CASE REPORTS

Assessment of Aspergillus-Specific T Cells for Diagnosis of Invasive Aspergillosis in a Leukemic Child with Liver Lesions Mimicking Hepatosplenic Candidiasis

Leonardo Potenza,† Patrizia Barozzi, Giulio Rossi, Giovanni Palazzi, Daniela Vallerini, Giovanni Riva, Monica Cellini, Monica Morselli, Francesco Volzone, Claudia Venturelli, Chiara Quadrelli, Lucia Di Pancrazio, Maria Carmen Cano, Paolo Paolucci, Giuseppe Torelli, and Mario Luppi*

Department of Oncology and Hematology, Section of Hematology, University of Modena and Reggio Emilia, Azienda Ospedaliera Policlinico di Modena, Modena, Italy

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A child with acute myeloid leukemia presented with multiple liver lesions mimicking hepatosplenic candidiasis during the neutropenic phase following the induction chemotherapy. All the available diagnostic tools showed repeatedly negative results, including galactomannan. An enzyme-linked immunospot (ELISPOT) assay showed a high number of Aspergillus-specific T cells producing interleukin-10 [TH2(IL-10)] and a low number of Aspergillus-specific T cells producing gamma interferon [TH1(IFN-γ)], revealing invasive aspergillosis (IA) before the confirmatory biopsy. A progressive skewing from the predominance of TH2(IL-10) to a predominance of TH1(IFN-γ) was observed close to the complete resolution of the infection and foreshadowed the outcome. The ELISPOT assay holds promise for diagnosing pediatric IA.

CASE REPORT

On day 9 of induction chemotherapy (10 mg/m² idarubicin, days 1 to 3; 200 mg/m² cytarabine, days 1 to 7; 100 mg/m² etoposide, days 1 to 5), an 11-year-old child with acute myeloid leukemia (AML) presented with febrile neutropenia. A complete cultural and molecular examination (CCME) of blood, urine, and stool was repeatedly unrevealing. The galactoman- nan antigenemia (GM) test, performed twice a week, and the complete pulmonary radiological study (PRxS) results were negative. Empirical antibiotic therapy was ineffective, and empirical intravenous fluconazole (12 mg/kg of body weight/day) was undertaken. On day 28, the febrile patient presented with pain in the right flank and abdominal tenderness. Laboratory tests showed neutrophil recovery, a slight increase in levels of transaminases, and a marked increase of alkaline phosphatase. On day 33, an abdominal ultrasound (AUS) disclosed multiple hypoechogenic liver lesions, with sparse hyperechoic features (Fig. 1A). PRxS results remained negative. Fluconazole was replaced by liposomal amphotericin B (L-AMB) (1.5 mg/kg endovenously), with improvement of the symptoms. On day 42, the patient, showing complete remission of AML, underwent a second chemotherapeutic course with the same schedule. Concomitantly, the lesions persisted without mutations as determined by AUS. On day 45, the fever again rose above 38°C. CCME and GM results remained negative. Empirical antibiotic therapy was reintroduced, and L-AMB was increased to 3 mg/kg of body weight/day. On day 51, AUS revealed a slight reduction in the sizes of the liver lesions. Nonetheless, fever persisted until bone marrow recovery. On day 62, a third course was undertaken (cytarabine, 3 g/m² twice a day, days 1 to 3; etoposide, 125 mg/m², days 2 to 5). On day 69, after a further febrile episode, AUS displayed an enlargement of the liver lesions (Fig. 1B). CCME and GM results continued to be negative. We judged it appropriate to explore the patient’s Aspergillus-specific T-cell responses by a recently described application of an enzyme-linked immunospot (ELISPOT) assay to definitely rule out invasive aspergillosis (IA) (9). On day 75, the peripheral blood sample collected for the ELISPOT assay was positive for a high number of Aspergillus-specific T cells producing interleukin-10 [TH2(IL-10)] and for a low number of Aspergillus-specific T cells producing gamma interferon [TH1(IFN-γ)], revealing invasive aspergillosis upon either a histologic (Fig. 2A and B) or a cultural (not shown) examination. Caspofungin (50 mg/day) was associated with L-AMB. On day 84, an ELISPOT assay confirmed a very high number of TH2(IL-10) and a low number of TH1(IFN-γ) (Fig. 1G). On day 95, the fever resolved. On day 102, an AUS showed a slight decrease in the sizes of the liver lesions (Fig. 1C). Eight days later, an ELISPOT assay demonstrated a sustained number of TH2(IL-10) and a stable number of TH1(IFN-γ)
FIG. 1. Outcomes of liver lesions and kinetics of the Aspergillus-specific T-cell responses in the patient. (A to E) Liver AUSs of the patient showing multiple hypoechoic nodular lesions (arrows) with scanty hyperechoic features (arrowheads) at the onset of symptoms on day 33 (A) and during the antifungal treatment on days 69, 102, 132, and 170 (B to E, respectively). (F) AUS showing a homogeneous liver at the complete resolution of IA on day 219 of the treatment. (G) Kinetics of Aspergillus-specific T-cell responses, determined by the IFN-γ and IL-10 ELISpot assay, in the patient during the entire course of liver IA. Black bars represent numbers of TH1(IFN-γ). Gray bars represent numbers of TH2(IL-10). Hatched bars represent T-cell responses in wells with phytohemagglutinin. Dotted bars represent T-cell responses in wells with human anti-CD3-stimulating antibody (Mabtech, Nacka Strand, Sweden). Results are shown as the means of results from triplicate wells ± standard deviations. SFCs, spot-forming cells; PBMCs, peripheral blood mononuclear cells.
FIG. 2. Histological and immunohistochemical findings. (A) Hematoxylin-eosin staining (magnification, ×200) showed an extensive granulomatous inflammation of the liver with dispersed birefringence filamentous structures (inset magnification, ×400). (B) Silver staining (magnification, ×200) showed septate, acutely branching, hyphae in a pattern typical of aspergillosis (inset magnification, ×400).
IA and invasive candidiasis represent the most common causes of infection-related mortality in pediatric patients with AML (11). Data from the Centers for Disease Control and Prevention show that the mortality associated with IA has increased 357% since 1980, due to the limitation of diagnostic tools in establishing a prompt and undoubted diagnosis (7). Also, testing for GM, the most widely used noncultural method, is hampered by having a variable diagnostic accuracy, especially for children (2, 8).

Clinically, pulmonary infiltrates are the most common presentation of IA, with liver involvement occurring in less than 15% of patients and only in the late phase of the infection (3). In contrast, visceral presentation is common with invasive candidiasis, especially in the chronic form, which is also termed hepatosplenic candidiasis (HSC). HSC occurs in 3% to 7% of neutropenic patients and persists for months, despite granulocyte recovery and antifungal treatment. Fever, upper gastrointestinal symptoms, and increased serum alkaline phosphatase characterize HSC, and it has four dominant ultrasonographic patterns: “wheel within a wheel,” “bull’s eye,” hypoechoic lesion, and echogenic foci (4).

Only four cases of primary liver IA have so far been reported; they have occurred in three adult patients and one pediatric patient, who presented with either a single lesion or multiple lesions. In all four patients, the diagnosis was proven by biopsy and preceded by a single positive GM test in only one case (5, 6, 12, 13).

This is a further case of liver IA mimicking HSC on clinical and radiological grounds. Of note, while all the other diagnostic tools, including GM testing, showed repeatedly negative results, a positive ELISPOT result provided the sole hint of IA and shortly preceded the confirmatory biopsy. We recently reported the potential of the ELISPOT assay to diagnose IA in adult patients with pulmonary infiltrates (9). Also, in this case of extrapulmonary IA, the ELISPOT assay provided the kinetics of the *Aspergillus*-specific T-cell responses during the course of IA by showing (i) a permissive response, polarized in TH2(IL-10), at the onset; (ii) a stable TH1(IFN-γ) response with a progressive decrease of the TH2(IL-10) response, which in turn was sufficient to counterbalance the TH1(IFN-γ) and avoid an excessive inflammatory response during the regression phase; and (iii) a protective response, polarized in TH1(IFN-γ), at the complete resolution (Fig. 2). This is reminiscent of the kinetics of the *Aspergillus*-specific T-cell responses in the mouse model of IA and consistent with the notion that the presence of *Aspergillus*-specific TH1(IFN-γ) responses provides a better chance for stem cell transplant patients to survive IA (1, 10).

In conclusion, liver IA should be considered in the differential diagnosis of liver lesions in neutropenic patients, as should HSC, tuberculosis, and staphylococcal infections. The ELISPOT assay may have a role in the diagnosis of IA in pediatric patients as well. The diagnostic accuracy of the ELISPOT assay needs larger confirmatory studies.

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L. Potenza, P. Barozzi, G. Torelli, and M. Luppi have applied for an international patent regarding clinical applications of the ELISPOT assay for the diagnosis of *Aspergillus* infection (Italian patent application WO2007IT00867).

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