, an equivocal result was interpreted as negative.

The predictive quality of the consensus global SAA staining result for each specimen was analyzed using standard measures of sensitivity and specificity. We computed kappa coefficients to gauge the reliability of the two pathologists' assessment of SAA staining positivity or negativity. We also computed kappa coefficients for the two pathologists' assessment of the intensity and distribution of SAA staining as well as the degree of inflammation, granuloma burden, necrosis, and hyalinization on H&E staining. We also performed a multivariate logistic regression estimating the probability of sarcoidosis diagnosis, based on SAA staining intensity and distribution. The R statistical computing platform was used for these analyses [7].

3. Results

One-hundred and six granulomatous biopsies from 100 patients underwent SAA staining and analysis. Ninety-five patients had one biopsy analyzed, four patients had 2 biopsies analyzed, and one patient had three biopsies analyzed. Excluding patient demographics, all analyses concerned the biopsy specimens. Thirty-six (34%) of the biopsies were from sarcoidosis patients while 70 (66%) biopsies were from patients with an alternative granulomatous disease. Table 3 shows the demographics of the patients, revealing that there was a higher percentage of black patients in the sarcoidosis group than in the non-sarcoidosis group. Table 4 shows the diagnoses of each biopsy. These diagnoses included numerous fungal, mycobacterial and other infections and vasculitides. Table 5 shows the organs that were biopsied, showing that the most common organs biopsied were the lung, skin/soft tissue, and mediastinal lymph node.

Figs. 1–4 display the SAA staining results on selected granulomatous tissues, true positive, false positive, true negative and false negative results for the diagnosis of sarcoidosis. e-Figs. 2–6 show various SAA stain results.

3.1. Various additional SAA stain results

Table 6 displays the Cohen Kappa correlations of the various assessments rendered by the two pathologists. The two