Blinded Comparison of a Direct Immunofluorescent Monoclonal Antibody Staining Method and a Giemsa Staining Method for Identification of Pneumocystis carinii in Induced Sputum and Bronchoalveolar Lavage Specimens of Patients Infected with Human Immunodeficiency Virus

JOHN S. WOLFSON,* MARY ANN WALDRON, AND LUZ STELLA SIERRA

Infectious Disease Unit, Medical Services, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114

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A new direct immunofluorescence monoclonal antibody (DFA) method (Genetic Systems, Inc., Seattle, Wash.) for identification of Pneumocystis carinii in induced sputum and bronchoalveolar lavage specimens was compared in a blinded study with an established Giemsa stain method. We evaluated 148 consecutive clinical specimens from 104 patients with the following results. For the 67 patients (64%) infected with the human immunodeficiency virus (HIV), 49 were initially negative by both the DFA and the Giemsa methods, none were negative by DFA and positive by Giemsa, 6 were positive by DFA and negative by Giemsa, and 12 were positive by both methods, for a sensitivity and a negative predictive value of >99%. For the six patients positive by DFA and negative by Giemsa, all were positive by both methods on evaluation of subsequently obtained clinical specimens, suggesting a specificity of >99% and a false-positive rate of <1%. For 37 patients whose HIV status was negative or unknown, 35 were negative by both methods and 2 were positive by DFA and negative by Giemsa. The DFA method was simple to perform and required less time for scoring of stained slides than the Giemsa method, but care had to be taken to avoid false-positive readings due to extraneous fluorescence. This study indicates that the DFA method represents an advance as a sensitive, simple, and rapid way to identify P. carinii in induced sputum and bronchoalveolar lavage specimens from HIV-infected patients and suggests greater sensitivity of the DFA than the Giemsa method in this patient population.

With spread of infection with the human immunodeficiency virus (HIV), the possibility of diagnosis of Pneumocystis carinii pneumonia has increased markedly (2, 7), resulting in the need for improved methods for identification of P. carinii which are rapid, simple, minimally invasive or noninvasive, and inexpensive. Currently, patients may be evaluated for P. carinii pneumonia by microscopic examination of induced sputum (IS) or material obtained by bronchoalveolar lavage (BAL) (3, 7, 10, 13). The more common stains used to visualize P. carinii include methylene silver (4, 12), Giemsa (3, 9, 11), toluidine blue (6, 8, 9), Gram-Weigert (15), Papanicolaou (5), Cellufluor (1), and immunofluorescent monoclonal antibody (4, 6, 8, 9). Potential advantages of staining with monoclonal antibodies include excellent sensitivity and specificity. Immunofluorescence methods reported to date, however, have used indirect staining methods, which are time consuming. We therefore performed a blinded study comparing a new rapid direct immunofluorescent monoclonal antibody (DFA) method (Genetic Systems, Inc., Seattle, Wash.) to our currently used Giemsa method for identification of P. carinii in IS and BAL specimens.

All IS and BAL clinical specimens submitted to the Parasitology Laboratory between 31 January 1989 and 18 June 1989 for diagnosis of P. carinii pneumonia were evaluated. Sputum was induced by inhalation of 100 ml of 3% NaCl from the reservoir of an ultrasonic nebulizer (Bennett US-1), with expectoration at 5-, 10-, and 15-min intervals. BAL was performed by instillation of 150 ml of normal saline in 25-ml aliquots into the right middle lobe bronchus and suctioning into a sterile trap. Specimens were processed immediately. With IS specimens, one part sputum and three parts dithiothreitol solution (Stat-Pack Sputolysin Test, Behring Diagnostics, La Jolla, Calif.) were mixed by inversion, diluted 10-fold in 6.7 mM phosphate buffer (pH 7.1) (14), incubated at ambient temperature for 10 min, mixed, and centrifuged (10 min, 1,875 × g). Sediment was removed and spread onto five or six slides, one or two of which were stored at room temperature. Material on four slides was fixed in absolute methanol for 1 min, stained with 2 ml of Giemsa stain in 40 ml of 6.7 mM phosphate buffer (pH 7.1)-0.1% Triton X for 20 min, rinsed in phosphate buffer, and mounted. IS specimens were examined at ×400 magnification and scored as positive if clusters of free trophozoites and/or precysts were seen; slides were scored as negative if parasites were not seen during examination of each slide for 15 min. BAL fluid was centrifuged, and sediment was processed as described above. Four Giemsa-stained BAL slides were evaluated and scored as positive if either clusters of mature cysts showing rosette formation of intracystic bodies or clusters of free trophozoites or precysts were seen; if no parasites were seen during 30 min of examination, slides were scored as negative. Stained unstained slides containing IS and BAL material were stained for DFA (Genetics Systems, Inc.) as recommended by the manufacturer, except that specimens were fixed for 1 min in absolute methanol rather than acetone, to reduce loss of specimens from slides during fixation. Three specimens which washed off slides during preparation for DFA were not included in
analyses. DFA slides were scored independently in a blinded fashion as coded unknown specimens by two investigators (M.A.W. and L.S.S.) using a Leitz epifluorescence microscope (excitation filter [band-pass filter; BP], 450 to 490 nm; mirror [reflection short-pass filter], 510 nm; barrier filter [long-pass filter; LP], 515 nm) or a Zeiss epifluorescence microscope (excitation filter [BP], 450 to 490 nm; mirror [dielectric reflector], 510 nm; barrier filter [LP], 520 nm) with a 40× dry objective. Slides containing three or more cysts were scored as positive. Each DFA slide was evaluated for a maximum of 3 min. There was complete concordance between the two investigators in the blinded independent scoring of specimens. To assess reproducibility of the DFA method, two slides were made for each of 62 specimens and included in the blinded evaluation. In all instances, reading of duplicate slides was concordant.

A total of 148 specimens (109 IS and 39 BAL) from 104 patients (90 IS and 14 BAL) were studied. Chart review revealed that 67 patients (64%) were infected with HIV (66 HIV-antibody-positive patients and one homosexual man with Kaposis sarcoma whose HIV status was not tested), 12 were HIV antibody negative, and 25 were not tested.

For the 67 HIV-infected patients (Table 1), 49 were initially negative by both the DFA and the Giemsa methods, none were negative by DFA and positive by Giemsa, 6 were positive by DFA and negative by Giemsa, and 12 were positive by both methods, for a sensitivity and a negative predictive value of >99% and a false-negative rate of <1%. For the six patients positive by DFA and negative by Giemsa, all were positive by both methods on evaluation of subsequently obtained clinical specimens, suggesting a specificity and a positive predictive value of <99% and a false-positive rate of <1%, assuming that the six discrepant results represented false-negative findings by the Giemsa method. Two patients initially negative by DFA and Giemsa in two and three IS studies (and scored above as such) were later positive by DFA and negative by Giemsa in a BAL study; neither patient was further studied.

For the 12 HIV-negative patients, all DFA and Giemsa tests were negative. For 25 patients for whom HIV status was not determined (including 9 with cancer and 7 with a kidney, heart, or liver transplant), 23 were negative by both the DFA and Giemsa methods. Material obtained by BAL from one was positive by DFA and negative by Giemsa. Material obtained in three BAL studies from another patient was positive in two of three studies by the DFA method and negative in three by the Giemsa method; subsequent studies were negative.

For the total 148 specimens studied, 84 specimens that were negative by the DFA method were negative by the Giemsa method, for a negative predictive value of >99%.

This study was the first to evaluate a DFA staining method for diagnosis of P. carinii pneumonia and to compare such a test in a blinded fashion to a Giemsa method. Results with the DFA method correlated well with those of the Giemsa method, as has been reported for indirect immunofluorescent monoclonal antibody methods compared with other methods (4, 6, 8, 9). For HIV-positive patients, results suggested that the DFA method was as or more sensitive than the Giemsa method. Specimens prepared by the DFA method required less time for microscopic examination than those prepared by the Giemsa method. Staining of specimens was more rapid and simpler for the DFA method than for an available indirect immunofluorescent monoclonal antibody staining method (unpublished observations). The DFA method had several deficiencies, however, including expense, requirement for epifluorescence microscopy, and need for careful evaluation of slides to avoid false-positive readings due to extraneous fluorescence. A question which remains unanswered for the DFA and all other methods for identification of P. carinii in specimens is whether every positive result represents clinically significant disease. In conclusion, the DFA method shows promise for use as a primary method for diagnosis of P. carinii pneumonia or as a rapid screen to exclude the presence of P. carinii in IS and BAL specimens.

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LITERATURE CITED


