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Varicella-Like Cutaneous Toxoplasmosis in a Patient with Aplastic Anemia

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A 60-year-old patient with aplastic anemia presented with vesicular varicella-like skin lesions on her face, arms, legs, back, and abdomen. However, diagnosis for herpetic infection was negative. Findings of a skin biopsy led to a tentative histologic diagnosis of toxoplasmosis, and infection with *Toxoplasma gondii* was confirmed by immunohistochemistry and PCR. Cutaneous toxoplasmosis is a rare finding in immunocompromised patients and might mimic other infectious diseases, and vesicular lesions associated with toxoplasmosis have not been reported previously.

CASE REPORT

A 60-year-old woman was diagnosed with aplastic anemia in April 2012 and referred to our hospital. Bone marrow analysis revealed highly deficient hematopoiesis and therapy with thymoglobulin, cyclosporin, and methylprednisolone was initiated. During her second week as an inpatient, a *Clostridium difficile* infection was treated with metronidazole. In week 4, the patient developed a fever due to a *Klebsiella pneumoniae* infection, as identified by a blood culture; additionally, cytomegalovirus (CMV) viremia was detected. In week 8, *Stenotrophomonas maltophilia* was detected in her sputum and therapy with cotrimoxazole was immediately initiated at 960 mg intravenously twice daily until the end of her hospital stay. In the further course, multiple disseminated small vesicles with little surrounding erythema were observed on the patient's face, arms, legs, back, and abdomen in week 8 of her hospital stay (Fig. 1A). Her C-reactive protein level increased to 415 mg/liter, the patient developed pneumonia, and thoracic computer tomography revealed images compatible with a fungal infection, i.e., infiltrates in the lungs, halo signs, and pulmonary nodules, typically seen in fungal pneumonia. This diagnosis was confirmed later when *Rhizomucor pusillus* was detected in a bronchoalveolar lavage specimen. Antifungal treatment with liposomal amphotericin was initiated, and mechanical ventilation was necessary because of respiratory failure. The patient died 10 weeks after admission.

Varicella-like skin lesions were highly suggestive of a herpetic infection with herpes simplex virus or varicella-zoster virus. The patient reported having had herpes and chickenpox during childhood; thus, a primary infection with these herpesviruses was excluded and reactivation of a latent herpesvirus infection was suspected. However, skin swabs of affected areas and skin biopsy specimens were repeatedly negative for herpes simplex virus and varicella-zoster virus by PCR analysis. In the skin biopsy specimen, intracytoplasmic particles with the shape of parasites (*Leishmania* sp., *Toxoplasma gondii*) were detected histologically. No antibodies against *Leishmania* sp. were detectable; however, *T. gondii* infection was verified by PCR analysis of DNA extracted from lesion material targeting the B1 region and the AF146527 repetitive DNA element in the *T. gondii* genome. The histological changes in this case were subtle, with only a few organisms visualized within epidermal keratinocytes. However, no kinetoplast was detectable within these organisms. No organisms were seen in the dermal endothelium. Single protozoal tachyzoites or aggregates thereof were mostly intracytoplasmatic and occasionally extracellular. *T. gondii* bradyzoites and tachyzoites were identified within the epidermal keratinocytes by hematoxylin-and-eosin (HE) staining (Fig. 1B) and confirmed by immunohistochemical analysis (Fig. 1C) with a polyclonal antibody derived from rabbits immunized with homogenized *T. gondii*-infected mouse brain.

In a retrospective analysis of several serum samples and one sputum sample, *T. gondii* DNA was detectable in sputum and several serum samples as early as week 5; the greatest parasite DNA loads were detected in week 8, when the skin lesions appeared; the DNA load declined in week 9; and samples were negative in week 10 (Fig. 2). Thus, infection with *T. gondii* was already systemic 3 weeks prior to the appearance of cutaneous lesions. The anti-*Toxoplasma* IgG titer was 255 IU/ml on admission and 977 IU/ml in week 8, when skin lesions were observed, and increased to 1,975 IU/ml in week 9.

Methods. For analysis of anti-*Toxoplasma* antibodies, the Architect Toxo IgG chemiluminescent microparticle immunoassay (Abbott, Wiesbaden, Germany) was used; specimens with concentrations of ≥3.0 IU/ml were considered reactive, according to the manufacturer's instructions. All sera were tested retrospectively in the same run. We used PCR to detect DNA from *T. gondii* and sequencing to confirm the identity of the PCR amplicon. For PCR, the DNA element AF146527 (1), which appears in 200 to 300

Received 25 October 2012 Returned for modification 21 November 2012
Accepted 25 January 2013
Published ahead of print 6 February 2013
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doi:10.1128/JCM.02851-12

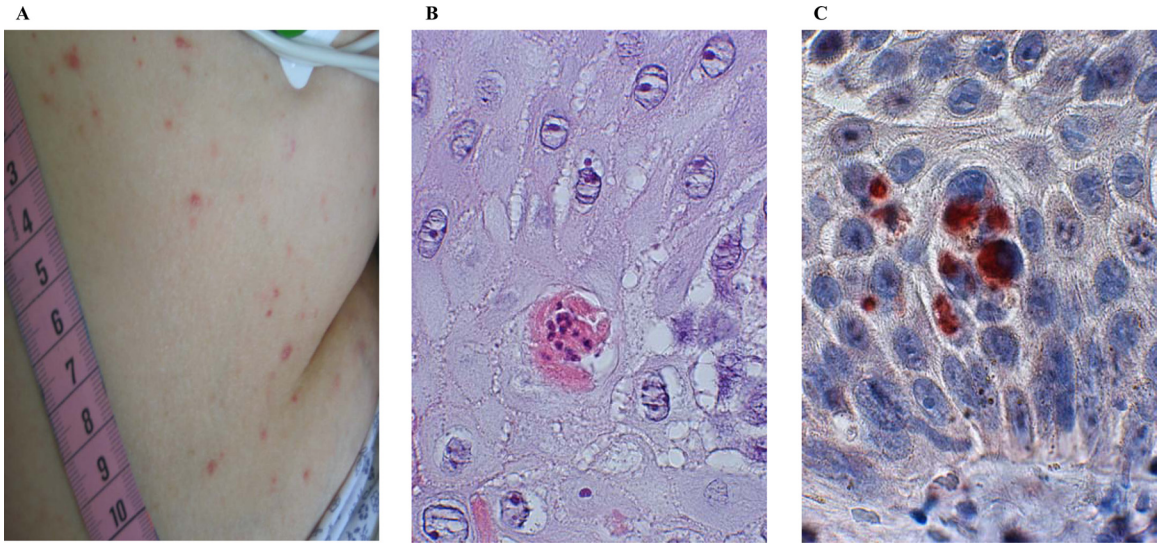

FIG 1 (A) Macroscopic view of disseminated vesicles with sparse surrounding erythema on the trunk. (B) Microscopic appearance of the skin lesion. It shows proliferative forms of *T. gondii* and intercellular edema of the epidermis without inflammatory reactions. HE staining was used. (C) Brown stain shows the presence of *T. gondii* detected by a polyclonal antibody. Original magnification, ×63.

copies, was chosen, as it was reported that PCR of this region is superior to that of the common B1 gene, which appears in 35 copies (2). This real-time PCR amplified an 83-bp fragment and was adapted to the fully automated BD MAX PCR device (Becton, Dickinson, Heidelberg, Germany). All of the *T. gondii* DNA amplicons derived from our patient showed 100% identity to the published sequence. AF146527 PCR was reported to be more sensitive than PCR–enzyme-linked immunosorbent assay for B1 and is known to amplify DNA from all three major lineages of *T. gondii* (3), as demonstrated for RH (type I), RMS-1995-ABE (type II), and NED (type III). AF146527 and B1 PCRs are not informative for strain typing. A recent report (4) indicated that strains without AF146527 or with mutated sequences might exist that can be missed when targeting this DNA region, thus decreasing the sensitivity of this PCR assay. Moreover, the B1 and AF146527 regions might appear in fewer copies, i.e., 5 and 12 instead of 35 and 230

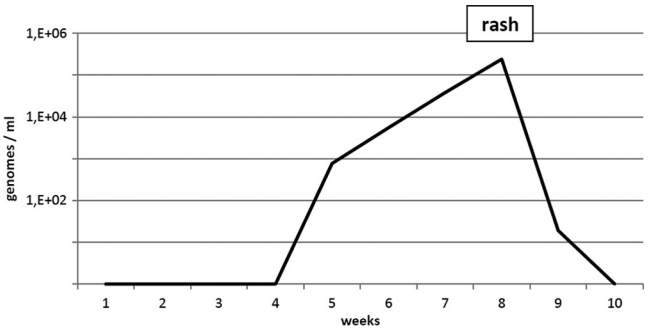

FIG 2 Detection of *T. gondii* in serum samples. *T. gondii* genomic DNA was detected by real-time PCR with hydrolysis probes targeting DNA sequence AF146527. The PCR assay included an internal control and was done on a BD MAX (Becton, Dickinson) in the PCR-only mode. Quantification was achieved by running DNA dilutions from manually counted *T. gondii* RH parasites. Indicated are the numbers of samples analyzed at the respective time points and the number of genome copies per milliliter in the case of positive detection.

(5). Additionally, we amplified *Toxoplasma* DNA by a second PCR targeting the B1 gene from the skin biopsy specimen by using a seminested PCR that amplified a 190-bp amplicon from B1 (P3, 5′ CTT CAA GCA GCG TAT TGT CG 3′; P7, 5′ TAA AGC GTT CGT GGT CAA CT 3′; P8, 5′ GGA ACT GCA TCC GTT CAT GA 3′) with P3/P7 followed by P8/P7 as follows: 95°C for 5 min; 30 cycles of 95°C for 50 s, 55°C for 50 s, and 72°C for 40 s; and 72°C for 10 min.

For quantification of *T. gondii* DNA, a real-time PCR targeting the repeated DNA region AF146527 (2) was performed. PCR analysis for detection of *Toxoplasma* in blood was performed retrospectively because only stored serum samples were still available. Forward (5′ CTT CGT CCA AGC CTC CG 3′) and reverse (5′ GAC GCT TTC CTC GTG GTG AT 3′) primers were combined with a 6-carboxyfluorescein-labeled hydrolysis probe targeting AF146527 (5′ CCC TCG CCC TCT TCT CCA CTC TTC AA 3′) and a Texas Red-labeled probe (5′ CTA GCA GCA CGC CAT AGT GAC TGG C 3′) that amplified the DNA sequence of an internal control plasmid. A thousand copies of the control plasmid with the same primer binding sites but encompassing a *tet* cassette were added. The PCR was performed in the PCR-only mode as follows: 95°C for 60 s, followed by 45 cycles of 98°C for 9 s and 60°C for 17.8 s. Quantification was achieved by running DNA dilutions from manually counted *T. gondii* RH parasites.

For genotyping, *T. gondii* DNA extracted from a skin biopsy sample was submitted to a genotyping analysis with 15 microsatellite markers distributed on 10 of 14 chromosomes of *T. gondii*, as described elsewhere (6). Briefly, for each primer pair, the forward one was 5′ end labeled with fluorescein to allow sizing of PCR products electrophoresed in an automated sequencer. PCR was carried out in a 25-μl reaction mixture consisting of 12.5 μl of 2× Qiagen Multiplex PCR Master Mix (Qiagen, Courtaboeuf, France), 5 pmol of each primer and 5 μl of DNA. The cycling conditions were 15 min at 95°C; 35 cycles of 30 s at 94°C, 3 min at 61°C, and 30 s at 72°C; and 30 min at 60°C. PCR products were diluted 1:10 with deionized formamide. One microliter of each

diluted PCR product was mixed with 0.5 μl of a dye-labeled size standard (ROX 500; Applied Biosystems) and 23.5 μl of deionized formamide (Applied Biosystems). This mixture was denatured at 95°C for 5 min and then electrophoresed with an automatic sequencer (ABI PRISM 3130xl; Applied Biosystems). The sizes of the alleles in base pairs were estimated with GeneMapper analysis software (version 4.0; Applied Biosystems).

An immunocompromised patient with aplastic anemia developed varicella-like cutaneous lesions due to the reactivation of a *T. gondii* infection. Protozoan organisms were detected by histopathology in skin lesions, and a reactivated *T. gondii* infection was shown by immunohistochemistry and direct PCR detection within the lesions and in various serum samples. The steep increase in anti-*Toxoplasma* antibodies is not an argument for the diagnosis in this case; this increase is common in patients after grafting. Cutaneous toxoplasmosis is a rare finding, and vesicular lesions associated with toxoplasmosis have not been reported previously. Severe infections in immunocompromised patients usually result from the reactivation of latent cysts into invasive tachyzoites and may provoke severe symptoms such as encephalitis, ocular toxoplasmosis, pneumonia, and disseminated infection (7–11). Localized and sometimes disseminated varicella-zoster virus infection is observed in transplant patients (12). Only a few cases of cutaneous toxoplasmosis have been reported, e.g., by Leyva and Santa Cruz (13) in a 53-year-old male hematopoietic stem cell transplant (HSCT) recipient; by Lee et al. (14) in a 37-year-old male HSCT recipient with acute lymphoblastic leukemia (ALL); by Amir et al. (15) in a 31-year-old female acute myelogenous leukemia patient; by Vidal et al. (16) in a 16-year-old male ALL patient; by Fong et al. (17) in a 49-year-old HIV-positive male patient. Thus, cutaneous toxoplasmosis has been observed in only a few immunocompromised patients and the incidence rate of cutaneous toxoplasmosis seems to be quite low.

The histological changes in our case were subtle; extracellular *T. gondii* parasites were identified within epidermal keratinocytes by HE staining and confirmed by immunohistochemistry. Cutaneous manifestation of toxoplasmosis is rarely encountered and difficult to diagnose with certainty because of the morphological similarity of *T. gondii* to other organisms, such as *Leishmania* sp. and *Histoplasma capsulatum*. There is great variability in the appearance of cutaneous toxoplasmosis; a generalized rash and maculopapular, purpuric, papulopustular, and erythema multiforme-like lesions have been described. Nodular lesions have been observed in the skin of an HIV-infected patient (17).

In fact, genotyping analysis with 15 microsatellite markers showed that the *T. gondii* strain involved in this unusual case of toxoplasmosis was not atypical but harbored a type III genotype. Only one mismatch between AF179871 and an RH strain (C→T at position 754) was detected in the 190 bp sequenced. A large study of immunocompromised patients showed that type III was the second most common genotype recovered from European patients, after type II (11). Our case is in accordance with the conclusion of that study, which reported the absence of a correlation between the parasite genotype and the site or outcome of the infection. In other words and contrary to what is observed in immunocompetent patients in whom unusual and severe toxoplasmosis involves atypical strains (18), a common strain such as one of type III may be responsible for an uncommon presentation such as cutaneous toxoplasmosis in immunocompromised patients.

T. gondii DNA was also detected in a serum sample prior to cutaneous manifestation and while the rash was present and in a sputum sample. Thus, it is highly probable that the cutaneous manifestations were part of a disseminated *T. gondii* infection. The decline in the parasite load after week 8 may have been the result of therapy with cotrimoxazole, which is also effective against toxoplasmosis (19). However, in none of the rarely published cases of cutaneous toxoplasmosis was a PCR assay of blood samples performed. De Medeiros et al. (20) reported on nine bone marrow transplant (BMT) patients with disseminated toxoplasmosis, eight of whom were serologically *Toxoplasma* positive prior to transplantation. Disseminated toxoplasmosis developed in these patients despite prophylaxis with trimethoprim-sulfamethoxazole. A BMT patient with multiple skin lesions after discontinuation of *Pneumocystis jirovecii* prophylaxis with cotrimoxazole was recently reported (16). Active CMV infection, as demonstrated in the case presented here, is associated with immunosuppression and predisposes to the development of superinfections in immunocompromised patients. In a mouse model, murine CMV infection induced the reactivation of dormant *T. gondii*-associated pneumonia, raising the possibility that CMV infection can play a role in the reactivation of *T. gondii* (9). The disease-promoting role of CMV in *Pneumocystis* pneumonia is well known and has been described previously (21, 22). The diagnosis of disseminated toxoplasmosis was made in retrospect; thus, no specific anti-*Toxoplasma* treatment was given during hospitalization or prior to the patient's death. However, because of bacterial infections, cotrimoxazole, which is also effective against toxoplasmosis, was administered. Because of concomitant infections with fungal and bacterial pathogens, the impact of *Toxoplasma* on pneumonia and fatal outcome is difficult to assess.

A possible strategy to identify patients at the greatest risk of toxoplasmosis, e.g., HSCT patients or patients with hematological malignancies, is screening for *Toxoplasma* IgG prior to the initiation of immunosuppression (23, 24). *Toxoplasma*-seropositive patients could be monitored closely, and toxoplasmosis could be investigated by PCR assay in a setting of unexplained fever or syndromes such as pneumonia or cutaneous or brain lesions. These patients could be monitored by PCR assay in the posttransplant period or after the initiation of immunosuppression according to Martino et al. (24), similar to the preemptive strategy used for CMV.

The manifestations of cutaneous toxoplasmosis vary greatly and have nonspecific characteristics. This case illustrates the possible confusion cutaneous toxoplasmosis might cause by mimicking varicella-like lesions. In the case reported here, the identity of *Toxoplasma* was confirmed by PCR. Cutaneous toxoplasmosis may be more common in heavily immunosuppressed patients since this parasite is easily overlooked.

ACKNOWLEDGMENTS

We acknowledge the technical assistance of M. Hofko and J. Schnitzler for graphical support.

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