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Cost effectiveness of the induced sputum technique for the diagnosis of *Pneumocystis carinii* pneumonia (PCP) in HIV-infected patients

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ABSTRACT: The purpose of this study was to assess: 1) the percentage of human immunodeficiency virus (HIV)-infected patients with suspected *Pneumocystis carinii* pneumonia (PCP) but unable to undergo the induced sputum procedure, together with the reasons involved; 2) the sensitivity and specificity of induced sputum procedure, using conventional stains and an immunofluorescence test; and 3) the cost of introducing induced sputum procedure for the diagnosis of PCP.

One hundred and thirty eight HIV-infected patients with suspected PCP underwent induced sputum procedure and bronchoalveolar lavage (BAL). *P. carinii* was identified in induced sputum and BAL samples using conventional and immunofluorescence staining. The economic analysis took into account the direct costs of the two procedures.

The induced sputum procedure was either not feasible or unsuccessful in 29% of the patients. The sensitivity of induced sputum, using conventional and immunofluorescence staining, was 0.27 and 0.56 respectively. The economic analysis showed that the two strategies (systematic BAL *versus* BAL only after negative induced sputum) are equivalent in cost terms when the induced sputum to BAL cost ratio is equal to the product of the prevalence of PCP by the sensitivity of induced sputum procedure.

We conclude that the immunofluorescence test should be the reference technique for induced sputum samples, whilst conventional stains are more clinically relevant for BAL samples. The cost of introducing induced sputum should take into account the sensitivity of induced sputum and the prevalence of PCP in the suspected population.

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Pneumocystis carinii (PCP) remains a common, life-threatening, opportunistic infection and is the first manifestation of acquired immune deficiency syndrome (AIDS) in one-third of human immunodeficiency virus (HIV)-infected patients in France. Even though the incidence of PCP should decrease with widespread prophylaxis, there were 2,500 new cases in France [1] and as many as 160,000 in the USA in 1991. There is thus an increased demand for bronchoalveolar lavage (BAL), which is currently the standard procedure for the diagnosis of PCP [2, 3]. However, BAL is invasive, time-consuming and expensive, and some centres have therefore proposed non-invasive diagnostic procedures such as induced sputum (IS), followed by BAL if IS is negative [4, 5]. The IS procedure, when performed with appropriate techniques for induction, processing, conventional staining and interpretation, has a sensitivity of 0.5–0.75 and is fully specific [4–7]. The use of immunofluorescence (IF) staining methods increases the sensitivity [8–13]. However, few authors have analysed the clinical limits of this procedure, *i.e.* the proportion of patients for whom it is inapplicable or unsuccessful. Moreover, its economic basis remains to be fully analysed. In a prospective study, we assessed: 1) the proportion of patients unable to undergo IS and the reasons involved: 2) the sensitivity and specificity of IS relative to BAL using conventional staining and IF; and 3) the relative cost of a strategy involving BAL only after negative IS.

Patients and methods

Patients

Over a 10 month period, all HIV-infected patients (n=138) with suspected PCP (suggestive clinical

features, abnormal chest X-ray, or hypoxaemia <80 mmHg (<11 kPa) on room air) were included in the trial. There were 123 men and 15 women and the mean age was 33.4 yrs (21–53 yrs); 87 patients were homosexuals, 17 were infected by blood transfusion or heterosexual intercourse and 34 were intravenous drug abusers. The mean CD4 lymphocyte count was 72·mm^{-3}. Forty seven patients were receiving primary prophylaxis with pentamidine isothionate aerosols, 300 mg every four weeks (n=37), or sulphamethoxazole-trimethoprim, 1 tablet *q.d.* (n=10). Twenty seven patients were receiving secondary prophylaxis with pentamidine isothionate aerosols, 300 mg every four weeks (n=21), or sulphamethoxazole-trimethoprim, 1 tablet *q.d.* (n=6). The patients receiving prophylaxis were not different from those not receiving prophylaxis in terms of clinical, radiological characteristics and room air arterial blood gas.

Sputum induction technique

Sputum production was induced by inhalation of an aerosolized 3% saline solution for 15 min using an ultrasound device (Ultra Neb 99, De Vilbiss, Arcueil, France).

BAL technique

Regardless of the results of IS, all of the patients subsequently underwent BAL on the same day, as described previously [14], by instillation of 240 ml of normal saline in 60 ml aliquots into the right middle lobe bronchus and then suctioning the solution into a sterile trap.

Parasite staining techniques

IS and BAL specimens were processed immediately. The sputum samples were liquified in a mucolytic solution (Digest EUR*, dihydroxydithiolbutane diluted 1/10 in sterile water) then vortexed, incubated at 37°C for 5 min, washed with phosphate-buffered saline (PBS) and centrifuged. Smears were made from the pellet, then fixed and stained. BAL specimens were centrifuged and 10 ml of the pellet was smeared, air dried and fixed with methanol. Two stains were used for each sample: Giemsa was used to stain trophozoites and intracystic bodies, and Musto, a silver stain [15], was used to stain the cyst walls. A specific monoclonal antibody for *P. carinii* was also used ("Monofluo" R Kit *P. carinii*, Diagnostics Pasteur, France). Positive specimens contained characteristic fluorescent oocysts. The monoclonal antibody used was specific for *P.carinii* and did not have cross-reactivity with other organisms. Test specimens containing five or more fluorescent oocysts were scored as positive, whilst those containing 1–5 oocysts were retested; if the second test confirmed the first, the specimens were scored as weakly positive. The slides were examined in blinded fashion, the pathologists being unaware of the patient's clinical status. During the course of the study, the IF results were not used in patient management.

Cost analysis

Only the direct costs (sum of hospital charges, technician time, materials costs, and professional fees) were taken into account; the cost ratio of IS:BAL is 1:10 in our institution.

Statistical methods

Statistical differences between the two sub-populations were analysed using a Chi-squared test. The results are expressed as mean±SEM.

Results

The IS procedure was either not feasible or unsuccessful in 40 of the 138 patients (29%). The reasons were either neurological disorders, major asthenia or dyspnoea in 30 patients, while in the remaining 10 patients, no sputum was obtained despite well-conducted induction. All of these patients underwent BAL, and PCP was diagnosed in 13 (32%).

Both IS and BAL were successfully performed in 98 of the 138 patients (71%). Using conventional stains, 26 cases of PCP were identified by BAL and 7 also by IS. The prevalence of PCP was, therefore, 0.26 and the sensitivity and specificity of IS were 0.27 and 1, respectively. None of the negative cases, followed for 3 months, showed clinical or radiological features of PCP. Using the IF technique, 30 cases of PCP were identified by BAL, and 17 also by IS, giving a prevalence of 0.30 and a sensitivity and specificity for IS of 0.56 and 1, respectively (table 1).

Table 1. – Sensitivity of IS using conventional and immunofluorescent staining; prevalence of *Pneumocystis carinii* pneumonia in the study population

Staining	Sensitivity	Prevalence
Conventional	0.27	0.26
Immunofluorescence	0.56	0.30

IS: induced sputum.

Using this IF technique, the prevalence of PCP and the sensitivity of IS were lower in the patients receiving prophylaxis (0.15 and 0.37, respectively) than in the patients not receiving prophylaxis (0.34 and 0.63, respectively) although the difference was not significant.

In four cases, BAL specimens negative with conventional staining were weakly positive with IF. As the IF results were only known at the end of the study, these patients remained without PCP-specific

treatment. None were receiving anti-*P. carinii* prophylaxis or had previously had PCP. The outcome of the acute lung disease, diagnosed as bacterial bronchopneumonia in three and lymphocytic interstitial pneumonia in one, was favourable in all four cases. With a mean follow-up of five months (range 2–7 months), none of these patients had developed clinical or radiological features of PCP.

To evaluate the economic advantage of introducing IS as the first-line diagnostic test for PCP, we calculated the threshold of prevalence at which the cost of the two strategies (BAL systematically *versus* BAL only if IS is negative) would be the same. Since the specificity of IS is 1, the IS:BAL cost ratio is the product of prevalence by sensitivity for an equivalent cost of both strategies (see appendix). This approach gives a simple evaluation of the cost of the introduction of IS. For a given IS:BAL cost ratio, and as a function of the sensitivity of IS and the prevalence of PCP in the study population, the cost-equivalent curves for the two diagnostic strategies can be drawn (fig. 1). This curve designates two zones: in the upper right zone (shaded for a 0.1 cost ratio) it is preferable to perform BAL only if IS is negative, while in the lower left zone it is preferable to perform BAL from the outset. In the present study, with an IS:BAL cost ratio of 0.10, the cost-effective threshold prevalence of PCP would be 0.38 using conventional staining but 0.17 using IF, since the higher the sensitivity, the lower the cost-effective threshold of prevalence.

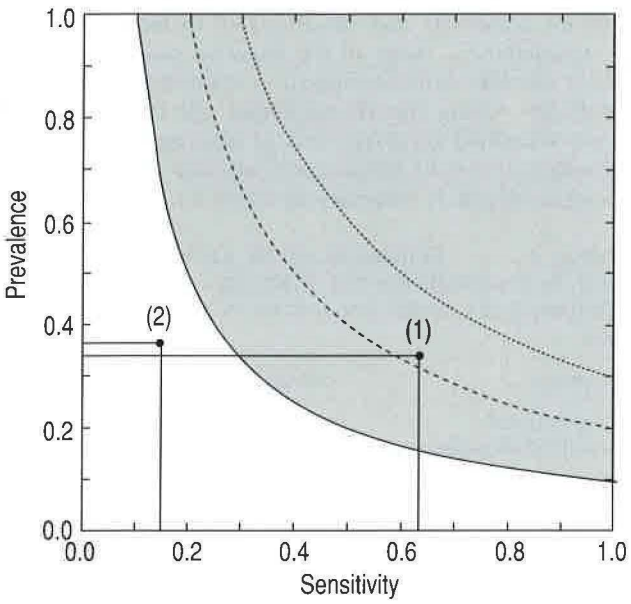

Fig. 1. – Cost equivalent curve of the two diagnostic strategies (systemic BAL *versus* BAL only after a negative sputum test). ·····:CR=0.3; ----: CR=0.2;—— : CR=0.1. Shaded area: for a 0.1 cost ratio it is preferable to perform BAL only if IS is negative (1): patients without specific prophylaxis. The introduction of IS procedure is cost-effective; point (2): patients with specific prophylaxis, the introduction of the IS procedure is not cost-effective. CR: induced sputum to bronchoalveolar lavage cost ratio; BAL: bronchoalveolar lavage; IS: induced sputum.

Discussion

Few authors have dealt with the clinical limitations of IS O'Brien *et al.* [16] stated that 27% of their patients were unable or refused to undergo the IS procedure, whereas, in another publication, this was the case in only three of 62 subjects [6]. In our study, the reasons for IS being unfeasible were numerous, but were mainly related to neurological disorders, major asthenia or dyspnoea. When used, the procedure was generally successful; failures were essentially due to a lack of co-operation by the patient. Although some authors have suggested that unsuccessful IS is correlated with the presence of PCP, we found that the prevalence in this group was not significantly different to that among the patients who underwent the procedure.

Immunofluorescence staining with monoclonal antibodies is a highly sensitive technique, as shown by the four cases in which IF gave weakly positive results for BAL specimens which were negative by conventional staining. The fact that the four patients concerned did not go on to develop PCP suggests that weakly positive IF staining in BAL samples may not be clinically relevant and should be considered as falsely positive if the clinical course is taken as the gold standard. Indeed, analysing 58 BAL samples from HIV-infected subjects, Beauvais *et al.* [17] found two cases in which a small number of cysts were detected by the same IF test, although the patients did not subsequently develop PCP. Using a direct monoclonal antibody IF method, Ng *et al.* [18] found one false-positive result, on the basis of the clinical course, among 163 specimens. Since conventional staining is perfectly specific and always correlates with the development of PCP, we consider that it should be the reference method for BAL samples. IF is useful for IS samples, which often contain a lower density of *P. carinii* than BAL specimens, increasing the sensitivity of the procedure from 0.26 to 0.56 in our hands. Other authors have reported that the use of IF increases the sensitivity from 0.5 to 0.9 [12, 17, 18], suggesting that IF should be the reference method for IS samples [19].

Despite its good sensitivity and full specificity, the usefulness of IS is highly dependent upon the prevalence of PCP in the study population. For example, in San Francisco [12] where the prevalence of PCP in the population submitted to the IS procedure was 0.75 and the sensitivity was 0.81, performing BAL only when IS was negative avoided 61% of bronchoscopies. In contrast, in our centre, where the highest IS sensitivity (IF staining), is 0.56 and the prevalence of PCP is 0.30, only 17% of BAL procedures would be avoided. This raises the question as to whether or not IS is justified on an economic basis, particularly with the widespread use of specific prophylaxis.

Using our economic analysis, if the San Francisco and Denver centers [13, 16], which report higher PCP

prevalence rates (0.75 and 0.64, respectively) and higher IS sensitivities (0.81 and 0.66, respectively), had the same IS/BAL cost ratio as ours, the use of IS would lead to a considerable cost saving (50 and 32%, respectively, of the cost of the systematic BAL strategy). In contrast, with a PCP prevalence of 0.43 and a reported IS:BAL cost ratio of 0.25, O'BRIEN *et al.* [16] did not recommend the use of IS, as their calculated cost-effective threshold of sensitivity, 0.24, was higher than the observed sensitivity (0.15). KIRSCH *et al.* [20] reported that the sensitivity and specificity of IS were 0.71 and 1, respectively, using Gomori-methenamine silver and modified Wright-Giemsa stains. With an IS:BAL cost ratio of 0.35, they concluded that 53,732,800 $ might be saved in the diagnosis of 160,000 PCP cases projected in the USA for the year 1991. However, this cost saving would clearly depend on the prevalence of PCP: with a prevalence in the test population below 0.48, the IS-based strategy would in fact be more expensive. Indeed, with the widespread use of specific prophylaxis, the sensitivity of IS may fall. Although in our study there was only a trend suggesting a lower yield of the IS procedure in patients on prophylaxis, a recent study [21] found a significantly decreased sensitivity of the IS procedure in patients receiving aerosolized pentamidine prophylaxis compared to those receiving no prophylaxis.

Each centre should, therefore, evaluate the wisdom of introducing or continuing the IS technique on the basis of its sensitivity in their hands and the local prevalence of PCP, after clinical screening of suspected patients.

In summary, our results emphasize the need for a careful clinical selection of patients who are likely to benefit from diagnostic testing for PCP, particularly those receiving specific prophylaxis. In situations where the cost effectiveness of the induced sputum technique is borderline, a major consideration will be patient comfort, given the stress and morbidity associated with BAL.

Appendix

For n patients, where P is the prevalence of PCP, BC the cost of BAL, SC the cost of sputum induction, Se the sensitivity and Sp the specificity of sputum induction:

- the cost of BAL for n patients is: $n \times BC$
- the cost of IS followed by BAL, if negative IS is: $SC \times n$ (cost of IS for all patients) plus $BC \times [n \times P \times (1\text{-}Se)$ (percentage of patients with PCP and negative-induced sputum test results) plus $n \times (1\text{-}P)$ (number of PCP-free patients)]

Thus the cost of the two strategies is the same when:

$n \times BC = n \times SC + BC \times [n \times P \times (1\text{-}Se) + n \times (1\text{-}P)]$

where $1 = SC/BC + P - Se \times P + 1 - P$

and $SC/BC = Se \times P$

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