Correspondence

Scrub Typhus: Prevalence and Diagnostic Issues in Rural Southern India

SIR—Recent reports from India and other neighboring countries suggest that there is a resurgence of scrub typhus infection caused by *Orientia tsutsugamushi* in these parts of the world and that the resurgence is associated with considerable morbidity and mortality [1–3]. At present, scrub typhus is rarely diagnosed because of its nonspecific clinical presentation that includes fever, rigors, headache, and occasionally rash, and because of a low index of suspicion and the lack of diagnostic facilities in India. We wish to draw the attention of readers to the magnitude of this disease in rural South India and to the validity of the widely used Weil-Felix test.

Using the Weil-Felix test and ELISA, we conducted a study to determine the prevalence of scrub typhus in the community at one of the block (administrative unit of a district) level community health centers of Christian Medical College and Hospital, Vellore district, Tamil Nadu, South India. This health center is the only hospital with inpatient treatment facilities in that block, which has 100,000 inhabitants. An average of 150 patients are seen daily in the outpatient department. During this study period at the tertiary care hospital, we also evaluated the validity of the Weil-Felix test for a range of titers using a panel of 125 serum samples collected by the microbiology department from the infected and control patients (according to diagnoses made using ELISA results). The institutional review board approved this study.

From mid-October 2002 to January 2003, all patients who reported to the outpatient department of the community health center with confirmed fever of 7–30 days duration were admitted and evaluated. Patients' histories were obtained and clinical examinations and routine investigations were done, which included a total WBC count, a differential count, microscopy of a urine sample, a Widal test, and chest x-ray when indicated. We found that findings of primary evaluations could yield a diagnosis of the most common causes of febrile illnesses (with durations between 7–30 days), such as enteric fever, respiratory tract infection, and urinary tract infection. Blood samples were collected from all patients who had undiagnosed febrile illness to test for rickettsial antibodies with the Weil-Felix test and ELISA. The Weil-Felix test was performed according to standard procedures with whole-cell antigens prepared from the Ox-19, Ox-2, and Ox-K strains of *Proteus vulgaris* [4]. For ELISA, we used the Scrub Typhus Group ELISA Kit (PanBio) to detect IgG and IgM antibodies. These kits use a 56-kDa recombinant antigen and have specificities and sensitivities of ~90% for detecting specific antibodies [5]. To determine whether the disease is endemic in the study area, a serosurvey was conducted among 100 apparently healthy individuals who did not report experiencing fever during the previous month and who resided in 5 different villages where cases of scrub typhus had occurred.

Thirty-three febrile patients were evaluated, and a specific diagnosis could not be established at that time for 18 of these patients clinically or by the routine investigations described earlier. Later, 8 (47%) of these patients received the diagnosis of scrub typhus based on ELISA results. One (12.5%) of these 8 patients tested positive for *P. vulgaris* Ox-K antigens with a titer of 1:80, two patients (25%) with a titer of 1:40, and two patients (25%) with a titer of 1:20. None of the 8 patients had eschars that would suggest a diagnosis of scrub typhus clinically. Two patients also had IgG antibodies for scrub typhus. Of the control subjects in the community, 4 (4%) had IgG antibodies, and 1 (1%) had IgM antibodies.

We attempted to validate the Weil-Felix test in the population using a panel of 125 serum samples. The sensitivity for Ox-K was 30% at a titer breakpoint of 1:80, but the specificity and positive predictive value were 100%. At a breakpoint of 1:20, the sensitivity was 61%, the specificity was 94%, and positive predictive value was 84%. At a breakpoint of 1:40, the sensitivity was 49%, the specificity was 96%, and positive predictive value was 88%.

This study highlights the finding that ~50% of the undiagnosed prolonged fevers that occur during the cooler months of the year in the rural areas of Tamil Nadu, South India, could be due to scrub typhus. Although the disease occurrence was high during the study period, the prevalence of IgG antibodies in the community was low, which suggests that the disease was probably relatively new to the area. Many studies done in the 1960s and 1970s have demonstrated the endemic nature of this disease in many parts of India [6]. However, in later years, the disease virtually disappeared, probably because of widespread use of insecticides to control other vectorborne diseases, empiric treatment of febrile illnesses with tetracyclines and chloramphenicol by practitioners, and changes in lifestyle. There seems to be a resurgence of the disease now [3].

Accurate and early diagnosis of scrub typhus remains a challenge in India because of its nonspecific presentation and the paucity of confirmatory diagnostic resources. We found that the Weil-Felix agglutination test had only 30% sensitivity at a titer of 1:80. However, it is worth noting that the specificity of this test was

high even at lower titers, so patients with low titers should also be evaluated for scrub typhus. It is known that Weil-Felix test results may be negative during the early stages of the disease because agglutinating antibodies are detectable only during the second week of illness [7]. ELISA, however, when performed with 56 KDa antigen, has 90% sensitivity and specificity, allows detection of IgG and IgM antibodies, and provides positive results within 3-4 days after the onset of illness. However, the availability and the cost of ELISA are major problems in India.

This study report emphasizes the need for increased awareness of rickettsial infections in rural Southern India. Because of current circumstances in India, we suggest that the diagnosis of scrub typhus should be largely based on a high index of suspicion and careful clinical, laboratory, and epidemiological evaluation. Use of empiric treatment should also be considered to reduce the high mortality observed with the disease. Introduction of improved diagnostic methods would allow greater appreciation for the prevalence of the disease.

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Positive Predictive Value of Epstein-Barr Virus DNA Detection in HIV-Related Primary Central Nervous System Lymphoma

Sir—Epstein-Barr virus (EBV) DNA PCR detection in CSF has been proven to be sensitive and specific for the diagnosis of HIV-related primary CNS lymphoma (PCNSL) [1–6]. In clinical practice, this test has been shown to be useful to achieve a "minimally invasive" diagnosis of PCNSL, in the presence of clinicoradiological findings and thallium 201 single-photon emission tomography findings consistent with PCNSL and no response to antitoxoplasmic treatment [7, 8].

In their recent article, Ivers et al. [9] reported the EBV DNA PCR results for CSF samples obtained from 26 HIV-infected patients with neurological problems. EBV DNA was found in samples from 7 of these 26 patients. Because only 2 of the patients received a diagnosis of PCNSL, the resulting low positive predictive value (PPV) led the authors to conclude that improved standardization may be required for the use of this test in clinical practice.

We would like to discuss 2 important issues that, in our opinion, have not been adequately addressed by Ivers et al. [9]. First, the diagnostic value of a test depends on a number of variables, including the diagnostic standard and the analytical sensitivity of the assay [10]. Nucleic acid amplification protocols may vary significantly between laboratories, because of the use of different nucleic acid extraction techniques, primers, and amplification technology. Assays with high analytical sensitivity may be associated with increased rates of false-positive results—although not necessarily with increased CSF lymphocyte counts—and, thus, with poor diagnostic specificity and a low PPV. It is unfortunate that methodological information was not provided by Ivers et al. [9], making it difficult to compare their results with the results obtained in previous studies.

Second, the positive and negative predictive values of a diagnostic test vary substantially, depending on the prevalence of

Table 1. Diagnoses given to 22 HIV-infected patients whose CSF samples were positive for Epstein-Barr virus DNA.

Diagnosis	No. of patients
PCNSL	
Histologically proven[a]	5
Probable[b]	7
Possible[c]	5
Lymphomatous meningitis	2
Other CNS disorder[d]	3

NOTE. PCNSL, primary CNS lymphoma.

[a] Determined by brain biopsy (3 cases) or at postmortem examination (2 cases).

[b] Defined by abnormalities in brain CT or MRI results that are consistent with PCNSL, lack of response to antitoxoplasmic treatment, and a positive result of thallium 201 single-photon emission tomography (SPECT) examination.

[c] Defined by abnormalities in CT or MRI results that are consistent with PCNSL, lack of response to antitoxoplasmic treatment, and either a negative result of SPECT examination or no performance of SPECT.

[d] CNS tuberculosis, HIV encephalitis, or CNS toxoplasmosis (1 case each).

the disease addressed. Although details about the anti-HIV treatment received by patients in the study by Ivers et al. [9] were not given, all of the patients were observed after HAART became available in the Western world. Declines in the incidence and prevalence of PCNSL have been observed since HAART became available and have been more relevant than the declines in incidence and prevalence of other opportunistic CNS disorders [11]. To evaluate the effect of HAART on the PPV of our EBV DNA amplification assays [1, 3], we reviewed the findings of tests performed on CSF samples obtained from 491 HIV-infected patients with neurological problems who were admitted to our clinics between January 1998 and November 2002—in other words, during the same period studied by Ivers et al. [9]. EBV DNA was found in the CSF samples from 22 patients (4.5%) (table 1). When only histologically proven cases of PCNSL and "probable" cases of PCNSL were considered as cases of disease, the PPV was 55%. The PPV increased to 77% when "possible" cases of PCNSL were also included as cases of disease. According to the Bayes formula, the estimated PPV (based on diagnostic specificity and sensitivity of 98% and 97%, respectively [4], and a PCNSL prevalence of 4% [12]) was 67%. The estimated PPV was 90% during 1988–1995 (i.e., before availability of HAART), given a PCNSL prevalence of 16% [4].

Thus, even when the epidemiological changes related to HAART are considered, one would not expect PPVs as low as those reported by Ivers et al. [9]. In this regard, false-positive results due to poor specificity of the assay cannot be excluded. Nevertheless, we believe that it is important to recognize the possible influence of HAART on the clinical significance of diagnostic tests and to interpret the results accordingly.

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Reply to Cinque et al.

Sir—We appreciate the letter from Cinque and colleagues [1] that emphasizes the variables that may have been at play in our evaluation of the operational characteristics of PCR for detection of Epstein-Barr virus (EBV) DNA in CSF samples to establish the diagnosis of primary CNS lymphoma in HIV-infected patients [2]. We reported the positive predictive value of EBV DNA PCR to be 29%. A number of factors should be considered when determining the value of any diagnostic test. These factors include the accuracy and precision of the test, the reference standard to which the test is compared, the receiver operating characteristics, and the prevalence of the disease in the population undergoing testing.

Cinque et al. [1] demonstrate a decline in the positive predictive value of EBV DNA PCR in their cohort during the same period as that considered in our study, and they attribute this decline to a decreased prevalence of disease. In our report, we noted that the prevalence of disease in our cohort was low, and we agree that this likely contributes to the low positive predictive value of the test in our study. The changing prevalence of disease over time is sometimes overlooked in the evaluation

of diagnostic tests, and it is appropriate to highlight its importance—particularly in the case of primary CNS lymphoma, which has decreased in prevalence since the advent of combination antiretroviral therapy [3–5].

Variability in nucleic acid amplification protocols between laboratories may result in varying levels of assay sensitivity. Our report aims, in part, to emphasize how EBV DNA PCR performs in a clinical setting that is commonly encountered in developed countries—specifically, in situations in which a commercial or commercially affiliated laboratory offers a novel diagnostic test for physicians in practice. These physicians often have no control over how the test is set up or performed. We believe our results have particular relevance for physicians practicing under such circumstances. We agree that, as for any nonstandardized test, the potential for variability should be carefully considered when EBV DNA PCR results are interpreted in the course of evaluation of patients with HIV infection and CNS disease.

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Confirmation of Results of Tests for West Nile Virus Infection in Organ Transplant Recipients

Sir—We refer to the recent article by Ravindra et al. [1], which describes 3 recipients of kidney or pancreas transplants who developed West Nile fever, 2 of whom had meningoencephalitis. We describe a renal transplant recipient who also developed West Nile fever with meningoencephalitis and who remained severely disabled after the development of infection. We also wish to point out the importance of confirming a positive result of an ELISA test with the plaque reduction neutralization test.

A 32-year-old man from Yemen who had received a renal transplant was admitted to the hospital in October 2003 with a 3-day history of fever, chills, and headache. He received immunosuppressive therapy that included prednisone, mycophenolate, and cyclosporine.

The patient had no history of blood product transfusion since the time of his transplantation. At admission, the patient was lethargic and had a fever (temperature, 38.8°C), and he became encephalopathic, with a deteriorating level of consciousness that required ventilator support. Lumbar puncture was performed, and analysis of CSF samples revealed a WBC count of 110 cells/mm^3 (23% polymorphs, 40% lymphocytes, 2% band form, and 22% monocytes), an RBC count of 15 cells/mm^3, a protein level of 117 mg/dL, and a glucose level of 59 mg/dL. IgM Capture ELISA (PanBio) detected IgM antibody to West Nile virus in serum samples but not in CSF samples. The serum specimen was also IgG antibody negative. Testing of the serum samples with capture ELISA was repeated 4 weeks later, and the results were positive for both IgG and IgM antibody. An electroencephalogram showed severe slowing bilaterally and frontal sharp waves consistent with encephalopathy. A CT scan did not reveal any significant abnormality. Results of confirmatory testing with a plaque reduction neutralization test (performed at New York state Department of Health) were negative at admission and were strongly positive 4 weeks later.

Management of the patient's illness involved supportive care. Immunosuppression was decreased by stopping treatment with mycophenolate and cyclosporine and by tapering the dose of prednisone. The patient required tracheotomy and receipt of long-term mechanical ventilation; his level of consciousness did not improve, and he died after 3 months.

In 2003 alone, 9858 cases of West Nile fever were reported across the United States, with a case-fatality rate of 2.6% [2]. Of the solid-organ transplant recipients with West Nile fever who were described in case reports, 8 of 9 had encephalitis, and 2 of the 8 died of the disease [1, 3]. Two of 3 patients in the report by Ravindra et al. [1] had encephalitis, and all recovered.

The patient whom we describe remained dependent on ventilatory support and did not recover consciousness for 3 months, at which time he died. This case emphasizes the serious consequences of West Nile fever, even if patients survive the illness [3].

West Nile virus infection is diagnosed by detection of IgM antibody to West Nile virus in serum or CSF samples by use of the IgM antibody capture ELISA as a sen-

sitive test. Of the patients with cases of West Nile virus identified in New York City in 1999 and 2000 and for whom a CSF sample was available, 95% had demonstrable IgM antibody (90% within 8 days of onset of symptoms) [4].

Residents in areas in which West Nile virus is endemic may have persistent IgM antibody from a previous infection that is unrelated to their current clinical illness, and, because most infected persons are asymptomatic and because IgM antibody may persist for ⩾6 months, an increase in the West Nile virus–specific neutralizing antibody titer between serum samples obtained in the acute phase and serum samples obtained in the convalescent phase is confirmatory of acute infection [5].

Serum samples for which ELISA demonstrates positive results should also be tested by plaque reduction neutralization test, the most specific test for arthropod-borne flaviviruses, to determine the specificity of antibodies to West Nile virus [6]. False-positive results of ELISA can occur because of the presence of other flaviviruses, such as St. Louis encephalitis virus, Japanese encephalitis virus, yellow fever virus, and dengue fever virus [7].

The close antigenic relationships among the flaviviruses may cause persons who were recently vaccinated with yellow fever vaccine or Japanese encephalitis vaccine or persons who had been recently infected with a related flavivirus (e.g., St. Louis encephalitis fever or dengue fever) to have a positive result of a test for IgM antibody to West Nile virus [7, 8]. The patient from Yemen whom we describe had resided in the United States for many years and had no history of recent travel or of recent vaccinations, making infection with other flaviviruses less likely.

In conclusion, West Nile virus infection in solid-organ transplant recipients can cause severe disability, and diagnosis of West Nile virus infection made on the basis of results of ELISA for antibodies should be confirmed with a plaque reduction neutralization test—the most specific test to help distinguish positive results of ELISA or other assays (e.g., an indirect immunofluorescence assay or a hemagglutination inhibition assay) from false-positive results that are due to cross-reactions with other flaviviruses.

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Domestically Acquired Fluoroquinolone-Resistant *Campylobacter* Infection

Sir—In a recent article, Kassenborg et al. [1] reported that, "When patients with domestically acquired fluoroquinolone-resistant *Campylobacter* infection were compared with matched healthy control subjects in a multivariate analysis, those infected were 10 times more likely to have eaten chicken or turkey cooked at a commercial establishment (18 [55%] of 33 case patients vs. 7 [21%] of 33 controls; matched OR, 10.0; 95% CI, 1.3–78).… This study provides additional evidence that poultry is an important source of domestically acquired fluoroquinolone-resistant *Campylobacter* infection" (p. S279).

The presented results are highly dependent on the specific model and variables selected, and they only achieve statistical significance if model uncertainty is improperly disregarded [2]. Our analysis of the same data reveals that the findings are highly sensitive to the subset of risk factors considered, the choice of variable-selection algorithms (e.g., forward vs. backward stepwise variable selection), the selection of a model form (e.g., logistic regression vs. nonparametric alternatives), and the treatment of missing data. The claimed 95% CI for the matched OR excludes 1 only because uncertainties have not been accounted for in these modeling choices [2]. Slight variations in modeling approach (e.g., using backward vs. forward stepwise variable selection vs. Bayesian model averaging) eliminate the claimed finding of a positive association between fluoroquinolone-resistant campylobacteriosis and poultry consumption. (Moreover, 55% is not usually considered "10 times more likely" than 21%. The matched OR of 10 is only a prediction from an unvalidated logistic regression model for which appropriate model diagnostics have not been presented [3], not an empirical finding.)

Nonparametric techniques, such as classification tree analysis, can help to avoid parametric model-selection biases

[4]. Kassenborg et al. [1] state, "In our final multivariate model, we examined the following risk factors: eating chicken or turkey cooked at a commercial establishment, eating in a non–fast food restaurant, using antacids, and eating nonpoultry meat at home. Using this model, we found that eating chicken or turkey at a commercial establishment was the only risk factor that remained independently associated with illness" (p. S281). By contrast, when we examined the same data set using classification tree analysis (which allows all variables to be considered), we found that exposure to ground beef outside of the home and exposure to raw milk both appear to be significant risk factors for fluoroquinolone-resistant campylobacteriosis. If all variables are considered, chicken consumption as a whole and chicken consumption in commercial establishments have nonsignificant negative associations with fluoroquinolone-resistant campylobacteriosis, whereas chicken consumption as a whole (of all types and at all venues) is associated with a statistically significantly lower risk of campylobacteriosis.

In summary, the findings presented by Kassenborg et al. [1] appear to be highly sensitive to specific modeling choices. Different choices—or use of nonparametric methods, to avoid having to make such choices—lead to very different conclusions. The reported significant positive association between poultry consumption and domestically acquired fluoroquinolone-resistant *Campylobacter* infection appears to be an implication of the particular model used that disappears when less restrictive models are used.

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Potential conflict of interest. L.A.C. has, in previous years, prepared comments on fluoroquinolone risk assessment for the US Food and Drug Administration's Center for Veterinary Medicine and the Animal Health Institute. He testified in 2003 for Bayer Animal Health on enrofloxacin use and campylobacteriosis. None of these parties was involved in the writing of this letter.

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Reply to Cox

Sir—Amplifying comments he made previously [1], Cox [2] has provided an interesting critique of our analysis of the FoodNet *Campylobacter* case-control study data [3]. We agree that multivariable analysis of epidemiologic data is inherently selective from a large number of exposures and the nearly infinite number of model forms. We agree that choosing an appropriate model is an essential part of data analysis and interpretation [4]. We followed standard epidemiologic principles to analyze the largest reported case-control study of sporadic *Campylobacter* infections and found a consistent, strong, and robust association between domestically acquired fluoroquinolone-resistant *Campylobacter* infection and the eating of poultry (chicken and turkey) outside of the home [3].

We do not agree that classification and regression tree (C&RT) analysis is an appropriate analytic tool for our data. The purpose of our analysis was to estimate the contribution of several independent exposures (risk factors) on the main outcome (fluoroquinolone-resistant *Campylobacter* infection). The hierarchical nature of the C&RT models does not allow estimation of the net effects of individual risk factors on the main outcome [5]. Lemon et al . [5] caution that, in situations like those in our study, which was designed to determine risk factors for *Campylobacter* infection, C&RT analysis should "not be used as a substitute for proven regression techniques" (p. 179). Moreover, the repeated use of "all variables" in describing a reanalysis of our data [2] leads us to believe that the conclusions of this reanalysis may be the result of the "data dredging," which Lemon et al. [5] specifically warn against in the application of C&RT.

Bayesian model averaging, which is distinct from C&RT, is an intriguing suggestion to account for uncertainty in our logistic model in a quite different fashion. As Viallefont et al. [6] discuss, when using Bayesian model averaging, the prior probability of the model form that was selected should take into account the available scientific knowledge. A Bayesian analysis of our data would use the large body of scientific evidence linking the use of fluoroquinolones (such as enrofloxacin) in poultry to the development of resistance in *Campylobacter* and the association between *Campylobacter* infection in humans and exposure to poultry to calculate a prior probability [7, 8]. Such an analysis would likely result in an even stronger measure of association between domestically acquired, fluoroquinolone-resistant *Campylobacter* infection in humans and eating chicken outside of the home.

Widespread use of the standards proposed by Bagley et al. [9] in the scientific literature would create greater transparency in describing what is done in multivariable analysis. Space limitations often limit such descriptions. Amplifying the description of the multivariable analysis in our study would not change the findings.

Readers interested in the legal context of this discussion, including the Administrative Law Judge's initial decision to up-

hold the US Food and Drug Administration's (FDA) proposed prohibition of fluoroquinolone use in poultry, are referred to FDA docket number 00N-1571 [1].

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Lack of Evidence That False-Positive *Aspergillus* Galactomannan Antigen Test Results Are Due to Treatment with Piperacillin-Tazobactam

Sir—Test results positive for circulating galactomannan (GM) in peripheral blood are a major criterion defining invasive aspergillosis [1]. Therefore, surveillance of patients with hematological malignancies who are at high risk for invasive aspergillosis by performing the GM assay on peripheral blood samples has become a standard method in many centers. Recent reports of false-positive results obtained with the Platelia *Aspergillus* GM ELISSA (Bio-Rad) in association with administration of piperacillin-tazobactam were published in *Clinical Infectious Diseases* and elsewhere [2, 3]. As a possible explanation, the investigators also reported on ELISA results positive for GM in most batches of piperacillin-tazobactam used during the study periods. We performed a study to survey the incidence of false-positive GM assay results associated with piperacillin-tazobactam therapy at our institution (Charité-Campus Benjamin Franklin; Berlin, Germany). From February 2003 through July 2003, we performed the *Platelia Aspergillus* GM assay twice weekly on peripheral blood samples obtained from neutropenic patients with hematological abnormalities who were receiving 13 different batches of piperacillin-tazobactam. Altogether, 40 neutropenic episodes (median duration, 14.3 days; range, 4–53 days) among 35 patients (median age, 51.6 years; range, 19–77 years) with acute leukemia (18 patients), lymphoma (8 patients), myeloma (4 patients), or other diseases (5 patients) were evaluated. During piperacillin-tazobactam treatment (total duration, 254 days; median duration, 6.4 days), 96 GM assays were performed. Ninety-four GM assays had negative results, and only 2 had positive results (optical density indexes, 1.6 and 2.2). Because these GM-positive samples were obtained from a patient who died from proven pulmonary aspergillosis within a week after the first positive GM assay test results, they were considered to be true-positive results.

Although we performed our investigation during a time period similar to that of previous reports (i.e., early 2003), we found no evidence of false-positive GM assay results in association with piperacillin-tazobactam treatment. This casts some doubt on the hypothesis of Adam et al. [2] that false-positive GM test results caused by contamination of certain piperacillin-tazobactam batches are the result of a recent modification of the drug production process. Thus, further investigations are warranted to precisely determine the origin of false-positive results.

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Clinical Infectious Diseases 2004; 39:1401–2

Platelia *Aspergillus* Assay and Potential Cross-Reaction

Sir—I read with interest 2 articles in the 15 March 2004 issue of *Clinical Infectious Diseases.* Both Adam et al. [1] and Viscoli et al. [2] reported their findings of "false-positive" results obtained with the Platelia *Aspergillus* ELISA test (Bio-Rad) among patients receiving piperacillin-tazobactam. Investigation revealed that these patients did not have any invasive fungal diseases.

The Platelia *Aspergillus* assay detects galactomannan (GM), a complex sugar, which is found in the *Aspergillus* cell wall. A positive reaction to the assay could be a sign of a possible *Aspergillus* infection. However, GM is a polysaccharide that is also found in many food products and is also present in other fungi. These other sources of GM may also give positive results with the Platelia *Aspergillus* test. GM is a normal by-product of the penicillin fermentation process. It is a noninfectious, nonpyrogenic carbohydrate.

As noted by Viscoli et al. [2, p. 915], "…cross-reactions of the monoclonal antibody (MAb) EB-A2, used in this test, have been described with other organisms (such as *Fusarium oxysporum, Rhodotorula rubra, Trichophyton rubrum, Trichophyton interdigitalis, Penicillium chrysogenum, Penicillium digitatum, Paecilomyces variotii,* and *Alternaria* species) and also with infant milk formulas, cyclophosphamide, and food." Piperacillin-tazobactam is manufactured in sterile manufacturing facilities and is subjected to rigorous sterility controls prior to its release to the market. Wyeth conducted laboratory testing of piperacillin-tazobactam product. The product is sterile and free of *Aspergillus* organisms. It is not "contaminated" with *Aspergillus* organisms or *Aspergillus* GM.

Because the Platelia *Aspergillus* test kit detects GM, not *Aspergillus* as its name implies, there can be a positive reaction to any GM, regardless of source. Therefore, the reaction is better characterized as either a positive reaction to GM or a "cross-reaction" by the test. It is not evidence of "contamination" of the sample being tested. Wyeth has taken steps to notify clinicians of this "cross-reaction" through a letter to health care providers, mailed in December 2003/January 2004, and through a modification of the Laboratory Interactions section of its worldwide product directional circular.

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Clinical Infectious Diseases 2004; 39:1402

Reply to Wu

Sir—We read with interest Dr. Wu's comments [1] about our article [2] on the relationship between false-positive results of the Platelia *Aspergillus* ELISA (Bio-Rad) and treatment with piperacillin-tazobactam. Careful investigation clearly showed an association between the false-positive reaction and administration of piperacillin-tazobactam, and no other explanation was found. We never doubted that piperacillin-tazobactam is a sterile product and certainly not contaminated with *Aspergillus* organisms. This is important and reassuring, both for patients and for clinicians. On the other hand, we never used the term "contamination" in our article [2].

We agree with Wu [1] that the molecule responsible for the cross-reaction of the Platelia *Aspergillus* test is so far of uncertain origin and that it does not necessarily originate from *Aspergillus* species. For this reason, we expect that Wyeth Pharmaceuticals will be able to provide the scientific community with an explanation for the phenomenon, including the molecular characterization and origin of this "extraneous" molecule, very soon.

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Clinical Infectious Diseases 2004; 39:1402

Reply to Penack et al. and Wu

Sir—In their letter, Penack et al. [1] question the reliability of the association between false-positive galactomannan (GM) antigenemia results obtained with use of

the Platelia *Aspergillus* ELISA (Bio-Rad) and piperacillin-tazobactam treatment. To date, outbreaks of false-positive results during surveillance of GM antigenemia have been reported by 3 European centers caring for patients with hematological abnormalities (2 centers in France [2, 3] and 1 in Italy [3]). In France, moreover, several other centers experienced the same phenomenon starting in May 2003 and alerted the Agence Française de Sécurité Sanitaire des Produits de Santé, which is the French health products safety agency.

To identify the risk factors for false-positive assay results, we designed a case-control study to compare exposures to risk factors of patients with and patients without false-positive results. Baseline characteristics and drug treatment histories were exhaustively collected from patient clinical charts. Multivariate analysis showed that administration of piperacillin-tazobactam was the only significant risk factor for false-positive assay results. A causal relationship was further suggested by positive GM assay results in 3 of 4 piperacillin-tazobactam batches recovered in our hematology unit. Viscoli et al. [4], in a similarly designed study, obtained the same results. Convergence between the conclusions of our study [2] and of the study by Viscoli et al. [4] is unlikely to be just a coincidence.

Piperacillin and tazobactam are penicillin derivatives and are obtained by semisynthesis from β-lactam compounds harvested from *Penicillium* mold cultures. GM is a fungal constituent of the cell wall of *Aspergillus* organisms but also of *Penicillium* molds, as described elsewhere [5] and as we stated in our article [2]. Ansorg et al. [6] have previously reported that some batches of piperacillin and amoxicillin-clavulanate may contain detectable level of GM antigen and have suggested that GM antigen may be carried through the production process into batches designed for therapeutic use. The Platelia *Aspergillus* assay was released to the market in 1997 in European countries, at which time piperacillin-tazobactam had already been a first-line drug for administration to febrile neutropenic patients for several years. We found, as did others [3], that the level of GM antigen recovered from piperacillin-tazobactam batches during the epidemic period could range widely according to the batch tested. However, the letter from Wu [7] is very elusive concerning the presence of GM antigen in piperacillin-tazobactam preparations. Because outbreaks of false-positive GM antigenemia related to piperacillin-tazobactam administration have occurred only recently and began to occur simultaneously in May 2003 in several independent hematological centers, we assume that a plausible cause may have been the presence of GM antigen in unusually high amounts in some of the piperacillin-tazobactam batches released during the epidemic period. However, we do not suggest that piperacillin-tazobactam preparations contained *Aspergillus* GM, viable *Aspergillus* organisms, or pyrogenic substances. The risk for occurrence of false-positive assay results due to piperacillin-tazobactam treatment in hematological centers could have depended on the level of GM antigen in the piperacillin-tazobactam batches locally supplied. We hope that Wyeth Pharmaceuticals will provide further information that will allow the causes for the recent implication of piperacillin-tazobactam administration in false-positive GM antigen ELISA results to be clarified.

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