Rapid Diagnosis of Human Brucellosis by Quantitative Real-Time PCR: a Case Report of Brucellar Spondylitis

Amalia Navarro-Martínez, Elena Navarro, María Jesús Castaño, and Javier Solera

Internal Medicine Department, University General Hospital, c/ Hermanos Falcó s/n, 02006, Albacete, Spain,
Experimental Research Unit, University General Hospital, c/ Hermanos Falcó s/n, 02006, Albacete, Spain

Received 28 June 2007/Returned for modification 20 August 2007/Accepted 15 October 2007

Blood samples and a bone biopsy specimen from one patient diagnosed with spondylodiscitis of unknown etiology were analyzed by quantitative real-time PCR to detect Brucella melitensis. The high sensitivity and specificity of this assay allowed the diagnosis of brucellar spondylitis within 24 h, a result that we were unable to obtain through the use of conventional methods.

CASE REPORT

An 81-year-old woman presenting with thrombophlebitis was admitted to the Vascular Surgery Department of the University General Hospital, Albacete, Spain. She had a history of diabetes, had suffered from lumbar pain for years, and lived in a geriatric residence. Physical examination showed edema and inflammation of the right leg, a positive Homans’ sign, and lumbar pain. Her body temperature was 36.5°C. All hemato logical values except the erythrocyte sedimentation rate were normal; the erythrocyte sedimentation rate was 51 mm/h. All blood chemistry values except the values for glucose, C-reactive protein, and rheumatoid factor were normal; the glucose concentration was 188 mg/dl, the C-reactive protein level was 59.6 mg/liter, and the rheumatoid factor value was 60 IU/ml. Plain radiograms of the spine showed degenerative changes. A computed tomography scan of the abdomen and pelvis revealed a diffuse spondylitis at vertebral levels L4 and L5, while magnetic resonance imaging showed typical findings for vertebral osteomyelitis involving the subchondral pars of L4 and L5. The patient was diagnosed with spondylodiscitis of unknown etiology.

Further evaluation of the patient was conducted in our Internal Medicine Department. Certain risk factors for brucellosis were present, such as the ingestion of unpasteurized dairy products and occupational exposure. Therefore, on suspicion of brucellar spondylitis, more blood samples as well as a bone marrow biopsy specimen were taken to obtain specimens for microscopic examination and specific culture for Brucella and Mycobacterium tuberculosis. Standard tube agglutination testing of the initial samples, performed by standard methods (13) with commercial reagents (Knickerbocker), yielded negative results for antibodies to Brucella (titers, <160). The rose bengal test for brucellosis was also negative, as was the Mantoux skin test screen for M. tuberculosis infection. All blood cultures, processed with a Bactec 9050 system (Becton Dickinson) and incubated for 30 days (13), were negative. However, at 14 days the bone marrow culture was positive for Brucella spp., which were identified by standard microbiological techniques (5). All of the strains isolated were sent to the Centro de Investigación y Tecnología Agroalimentaria (Zaragoza, Spain) for definitive identification and biotyping.

Apart from the conventional techniques for testing for brucellosis described above, we also analyzed the blood samples and the one bone marrow sample by quantitative real-time PCR (Q-PCR) with specific primers and a TaqMan probe to detect Brucella melitensis. We chose to use the LightCycler system (Roche Diagnostics, Mannheim, Germany), an ultra-rapid system based on real-time fluorometric quantification of PCR products, for this analysis. The use of a single exonuclease hydrolysis probe for the detection of the PCR products in this assay ensured high specificity. The Q-PCR assay specific for Brucella melitensis was performed as described elsewhere (8). After 24 h, the bone marrow sample was positive, with a B. melitensis burden of 57 copies/mg of tissue. Several blood samples were also positive. The chronological results of the agglutination tests, blood cultures, and Q-PCR for 13 blood samples are shown in Table 1.

Specific treatment for infection with Brucella spp. with doxycycline (100 mg every 12 h) and trimethoprim-sulfamethoxazole was initiated. By the time that we received the microbiological results of the blood and bone marrow cultures, the patient had already been in treatment for 1 month, with clinical improvement of the pain. The bone marrow culture turned positive after 14 days, and the blood cultures were negative after 30 days. The duration of antimicrobial therapy was 90 days. Patient follow-up at 1 year has shown a favorable evolution, with no relapse of symptoms.

Brucellosis is a systemic, infectious disease caused by bacteria of the genus Brucella and may involve multiple organs and tissues (10, 15). The intracellular location of the bacteria protects it from some of the basic mechanisms of the host’s immune system and from antimicrobial therapy (9). This could be the reason for the appearance of chronic disease, focal complications, and relapses (1, 11, 12). Spondylitis is the most important form of focal complication in brucellosis and usually involves the lumbar spine. It may be difficult to diagnose and can be complicated by neurological or vascular conditions (10).
The absolute diagnosis of brucellosis requires isolation of the bacterium from blood or tissue samples (9). The new automatic blood culture systems, such as the Bactec system, have proven very sufficient for isolation of the microorganism and are usually able to isolate the microorganism in the course of the first week (1). Several specimens for culture should always be taken, given the potential for false-negative results. In addition to blood, cultures of disk or bone marrow specimens that have been removed surgically or by needle aspiration (10) may reveal the presence of the organism (10). However, harvesting of bone marrow for culture remains an invasive and painful technique; therefore, blood samples are usually employed (9).

A presumptive diagnosis of brucellar spondylitis can be made in clinically compatible cases with either an epidemiologic link to a confirmed case or supportive serology. *Brucella* agglutination titers of greater than or equal to 160 in one or more serum specimens obtained after the onset of symptoms (10) usually indicate *Brucella* infection, although low titers determined by standard tube agglutination testing have been reported with this disease. Seroconversion and evolution of the titers can also be used for diagnosis (9, 10). Negative serologic and culture tests are very unusual in brucellosis and also in *Brucella* spondylitis (10).

The development of a rapid, definitive diagnostic test for brucellosis has remained an elusive target (1). However, the discovery of a real-time PCR assay specific for *Brucella* species is a recent advance. This molecular method, which amplifies and detects target nucleic acids, has been found to have a sensitivity and a specificity superior to those of conventional methods of detection, including time-consuming blood culture and serological tests (8). Moreover, compared with conventional PCR, real-time PCR may be valuable for the quantification of nucleic acids in individual blood samples, as well as for automation and computerization of the data (3, 6, 7). Although this technique may be applied to other clinical samples (serum, biopsy, cerebrospinal fluid, etc.), it is not yet being used in daily clinical practice. The extraction methods and the experimental setup lack standardization, and there is need for a better understanding of the clinical significance of real-time PCR as a diagnostic test (8, 9).

This case report demonstrates the utility of the real-time PCR assay for the rapid diagnosis of brucellar spondylitis in a patient with a negative serum agglutination test result and negative blood culture results.

Spondylitis is an osteoarticular focal complication of brucellosis that often results in residual damage. Although in the region covered by our health care system, 84% of the brucellosis patients have a history of epidemiological exposure to the bacterium, the focal forms of brucellosis, especially brucellar spondylitis, are associated with longer-than-average delays in diagnosis. The major obstacle to establishing a clinical diagnosis of spondylitis early in the course of brucellosis, is the non-specific nature of the signs and symptoms at presentation, such as spinal pain, fever, and constitutional symptoms (2, 4, 10, 14). Additionally, brucellar spondylitis tends to affect older individuals, who may display evidence of general osteoarticular degeneration unrelated to *Brucella*.

The presence of *Brucella* organisms in a blood culture is diagnostic. The new automatic blood culture systems, such as the Bactec system, turn positive in 7 to 10 days, but they should be preserved for a minimum of 3 weeks before it is determined that the results are negative (1, 9). In addition to blood cultures, culture of disk or bone tissue specimens that have been removed surgically or by needle aspiration may reveal the presence of the organism (10). The case described here is a typical chronic one, with a positive bone tissue culture result but negative blood culture results.

The serum agglutination test remains the most popular tool for the diagnosis of brucellosis, but it has the shortcomings referred to above. Negative serological and culture test results are very unusual in *Brucella* spondylitis. Solera et al. described 35 cases, all of which (100%) were positive by the rose bengal test, most of which (91%) were positive by tube agglutination, and about half of which were positive by culture (10). These findings show the exceptionality of the present case that was both rose bengal and agglutination test negative.

Q-PCR with the LightCycler instrument is a new option which can be employed for the diagnosis of brucellosis disease and which may become a method of choice, as it is fast and provides a quantitative measure. With respect to Q-PCR as a diagnostic tool for brucellosis, the results of previous studies have demonstrated that they have 100% sensitivity and 100%
Specificity for the diagnosis of both initial infections and relapse episodes (8).

For our patient, real-time PCR allowed the rapid diagnosis and the initiation of adequate treatment within only 24 h. It is worth noting that conventional serology and blood cultures were negative; the result for the bone marrow culture confirmed the diagnosis. By the time that we received the microbiological result, the patient had been in treatment for 1 month, with a favorable clinical evolution and an improvement in the pain.

In summary, the high sensitivity and the high specificity of this real-time PCR assay, together with its speed and versatility, make this technique a very useful tool for the diagnosis of human brucellosis, especially in patients with a condition that is difficult to diagnose. Our case demonstrates that brucellar spondylitis can be diagnosed with a histological specimen by real-time PCR in a time span of 24 h, a result we were unable to obtain by the use of conventional methods.

REFERENCES