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 methamine silver (4, 12), Giemsa (3, 9, 11), toluidine blue (6, 8, 9), Gram-Weigert (15), Papanicolaou (5), Cellufluor (1), and immunofluorescent monoclonal antibody (DFA) method (Genetic Systems, Inc., Seattle, Wash.) which were currently used in this study. 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.

* Corresponding author.
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 [dichroic 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's 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 is 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

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<th>Table 1. Comparison of two methods for identification of P. carinii in IS and BAL specimens from HIV-infected patients</th>
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<td>DFA result/Giemsa result</td>
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*a For patients evaluated by both IS and BAL, the first test performed was tallied.

*b Discrepancies between the DFA and the Giemsa method which occurred on evaluation of different clinical specimens from the same patient or a discrepancy which occurred in evaluation of a clinical specimen obtained after evaluation of a specimen for which there was no discrepancy included.


