JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2000, p. 1461–1467 Vol. 38, No. 4
0095-1137/00/$04.00+0

Evaluation of Diagnostic Value and Epidemiological Implications of PCR for *Pneumocystis carinii* in Different Immunosuppressed and Immunocompetent Patient Groups

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Received 14 October 1999/Returned for modification 29 November 1999/Accepted 29 January 2000

To evaluate the value of single and nested PCRs for diagnosis of *Pneumocystis carinii* pneumonia (PCP) in a variety of respiratorily distressed patient groups, 574 respiratory samples from 334 patients (89 human immunodeficiency virus [HIV]-positive patients, 61 transplant recipients, 66 malignancy patients, 34 otherwise immunosuppressed patients, and 84 immunocompetent patients) were prospectively examined by microscopy and single and nested PCRs. The resulting data were correlated with clinical evidence of PCP. Microscopy and single PCR of bronchoalveolar lavage (BAL) specimens from HIV patients were 100% sensitive and specific in detecting PCP, whereas nested PCR, although being 100% sensitive, reached a specificity of only 97.5%. In the three non-HIV immunosuppressed patient groups, both single and nested PCR invariably produced lower positive predictive values than microscopy. Among immunocompetent patients, the positive predictive values of both PCRs were 0%. Therefore, the diagnostic values of the PCR methods tested do not seem to offer any additional advantage compared to that of conventional microscopy for these patient groups. However, nested PCR identified a significant percentage of clinically silent *P. carinii* colonizations in about 17 to 20% of immunocompetent and immunosuppressed non-HIV patients.

Pneumocystis carinii is an opportunistic eukaryotic pathogen causing life-threatening pneumonia (*P. carinii* pneumonia [PCP]) in immunosuppressed patients. Since its discovery in the early 1900s, it was thought to be a protozoon. Then in the 1980s, DNA analysis showed that this organism is, in fact, a fungal species (for a review, see reference 25). PCP is still the most common initial AIDS manifestation but has also been described for immunocompromised patients, i.e., malignancy patients, transplant recipients, and patients receiving immunosuppressive therapy.

As *P. carinii* cannot be grown in culture from clinical specimens, laboratory diagnosis of PCP has relied mainly upon microscopic demonstration with conventional cytochemical staining, i.e., with toluidine blue O, Grocott's methenamine silver, calcofluor for detection of the historically termed cyst stage, and Giemsa and Wright stains for identification of the traditionally termed trophozoites. Immunocytochemical staining procedures with monoclonal or polyclonal antibodies have been developed to increase sensitivity and specificity (2, 4, 10, 19, 20). All these methods, however, depend to a high degree on the quality of the specimens obtained for diagnosis. Therefore, invasively obtained respiratory samples such as bronchoalveolar lavage (BAL) specimens are still the material of choice for the diagnosis of PCP.

PCR technology was first applied to the diagnosis of PCP by Wakefield et al. to improve sensitivity and specificity (39). This promising technology allows PCP diagnosis from less or not invasively obtained clinical specimens such as induced sputa or oral washings (14, 16, 17). A nested-PCR approach (42) offers an even more sensitive and specific tool than the widely used single-PCR method (39) for detecting *P. carinii* DNA. However, the role of PCR in the laboratory diagnosis of PCP has yet to be defined, especially with regard to patient groups presenting with different grades or causes of immunosuppression. Furthermore, the diagnostic value of PCR has yet to be evaluated for differing types of clinical specimens.

Besides these more technical aspects influencing PCP diagnosis, the not yet completely understood biology of this ubiquitous pathogen and its still unclear pathology create difficulties in interpreting a positive PCR result. The issues of whether *P. carinii* giving rise to a specific serological antibody response early in childhood (31) persists for life and whether PCP might result from reactivation of a persistent infection have not yet been resolved. Therefore, respiratory samples from latently infected or colonized persons might yield positive PCR results for *P. carinii* DNA not causing overt PCP.

To evaluate the usefulness of PCR for PCP diagnosis, we studied four patient groups with different degrees or causes of immunosuppression as well as immunocompetent patients presenting with acute respiratory symptoms.

MATERIALS AND METHODS

Clinical specimens. Six hundred twenty-six respiratory specimens, including 461 BAL specimens, 68 endotracheal aspirates (ETA), 78 expectorated or induced sputa, and 19 other specimens, were prospectively obtained from 375 patients (126 females and 249 males; mean age, 45.7 years; range, 2 months to 90 years) from 1 January 1997 to 31 December 1997. Three hundred thirty-four patients with 574 clinical samples (Table 1) could be divided into five groups: 89 HIV-positive patients; 61 transplant recipients (21 bone marrow, 16 lung, 14 heart, 5 heart and lung, and 5 kidney transplant patients); 66 patients with malignancies, including leukemia, lymphoma, and solid tumors; 34 immunosuppressed patients (either suffering from some kind of immunodeficiency or receiving cytotoxic or immunosuppressing medication); and 84 immunocompetent

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TABLE 1. Numbers of clinical samples and patients from different patient groups analyzed in this study

Sample	No. of clinical samples (no. of patients) from patient group:				
	HIV	Transplant	Malignancy	Immuno-suppressed	Immuno-competent
BAL	84 (63)	121 (55)	78 (46)	40 (27)	104 (66)
ETA	9 (7)	12 (9)	18 (8)	5 (3)	18 (15)
Sputum	36 (25)	5 (4)	16 (13)	5 (5)	5 (5)
Others[a]	5 (4)	6 (6)	3 (3)	2 (1)	2 (2)
Total	134 (89)	144 (61)	115 (66)	52 (34)	129 (84)

[a] Includes oral washings and lung biopsies.

patients. Immunocompetence was defined by normal immunological function, no immunosuppressive or cytotoxic therapy, no HIV seropositivity, no systemic disease, and no malignancy. All patients were investigated for pulmonary symptoms characterized by dyspnea, cough, and fever and possibly accompanied by abnormal chest radiographs. Exact clinical data were obtained by medical chart review in cases in which conventional staining and/or PCR yielded positive results. These clinical data included the final diagnosis of the underlying primary pulmonary disease, microbiological and (cyto)pathological results of the respiratory specimen under evaluation and of subsequently obtained material, $CD4^+$ lymphocyte count (if performed), serum lactate dehydrogenase level, results of antibiotic treatment, prior or subsequent PCP episodes, use of antipneumocystic prophylaxis, and clinical outcome. Patients with a negative staining result but positive PCR were thought of as true-PCP patients if clinical findings were consistent with PCP, no other microbial pathogen was isolated from respiratory samples, serum lactate dehydrogenase levels were elevated, and empiric antimicrobial therapy included an antipneumocystic agent that led to resolution of the respiratory symptoms. If one or two of the last three criteria were not met, the patient was considered a possible-PCP patient, and statistical values were calculated separately for both PCP and non-PCP scenarios.

Only positive microscopical findings were reported to the clinicians. Therefore, diagnosis and therapy were not based on positive PCR results without microscopical corroboration.

Specimen processing. Clinical specimens were centrifuged at 3,430 × *g* for 10 min. A portion of the pellet was smeared on slides and Giemsa and Grocott stained for microscopic evaluation. Smears were examined by two microscopists experienced in *P. carinii* diagnosis.

DNA extraction and PCR procedures. Another part of the pellet was stored at −20°C until required for PCR analysis. Following overnight proteinase K digestion at 55°C, DNA was extracted using a Qiagen (Hilden, Germany) tissue kit. A two-step protocol was applied for nested PCR, as described elsewhere (42); external primers pAZ 102E and pAZ 102H were first used, which yielded a 340-b p fragment (39), after which a second round of amplification was performed using the nested primers pLE1 (5′-TCGGACTAGGATATAGCTGG-3′) and pLE2 (5′-CCCTTTCGACTATCTACC-3′), which resulted in a 193-bp product. Primary PCR was performed on 5 μl of proteinase K-treated samples in 45 μl of PCR reagent mixture containing 20 mM (each) dATP, dCTP, dGTP, and dTTP (Pharmacia LKB Biotechnology, Freiburg, Germany); 20 pmol of each primer (Metabion, Planegg-Martinsried, Germany); 2.5 U of *Taq* polymerase (AmpliTaq; PE Applied Biosystems, Weiterstadt, Germany); 5 μl of 10× *Taq* buffer (Perkin-Elmer [PE] Applied Biosystems); and sterile water. For a hot start, the PCR reagent mixture was preheated to 85°C for 2 min and subsequently to 80°C for 5 min before DNA was added. Thirty-five cycles consisting of denaturation at 94°C for 60 s, annealing at 55°C for 60 s, and extension at 72°C for 90 s were performed. Thereafter, a final extension for 7 min at 72°C was done. Two microliters of the primary PCR product was used for the same hot-start PCR program with 30 cycles. Products of both primary and nested PCR were investigated by agarose gel electrophoresis, stained with ethidium bromide, and analyzed under UV light. PCR analysis was performed without prior knowledge of the conventional-staining diagnosis. Contamination precautions included use of aerosol barrier pipette tips and the performance of master mix preparation, DNA extraction, PCR procedure, and specimen detection in separate rooms. Several positive (from BAL specimens of PCP patients) and negative (autoclaved water and the PCR mixture minus the DNA template) controls were tested simultaneously. For PCR-positive samples with negative conventional-staining results (PCR-positive samples were reexamined microscopically by two different persons) DNA isolation was repeated and an additional PCR was performed. A positive result was accepted when this PCR determination was also positive.

To show the specificity of the nested PCR for *P. carinii*, DNA sequencing of the amplified gene products of 12 nested-PCR-positive samples from the four non-HIV groups and from two control specimens of two different patients microscopically proven to have PCP was performed by the *Taq* cycle DyeDeoxy terminator method with an ABI PRISM 373A automatic sequencer (PE Applied Biosystems). The obtained sequences were compared with known nucleotide sequences by using the BLAST program (1).

RESULTS

Three hundred thirty-four of 375 patients could clearly be attributed to one of the five patient groups under study. From these 334 patients with respiratory disease, 574 samples were analyzed (Table 1). For evaluation of the differing diagnostic methods, samples and patients were analyzed separately for several reasons. First, material obtained by the same method from different locations during the same disease episode, e.g., by BAL of various regions of the bronchial tree, may yield different results. Second, samples obtained at different points in time during a single episode might differ in their diagnostic outcome. Third, samples obtained by different means may produce differing diagnostic results.

Within the HIV-positive group, conventional microscopy of routinely stained samples and single PCR correctly identified all patients with clinically proven PCP without producing false-positive results (Table 2). The nested PCR also detected all PCP patients within the HIV-positive group. One HIV-positive patient who was found to be *P. carinii* DNA positive in nested PCR of BAL material neither had suffered previously from PCP nor developed PCP in a follow-up period of 18 months. In the three non-HIV immunocompromised groups, microscopy detected all clinically proven PCP patients. Relying on microscopy alone, however, would have caused us to miss four possible-PCP patients (according to the definition given above) from within the three patient groups. On the other hand, both single and nested PCR produced a high number of false-positive results for these three patient groups. For 14 immunocompetent patients without clinically proven PCP and negative microscopic results, nested PCR yielded false-positive results, while for two immunocompetent non-PCP patients, single PCR yielded positive results.

A good correlation between the results of microscopy and single and nested PCRs was found for respiratory samples of HIV-positive patients, especially when we focused on clinical cases rather than on samples alone (Table 3). The three sputa positive by nested PCR only were obtained from a single patient as follow-up to a PCP episode previously diagnosed from BAL specimens by all three methods analyzed in this study. Therefore, the patient would have been missed only by conventional staining or single PCR if no BAL had been performed. In the other four patient groups, however, microscopy and PCR results differed markedly, mainly with BAL samples but also with sputum and ETA specimens. The discrepancies between the three diagnostic methods performed on differing

TABLE 2. Results of conventional microscopy and single and nested PCRs and correlation with clinical evidence for PCP in patients with acute respiratory symptoms for samples and patients

Patient status	Total no. of samples (total no. of patients)	No. of samples (no. of patients) that were:			
		Nested-PCR positive	Single-PCR positive	Microscopy positive	Positive clinically for *P. carinii*[a]
HIV positive	134 (89)	39 (21)	31 (20)	28 (20)	38 (20)
Transplant recipient	144 (61)	20 (10)	10 (5)	6 (3)	6 + 2 (3 + 1)
Malignancy	115 (66)	17 (11)	10 (6)	3 (2)	4 + 2 (2 + 2)
Immunosuppressed	52 (34)	11 (7)	4 (2)	3 (1)	3 + 1 (1 + 1)
Immunocompetent	129 (84)	16 (14)	2 (2)	0 (0)	0 (0)

[a] Figures added by a plus sign represent possible cases of *P. carinii* infection as defined in Materials and Methods.

respiratory materials from the five different patient groups are presented in Table 4. Discrepancies between conventional staining and nested PCR were highest among the immunocompromised groups, with results differing in nearly every sixth case, and were lowest among the HIV patients. In all five patient groups—almost independently of the respiratory material investigated—the discrepancies were largely due to differences between single- and nested-PCR results, while the results of conventional staining and single PCR differed less frequently. A detailed list of the statistical values representing sensitivity, specificity, positive predictive value, and negative predictive value for the five patient groups and the investigated patients' respiratory specimens is presented in Table 5. With respiratory disease episodes of HIV patients being considered, microscopy and single PCR were 100% sensitive and specific, reaching positive and negative predictive values of 100%, whereas nested PCR was 100% sensitive and 97.5% specific, reaching positive and negative predictive values of 97 and 99%. If confirmed- and possible-PCP patients are taken together, both single and nested PCR did not increase the sensitivity for samples of any patient group compared to the sensitivity of microscopy, irrespective of the type of respiratory material examined. However, at the level of individual samples, the sensitivity with samples of HIV and malignancy patients could be raised by using both single and nested PCR compared to that of conventional microscopy alone. In HIV-positive and transplanted patients, sensitivity rates for BAL specimens were impressively higher than for sputum and ETA specimens, whereas this could not be observed in the other three patient groups. Furthermore, specificities and positive predictive values in all patient groups and for all differing specimens examined were lower for nested PCR than for conventional microscopy.

DISCUSSION

To evaluate the usefulness of single and nested PCRs as diagnostic tools for the diagnosis of PCP and to compare them to conventional microscopy, we analyzed 334 patients presenting with acute respiratory symptoms who could clearly be separated into one of five patient groups based on different causes of immunosuppression such as HIV, posttransplantation therapy, malignancies, and other immunocompromising conditions.

In the HIV patient group, all confirmed-PCP cases were detected by each of the three diagnostic methods examined—microscopy and single and nested PCRs—reaching sensitivities of 100%. However, nested PCR exhibited a higher sensitivity rate with BAL specimens as well as sputum and ETA samples than those of single PCR or microscopy. On the other hand, specificities and positive predictive values were higher for microscopy and single PCR than for nested PCR in this patient group. Therefore, by relying on microscopy alone, no HIV-positive PCP patient would have been missed while by nested PCR alone, one patient out of 89 would have wrongly identified as suffering from PCP.

In the other three immunocompromised patient groups, the discrepancies between the results obtained by conventional and molecular methods were even more profound. If only clinically confirmed cases of PCP had been considered, both PCR methods would have produced a significant percentage of false-positive results while microscopy would have correctly identified all clinically confirmed PCP cases without yielding a false-positive result. If only confirmed- and possible-PCP cases had been considered, microscopy would have missed several PCP episodes; in this scenario, however, both PCRs would have also yielded a remarkable number of false-positive findings. For nested PCR, the positive predictive values for the three immu-

TABLE 3. Correlation of positive findings by microscopy and PCR for samples and patients from different patient groups

Material	No. of positive samples (patients)/total no. of samples (patients) by test type for patient group:															
	HIV			Transplant			Malignancy			Immunosuppressed			Immunocompetent			
	Nested PCR	Single PCR	Microscopy	Nested PCR	Single PCR	Microscopy	Nested PCR	Single PCR	Microscopy	Nested PCR	Single PCR	Microscopy	Nested PCR	Single PCR	Microscopy
BAL	36/84 (21/63)	31/84 (20/63)	28/84 (20/63)	15/121 (7/55)	10/121 (5/55)	6/121 (3/55)	16/78 (11/46)	9/78 (6/46)	3/78 (2/46)	8/40 (5/27)	2/40 (1/27)	2/40 (1/27)	13/104 (12/66)	2/104 (2/66)	0/104 (0/66)
ETA	0/9 (0/7)	0/9 (0/7)	0/9 (0/7)	3/12 (3/9)	0/12 (0/9)	0/12 (0/9)	0/18 (0/8)	0/18 (0/8)	0/18 (0/8)	1/5 (1/3)	1/5 (1/3)	1/5 (1/3)	2/18 (2/15)	0/18 (0/15)	0/18 0/15)
Sputum	3/36 (1/25)	0/36 (0/25)	0/36 (0/25)	0/5 (0/4)	0/5 (0/4)	0/5 (0/4)	0/16 (0/13)	0/16 (0/13)	0/16 (0/13)	2/5 (2/5)	1/5 (1/5)	0/5 (0/5)	1/5 (1/5)	0/5 (0/5)	0/5 (0/5)
Total	39/134 (21/89)	31/134 (20/89)	28/134 (20/89)	20/144 (10/61)	10/144 (5/61)	6/144 (3/61)	17/115 (11/66)	10/115 (6/66)	3/115 (2/66)	11/52 (7/34)	4/52 (2/34)	3/52 (1/34)	16/129 (14/84)	2/129 (2/84)	0/129 (0/84)

TABLE 4. Discrepancies between results of conventional staining and single and nested PCRs for *P. carinii* from different patient groups for samples and patients

Patient group	Material	Compared methods[a]	No. of discrepancies/total no. of samples (%)	No. of differing results in patients/ total no. of patients (%)
HIV	BAL	Nested PCR and CS	8/84 (9.5)	1/63 (1.6)
		Single PCR and CS	3/84 (3.6)	0/63 (0)
		Nested and single PCR	5/84 (5.9)	1/63 (1.6)
	Sputum and ETA	Nested PCR and CS	3/45 (6.7)	1/31 (3.2)
		Single PCR and CS	0/45 (0)	0/31 (0)
		Nested and single PCR	3/45 (6.7)	1/31 (3.2)
	Total	Nested PCR and CS	11/134 (8.2)	1/89 (1.1)
		Single PCR and CS	3/134 (2.2)	0/89 (0)
		Nested and single PCR	8/134 (5.9)	1/89 (1.1)
Transplant	BAL	Nested PCR and CS	9/121 (7.4)	4/55 (7.3)
		Single PCR and CS	4/121 (3.3)	2/55 (3.6)
		Nested and single PCR	5/121 (4.1)	2/55 (3.6)
	Sputum and ETA	Nested PCR and CS	3/17 (17.6)	3/13 (23.1)
		Single PCR and CS	0/17 (0)	0/13 (0)
		Nested and single PCR	3/17 (17.6)	3/13 (23.1)
	Total	Nested PCR and CS	14/144 (9.7)	7/61 (11.5)
		Single PCR and CS	4/144 (2.8)	2/61 (3.3)
		Nested and single PCR	10/144 (6.9)	5/61 (8.2)
Malignancy	BAL	Nested PCR and CS	13/78 (16.7)	9/46 (19.6)
		Single PCR and CS	6/78 (7.7)	4/46 (8.7)
		Nested and single PCR	7/78 (8.9)	5/46 (10.9)
	Sputum and ETA	Nested PCR and CS	0/34 (0)	0/21 (0)
		Single PCR and CS	0/34 (0)	0/21 (0)
		Nested and single PCR	0/34 (0)	0/21 (0)
	Total	Nested PCR and CS	14/115 (12.2)	9/66 (13.6)
		Single PCR and CS	7/115 (6.1)	4/66 (6.1)
		Nested and single PCR	7/115 (6.1)	5/66 (7.6)
Immunosuppressed	BAL	Nested PCR and CS	6/40 (15.0)	4/27 (14.8)
		Single PCR and CS	0/40 (0)	0/27 (0)
		Nested and single PCR	6/40 (15.0)	4/27 (14.8)
	Sputum and ETA	Nested PCR and CS	2/10 (20.0)	2/8 (25.0)
		Single PCR and CS	1/10 (10.0)	1/8 (12.5)
		Nested and single PCR	1/10 (10.0)	1/8 (12.5)
	Total	Nested PCR and CS	8/52 (15.4)	6/34 (17.6)
		Single PCR and CS	1/52 (1.9)	1/34 (2.9)
		Nested and single PCR	7/52 (13.5)	5/34 (14.7)
Immunocompetent	BAL	Nested PCR and CS	13/104 (12.5)	12/66 (18.2)
		Single PCR and CS	2/104 (1.9)	2/66 (3.0)
		Nested and single PCR	11/104 (10.6)	10/66 (15.2)
	Sputum and ETA	Nested PCR and CS	3/23 (13.0)	3/20 (15.0)
		Single PCR and CS	0/23 (0)	0/20 (0)
		Nested and single PCR	3/23 (13.0)	3/20 (15.0)
	Total	Nested PCR and CS	16/129 (12.4)	14/84 (16.7)
		Single PCR and CS	2/129 (1.6)	2/84 (2.4)
		Nested and single PCR	14/129 (10.9)	12/84 (14.3)

[a] CS, conventional staining.

nocompromised patient groups were less than 40%. Furthermore, in all five patient groups and for almost every respiratory material analyzed, the discrepancies between the different diagnostic methods were largely due to the differences between single- and nested-PCR results, while the results differed less frequently between conventional microscopy and single PCR. This further underscores the vague value of nested PCR in the diagnosis of PCP. In addition, it might be wise to consider that conventional microscopy of a Giemsa-stained BAL specimen obtained from a bone marrow transplant recipient revealed a diagnosis of *Toxoplasma gondii* pneumonia which would have been missed by performing *P. carinii* PCR alone.

In most studies comparing microscopy- and PCR-based methods for PCP diagnosis (2, 4, 6, 7, 8, 10, 12, 13, 15, 18, 19, 21, 22, 24, 26, 27, 28, 29, 32, 33, 34, 35, 36, 37, 38, 40, 41), no significant advantage of nested PCR, with regard to sensitivity and specificity compared to those of morphological diagnosis, could be established when BAL specimens were examined. For HIV patients, however, the majority of studies report higher sensitivity rates for single and nested PCR than for microscopy when induced sputum is the material examined. This observation was corroborated in our study for nested PCR only, although no PCP patient was missed by relying on microscopy alone. Interestingly, in contrast to results from studies of postmortem lung material (11, 23, 30) or of respiratory samples from immunocompetent patients with acute respiratory dis-

TABLE 5. Sensitivity, specificity, and positive and negative predictive values of microscopy and single and nested PCRs for samples and patients of different patient groups

Patient group	Material	% for samples (% for patients)[a]											
		Nested PCR				Single PCR				Microscopy			
		SENS	SPEC	PPV	NPV	SENS	SPEC	PPV	NPV	SENS	SPEC	PPV	NPV
HIV	BAL	100 (100)	97.9 (97.5)	97 (96)	100 (100)	88.6 (100)	100 (100)	100 (100)	93 (100)	80.0 (100)	100 (100)	100 (100)	88 (100)
	Sputum and ETA	75.0 (100)	100 (100)	100 (100)	98 (100)	0 (0)	100 (100)	ND (ND)	91 (97)	0 (0)	100 (100)	ND (ND)	91 (97)
	Total	97.4 (100)	98.9 (98.5)	97 (96)	99 (100)	79.5 (100)	100 (100)	100 (100)	92 (100)	71.8 (100)	100 (100)	100 (100)	90 (100)
Transplant	BAL	100 (100)	92.9 (94.6)	47 (60)	100 (100)	100 (77.8)	97.4 (97.3)	70 (70)	100 (98)	85.7 (66.7)	100 (100)	100 (100)	99 (97)
		100 (100)	92.3 (94.1)	43 (57)	100 (100)	100 (75.0)	96.2 (96.1)	60 (60)	100 (98)	100 (75.0)	100 (100)	100 (100)	100 (98)
	Sputum and ETA	0 (0)	81.3 (75.0)	0 (0)	93 (90)	0 (0)	100 (100)	ND (ND)	94 (92)	0 (0)	100 (100)	ND (ND)	94 (92)
	Total	85.7 (88.9)	89.9 (91.2)	30 (40)	99 (99)	85.7 (66.7)	97.1 (97.1)	60 (60)	99 (98)	85.7 (66.7)	100 (100)	100 (100)	99 (98)
		100 (100)	87.9 (89.5)	30 (40)	100 (100)	100 (75.0)	96.6 (96.5)	60 (60)	100 (98)	100 (75.0)	100 (100)	100 (100)	100 (98)
Malignancy	BAL	100 (100)	82.7 (84.9)	19 (31)	100 (100)	100 (100)	92.0 (94.5)	33 (56)	100 (100)	100 (60.0)	100 (100)	100 (100)	100 (97)
		100 (100)	79.5 (83.3)	18 (36)	100 (100)	100 (100)	90.9 (95.2)	33 (67)	100 (100)	100 (50.0)	100 (100)	100 (100)	100 (96)
	Sputum and ETA	ND (100)	ND (100)	ND (100)	ND (100)	ND (100)	ND (100)	ND (100)	ND (100)	ND (100)	ND (100)	ND (100)	ND (100)
		ND (100)	ND (100)	ND (100)	ND (100)	ND (100)	ND (100)	ND (100)	ND (100)	ND (100)	ND (100)	ND (100)	ND (100)
	Total	100 (100)	88.3 (89.9)	24 (35)	100 (100)	100 (100)	94.6 (96.3)	40 (60)	100 (100)	75.0 (50.0)	100 (100)	100 (100)	99 (97)
		100 (100)	85.9 (88.7)	18 (36)	100 (100)	100 (100)	93.8 (96.2)	33 (67)	100 (100)	100 (50.0)	100 (100)	100 (100)	100 (97)
Immunosuppressed	BAL	100 (100)	84.2 (84.6)	25 (20)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)
	Sputum and ETA	100 (100)	77.8 (87.5)	33 (67)	100 (100)	100 (100)	88.9 (100)	50 (100)	100 (100)	100 (50.0)	100 (100)	100 (100)	100 (89)
		100 (100)	71.4 (83.3)	33 (67)	100 (100)	100 (100)	85.7 (100)	50 (100)	100 (100)	100 (50.0)	100 (100)	100 (100)	100 (86)
	Total	100 (100)	83.7 (85.4)	27 (36)	100 (100)	100 (100)	97.9 (100)	75 (100)	100 (100)	100 (75.0)	100 (100)	100 (100)	100 (98)
		100 (100)	81.8 (84.4)	14 (29)	100 (100)	100 (100)	96.9 (100)	50 (100)	100 (100)	100 (50.0)	100 (100)	100 (100)	100 (97)
Immunocompetent	BAL	ND (ND)	87.5 (81.8)	0 (0)	100 (100)	ND (ND)	98.1 (96.9)	0 (0)	100 (100)	ND (ND)	100 (100)	ND (ND)	100 (100)
	Sputum and ETA	ND (ND)	86.9 (85.0)	0 (0)	100 (100)	ND (ND)	100 (100)	ND (ND)	100 (100)	ND (ND)	100 (100)	ND (ND)	100 (100)
	Total	ND (ND)	87.6 (83.3)	0 (0)	100 (100)	ND (ND)	98.4 (97.6)	0 (0)	100 (100)	ND (ND)	100 (100)	ND (ND)	100 (100)

[a] SENS, sensitivity; SPEC, specificity; PPV, positive predictive value; NPV, negative predictive value. Underlined numbers indicate statistical values, if proven and possible-PCP patients are treated as true PCP patients.

eases (41, 42), a significant percentage (17%) of immunocompetent non-PCP patients in our study were identified as *P. carinii* DNA positive by nested PCR, suggesting colonization within the immunocompetent patient group. Similar colonization rates have been found for chronic obstructive pulmonary disease patients (5), immunocompetent patients with respiratory diseases (3), and children with chronic respiratory disorders in the absence of underlying immunodeficiencies (9). Similar percentages of *P. carinii* carriage ranging from 17 to 20% were detected by nested PCR in all three non-HIV immunocompromised groups, despite varying degrees of immunosuppression. In the HIV group, however, colonization with *P. carinii* as defined by a positive nested-PCR result was found only for a single patient in the absence of clinically manifested PCP (1.1% of HIV patients in our study population). These findings indicate that *P. carinii* colonization can exist in different immunocompetent and -compromised patient groups without causing overt PCP. The observation that nested-PCR results correlated very well with the absence or presence of PCP in HIV patients with acute respiratory symptoms further suggests that *P. carinii* colonization occurs rarely in association with HIV infection without leading to PCP. Therefore, it may be concluded that PCP does not usually result from reactivation of a latent infection during immunosuppression, since (with one exception) in almost no non-PCP HIV-infected patient could *P. carinii* DNA be detected by nested PCR. The immunological status of the HIV patients in our study was of such quality that *P. carinii* DNA detection by nested PCR almost invariably correlated with clinical PCP, underscoring the pathogenetic potential of *P. carinii* in this patient group.

Taken together, the data of this study indicate that nested PCR, albeit useful in epidemiological studies, has only limited value in the diagnosis of overt PCP due to the high number of false-positive results and low positive-predictive values obtained for non-HIV immunocompromised and immunocompetent patients. Even with HIV patients, for whom the differences between the diagnostic results of microscopy and single and nested PCRs were quite marginal, microscopy and single PCR performed better than nested PCR with regard to specificity and positive predictive value, when patients rather than individual specimens were analyzed. Therefore, the timing of collection, amount, and quality of respiratory material seems to be more important for obtaining rapid and significant laboratory results in the diagnosis of PCP than the replacement of conventional microscopy by molecular methods.

ACKNOWLEDGMENT

We thank Randy Caldwell for critically reading the manuscript.

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