

Application of T-SPOT.TB in the Diagnosis of Cutaneous *Mycobacterium marinum* Infections and Evaluation of Treatment Efficacy

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This study evaluated the diagnostic value and therapeutic efficacy assessment potential of the interferon- γ release assay T-SPOT.TB in cutaneous *Mycobacterium marinum* infections. A total of 145 patients diagnosed at the Institute of Dermatology, Chinese Academy of Medical Sciences (2020–2024), excluding tuberculosis cases, were retrospectively analysed. Clinical data including demographics, underlying diseases, pathology, microbiological culture, molecular identification, T-SPOT.TB results, treatment regimens, and outcomes were collected. T-SPOT.TB results before treatment, and at 3 and 6 months post-treatment, were compared to assess its diagnostic and monitoring utility. The baseline T-SPOT.TB positivity rate was 71% (95% CI: 63.2–77.8%). Among 17 patients retested at 3 months, positivity was 64.7% ($p=0.125$), with median spot-forming cells (SFCs) significantly decreasing from 20.0 (IQR 8.5–41.0) to 8.0 (IQR 2.5–28.0) ($p=0.0007$). Among 27 patients retested at 6 months, positivity was 59.3% ($p=0.344$), with median SFCs decreasing from 12.0 (IQR 5.0–30.0) to 8.0 (IQR 3.0–13.0) ($p=0.0006$). No significant difference in positivity rates was observed between 3 and 6 months post-treatment. The T-SPOT.TB assay shows significant diagnostic value for cutaneous *M. marinum* infections and facilitates early diagnosis. Declining SFC counts post-treatment provide useful reference for evaluating therapeutic response.

Key words: T-SPOT.TB; cutaneous *Mycobacterium marinum* infections; diagnostic value.

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Cutaneous *Mycobacterium marinum* infection is caused by a slow-growing non-tuberculous mycobacterium (NTM) widely present in freshwater and marine environments. It infects humans opportunistically through minor skin abrasions, causing chronic granulomatous skin and soft tissue infections (1, 2). First isolated from fish in 1926, its zoonotic potential was confirmed by the first human case reported in 1951 (3, 4). Epide-

SIGNIFICANCE

This study demonstrates that T-SPOT.TB, a standardized interferon- γ release assay (IGRA), can serve as an adjunctive tool for rapid diagnosis and treatment monitoring of cutaneous infections caused by *M. marinum*. Monitoring dynamic changes in T-SPOT.TB reactivity during treatment provides clinicians with objective immunological data to supplement clinical evaluation of treatment efficacy. This approach may facilitate earlier and more precise diagnosis, enabling optimized treatment strategies that reduce therapeutic delays and improve clinical outcomes – particularly in high-risk populations with aquatic exposures, such as aquaculture workers.

miological data remain limited, especially in China, with reported incidence rates varying globally: 0.04/100,000 annually in France (1996–1998) (5), 0.05–0.13 in Denmark (2010–2016) (5), and 0.27 in the United States (6). Recent increases in aquaculture, aquarium ownership, and immunocompromised populations have contributed to rising case numbers (7), suggesting underdiagnosis and underscoring the need for improved surveillance.

Clinical manifestations vary with host immunity, necessitating laboratory confirmation. Traditional diagnostics include acid-fast staining, histopathology, culture, and biochemical identification, each with limitations: low sensitivity and lack of species specificity for staining and histology; prolonged culture times (weeks to months) and susceptibility to prior antibiotics; and molecular methods (PCR, sequencing) offering improved specificity but limited sensitivity on direct specimens and dependence on culture (8, 9). These challenges highlight the need for rapid, sensitive, and specific diagnostic tools.

The T-SPOT.TB assay, an interferon- γ release assay (IGRA), detects T-cell responses to *M. tuberculosis*-specific antigens ESAT-6 and CFP-10 in peripheral blood mononuclear cells (10). While established for tuberculosis diagnosis and monitoring (11), its role in NTM infections is unclear. Notably, *M. marinum* shares >85% genomic similarity with *M. tuberculosis* (12) and expresses the same antigens, explaining frequent false-positive T-SPOT.TB results in cutaneous *M. marinum* infections (13). This study aims to rigorously evaluate the clinical utility of T-SPOT.TB for diagnosing and monitoring cutaneous *M. marinum* infections.

MATERIALS AND METHODS

Data collection

We retrospectively reviewed patients diagnosed with cutaneous *M. marinum* infections at the Dermatology Hospital of the Chinese Academy of Medical Sciences (January 2020–December 2024). Data collected included demographics, underlying diseases, pathology, culture, molecular identification, T-SPOT.TB results, treatment, and outcomes. Inclusion criteria: (i) cutaneous lesions (erythema, nodules, ulcers, abscesses); (ii) histopathology consistent with infectious granulomas or atypical mycobacterial infection; (iii) positive tissue culture with molecular confirmation of *M. marinum*; (iv) T-SPOT.TB performed before antimycobacterial therapy; (v) no history or evidence of tuberculosis (negative *M. tuberculosis* qPCR on skin). Exclusion criteria: (i) positive *M. tuberculosis* qPCR; (ii) history of visceral tuberculosis; (iii) uncertain clinical diagnosis; (iv) prior antimycobacterial treatment. Ethical approval was obtained (Approval No. 2021-KY-013).

T-SPOT.TB assay

T-SPOT.TB was performed at baseline (pre-treatment), 3 months, and 6 months post-treatment, measuring spot-forming cells (SFCs) responding to ESAT-6 (Antigen A) and CFP-10 (Antigen B). Assays and supplementary mycobacteriological tests were conducted in an accredited laboratory. For the interpretation criteria, a result was classified as positive when either the negative control had 0–5 SFCs and the SFC count in Antigen A or Antigen B exceeded the negative control by ≥ 6 , or the negative control had 6–10 SFCs and the SFC count in Antigen A or Antigen B was at least twice that of the negative control.

Statistical analysis

SPSS 22.0 (IBM Corp, Armonk, NY, USA) and GraphPad Prism 10.1 (<https://www.graphpad.com/features>) were used. Categorical variables were frequencies/percentages; continuous variables were mean $\pm$ SD or median (IQR). McNemar test compared paired positive rates; Mann–Whitney *U* test compared independent groups; Wilcoxon signed-rank test compared paired SFCs. $P < 0.05$ was significant.

RESULTS

Baseline clinical characteristics

Among 372 culture-positive cutaneous *M. marinum* cases, 145 met the inclusion criteria (44 males [30.3%], 101 females [69.7%]; age 21–78 years, mean 56.23 ± 11.42). Lesions were localized mainly to the hands/upper limbs (95.9%). Rare sites included lower limbs ($n=2$), face ($n=2$), gluteal region ($n=1$), and 1 disseminated case.

T-SPOT.TB assay results

Baseline T-SPOT.TB was positive in 103/145 (71.0%; 95% CI: 63.2–77.8%). All patients received clarithromycin, moxifloxacin, and rifampin combination therapy. Follow-up T-SPOT.TB was performed in 17 patients at 3 months and 27 at 6 months.

T-SPOT.TB at 3 months post-treatment

Among 17 retested, baseline positivity was 88.2% (15/17), decreasing to 64.7% (11/17) at 3 months ($p=0.125$). Median SFCs dropped significantly from 20.0 (IQR 8.5–41.0) to 8.0 (IQR 2.5–28.0) ($p=0.0007$) (Fig. 1).

T-SPOT.TB at 6 months post-treatment

Among 27 retested, baseline positivity was 74.1% (20/27), decreasing to 59.3% (16/27) at 6 months ($p=0.344$). There was no significant difference in positivity between 3 and 6 months ($p=0.645$). Median SFCs decreased from 12.0 (IQR 5.0–30.0) to 8.0 (IQR 3.0–13.0) ($p=0.0006$) (Fig. 2).

DISCUSSION

Cutaneous *M. marinum* is the leading cause of cutaneous NTM infections, accounting for 50–80% of cases (14, 15), with higher incidence in coastal/lakeside areas and increasing reports worldwide (7, 14, 16). Ageing populations, rising immunocompromised individuals (e.g., diabetes, HIV/AIDS), and expanded immunosuppressive therapies have increased the susceptibility to and severity of infections (17). Aquaculture growth and aquatic recreational activities elevate exposure risk via skin microabrasions. Molecular diagnostics and heightened clinical awareness have improved detection, yet public knowledge and preventive measures remain inadequate, contributing to infection risk.

Misdiagnosis is common; fewer than 60% receive timely diagnosis and treatment within 3 months of symptom onset (5, 18), leading to prolonged disease and resource waste. Diagnosis requires comprehensive assessment including exposure history, lesion morphology, histology, microbiology, molecular tests, and treatment response (19). Culture remains the gold standard but is limited by variable positivity (2.9–96.8%) and long incubation times (19). Molecular methods offer rapid, sensitive species identification but are costly and require specialized facilities, limiting widespread use (8, 20).

The T-SPOT.TB assay, measuring interferon- γ release to ESAT-6 and CFP-10, is a sensitive and specific immunological tool for *M. tuberculosis* diagnosis and monitoring (11, 21, 22). Given the shared RD1 region encoding these antigens in *M. marinum* and other NTMs (23), T-SPOT.TB positivity is common in cutaneous *M. marinum* infections (24), consistent with our 71% posi-

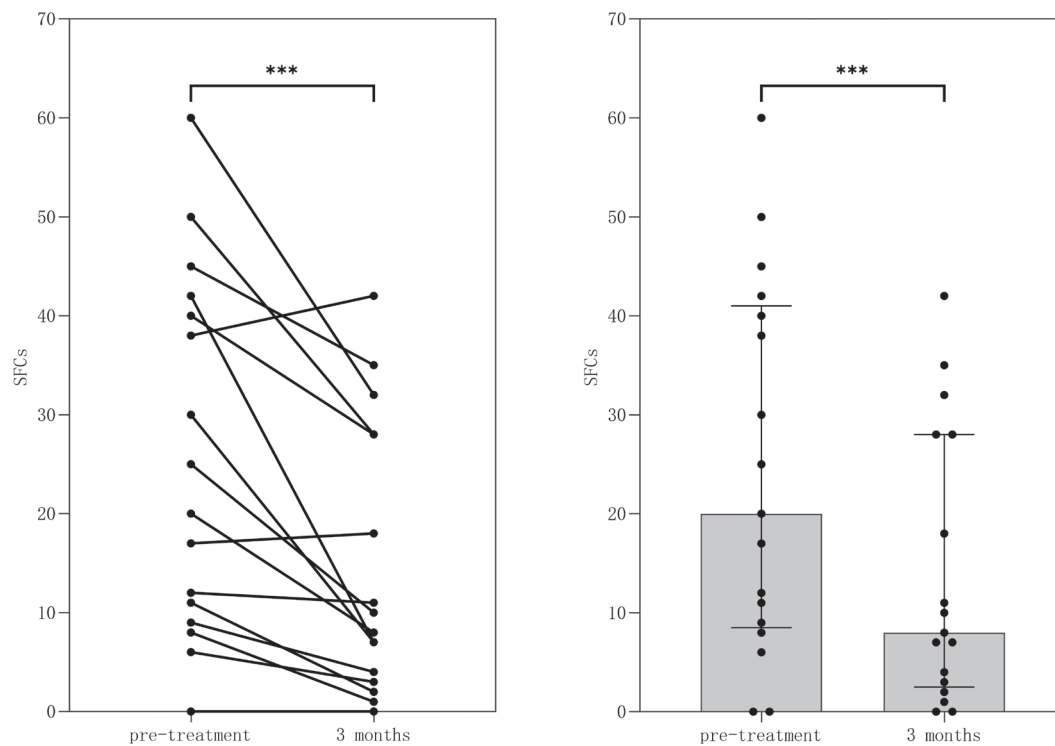


Fig. 1. Changes in SFCs before treatment and at 3 months post-treatment.

tivity rate. Skin infections by other RD1-positive NTMs are rare clinically, making T-SPOT.TB a valuable early screening tool for *M. marinum* infection in patients presenting with granulomatous lesions, a history of aquatic exposure, distal upper limb involvement, and no evidence of pulmonary involvement. Positive results can guide

early empirical therapy pending confirmatory tests (see Fig. 3). However, in tuberculosis (TB) endemic regions, the interpretation of T-SPOT.TB results for *M. marinum* diagnosis requires particular caution due to antigenic similarities between *M. tuberculosis* and *M. marinum*. Before considering T-SPOT.TB as an adjunctive test for

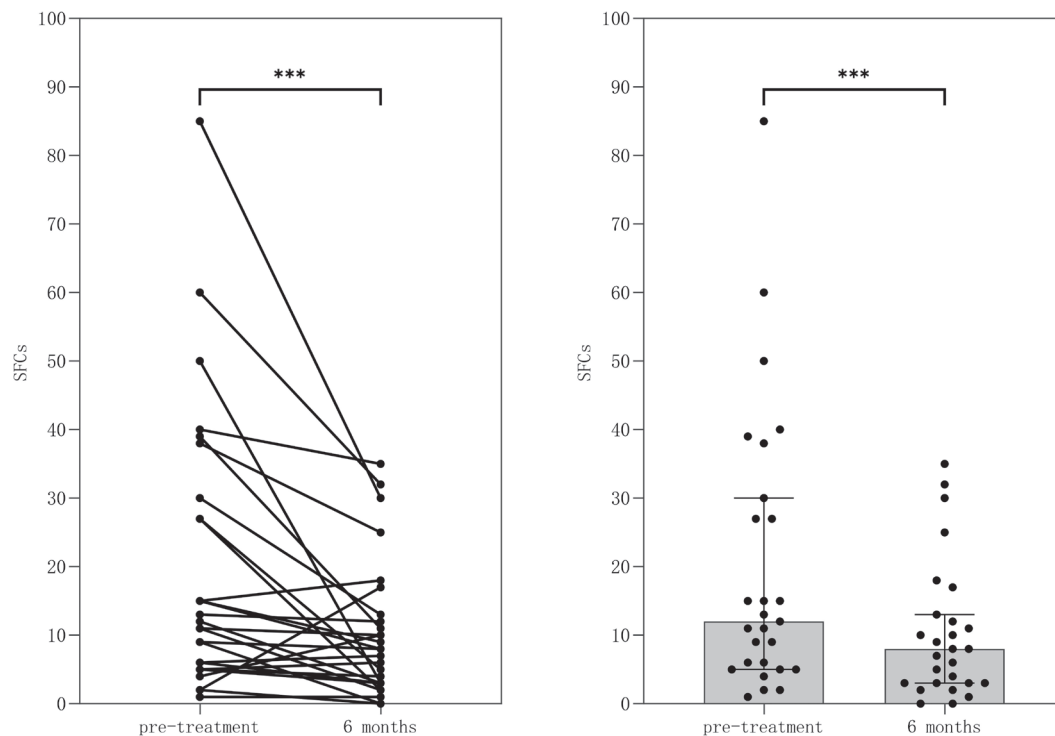


Fig. 2. Changes in SFCs before treatment and at 6 months post-treatment.

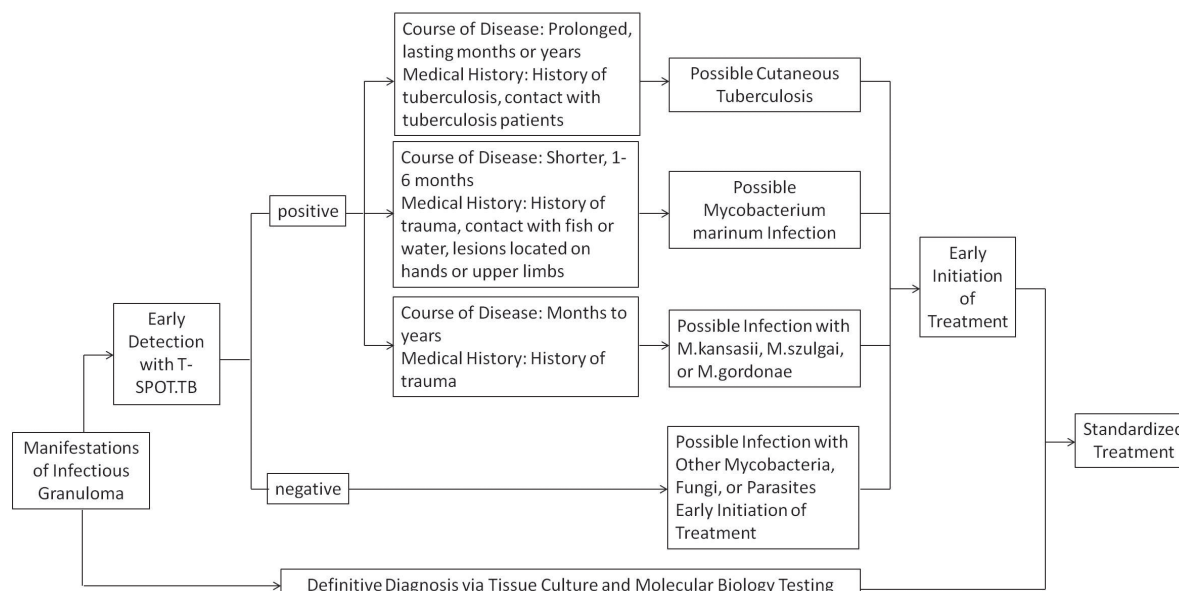


Fig. 3. Diagnostic flowchart for cutaneous *M. marinum* infections.

M. marinum infection, clinicians must systematically rule out tuberculosis through detailed exposure history and chest CT imaging. This exclusion protocol is critical given the assay's inability to differentiate between tuberculous and non-tuberculous mycobacterial infections due to shared RD1 antigens.

Standard treatment includes clarithromycin, moxifloxacin, and rifampin or ethambutol for 4–6 months, continuing 1–2 months after lesion resolution (25–28). While T-SPOT.TB positivity rates did not significantly change post-treatment, SFC counts declined significantly, especially in patients with higher baseline values, suggesting potential utility in monitoring therapeutic response. In clinical management, T-SPOT.TB assay can be performed every 3 months in patients with *M. marinum* infection undergoing standardized therapy. Monitoring SFCs alongside clinical symptom improvement provides a reliable assessment of therapeutic efficacy and informs the determination of the optimal treatment duration. Some patients showed paradoxical SFC increases despite clinical resolution, possibly due to immune reconstitution or residual antigen stimulation (29, 30), warranting further study. Prior research indicates that dynamic T-SPOT.TB changes do not consistently correlate with clinical improvement or pathogen clearance (31, 32), limiting its role as a definitive treatment marker. Our follow-up sample size was limited, and individual variability may bias results. Larger multicentre studies are needed to clarify T-SPOT.TB's role in treatment monitoring.

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The authors have no conflicts of interest to declare.

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