

## Use of Molecular Biology Techniques in Sarcoidal Granulomatous Dermatitis: A Clinicopathological and Molecular Approach with Diagnostic Implications

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Several epidemiological, immunological and molecular studies have been carried out in sarcoidosis; however, there have been no studies on sarcoidal granulomatous dermatitis, its infectious causes and differential diagnoses.

Using molecular biology techniques, the aim of this study was to evaluate the potential role of mycobacteria, bacteria and *Leishmania* in the aetiology of sarcoidal granulomatous dermatitis.

### MATERIALS AND METHODS

A total of 48 skin biopsies, diagnosed as sarcoidal granulomatous dermatitis, were initially retrieved retrospectively (1991–2014). Only those cases with demonstrable negative results in cultures and special stains for bacteria, mycobacteria and fungi were included in the study.

The 16S rRNA primers used were of broad range for all bacteria. *Mycobacterium* spp. DNA was detected with hsp65 and/or 16S-23S rRNA gene primers. FluoroType<sup>®</sup> MTB (Hain Life Science, Nehren, Germany), a newly commercialized fluorescence-based molecular genetic test system, was also used for detection of *Mycobacterium tuberculosis* complex. The presence of *Leishmania* spp. DNA was analysed by amplifying the kinetoplast minicircle DNA sequence via real-time PCR.

### RESULTS

Twelve of the 48 histological specimens were positive for microorganism DNA detection by PCR (Table S1<sup>1</sup>). Mycobacteria DNA was identified in 8 samples, *Leishmania*-specific DNA was identified in 3 cases, and *Pseudomonas aeruginosa* DNA was found in one sample. This result was likely due to contamination during processing.

**Mycobacteria-positive cases.** All 8 cases of mycobacteria were detected only via amplification of the 16S-23S rRNA gene and/or with the FluoroType<sup>®</sup> Kit. Six of these exhibited DNA sequences related to *M. tuberculosis* complex (MTBC), and the remaining 2 cases (patients 4 and 5) showed >90% homology with *M. xenopi*.

Clinically, most of these patients had multiple chronic skin lesions (at least a 2-year history), consisting of nodules, plaques and lupus pernio located predominantly on the face (Fig. S1A, B<sup>1</sup>). Bilateral hilar lymphadenopathy was frequently observed in these patients and they were diagnosed with sarcoidosis. However, case 6 is noteworthy<sup>2</sup>.

Histologically, all of the specimens exhibited epithelioid granulomas without necrosis and a dermal inflammatory infiltrate of lymphocytes and histiocytes throughout the reticular dermis. Multinucleated giant cells were present in the granulomas of all cases; however, plasma cells were absent. Only one biopsy (patient 2) demonstrated focal necrosis.

**Leishmania-positive cases.** Clinically, these 3 patients (1, 3 and 12) had chronic cutaneous lesions with at least a 2-year history, but with no other organ involvement. Cutaneous lesions were single or few nodules, without ulceration, situated predominantly on the extremities (Fig. S1C<sup>1</sup>). All cases were diagnosed as cutaneous sarcoidosis. A clinical diagnosis of cutaneous leishmaniasis was not made in any of the patients.

Histologically, all 3 cases shared the same features: the presence of naked granulomas with few lymphocytes around them; the inflammatory infiltrate of lymphocytes and histiocytes was diffuse and observed in the superficial or mid-dermis; and the presence of multinucleated giant cells and plasma cells was the rule (Fig. S1C<sup>1</sup>).

### DISCUSSION

**Leishmania-specific DNA.** Consistent with previous studies (1–4), the results of the current study show that cutaneous leishmaniasis may be misinterpreted as other granulomatous disorders, especially in chronic forms. Real-time PCR for *Leishmania* spp. enabled the diagnosis of 3 cases of cutaneous leishmaniasis that had been missed on clinicopathological correlation and with haematoxylin and eosin (H&E) and Giemsa stains<sup>3</sup>.

<sup>2</sup>A 53-year-old male, with a past history of hepatitis C virus (HCV) infection, presented with multiple plaque-type skin lesions on his face and ears. The chest X-ray revealed bilateral hilar lymphadenopathy, without ring enhancement or caseation. His serum angiotensin converting enzyme (ACE) level was normal and tuberculin test was negative. Skin biopsy revealed non-caseating granulomas consistent with the diagnosis of sarcoidosis. He was managed with oral corticosteroids with partial clinical response. Considering his underlying condition (HCV) as well as the successive recurrences of skin lesions, a treatment with RIPE (rifampicin, isoniazid, pyrazinamide and ethambutol) was added over the course of 6 months; he subsequently achieved a good clinical response.

<sup>3</sup>All of the cases were misinterpreted as cutaneous sarcoidosis, but clinical and histological re-evaluation of these cases revealed some subtle elements that served as a guide for establishing the correct diagnosis: (i) the predominance of few cutaneous lesions (<3 lesions), with chronic evolution, without systemic involvement and located on the extremities; (ii) the presence of unusual histopathological features, such as sarcoidal granulomas, does not exclude the diagnosis of cutaneous leishmaniasis, as evidenced in our study; (iii) the presence of a diffuse infiltrate of lymphocytes and histiocytes in the superficial dermis, multinucleated giant cells and, especially, plasma cells are important subtle histological features that can lead to the correct diagnosis.

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*Mycobacterium xenopi* DNA. When *M. xenopi* is identified by molecular techniques from a human sample, its clinical significance needs to be determined (5). A clinical and histological re-evaluation of our cases allowed us to find some features that served to confirm that the molecular isolation of *M. xenopi* was not clinically relevant and consequently did not correspond to a true infection. Features that served to confirm that the molecular isolation of *M. xenopi*: (i) the clinical appearance of the lesions strongly resembles sarcoidosis; (ii) the 2 patients were immunocompetent, had no history of chronic exposure to water and the Mantoux test was negative in both of them; (iii) no demonstrable signs of pulmonary involvement or pre-existing pulmonary disease; (iv) treatment with oral corticosteroids resulted in complete resolution of skin lesions.

*Mycobacterium tuberculosis complex* DNA. Our recent meta-analysis (6) compiled the published evidence for the presence of microbial DNA in sarcoidosis patients via molecular biological techniques. We concluded that the balance of evidence from pooled analysis favours association between some microorganisms (i.e. mycobacteria and *Propionibacterium acnes*) and sarcoidosis. However, many of those studies have been criticized for their small sample size and high incidence of false-positive PCR (7, 8). To date, there is a lack of evidence that sarcoidosis is associated with an active, replicating mycobacterial infection (9).

An alternative hypothesis to explain these observations would be that sarcoidosis and tuberculosis could coexist in the same patient. Concerning our study, there was no past history of tuberculosis and the Mantoux test was negative in all of the patients in whom MTBC DNA was identified. In addition, almost all of them had the presence of symmetrical hilar lymphadenopathy, without necrosis or cavitation, which is a radiological finding frequently observed in sarcoidosis and uncommon in tuberculosis.

Finally, in view of the current scarce evidence available, it might be appropriate to consider that sarcoidosis and tuberculosis may represent a spectrum of the same disease. Analogous to leprosy, sarcoidosis might represent the tuberculoid form of the pathological responses to mycobacteria, while pulmonary tuberculosis would represent the opposite end of the same spectrum.

This viewpoint has been suggested previously (10) and was recently taken up by Agrawal et al. (11), who proposed the development of a subdivision classification system for tuberculosis and sarcoidosis. According to the authors, S (sarcoidosis) and TB (tuberculosis) would comprise the opposite pure forms of the same spectrum, whereas ST (sarcoid-tuberculous) and TS (tuberculous-sarcoid) would represent mixed features of both diseases (11).

Such a classification system may, in part, explain the results obtained in our study. The clinical and histopathological manifestations of our 6 patients strongly resemble sarcoidosis. However, in the presence of chronic cutaneous lesions, bilateral hilar lymphadenopathy, positive

PCR results for MTBC DNA and an absence of response to classical treatments for sarcoidosis, one should consider the “grey zone” entities, such as TS and ST. These patients should be treated with combination therapy, with both immunosuppressants and anti-tubercular therapy, so as to allow treatment of the underlying alternative disease adequately, such as it was possible to verify in one of our patients (case 6). The main limitation of this study includes its retrospective design.

In conclusion, PCR techniques are a reliable tool for obtaining an accurate diagnosis, especially for MTBC and *Leishmania* spp. However, not all PCR techniques have the same sensitivity and specificity. This study demonstrates that the 16S-23S rRNA gene primers and the FluoroType® Kit have a high degree of accuracy for detection and identification of *M. tuberculosis* complex. In endemic regions, in particular, PCR for *M. tuberculosis* and *Leishmania* should be performed in the case of any sarcoidal granulomatous skin disease, regardless of its clinical presentation, even when dermatologists and dermatopathologists do not suspect an infectious cause.

The authors declare no conflicts of interest.

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