

Clinical Utility of Polymerase Chain Reaction in the Detection of *Mycobacterium Tuberculosis* in Different Types of Cutaneous Tuberculosis and Tuberculids

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Abstract

Introduction: The role of polymerase chain reaction (PCR) in the diagnosis of cutaneous tuberculosis in clinical practice has not been defined as no PCR assay has been tested in a large-scale clinical study. The objective of this study was to test the clinical utility of a PCR assay in the diagnosis of different types of cutaneous tuberculosis and tuberculids. **Materials and Methods:** Analysis of archival biopsy specimens by a nested PCR assay targeting IS6110 of *Mycobacterium tuberculosis* (M.tb) DNA was performed in a tertiary-care skin hospital in Singapore. PCR results were compared with cultures and concordance with final diagnosis. **Patients and Specimens:** One hundred and nineteen skin biopsies from 105 patients comprising 58 cases of confirmed or highly probable cutaneous tuberculosis, ranging from multibacillary infections to paucibacillary forms and 47 cases of possible tuberculids were analysed. Twenty-four subjects with non-tuberculous granulomas and normal skin controls were included. **Results:** In 14 immunocompromised patients with multibacillary mycobacterial infections (AFB+ on biopsy), PCR was positive in 9 patients. Correlating PCR results with the final diagnosis, the PCR technique was 100% sensitive and specific in this group. In paucibacillary tuberculosis, PCR positivity rates were 55% for tuberculosis verrucosa cutis (38 cases) and 60% for lupus vulgaris (5 cases). When confirmed cases of tuberculosis were considered, the overall sensitivity was 73%. In 26 cases of erythema induratum, PCR was positive in 54% and correlated with a documented response to anti-tuberculous treatment in 80%. **Conclusions:** The use of PCR in the routine diagnostic panel for cutaneous tuberculosis should take into consideration the differential sensitivities for different clinical types. In the setting of an immunocompromised patient with AFB+ lesions, PCR has a definite role in rapid diagnosis and in differentiating atypical mycobacterial infection from tuberculosis. Where paucibacillary tuberculosis is suspected, clinical decision should not be based on PCR results alone. In erythema induratum, we found some correlation between PCR results and response to anti-tuberculous therapy.

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Introduction

Laboratory confirmation of cutaneous tuberculosis has remained difficult using conventional techniques which include the demonstration of acid-fast bacilli (AFB) on histopathologic examination and culture. In tuberculosis verrucosa cutis and lupus vulgaris which are paucibacillary, direct microscopy and tissue cultures lack the specificity and/or sensitivity for the detection of *Mycobacterium tuberculosis* (M.tb). These conditions are often difficult to differentiate from other granulomatous processes of the skin, such as atypical mycobacterial infections and sarcoidosis.

In the last decade, the use of polymerase chain reaction (PCR) for the amplification of M.tb DNA appeared promising in terms of speed and diagnostic applicability in

clinical practice.¹⁻⁷ Various PCR assays have been developed with sensitivity and specificity ranging from 60% to 100%.^{8,9} Published data, indicating the usefulness of PCR in the diagnosis of different forms of cutaneous tuberculosis, have so far been case reports and small case series.¹⁰⁻¹⁵ Using PCR methodology, the identification of M.tb DNA in erythema induratum and papulonecrotic tuberculid, which are believed to be tuberculids, has also confirmed a tuberculous origin in some cases.¹⁶⁻²²

The role of PCR in the diagnosis of cutaneous tuberculosis in clinical practice remains to be defined as no PCR assay has been tested in a large-scale clinical study. There is also little published data on the diagnostic performance of a PCR assay in the different types of cutaneous tuberculosis. The aim of this study was to compare the relative sensitivity

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and specificity of an optimised PCR protocol in different forms of cutaneous tuberculosis as seen in a tertiary skin hospital in Singapore, using archival skin biopsy specimens. We have also analysed cases diagnosed as erythema induratum or nodular vasculitis and erythema nodosum for the presence of *M.tb* DNA.

Materials and Methods

Patients and Specimens

A total of 143 skin biopsy specimens from 129 patients were selected from the histopathology files of the National Skin Centre for PCR analysis. This number included 58 cases of confirmed or highly probable cases of cutaneous tuberculosis and 47 cases of possible tuberculids diagnosed in our institution between 1984 and 1999. We defined confirmed cases of tuberculosis based on positive tissue cultures and/or positive clinical response to treatment with 3- or 4-drug anti-tuberculous treatment. Highly probable cases were those in whom the diagnosis was based on clinicopathologic criteria^{23,24} but which did not fulfil the above criteria. This series of cases also included specimens used in an earlier study in our institution using a different PCR protocol.²⁵ Tissue cultures were performed with Lowenstein Jensen culture medium using standard techniques, with the addition of the Bactec system since 1990.

We categorised the specimens into 4 groups based on the clinicohistological diagnoses:

1. Multibacillary mycobacterial infections (AFB-positive on biopsy) in immunocompromised patients

There were 14 patients from whom 18 specimens were submitted. All of these patients were immunocompromised patients. The medical conditions were as follows: renal transplant (6), HIV infection (2), systemic lupus erythematosus [SLE] (3), dermatomyositis (1), chronic generalised exfoliative dermatitis (1), metastatic nasopharyngeal carcinoma (1) and breast carcinoma (1). These patients were immunocompromised either from the systemic illness and/or therapy they were receiving. One patient acquired HIV infection through a renal transplant overseas. Cutaneous presentations were nodules or plaques, usually multifocal, panniculitis or cellulitis. The skin biopsies were done under the following clinical circumstances: (i) evidence of active tuberculosis elsewhere e.g. in the chest or bone, (ii) past history of tuberculosis including cutaneous tuberculosis in 1 patient, (iii) history of exposure to tuberculosis, (iv) in at least 3 cases, the clinical suspicion of an atypical mycobacterial infection was also considered. All of these cases had histologic features of diffuse or loosely formed granulomas in the dermis or subcutaneous fat. Variable features were dermal or subcutaneous abscesses, caseous necrosis and fibrinoid

necrosis. Abundant AFB were demonstrable on Ziehl-Neelsen stain in all of the cases. Bacterial cultures were done in 15 specimens for both *M.tb* and *Mycobacterium other than tuberculosis* (MOTT). This was positive for *M.tb* in 1 case and MOTT in 2 cases.

2. Paucibacillary tuberculosis

These were cases with a clinicohistological diagnosis of tuberculosis verrucosa cutis (TBVC) and lupus vulgaris (LV), diagnosed by experienced dermatologists. Lesions were classified clinically as TBVC in 38 patients (yielding 44 specimens) and lupus vulgaris in 5. One patient presented with granulomatous facial plaques associated with cervical lymphadenopathy which was AFB-positive on histology but culture-negative. Histopathological findings include typical tuberculoid granulomas consisting of epithelioid cells with or without giant cells, dermal abscesses and dermal necrosis—caseous or fibrinoid. AFBs were not seen on biopsy in all the cases. Cultures were not performed in most of these cases as past experience had shown a poor yield.^{26,27} In 14 cases of TBVC where cultures were done, only 1 case was culture-positive for *M.tb*. Cultures for the 5 cases of lupus vulgaris were not done or not available.

3. Possible tuberculids

(a) Nodular vasculitis or erythema induratum

There were 26 cases selected on the basis of histological diagnosis. The cases of nodular vasculitis had unequivocal features of lobular panniculitis with granulomatous infiltrate and vascular involvement, either infiltration by chronic inflammatory cells or fibrinoid necrosis. Variable features were fat necrosis and neutrophilic abscesses. Those cases diagnosed as erythema induratum had indistinguishable histologic features, except for the absence of vascular involvement. In 3 cases, there was radiological evidence of active or old pulmonary tuberculosis: 1 case showing a cavitating lesion and another 2 scarring in the apices.

(b) Papulonecrotic tuberculid

This was diagnosed in 1 patient who had disseminated papular lesions on the forearms and thighs, whose histology showed the presence of tuberculoid granulomas, caseous necrosis and dermal abscesses.

(c) Erythema nodosum

Twenty cases of histologically proven erythema nodosum were also included in the study. Nine were cases of chronic recurrent erythema nodosum with a duration greater than 6 weeks. None had abnormal chest X-rays.

4. Non-tuberculous granulomas

These cases were included to test the specificity of the PCR assay:

(a) fish-tank granulomas or atypical mycobacterial infections

There were 18 cases selected with characteristic clinical features and majority gave a history of either recreational or occupational exposure to aquarium fish. Two cases had sporotrichoid lesions on the forearm. Two cases showed the presence of a few AFB on biopsy and both were culture-negative for *MOTT*. Another 4 were AFB-negative but culture-positive for *Mycobacterium marinum*.

(b) sarcoidosis – 3 cases

(c) granuloma annulare – 3 cases

Preparation of Specimens for PCR

DNA extraction: 10- μ m thick sections were cut from formalin-fixed paraffin-embedded tissue blocks using ethanol swabbed microtome blades for each section. After deparaffinisation, tissue was pelleted and vacuum-dried before incubation overnight in lysis buffer containing proteinase K at 50°C. DNA was extracted by heat inactivation at 95°C and the supernatant recovered for use as template. 10 μ L of sample was used for each reaction.

PCR protocol: Nested PCR was performed to amplify an insertion sequence IS6110 using 2 sets of primers, as described elsewhere.²⁸ The outer primers were J: 5' – CGG GACCACCCGCGGCAAAGCCCGCAGGAC-3' and K: 5' – CATCGTGGAAGCGACCCGCCAGCCCA GGAT-3' and inner primers IS1: 5'-CCTGCGAGCGT AGGCGTCGG-3' and IS2: 5'-CTCGTCCAGCGCCGCT TCGG-3' to amplify a 123-bp DNA fragment in the second round amplification. The nested protocol was used as previous work in our laboratory using the same set of primers IS1 and IS2 had suggested a suboptimal sensitivity of the PCR protocol when used for paucibacillary tuberculosis.²⁵

The reactions were performed in an automated thermal cycler (Perkin-Elmer). In the first round, samples were denatured at 94°C for 3 min, followed by 45 cycles at 94°C for 1 min, 63°C for 1 min, 72°C for 2 min and a 5-min final extension at 72°C. The second round amplification was performed by denaturation at 94°C for 3 min, followed by 25 cycles at 94°C for 1 min, 72°C for 2 min and a 5-min final extension at 72°C. The PCR product was analysed by electrophoresis in ethidium bromide stained gel and visualised under UV illumination. The PCR products were confirmed by Southern blot hybridisation using a digoxigenin-labelled probe (DIG Nucleic Acid detection Kit, Boehringer Mannheim). The probe sequence was 5'-CTGCCAGGTCGACACAT-3'.

PCR controls: DNA extracted from *M.tb* culture was used as a positive control and negative control was obtained from normal human skin. A reagent control without any template was also run concurrently after every 2 samples. For each sample, a 110-bp human β -globin DNA band was amplified with two specific primers for human β -globin to

verify the DNA quality extracted from the tissue sections.

Skin biopsies from either normal skin or non-tuberculous lesions from the cohort of patients were not available for PCR.

Evaluation of Efficacy of PCR

The sensitivity of the method was tested with purified DNA of a *M.tb* strain and the detection limit was 1.3×10^{-5} ng of *M.tb* DNA or 2 copies of the IS6110 sequence in 50 μ L reaction volume.

PCR results were compared with the culture results, response to therapy and the final diagnosis. The final diagnosis of cutaneous tuberculosis was confirmed when the following criteria were met: (1) positive tissue cultures for *M.tb* or (2) clinical response to anti-tuberculous treatment.

Results

A summary of the PCR results in each patient group is shown in Table I, in comparison with culture results and final diagnosis. All positive PCR results were confirmed by Southern hybridization (Fig. 1).

In group 1, a total of 18 specimens from 14 immunocompromised patients were analysed and a summary of the clinical data and results of microscopy, culture, PCR and the clinical course is shown in Table II. All tissue specimens from patients in group 1 were AFB-positive with the exception of 1 specimen from patient 5, where the lesion from the chest was later diagnosed to be due to a drug eruption. Out of these 14 patients, cultures were positive in

Fig. 1. Agarose gel electrophoresis (A) and southern blot hybridisation (B) of PCR-amplified DNA products. Lanes 1,2, molecular weight (50bp) markers and digoxigenin-labelled molecular weight markers; Lane 3, *M.tb* positive control; Lane 4, normal skin tissue (negative control); Lane 5, reagent control; Lanes 6,7, AFB+ cases in immunocompromised patients (Patients 1 and 3); Lane 8, Atypical mycobacterial infection (*M. marinum* culture+); Lanes 9,10, Tuberculosis verrucosa cutis, with positive clinical response to anti-tuberculous therapy; Lane 11, Erythema nodosum; Lane 12, Erythema induratum, with positive clinical response to anti-tuberculous therapy; Lane 13, Atypical mycobacterial infection (culture -).

TABLE I: GROUPS 1 TO 4—GENERAL SUMMARY OF RESULTS

Group	Clinical diagnosis	No. of patients	AFB+ (No.)	Culture+ (No.)	PCR+ (No.)	Final diagnoses of TB (No.)
1.	Multibacillary mycobacterial infections in immunocompromised patients	14	14	MTB-1 MOTT-2	9	6
2.	Paucibacillary tuberculosis					
	Tuberculosis verrucosa cutis	38	0	1/14	21	8
	Lupus vulgaris	5	0	0	3	1
	Facial plaques in patient with AFB + lymph nodes (C/S -)	1	0	0	1	1
3.	Possible tuberculids					
	Erythema induratum/Nodular vasculitis	26	0	0	14	5
	Erythema nodosum	20	0	0	0	0
	Papulonecrotic tuberculid	1	0	0	0	Data not available
4.	Non-tuberculosis cases					
	MOTT infections	18	2 (C/S MOTT-)	MOTT-4	0	0
	Granuloma annulare	3	0	0	0	0
	Sarcoidosis	3	0	0	0	0
Total		129				

C/S: culture; -: negative; MOTT: *Mycobacterium other than tuberculosis*; MTB: *Mycobacterium tuberculosis*

only 3; 1 for *M.tb* and 2 for *MOTT*. PCR was positive in 9 cases; 1 was culture-positive for *M.tb* and 6 were culture-negative (cultures not sent in 2 cases). The final diagnosis of tuberculosis was confirmed in 6 cases and all were PCR-positive, giving a sensitivity of 100%. The final diagnosis of atypical mycobacterial infection, based on positive cultures for *MOTT* and/or clinical response to appropriate antimicrobial therapy, was made in 3 cases. All were PCR-negative, giving a specificity of 100%. In the remaining cases, the final diagnosis was uncertain as follow-up data were unavailable or inadequate. In patient 6, there was a relapse of lesions after initial response to treatment with clarithromycin and ciprofloxacin. These responded to addition of therapy with rifampicin, isoniazid and ethambutol. The subsequent course was further complicated by a second relapse and the patient is currently on clarithromycin, ciprofloxacin and ethambutol.

In group 2, the group of paucibacillary tuberculosis, 38 patients were diagnosed as TBVC and 5 patients as LV. Out of 14 cases where tissue cultures were done, only 1 case was culture-positive. PCR was positive in 21 cases (55%) of TBVC, including the culture-positive case and 3 cases (60%) of LV. Out of these, the diagnosis of TBVC was confirmed in 8 cases and lupus vulgaris in 1 case. In the remaining cases, no follow up data were available. An interesting case of a patient with granulomatous facial plaques and AFB+ (culture-negative) from the cervical lymph node was also PCR-positive from the skin lesion. She received anti-tuberculous treatment with rifampicin, isoniazid and ethambutol for 2 years with full resolution of

lymphadenopathy and facial lesions. A definite diagnosis of TBVC was made in 3 cases where PCR was negative. The overall sensitivity of the PCR assay in this group of paucibacillary tuberculosis was 73%.

In group 3 comprising possible tuberculids, 26 cases were diagnosed as erythema induratum or nodular vasculitis, out of which 14 (54%) were PCR-positive. In 5 cases where a positive response to anti-tuberculous treatment was documented, PCR was positive in 4 cases. All 20 cases of erythema nodosum and the case of papulonecrotic tuberculid tested were PCR-negative.

Group 4 consisted of a group of non-tuberculous cases comprising 18 cases of atypical mycobacterial infection such as fish-tank granuloma, including 4 culture-positive for *M. marinum*, 3 cases of granuloma annulare and 3 cases of sarcoidosis, were all PCR-negative for *M.tb*.

Discussion

In the past decade, molecular approaches to diagnosis are transforming the investigation of tuberculosis. Rapid diagnoses by PCR with a number of different targets have been described.^{1,3,29-31} The clinical heterogeneity and difficulties in diagnosing cutaneous tuberculosis are well recognised by dermatologists.^{32,33} Although PCR appears to be a promising diagnostic tool in cutaneous tuberculosis, its role in the routine diagnostic panel is uncertain. To our knowledge, there has not been any large-scale study which addresses the comparative usefulness of PCR in the diagnosis of different types of cutaneous tuberculosis. In this study, our aim was to test the utility of a PCR assay with

TABLE II: GROUP 1—AFB+ CASES IN IMMUNOCOMPROMISED PATIENTS
SUMMARY OF CLINICAL DATA, RESULTS OF MICROSCOPY, CULTURE, PCR AND FINAL DIAGNOSES

Patient	Sex/Age	Clinical data	Biopsies	Microscopy* (AFB)	C/S**	PCR	Clinical course	Final diagnosis
1.	F/53	Renal transplant with vulval ulcer	1	+	+	+	Responded to anti-TB treatment	TB
2.	M/40	SLE with nodules on legs and thighs	1	+	-	+	Responded to anti-TB treatment	TB
3.	M/42	Renal transplant with ulcerating nodules on legs	1	+	-	+	Responded to anti-TB treatment	TB
4.	F/45	Metastatic nasopharyngeal carcinoma with vasculitic lesions on calf	1	+	-	-	Data not available	Uncertain
5.	F/44	Renal transplant with tuberculous osteomyelitis on left foot	2 Leg Chest	+ - -	ND ND	+ -	Responded to anti-TB treatment	TB
6.	F/52	SLE with nodules on legs, right forearm P/H cutaneous TB 3 years ago (culture +) treated with 9 months of anti-TB drugs	3 Forearm Thigh Leg	+ + +	- - -	- - -	Relapsed after initial response to clarithromycin and ciprofloxacin. Cleared with addition of anti-TB treatment. Second relapse—currently on clarithromycin, ciprofloxacin and ethambutol	Uncertain
7.	M/69	Generalised exfoliative dermatitis on prednisolone for 3 years. Nodule on left foot	1	+	ND	+	Data not available	Uncertain
8.	M/76	Renal transplant with panniculitis right calf	2	+	-	+	Treated with doxycycline, clarithromycin and ciprofloxacin with no response. Lost to follow-up.	Uncertain
9.	F/43	SLE with nodules on thighs and legs	1	+	-	+	Responded to anti-TB treatment	TB
10.	M/42	HIV infection. Past history of pulmonary TB. Nodules on forearms and legs	1	+	-	+	Responded to anti-TB treatment (Died of AIDS)	TB
11.	F/80	Dermatomyositis on prednisolone Right forearm cellulitis	1	+	-	+	Data not available	Uncertain
12.	M/40	HIV infection Plaque on face	1	+	-	-	Responded to doxycycline and clarithromycin (Died of AIDS)	MOTT
13.	F/33	Renal transplant Nodules right shin	1	+	MOTT	-	Responded to ciprofloxacin	MOTT
14.	F/63	Sporotrichoid nodules right forearm History of breast carcinoma, diabetes mellitus	1	+	MOTT	-	Responded to bactrim	MOTT

MOTT: *Mycobacterium other than tuberculosis*; MTB: *Mycobacterium tuberculosis*; ND: not done; PCR: polymerase chain reaction; SLE: systemic lupus erythematosus

* Microscopy (AFB): microscopy for acid-fast bacilli

** C/S: culture; -: negative for MTB and MOTT

the optimisation of a PCR protocol, in different clinical settings. The PCR assay used detects the insertion sequence IS1160 of *M.tb* which has been found to be very sensitive and specific when compared with bacteriologic and clinical data.^{30,34,35} However, previous experience in our laboratory using the same PCR assay shows that the PCR protocol was not sensitive for paucibacillary tuberculosis. By a nested

PCR procedure, which has been shown to enhance sensitivities of PCR assays,^{28,36} we were able to combine high sensitivity and specificity, as confirmed by the clinical data and treatment response. Our experience shows that archival specimens, some dating back 15 years ago, could be processed for successful PCR amplification.

Studies which evaluate the sensitivity and specificity of

PCR for *M.tb* are often based on culture results for the diagnosis of active tuberculosis. In clinical practice however, the gold standard culture-positive diagnosis of cutaneous tuberculosis cannot be applied. We therefore included cases where clinical suspicion of cutaneous tuberculosis was high enough to warrant anti-tuberculous treatment even when cultures were negative. Cases showing a response to anti-tuberculous therapy were considered true positive cases.

In the group of immunocompromised patients with multibacillary mycobacterial infections, the difficulty of differentiating cutaneous tuberculosis and atypical mycobacterial infections clinically and histologically is well-illustrated in this study. It is noteworthy that even in these AFB+ cases, culture positivity rates were low for both *M.tb* and *MOTT*. In this group of patients, the PCR assay is most useful, being 100% sensitive and 100% specific. In the clinical context of an immunocompromised host presenting with cutaneous lesions which are AFB+ on biopsy, PCR allows the differentiation of cutaneous tuberculosis from atypical mycobacterial infections. In addition, the PCR method also offers rapid diagnosis; the processing time takes 2 working days while conventional cultures by Bactec take at least 2 weeks. This will allow early institution of appropriate treatment which reduces morbidity and mortality.

In the group of paucibacillary tuberculosis, although the clinical forms are well recognised by the clinician, the demonstration of AFB on biopsy and isolation of *M.tb* in culture can be negative in a significant number of cases, as is also our past experience.^{26,27,37,38} In this study, the PCR positivity rates for TBVC and lupus vulgaris were 55% and 60%. Correlating PCR results with the confirmed diagnosis of cutaneous tuberculosis based on our criteria, 8 cases were PCR-positive and 3 were PCR-negative. The overall sensitivity of PCR in this group was 73% and clearly better than that of culture and biopsy demonstration of AFB. Our PCR positivity rates also compare favourably with two other series of lupus vulgaris where cases were analysed by PCR with positivity rates of 53% and 75%.^{14,15}

The role of tuberculosis in the aetiology of erythema induratum/nodular vasculitis and papulonecrotic tuberculid has been confirmed by other workers.¹⁶⁻²² In our experience, out of the 26 cases of erythema induratum/nodular vasculitis that were submitted for PCR, 14 cases (54%) were positive. In the 5 cases where there was documented response to anti-tuberculous treatment, 4 cases were PCR-positive. The case diagnosed to have papulonecrotic tuberculid was PCR-negative. We also explored the possibility of erythema nodosum as a tuberculid and all 20 cases of histologically-proven erythema nodosum were PCR-negative. The prevalence of tuberculosis-related erythema induratum/

nodular vasculitis in our series is similar to a previous report from Spain.²⁰ However, the authors found no correlation between PCR results and response to anti-tuberculosis therapy, contrary to our experience.

The specificity of our PCR assay was also subjected to rigorous testing by including 18 consecutive cases diagnosed as atypical mycobacterial infections, predominantly fish-tank granulomas, 4 cases of which were culture-positive for *M. marinum*. This was of importance as the homology between IS6110 of *M.tb* and DNAs of other *Mycobacterium spp.* was demonstrated in one study.³⁹ We did not find any positive PCR results in these cases.

The data from our study have demonstrated the sensitivity and specificity of an optimised PCR assay based on IS6110 in the diagnosis of cutaneous tuberculosis. The sensitivity varies for different types of lesions within the spectrum of cutaneous tuberculosis and should be borne in mind when a clinician incorporates PCR in the diagnostic panel in cases of suspected tuberculosis. In the context of an immunocompromised host with mycobacterial infections which are AFB-positive on biopsy, the PCR has a clearly defined role as the test is highly sensitive and specific and rapid diagnosis is an added advantage in the management of such patients. In this clinical scenario, differentiation between cutaneous tuberculosis and atypical mycobacterial infection is crucial and early institution of appropriate treatment is important. In paucibacillary cases such as TBVC and lupus vulgaris, the sensitivity of our PCR assay is 73% and thus a negative PCR test does not rule out the diagnosis of cutaneous tuberculosis. In cases where PCR test is positive however, this can enhance the clinician's confidence in instituting anti-tuberculous treatment and reinforcement of patient's compliance with a prolonged treatment regimen. It is interesting to compare the experience from pulmonary tuberculosis where PCR tests are most widely evaluated, that sensitivities of 40% to 70% (overall 60%) and specificity of above 95% have been reported for smear-negative (paucibacillary) disease.⁴⁰

In conclusion, this study to our knowledge, is the largest series which confirms the clinical efficacy of PCR using a nested protocol in the diagnostic panel of cutaneous tuberculosis. We would emphasise that this requires meticulous precautions in the preparation of specimens and experienced personnel in the laboratory. We also recommend the appropriate use of the PCR test with a clear understanding of the differential sensitivities, taking into consideration the clinical setting and information and the individual patient in question. The final diagnosis of cutaneous tuberculosis cannot be made or excluded solely on the PCR result and careful clinicopathological correlation is essential in all cases.

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