False-Positive IgM Serology in Coccidioidomycosis

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The clinical observation has been made that there might be an unacceptable number of false-positive enzyme immunoassay (EIA) test results for IgM among persons suspected of having coccidioidomycosis. Patients with a positive result for IgM by EIA are thought to have a diagnosis of acute coccidioidomycosis. However, this study found that 82% of patients with an IgM-positive and IgG-negative EIA result did not have coccidioidomycosis.

The Premier Coccidioides enzyme immunoassay (EIA; Meridian Bioscience, Inc., Cincinnati, OH) is a commercially available test used for the serological diagnosis of coccidioidomycosis. Clinical laboratories use that EIA for the detection of both IgM and IgG antibodies directed against Coccidioides. A positive EIA result for IgM is believed to be associated with the initial antibody response seen in an acute Coccidioides infection (3). The EIA test is also used to determine the presence of IgG antibody, which is diagnostic of an infection with Coccidioides. Clinicians use the various IgM and IgG antibody responses to determine the presence or the absence of an acute or a chronic Coccidioides infection (5).

The Premier assay may be subject to false-positive results for IgM, as suggested by prior studies (2, 3). In contrast, those clinical and serological studies found a good correlation between a positive result for IgG by the Premier assay and infection with Coccidioides. A false-positive EIA result for IgM occurs when patients are thought to be experiencing acute coccidioidomycosis but do not have a Coccidioides infection. Clinical observations have raised concern that there may be an unacceptable number of false-positive IgM results with the Premier assay. A false-positive serological result can create clinical problems, resulting in the treatment of patients for a disease that they do not have or thinking that the patient has coccidioidomycosis and missing another medical condition. The study described here was done to correlate clinical findings and serological results for patients who had a positive result for IgM and a negative result for IgG by the Premier Coccidioides EIA.

CASE REPORT

A man with chronic lung disease and an aortic valve replacement who was receiving warfarin was admitted for hemoptysis. A month previously he had been diagnosed as having acute pulmonary coccidioidomycosis on the basis of positive results for IgM and negative results for IgG by EIA. He was placed on oral fluconazole at 400 mg daily. Fluconazole is known to alter the metabolism of warfarin. The fluconazole caused an increase in the anticoagulation effect of warfarin, which led to bleeding and hemoptysis. The problem resolved following discontinuation of the fluconazole and the finding of no confirmatory evidence for coccidioidomycosis.

MATERIALS AND METHODS

The laboratories at John C. Lincoln Hospitals (the clinical laboratory) have routinely used the Premier EIA kit since 1995 for the detection of IgM and IgG antibodies in patients suspected of having an infection due to Coccidioides. The EIAs were performed by using the standard instructions provided by the manufacturer. A split sample of serum specimens from 17 patients whose EIA test results were positive for IgM and negative for IgG was sent for confirmation of the results to the Department of Medical Microbiology and Immunology, University of California School of Medicine at Davis, Davis, CA (the reference laboratory). These sera were sent to the reference laboratory blinded as to the serological results from the clinical laboratory. The specimens were tested for IgM and IgG antibodies by immunodiffusion (ID) by use of the protocol of the reference laboratory (3, 5, 6). A sample of 15 serum specimens from patients with positive results for both IgM and IgG by EIA was evaluated in the same manner. The medical records for the 32 patients were reviewed by one of the authors (T.K.), and a consensus diagnosis as to whether the patient had coccidioidomycosis was made. This was done before the reference laboratory results were known. The clinical information was then correlated with the reference and clinical laboratory serological results.

RESULTS

The results of the serological assay and the review of the medical records of the 17 patients who had positive results for IgM and negative results for IgG by EIA are summarized in Table 1. There were three patients (patients 3, 6, and 16) who may have had an acute Coccidioides infection, as judged by chart review. Of the three, only one patient (patient 16) had IgM-positive and IgG-negative results that were confirmed by the reference laboratory. The two other patients (patients 3 and 6) were believed, as a result of the chart review, to have pneumonia indistinguishable from either coccidioidomycosis or any other community-acquired pneumonia; however, the positive IgM and negative IgG results were not confirmed by the reference laboratory. These two patients may or may not have had coccidioidomycosis, since no confirmatory cultures or follow-up serologies were done. Of the 17 patients, 3 (18%)
may have had coccidioidomycosis on the basis of the medical record review. In contrast, of the 15 patients with both IgM- and IgG-positive EIA results, 12 (80%) were believed to have had coccidioidomycosis.

The principal diagnoses determined from the medical records for each of the 17 patients with IgM-positive and IgG-negative results are also given in Table 1. Seven (41%) had a diagnosis of pneumonia, five (29.5%) had fever of unknown origin (FUO), and five (29.5%) had other diagnoses. For five patients, the medical record indicated a coded discharge diagnosis of coccidioidomycosis on the basis of the IgM-positive and IgG-negative EIA results; none of the five was believed to have had coccidioidomycosis.

The clinical laboratory did 2,139 serologies for coccidioidomycosis by EIA in 2008; of these, there were 104 (5%) with a positive IgM serological result for coccidioidomycosis. Early studies with a large sample of patients with and without coccidioidomycosis showed that tests for IgG and IgM in combination were accurate in identifying this disease (3). However, there were 8/362 (2.2%) serum samples from these patients who had a noncoccidiodal illness or a false-positive result for IgM. If the EIA test is falsely positive for IgM, it can create problems in a variety of ways. Physicians may have an index of suspicion for a false-positive test result. However, the correlation between the control group of IgM-positive and IgG-positive serological results by EIA was good (80%) and the correlation between the IgM-positive and IgG-negative results by EIA was poor (18%). In general, serology for Coccidioides was not indicated in the group of patients with false-positive results, reflecting the diverse levels of expertise of physicians ordering an EIA serology test for coccidioidomycosis in a community hospital.

If the estimated 82% rate of false-positive results for IgM can be confirmed, this would have a significant impact on the usefulness of this test. Clinicians who see mainly hospitalized cases of coccidioidomycosis usually see patients with more chronic disease. Acute coccidioidomycosis is usually seen by primary care or emergency room physicians, who are less likely to be aware of the false-positive IgM issue. However, from the analysis of the series of 17 consecutive patients with IgM-positive and IgG-negative EIA results performed in the present study, the impact of this observation seems evident. If the rate of false-positive IgM test results from this study is multiplied by the many hospitals in the region of endemicity that use this test for the serological diagnosis of coccidioidomycosis, the clinical impact would be significant. The magnitude of the impact would be difficult to assess because of the variety of clinical circumstances that could be influenced. However, the results obtained with this small sample suggest that a positive EIA test result for IgM, as it is currently performed, can be clinically misleading.

In a review of the Coccidioides serologies done at one institution from 1994 to 2002, 18% of the patients with a positive EIA result for IgM were believed to have a false-positive test result (2). That study noted about 22% of the false-positive results were in HIV-infected patients; 2 of 17 (12%) patients in our series were HIV positive. In contrast, a retrospective study of isolated IgM-positive serological results found no false-positive EIA results for IgM in 28 patients with presumptive coccidioidomycosis (1). However, of the 28 patients, Coccidioides infection was confirmed in only 7 (25%), which would approximate our findings of a rate of 3/17 (18%).

What causes a falsely positive IgM EIA result is not known. Several patients in the present series had negative IgM and IgG EIA results on repeat testing several days later, suggesting that the initial EIA test was falsely positive. The rapid change in results implies interference from such things as other diseases, especially other fungal infections; the use of mediations; or technical issues with the performance of the test for IgM. It should be noted that there appears to be a good correlation between having coccidioidomycosis and an EIA test result positive for IgG (3). Our results for patients who had both a positive result for IgM and a positive result for IgG by EIA found that they were likely to have coccidioidomycosis, and these results were unlikely to be falsely positive. Clinicians need to be advised to interpret with care an isolated positive result for IgM by EIA in light of the clinical circumstances and have an index of suspicion for a false-positive test result.

If it can be substantiated that there is a poor correlation between the result for IgM by EIA and disease, it should...

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**DISCUSSION**

This study was performed to evaluate the impact of a false-positive IgM serological result for coccidioidomycosis by use of the Premier EIA test. Early studies with a large sample of patients with and without coccidioidomycosis showed that tests for IgG and IgM in combination were accurate in identifying this disease (3). However, there were 8/362 (2.2%) serum samples from these patients who had a noncoccidiodal illness or a false-positive result for IgM. If the EIA test is falsely positive for IgM, it can create problems in a variety of ways. Physicians new to the area of endemicity may not be aware of this observation, until they find out by trial and error that they are treating someone for coccidioidomycosis who did not have the disease. This would also be at the expense of neglecting other...
influence the clinical utility of the test. Five of the hospitalized patients with a false-positive result for IgM had a discharge diagnosis of primary coccidioidomycosis but did not have this disease. An error in the coding of acute coccidioidomycosis as a discharge diagnosis can influence reimbursement and disease surveillance. In addition, the expense of a workup for a disease that does not exist and the unnecessary treatment with antifungal medications would obviously not be cost-effective.

The rates of false-positive results reported for the EIA test for IgM ranged from 0% (1) and 2.2% (3) to 18% (2). Our rate of 82% of false-positive results for IgM suggests that there is a problem with the EIA test for IgM. The rate may be higher because of the diversity of clinicians who order this serology in our community hospitals. IgM-positive and IgG-negative results were found for only 104/2,139 of tests done in 2008. This represents a relatively infrequent occurrence, and the majority of experienced clinicians have learned to interpret this result in the context of the clinical circumstances. In reviewing the records for the IgM-positive and IgG-negative patients, it was apparent that most of these tests were requested by physicians who were the least experienced and new to the area of endemicity. Under those circumstances, it might be more likely for problems to be created in the diagnosis for, and the treatment of, patients unlikely to have acute coccidioidomycosis.

The findings of Blair and Currier (1), which showed no false-positive results for IgG for 28 patients, are difficult to reconcile with those of the present study. Their results may reflect a selection bias, in that their patients were from a referral population and the diagnosis of coccidioidomycosis was determined retrospectively. Our study was prospective and more likely to identify a true false-positive result for IgM. In addition, 57% of their IgM-positive and IgG-negative patients were said to have complement-fixing antibody. Our experience shows a good correlation between a positive EIA result for IgG and the presence of complement-fixing antibody, suggesting that over half of their patients would have been IgM and IgG positive by EIA if the serology had been done in our laboratory. In our study, the presence of a positive EIA result for IgG was correlated with having coccidioidomycosis (80%). We can offer no other explanation for their results, other than it is not the experience of ourselves or others.

The EIA test for IgM appears to need refinement if it is to have clinical usefulness. The ongoing epidemic of coccidioidomycosis in the areas of endemicity warrants the use of a test that can accurately identify patients with acute coccidioidomycosis. This could be assisted by the appropriate use of the immunodiffusion test as an alternative for the detection of IgM antibody. Alternatively, identifying the presence of circulating antigen during acute coccidioidomycosis may help to improve the reliability of the serological diagnosis of acute coccidioidomycosis (4).

REFERENCES