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LETTER TO THE EDITOR

Diagnosis of occult tuberculosis in hematological malignancy by enumeration of antigen-specific T cells

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Hairy cell leukemia is a rare indolent lymphoproliferative malignancy characterized by splenomegaly and pancytopenia. Patients are prone to acquire life-threatening infections and can develop mycobacterial diseases.[1] Traditional tools for tuberculosis diagnosis rely on the identification of mycobacteria through microscopy and culture. These methods have low sensitivity and so cannot usefully rule out a diagnosis of tuberculosis where this is suspected. Moreover, mycobacterial culture can take several weeks, often delaying diagnosis and initiation of therapy. As a result, a significant proportion of tuberculosis patients are diagnosed only at autopsy. This diagnostic delay, and its adverse consequences, are even greater in immunosuppressed patients in whom the clinical presentation is frequently nonspecific and the progression of tuberculosis is more rapid.

The tuberculin skin test (TST) confirms infection and not active disease, but in low burden countries (where the prevalence of latent tuberculosis infection in the general population is low), a positive TST result can help in the decision to start treatment while mycobacterial culture results are awaited, or support a clinical diagnosis of tuberculosis when bacteriological confirmation is lacking. Conversely, a negative result might, in theory, help to rule out a diagnosis of tuberculosis. However, the TST is of little help in immunosuppressed patients, such as those with hematological malignancies, who often have falsely negative TST results.[2] A test of *Mycobacterium tuberculosis* infection with high diagnostic sensitivity would therefore be useful in the evaluation of immunosuppressed patients where tuberculosis is included in the differential diagnosis. The *ex vivo* enzyme-linked immunospot (ELISPOT) assay is a very sensitive method for detecting low frequencies of antigen-specific interferon-γ-producing T cells direct from peripheral blood. Thus, it may provide a more sensitive means of detecting *M. tuberculosis*-specific immune responses than the TST, which measures cutaneous delayed-type hypersensitivity to purified protein derivative (PPD). Two *M. tuberculosis* gene products, early secretory antigen target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10), are strong targets of cellular immune response in tuberculosis patients and contacts.[3] Recently, the ELISPOT-RD1 assay for interferon-γ-producing T-cells specific for ESAT-6 or CFP-10 peptides, has been demonstrated to be a rapid, highly sensitive and specific diagnostic tool for the detection of *M. tuberculosis* infection, including in immunosuppressed individuals.[4–6] Here, we describe how the ELISPOT-RD1 accelerated the diagnosis of disseminated tuberculosis in a leukemic patient with persistent fever and a negative TST.

A 68-year-old Caucasian woman presented with fatigue, weight loss, night sweats and fever. Her medical history revealed that her mother died of tuberculous osteomyelitis. Physical examination showed splenomegaly, without peripheral lymphadenopathy. The peripheral blood count showed hemo-

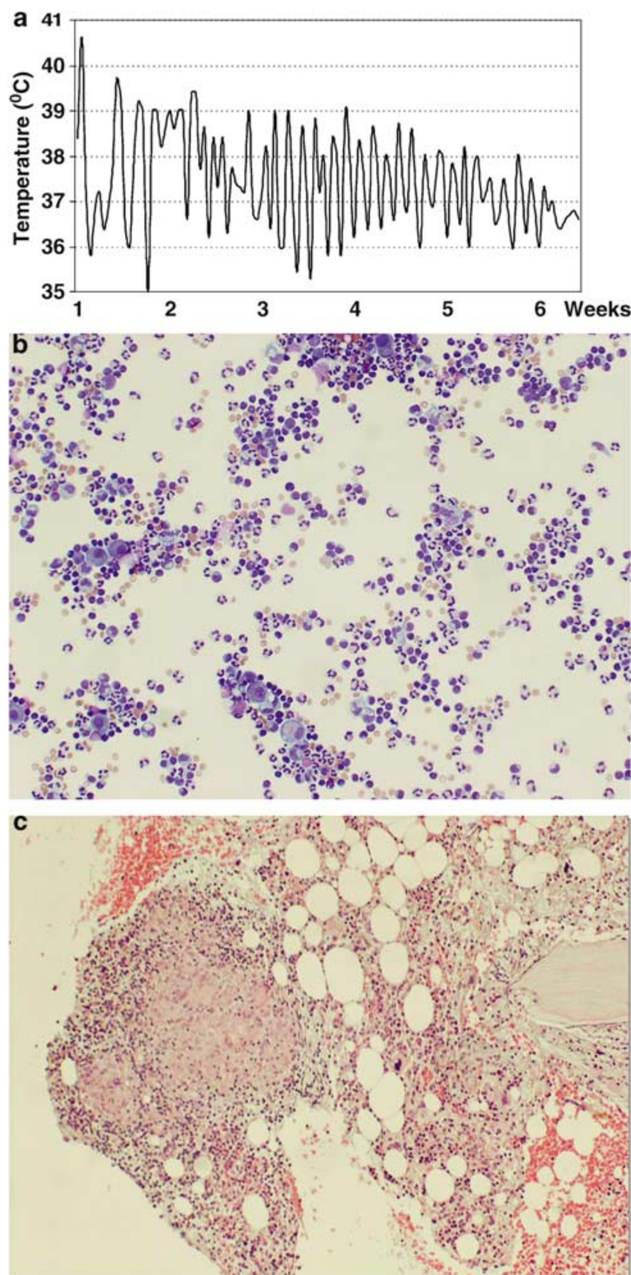

Figure 1 (**a**) Fever chart, showing the intermittent course of body temperature, and the progressive slow down after starting of the anti-TB treatment. (**b**) Pleural fluid staining (May–Grunwald–Giemsa, 130 ×), showing a prevalence of neutrophils, with few lymphocytes, mesothelial cells and rare red blood cells. No malignant cells are present. (**c**) Bone marrow biopsy (Hematoxylin–Eosin staining, 100 ×) with a 30% infiltration of HCL cells (significantly reduced, compared to the 90% infiltration in the bone marrow biopsy before starting interferon-α 2β therapy); two noncaseating granulomas are present. Acid-fast staining was negative.

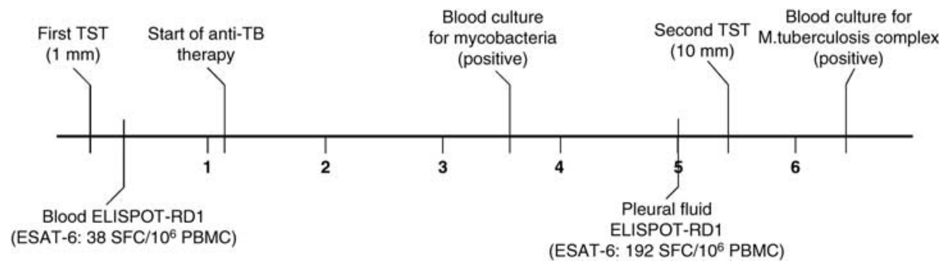

Figure 2 Timeline of the clinical events in the case reported. Dates are reported as weeks from first TST. ELISPOT-RD1 results are given as frequency of ESAT-6-specific interferon-gamma-secreting T cells (spot forming cells (SFC)) per million peripheral mononuclear cells (PBMC). All test conditions, including positive control (PHA), negative control (medium only) and test wells for recombinant ESAT-6 antigen and recombinant CFP-10 antigen, were performed in duplicate wells containing 250 000 PBMC each, as previously described.[4]

globin 9.0 g/dl, white blood cells 2.2×10^9/l and platelet count 69×10^9/l. A bone marrow biopsy revealed 90% of cells expressing CD20, CD25, CD11c and CD103, consistent with a diagnosis of hairy cell leukemia. An abdominal computed tomography (CT) scan confirmed the splenomegaly, with multiple hypodense lesions. A chest radiogram was normal. Therefore, the patient was treated with interferon-α2b, 2×10^6/m^2 IU three times a week. After 2 months, she was readmitted to the hospital for persistent fever. Despite improvement in the blood count, an abdominal CT scan showed the splenic lesions to be unchanged. Chest CT scan revealed minimal bilateral pleural effusions. At that time, interferon therapy was withdrawn. The day after admission, samples from blood, urine, faeces and pleural fluid were obtained for microbiologic examination. The following day the patient underwent bronchoscopy with bronchoalveolar lavage (BAL). TST was negative (induration 1 mm after 72 h, using 5 IU of PPD). At 1 week after admission, despite step-wise usage of empirical antimicrobial treatment the fever persisted and the patient's condition worsened (Figure 1a). All tests for mycobacterial, bacterial, fungal and viral pathogens were negative. Similarly, microscopic examination for acid-fast bacilli was negative. Pleural fluid microscopy yielded an inflammatory effusion with a prevalence of neutrophils together with a small number of macrophages, lymphocytes and activated mesothelial cells (Figure 1b). Recognising the possibility that the TST result in this immunosuppressed patient might be falsely negative, we performed an *ex vivo* ELISPOT-RD1 test on blood, as previously described.[4]

The ELISPOT-RD1 assay enumerated 38 spot forming cells (SFC) per million peripheral blood mononuclear cells (PBMC) in response to recombinant ESAT-6 in the blood sample. Although slightly below the usual cutoff of 40 SFC per million PBMC, this level of T-cells specific for ESAT-6 in this patient strongly suggested *M. tuberculosis* infection. This is because T cells in leukemic patients show phenotypic and functional abnormalities,[7] with low numbers of CD4+/CD45RO+ memory T cells,[8] the main contributors to IFN-γ production. Indeed, in our patient, the overall number of peripheral blood T cells was approximately one third of normal (13 vs 25–35% in normal individuals). Thus, the fact that this ELISPOT-RD1 assay was performed with about one third of the number of T cells usually used justified the use of a lower threshold for a positive test result. This was also reflected in the weak result obtained in the positive control wells (Phytohemaglutinin: 140 SFC/million PBMC), which usually have more than 500 SFC/million PBMC. Therefore, the ELISPOT-RD1 response to ESAT-6 was interpreted as indicative of tuberculosis infection and accelerated the diagnosis, prompting the initiation of antituberculosis therapy. After 4 weeks, the patient developed a large pleural effusion. Although the cellular infiltrate was atypical (Figure 1b), an ELISPOT-RD1 test on a sample of pleural fluid tested strongly positive (ESAT-6: 192; CFP-10: 424 SFC/million PBMC). Bone marrow histological examination showed reduction of leukemic infiltration to 30% but revealed the presence of a noncaseating granulomatous reaction (Figure 1c). Acid-fast staining was negative. At 18 days after initiation of anti-TB therapy based upon ELISPOT-RD1 results, a peripheral blood sample that had been taken the day of admission grew a mycobacterial species, which was subsequently identified 3 weeks later as *M. tuberculosis*, thus confirming the diagnosis of disseminated tuberculosis (Figure 2). This was the only microbiologic evidence of tuberculosis, since culture of pleural fluid, BAL and bone marrow were all negative. At 1 month after starting anti-tuberculosis therapy the TST turned positive (10 mm) and the ELISPOT-RD1 remained persistently positive both in blood and in pleural fluid. After 2 months of anti-tuberculosis therapy, the patient was apyrexial and CT scan showed the resolution of the pleural effusions. Abdominal CT scan revealed the disappearance of most and the reduction in size of the remaining splenic lesions, indicating that the splenic abnormalities were secondary to disseminated tuberculosis.

The ELISPOT-RD1 detected *M. tuberculosis* infection, and enabled an early diagnosis of active tuberculosis in our immunosuppressed TST-negative patient in the absence of any other finding suggestive of active tuberculosis and before the identification of mycobacteria in blood cultures. Therefore, the ELISPOT-RD1 accelerated the correct diagnosis. Moreover, the pleural fluid, with a cellular infiltrate that was atypical for tuberculosis, also tested strongly positive by ELISPOT-RD1, consistent with homing of ESAT-6-specific IFN-γ-secreting T cells to sites of active TB disease. The results reported here represent the first application of the ELISPOT-RD1 to the timely diagnosis of disseminated tuberculosis in patients with hematological malignancies. Large prospective studies are now warranted to validate the clinical utility of ELISPOT-RD1 in hematological patients with suspected tuberculosis in routine practice.

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References

1 Weinstein RA, Gelmann E, Golomb HM. Disseminated atypical mycobacterial infection in hairy-cell leukemia. *Lancet* 1978; **2**: 1052–1053.
2 Huebner RE, Schein MF, Bass Jr JB. The tuberculin skin test. *Clin Infect Dis* 1993; **17**: 968–975.
3 Lalvani A, Pathan AA, McShane H, Wilkinson RJ, Latif M, Conlon CP *et al.* Rapid detection of Mycobacterium tuberculosis infection by enumeration of antigen-specific T cells. *Am J Respir Crit Care Med* 2001; **163**: 824–828.
4 Richeldi L, Ewer K, Losi M, Hansell DM, Roversi P, Fabbri LM *et al.* Early diagnosis of subclinical multidrug-resistant tuberculosis. *Ann Intern Med* 2004; **140**: 709–713.
5 Ewer K, Deeks J, Alvarez L, Bryant G, Waller S, Andersen P *et al.* Comparison of T-cell-based assay with tuberculin skin test for diagnosis of Mycobacterium tuberculosis infection in a school tuberculosis outbreak. *Lancet* 2003; **361**: 1168–1173.
6 Liebeschuetz S, Bamber S, Ewer K, Deeks J, Pathan AA, Lalvani A. Diagnosis of tuberculosis in South African children with a T-cell-based assay: a prospective cohort study. *Lancet* 2004; **364**: 2196–2203.
7 Ruco LP, Procopio A, Maccallini V, Calogero A, Uccini S, Annino L *et al.* Severe deficiency of natural killer activity in the peripheral blood of patients with hairy cell leukemia. *Blood* 1983; **61**: 1132–1137.
8 van der Horst FA, van der Marel A, den Ottolander GJ, Kluin-Nelemans HC. Decrease of memory T helper cells (CD4+ CD45R0+) in hairy cell leukemia. *Leukemia* 1993; **7**: 46–50.