

# Usefulness of Interferon- $\gamma$ Release Assays in the Diagnosis of Erythema Induratum

Cristián Vera-Kellet, MD; Laurena Peters, MD; Kevin Elwood, MD, FRCPC; Jan P. Dutz, MD, FRCPC

**Background:** Erythema induratum (EI) manifests as tender indurated nodules on the lower legs. It may be associated with concomitant active tuberculosis (TB) and is considered a hypersensitivity reaction to mycobacterial antigens. However, the results of *Mycobacterium* cultures are rarely positive, and the tuberculin skin test is of limited usefulness in populations exposed to bacille Calmette-Guérin (BCG). Interferon- $\gamma$  release assays (IGRAs) are alternatives to the tuberculin skin test and have high specificity. We explored the use of IGRAs as an adjunct in the diagnosis of EI. We describe 5 patients with positive tuberculin skin test results and a history of vaccination against BCG or TB in whom IGRAs supported the diagnosis of EI.

**Observations:** All patients were initially seen with tender nodules on the lower legs and a history of BCG vaccination or TB. Tuberculin skin test results were positive, and chest radiographic results were normal. The results of *Mycobacterium* cultures were negative in all patients, and biopsy specimens were compatible with EI. Interferon- $\gamma$  release assays were performed in all patients and supported initiation of anti-TB treatment in 4 of 5 patients.

**Conclusion:** Interferon- $\gamma$  release assays may have value as an adjunct in the diagnosis of EI, particularly in the setting of prior BCG exposure.

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**E**RYPHEMA INDURATUM (EI) IS an uncommon condition that manifests as tender indurated nodules on the lower legs. It was originally described by Ernest Bazin in 1861 as nodular lesions on the legs in association with tuberculosis (TB).<sup>1</sup> The term *tuberculid* refers to a cutaneous eruption that arises in response to *Mycobacterium tuberculosis* or *M tuberculosis* antigens and is suspected to be immunologic in origin. Unlike cutaneous TB (ie, chancre, verrucosa, and scrofuloderma), *M tuberculosis* is seldom found by staining or culture of biopsy specimens in tuberculids; hence, a presumptive diagnosis of EI is made on the basis of characteristic clinical morphologic appearance, positive tuberculin skin test (TST) result, compatible histological findings, and historical evidence of TB exposure.

Erythema induratum is uncommon in North America but is prevalent in India, Hong Kong, and some areas of South Africa. For example, the most common cutaneous manifestation of TB in Hong Kong between 1993 and 2002 was EI (86.4% [127 of 147 TB cases]).<sup>2</sup> These areas have a higher incidence of TB, and vaccination against bacille Calmette-Guérin (BCG) is common. The TST can be used in non-vaccinated populations to investigate the

presence of active or latent TB. However, it is of limited usefulness in populations exposed to BCG. Interferon- $\gamma$  release assays (IGRAs) are alternatives to the TST and have high specificity for identification of *M tuberculosis* infection. The US Centers for Disease Control and Prevention<sup>3</sup> has recently determined that IGRAs are the preferred method of testing for TB in individuals who have received BCG vaccination. Therefore, we examined the use of IGRAs as an adjunct in the diagnosis of EI among individuals previously exposed to BCG vaccination or TB.

## METHODS

We describe 5 patients with suspected EI, a positive TST result, and previous BCG vaccination or TB. Interferon- $\gamma$  release assays (T-SPOT Assay TB test [T-SPOT]; Oxford Immunotec, Oxford, England; and QuantiFERON-TB Gold test [QFT]; Cellestis, Inc, Valencia, California) were performed in all patients. Erythema induratum was diagnosed when a patient was seen with a history of a painful nodular eruption on the lower limbs, pathologic findings were consistent with nodular vasculitis, the TST result was positive, and the eruption responded to anti-TB medication. Polymerase chain reaction (PCR)-based diagnosis of TB was unavailable in our jurisdiction. Interferon- $\gamma$  re-

### Author Affiliations:

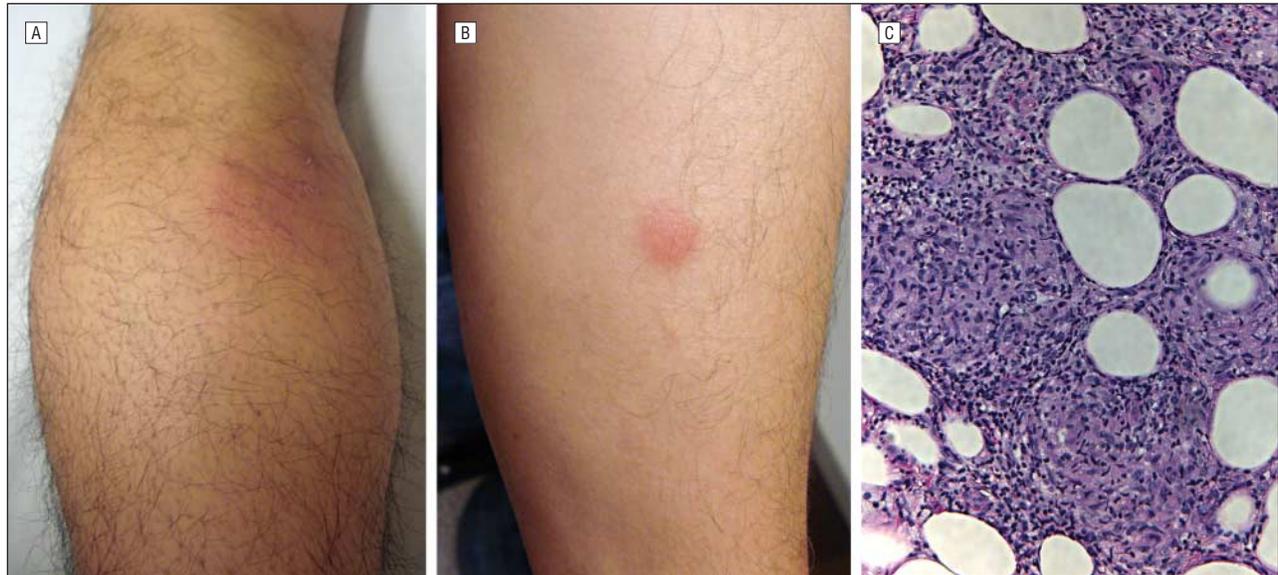
Department of Dermatology and Skin Science, University of British Columbia (Drs Vera-Kellet and Dutz), and Division of Tuberculosis Control, British Columbia Centre for Disease Control (Drs Peters and Elwood), Vancouver, Canada; and Department of Dermatology, Pontificia Universidad Católica de Chile, Santiago (Dr Vera-Kellet).

**Table. Clinical Characteristics of the Patients<sup>a</sup>**

Patient No./ Sex/Age, y	History	Biopsy Findings	Tuberculin Skin Test Result, mm	T-SPOT Result	QTF Result, IU/L	BCG Exposure	Outcome
1/F/51	10-y Recurrent tender nodules on left thigh	Necrotizing panniculitis	+, Size not documented	+	+, 7.86	+	6-mo Anti-TB treatment, with complete resolution
2/F/50	7-y Recurrent mildly atrophic plaque on medial aspect of right shin	Mixed lobular and septal panniculitis	10	+	-, 0.08	+	6-mo Standard treatment, with a decrease in frequency of nodular eruptions (ie, partial response)
3/F/49	8-mo Tender nodules on lower legs, pulmonary TB 22 y earlier	Mixed lobular and septal panniculitis	22	Strongly +	Not available	No	6-mo Anti-TB treatment, with complete resolution
4/F/35	3-y Recurrent nodules on extensor aspect of left lower leg	Granulomatous panniculitis	17	+	+, 1.97	+	9-mo Isoniazid and vitamin B <sub>6</sub> treatment, with complete resolution
5/M/51	6-mo Recurrent tender nodules on posterior legs and lateral thighs	Necrotizing lobular granulomatous panniculitis	10	+	+, 7.77	+	9-mo Isoniazid and vitamin B <sub>6</sub> treatment, with complete resolution

Abbreviations: BCG, bacille Calmette-Guérin; QTF, QuantiFERON-TB Gold test (Cellestis, Inc, Valencia, California); TB, tuberculosis; T-SPOT, T-SPOT Assay TB test (Oxford Immunotec, Oxford, England); +, positive; -, negative.

<sup>a</sup>All patients had normal chest radiographic results.



**Figure.** Patient 5 (Table). A, Posterior left calf showing a proximal subcutaneous nodule with overlying erythema. B, Volar left forearm at 48 hours after tuberculin skin test. C, Histopathologic findings of granulomatous lobular panniculitis (hematoxylin-eosin, original magnification  $\times 200$ ).

lease assays were interpreted according to guidelines published by the US Centers for Disease Control and Prevention.<sup>3</sup>

## RESULTS

Five patients with suspected EI were followed up by 2 of us (C.V.-K. and J.P.D.) between January 1, 2005, and December 31, 2009. Clinical characteristics of the patients are given in the **Table**.

An example of a case with prior BCG exposure is patient 5 (Table), a 51-year-old man with a 6-month history of recurrent tender nodules on the posterior aspects of his legs (**Figure**, A) and the lateral aspects of his thighs. His TST result was positive (Figure, B). Results of a skin biopsy specimen were compatible with nec-

rotizing panniculitis (Figure, C). *Mycobacterium* culture results were negative. His chest radiographic results were normal. He had been vaccinated against BCG. Interferon- $\gamma$  release assays were performed, and the results of the T-SPOT and the QTF were positive. The patient received 9 months of isoniazid and vitamin B<sub>6</sub> therapy and experienced complete resolution of the lesions.

An example of a case without prior BCG exposure is patient 3 (Table), a 49-year-old woman with an 8-month history of tender nodules on her lower legs. In 1987, the results of a sputum culture had been positive for *M tuberculosis*, and she had completed standard treatment that year. Erythema induratum was suspected 22 years later, and a skin biopsy specimen showed mixed lobular and septal panniculitis compatible with EI. Results of her My-

*cobacterium* culture were negative, her TST result was strongly positive, and her T-SPOT result was positive. Her condition improved after a further course of anti-TB therapy.

## COMMENT

Erythema induratum usually manifests as recurrent flares of violaceous nodules on the legs in young to middle-aged women. The lesions are cold, are sometimes painless, have a tendency to manifest central ulceration, and may heal with scars. The most frequent locations of these lesions are the posterior aspect (ie, the calves) and anterolateral areas of the legs. The feet, thighs, arms, and face can be involved.<sup>4</sup> The histologic features of EI include lobular panniculitis associated with granulomatous inflammation with focal necrosis, vasculitis, and septal fibrosis. These findings are not specific; they vary depending on the age of the lesion and overlap with other forms of panniculitis.<sup>4</sup> Nevertheless, the presence of septal granulomatous inflammation and lobular granulomatous inflammation is characteristic of EI and contrasts with the primary septal inflammation in erythema nodosum.

The association of EI with TB used to be controversial because mycobacteria are rarely cultured from skin lesions.<sup>1</sup> The term *nodular vasculitis* is used to describe chronic inflammatory nodules of the legs that show histopathologic changes similar to those of EI but are unassociated with TB.<sup>5</sup> The absence of acid-fast bacilli, detectable by microscopy, in tuberculid skin lesions may be the result of few bacilli. Another theory is that the lesions are secondary to a systemic reaction induced by circulating *M tuberculosis* fragments. Evidence that *M tuberculosis* is involved in the pathogenesis of EI is supported by the detection of *M tuberculosis* DNA in skin lesions, a strongly positive TST result, and resolution of the skin lesions with anti-TB therapy.<sup>6</sup> If skin biopsy specimens are evaluated for *M tuberculosis* using pathogen-specific DNA testing by PCR with primers for the repeat sequence IS6110, *M tuberculosis* is found in 25% to 100% of cases.<sup>7-9</sup> False-negative PCR test results have been ascribed to degraded target DNA, diminished PCR amplification secondary to the formalin used, or insufficient target DNA.<sup>10</sup> In the series of cutaneous TB cases from Hong Kong, 5 of 7 patients with EI who underwent PCR testing had positive results.<sup>2</sup>

The standard test for active or latent infection with *M tuberculosis* is the TST. Previous BCG vaccination can confound the results of a TST interpretation because it increases the likelihood of a positive TST result.<sup>11</sup> A meta-analysis<sup>12</sup> of the evidence from 1966 to 1999 for the effect of BCG vaccination on TSTs in individuals without active TB showed that positive TST results with indurations of at least 15 mm are more likely to be the result of TB infection than of BCG vaccination. Four of our 5 patients had a history of BCG vaccination, and 2 of 4 had TSTs with indurations less than 15 mm, rendering the results uninterpretable.

Interferon- $\gamma$  release assays are a reasonable option for patients who want more specific evidence of latent infection before undergoing a complete course of anti-TB treat-

ment, which has potentially toxic effects. Two different IGRAs are available to measure responses to the *Mycobacterium*-specific antigens: early secretory antigenic target 6 (ESAT-6) and cultured filtrate protein 10 (CFP-10). The QTF is based on a whole-blood enzyme-linked immunosorbent assay, and the T-SPOT is based on an ex vivo overnight interferon- $\gamma$  enzyme-linked immunospot technique to measure peripheral blood mononuclear cell-derived interferon- $\gamma$ .<sup>13</sup> The tests are similar in terms of antigens (ie, ESAT-6 and CFP-10) and incubation time (ie, overnight or 16-24 hours). Because the genes encoding these antigens are found in the region of difference 1 of the *M tuberculosis* genome, which is not present in the genomes of BCG, *Mycobacterium bovis*, or *Mycobacterium avium*, IGRAs do not yield false-positive results in populations exposed to BCG or *M avium*.<sup>14,15</sup> In contrast, infections with *Mycobacterium marinum*, *Mycobacterium kansasii*, and *Mycobacterium szulgai* cannot be distinguished from *M tuberculosis* using IGRAs because these organisms share the same antigen set.<sup>16</sup> The TST uses a mix of antigens from *M tuberculosis*, causing false-positive reactions in patients with BCG vaccination or *M avium* sensitization. Further advantages of IGRAs compared with the TST are that IGRAs require only 1 patient visit and that these assays are ex vivo tests, reducing the risk for adverse effects and eliminating potential boosting when testing is repeated.<sup>17</sup> The disadvantages of IGRAs compared with the TST include higher cost, the need for careful handling of drawn blood to maintain the viability of lymphocytes, and the requirement of a fully equipped laboratory with appropriately trained staff.

The number of interferon- $\gamma$ -producing antigen-specific effector T cells decreases significantly in most patients with TB between diagnosis and treatment completion, but IGRA reversion is exceptional.<sup>18-20</sup> Patients without IGRA reversion have a significantly higher baseline TST result.<sup>19</sup> It has been suggested that IGRAs may be used to monitor treatment efficacy or risk of relapse. In this regard, it is notable that patient 3 (Table), who had previously been treated for TB and thereafter had developed EI, had a strongly positive T-SPOT result. Patients with QTF scores in our series had a high level of response (range, 1.97-7.86 IU/L; positive cutoff, >0.35 IU/L), suggesting that high immune reactivity to *M tuberculosis* may correlate with the development of EI.

Chee et al<sup>21</sup> demonstrated a disparity in the performance of IGRAs among different racial/ethnic groups, with an increased likelihood of indeterminate QTF results among Malays and Indians compared with Chinese patients, as well as diminished T-cell responses to the *M tuberculosis*-specific antigens in Malays (with T-SPOT assay) and Indians (with T-SPOT and QTF assays). In their study, factors independently associated with indeterminate QTF results and with positive T-SPOT results were female sex, age older than 60 years, and non-Chinese (ie, Indian or Malay) race/ethnicity. Of note, our patient with discordant T-SPOT and QTF results (patient 2 in the Table) was a woman from India. Furthermore, she had an indeterminate response to anti-TB therapy and could not unequivocally be diagnosed as having EI.

Interferon- $\gamma$  release assays are being evaluated as tools to aid in the detection of occult *M tuberculosis* infection,

such as in the setting of tumor necrosis factor inhibitor therapy. Findings in our patient series suggest that IGRAs may have value as an adjunct in the diagnosis of EI, particularly in the setting of prior BCG exposure. All patients with suspected EI and a positive QTF test result responded to anti-TB therapy. The relative value in the cost-efficacy and specificity-sensitivity of DNA testing compared with IGRA testing for EI remains to be studied. Furthermore, the occurrence of prior BCG vaccination in 4 of 5 patients studied herein suggests that this may be a risk factor for the development of EI. Alternately, provenance from an area where TB is endemic (and where BCG vaccination is administered) may be the sole risk factor for the development of EI. A comparison of EI in TB-exposed populations with vs without prior BCG exposure may elucidate this possibility. Interferon- $\gamma$  release assays indicate only sensitization to TB and are not used to diagnose active infection as a rule. Although none of our patients had clinically active TB infection, positive TST and IGRA results should prompt clinical examination to rule out active infection. Finally, although we highlight the usefulness of IGRAs to support a diagnosis of EI in the setting of known previous BCG or TB exposure, TSTs remain a valuable tool for the diagnosis of EI in the absence of BCG exposure.

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**Correspondence:** Jan P. Dutz, MD, FRCPC, Department of Dermatology and Skin Science, University of British Columbia, 835 W 10th Ave, Vancouver, BC V5Z 4E8, Canada (dutz@interchange.ubc.ca).

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