Eur Respir J 2003; 21: 204–208
DOI: 10.1183/09031936.03.00035303

European Respiratory Journal
ISSN 0903-1936

CLINICAL FORUM

Induced sputum for diagnosing *Pneumocystis carinii* pneumonia in HIV patients: new data, new issues

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Induced sputum for diagnosing Pneumocystis carinii pneumonia in HIV patients: new data, new issues. D. Turner, Y. Schwarz, I. Yust. ©ERS Journals Ltd 2003.

ABSTRACT: The complexity of bronchoalveolar lavage (BAL) has motivated the search for noninvasive methodology to retrieve specimens for detecting the presence of various pulmonary diseases. Induced sputum (IS) has been shown to be a reliable tool in terms of sensitivity and specificity comparable to BAL. Investigators from institutions worldwide have published several reports providing evidence in support of one or the other or a combination of both approaches. Among them are studies demonstrating the sensitivity and specificity of IS in diagnosing *Pneumocystis carinii* pneumonia (PCP) in patients with acquired immunodeficiency syndrome (AIDS). In 1996, highly active antiretroviral therapy was introduced for routine use and the morbidity from opportunistic infections decreased sharply.

An earlier study showed that cost-effectiveness depends on the prevalence of a given condition in the population. More recent studies have confirmed that prophylaxis against PCP can be stopped after increasing the CD4 cell count, thus reducing the attractiveness of IS as a preferred method for monitoring the course of disease.

This review presents a brief description of the evolution of the bronchoalveolar lavage *versus* induced sputum controversy and reconsiders the strengths and weaknesses of the earlier arguments in light of newer data that have emerged with regard to *Pneumocystis carinii* pneumonia in acquired immunodeficiency syndrome.

Eur Respir J 2003; 21: 204–208.

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Keywords: Bronchoalveolar lavage
induced sputum
Pneumocystis carinii

Received: April 30 2002
Accepted after revision: October 3 2002

Bronchoalveolar lavage (BAL) has traditionally been the definitive approach to retrieving bronchial and alveolar cells for the differential diagnosis of lung diseases. It is, however, an invasive procedure and a search is therefore underway for other approaches that could yield comparable diagnostic information with less risk. One of the more successful procedures that is widely being accepted is induced sputum (IS).

Pulmonary infection is one of the most prominent human immunodeficiency virus (HIV)-related diseases [1] (table 1) and *Pneumocystis carinii* pneumonia (PCP) is one of the most important infections that can occur when the CD4 value falls below the level of 200 mm^{-3} [2]. The prevalence of PCP had reached 80% in this population prior to the introduction of prophylactic treatment against it.

Table 1. – Common pulmonary diseases in human immunodeficiency virus patients

Community-acquired pneumonia
Pneumocystitis carinii pneumonia
Tuberculosis
Mycobacterium avium
Cryptococcus
Histoplasmosis
Non-Hodgkin's lymphoma
Kaposi's sarcoma
Lymphocytic interstitial pneumonitis (in children)

The IS technique is very familiar to HIV clinicians who use it for diagnosing PCP. This review will describe the use of IS in HIV and evaluate the advantages and disadvantages of this procedure in diagnosing pulmonary diseases, especially PCP, in acquired immunodeficiency syndrome (AIDS) patients. This review also presents recent findings that seem to complicate, rather than resolve, the ongoing controversy of the comparative efficacy and value of these diagnostic approaches.

Methods

Collation data

The authors searched the MEDLINE database for literature published between 1960–2002. The medical subject headings terms used, in separate searches, were: induced sputum, HIV, *Pneumocystis carinii* and AIDS. The primary criteria for the inclusion of studies in this review were: 1) the authors' judgement of whether the studies were qualitatively representative of current lines of reasoning; and 2) the studies' provision of clinically supportive evidence for the position taken in the debate on the use of IS in diagnosing PCP in AIDS.

Specimen collecting

The inflammatory response of PCP in AIDS patients was studied by LIMPER *et al.* [3] who used BAL to compare AIDS patients with other immunosuppressed individuals. The results showed the inflammatory response in the lower respiratory tract of AIDS patients to be markedly diminished when compared to the inflammatory response of immunosuppressed patients without AIDS. The median of neutrophils in AIDS patients was 2.3% compared to 18.5% in non-AIDS patients.

A difference between IS and BAL is the derivation of the specimen. For IS, the majority of the specimen comes from the central proximal airway, whilst the BAL specimen comes more from the peripheral airways and the alveolar compartment [4]. IS has been implemented as a diagnostic tool for microbiological agents in the diagnosis of tuberculosis (TB) since the sixties. Several studies comparing the diagnostic yields of mycobacterial culture with those of conventionally expectorated or gastric aspirated sputa in adults showed contradictory results [5–8]. The yield of sputum for diagnosing PCP in non-HIV patients is apparently very low. A retrospective review, conducted between 1969–1972, of 194 cases of PCP in patients without AIDS estimated the diagnostic yield for sputum to be 6% [9]. HIV patients, however, were shown to have a higher PCP organism burden than other immunocompromised patients [3]. This fact may explain the higher sensitivity of sputum analysis for diagnosing PCP in HIV patients compared to non-HIV immunocompromised patients. Moreover, this high organism burden could explain the low volume of sputum needed for diagnosing PCP in HIV patients, as shown in a study by KISKA *et al.* [10]. Their study compared the diagnosis of PCP in sputum volumes <2> cc.

Many published studies compared the sensitivity of IS to BAL. The pioneers of these studies were PITCHENICK *et al.* [11] and BIGBY *et al.* [12]. In 1986, PITCHENICK *et al.* [11] performed IS immediately prior to transbronchial bronchoscopy (TBB) in 43 patients with AIDS. Gomori methamine silver stain was used to diagnose PCP. A total of 20 patients were found positive for PCP by one or more of the procedures. IS was positive in 11 of these 20 patients, providing a yield of 55%. BAL was positive in 11 of the 14 patients tested, *i.e.* a yield of 79%, and TBB was positive in 18 of the 20 patients tested, *i.e.* a yield of 90%. In the study by BIGBY *et al.* [12], a sensitivity of 56% was shown. The specimens in this study were stained by modified Giemsa. The negative predictive value was 39%, which meant an unsubstantiated negative result could not be relied upon alone. A sensitivity of 78% was found for 113 individuals who had undergone IS after completion of the study (*i.e.* 73 patients who had PCP were identified from their sputum specimen amongst 93 patients who were proven to have PCP), and the negative predictive value (NPV) was 50%. A technique using liquefaction of mucus and the Gram stain method, which allowed the concentration of alveolar casts, increased the sensitivity to 78% [13]. A study by LEIGH *et al.* [14] published a few years later, showed that the sensitivity could be increased to 94% and the NPV to 96% by using strict protocols, which included close adherence, fasting overnight before IS and brushing the oral mucosa. The specimens in this study were stained by a modified silver technique [14].

Less data is available that compares IS to expectorated sputum for diagnosing PCP. One retrospective study by METERSKY *et al.* [15] showed that the level of sensitivity between the two methods was similar using direct immunofluorescence. Furthermore, a high level of sensitivity was reached if polymerase chain reaction (PCR) assays were used to for detect PCP in an oral wash [16–18].

Laboratory diagnosing techniques

The laboratory methods currently in common use for diagnosing PCP include silver stain, Diff-Quik (a modified Giemsa stain) modified toluidine blue (T. blue), immunofluorescence and PCR assay [19–21]. The value of each method may influence the yield when diagnosing PCP by IS.

Staining. Silver stain, considered to be the "gold standard", is a cyst wall-staining agent. Disadvantages of this method are that it does not detect the trophozoite form of PCP and it has high background staining, including positive staining of fungal elements. Diff-Quik is relatively inexpensive and takes only a few minutes to prepare. It detects all stages of the PCP cycle. The disadvantage of this technique is the high background staining, which makes it difficult to distinguish PCP and therefore requires a high level of expertise. In addition, it may identify other pathogens, such as cryptococcus, toxoplasma and other microorganisms. Modified T. blue is specific for cysts and more rapid than silver stain. The disadvantage of T. blue is the use of noxious chemicals that necessitate a fume hood. There is also the problem of nonspecific staining and the possibility that yeast could be mistaken for PCP. The advantages of immunofluorescence are the ease at which the procedure can be undertaken and the rapid identification of PCP. Due to background fluorescence, this procedure also requires technical expertise. Direct fluorescent antibody (DFA) and indirect fluorescent antibody (IFA) tests are also available, with some being specific for cysts and others for a combination of cysts, trophozoites and sporozoites.

Few studies have compared the yield of different laboratory techniques for diagnosing PCP. In 1988, KOVACKS *et al.* [20] conducted a prospective study on 49 PCP positive patients, 46 of whom had been diagnosed by IS using three different staining techniques. PCP was confirmed from a positive result by two or more sputum-staining techniques or by one of these techniques and a subsequent BAL or biopsy. Sensitivity for IFA was 92%, 76% for modified Giemsa, and 80% for T. blue. IFA was significantly more sensitive than modified Giemsa ($p=0.008$) and T. blue ($p=0.07$), and yielded no false-positive results.

In a separate study, four methods of staining, which included silver, direct immunofluorescence, indirect

fluorescence and modified Giemsa, were compared in 37 PCP-positive patients amongst 50 IS specimens and in 21 PCP-positive patients amongst 50 BAL specimens [19]. A true-positive was defined when two out of four stains were found to be positive. Of the four methods studied, Giemsa was the least sensitive (92% for IS and 81% for BAL) and the DFA and IFA assays were the most sensitive (97% for IS). DFA was the least specific (85% for IS and 90% for BAL) and all three of the other methods approached a specificity of 100%. However, none of the differences were statistically significant. The better sensitivity of IS compared to BAL was explained by the authors to be an artifact, since BAL was used to evaluate the more difficult cases, such as patients who had been previously diagnosed as negative by IS. It is also important to note the stain preparation time. For the Giemsa stain, preparation was only 3 min, although the time taken to read the slide was the longest, *i.e.* 10–30 min. The preparation time for the direct immunofluorescence method was 45 min; however, it could be read <5 min.

Polymerase chain reaction. PCR was also evaluated as a laboratory technique for diagnosing PCP. It was shown to increase the sensitivity of IS. In a study by CALIENDO *et al.* [21], PCR was compared to DFA in 120 IS specimens and 112 BAL specimens in various immunocompromised patients (not only patients with HIV). The sensitivity of PCR for the BAL specimens was 100% and the specificity was 98%. For IS, the sensitivity of PCR was 94% and the specificity was 90%. The negative predictive value was 99%, which meant that a negative PCR test on an IS specimen could rule-out PCP without the need for bronchoscopy. The results of the analysis of the 10 specimens, which were negative for DFA but positive for PCR, were as follows: among 10 specimens from eight patients, three were true-positives confirmed by BAL, four were false-positives and one was undetermined.

What is the probability that a positive PCR result from asymptomatic HIV patients would predict the future disease expression in these patients? ELVIN *et al.* [22] described 13 samples that had been taken from asymptomatic patients among 25 PCR-positive samples collected by IS. The 13 samples came from eight patients, six of whom developed PCP within 164–352 days of the PCR-positive result and two of whom who were started on PCP prophylactic therapy. These results suggest that PCR may identify asymptomatic patients with a high risk of developing PCP later. However, the results must be examined with caution. For example, in a prospective study, another group from Copenhagen showed that the PCR sensitivity on IS was equal to the routine methods [23]. The advantages and disadvantages of PCR for diagnosing PCP are summarised in table 2.

Recent data has been published on diagnosing PCP using the PCR technique in non-HIV-infected patients, including immunocompetent individuals and patients suffering from chronic lung diseases. Whether a positive result by PCR is indicative of the presence of a clinical disease is controversial [24–30]. VARGAS *et al.* [31] reported a healthcare worker who was an asymptomatic carrier of PCP after being in contact with a patient confirmed with PCP.

Table 2. – The advantages and disadvantages of the polymerase chain reaction (PCR) assay

Advantages
High sensitivity and specificity
May indicate colonisation and the need for prophylaxis
The PCR is negative when the cyst is empty (*e.g.* after treatment)
Disadvantages
There is no standardised assay
Takes long time to complete
Discrimination between true reinfection and slow clearance may be difficult
Expensive

Cost effectiveness

By virtue of it being a noninvasive technique, it would follow that IS is a less expensive procedure. However, the cost-effectiveness of IS is open to debate. CHOUAID *et al.* [32] showed that cost-effectiveness was dependent on the prevalence of PCP within the population. Using a statistical model, they demonstrated that an increased cost-effectiveness only occurred when the prevalence of the disease was greater in the population. For example, in San Francisco (CA, USA), the prevalence of PCP in the population who underwent the IS procedure was 0.75 and the sensitivity to IS was 0.92. The performance of BAL is only necessary in cases where IS is negative, avoiding the need for a bronchoscopy by 61%. In contrast, in Paris, where the prevalence in the population studied was only 0.3 and the sensitivity was 0.56, only 17% of BALs would be avoided. This model showed that with a prevalence <0.48, IS would be more expensive. GLENNY and PIERSON [33] arrived at similar conclusion in a study they conducted in 1992.

A cost-analysis study on the PCR technique for IS samples in the diagnosis of PCP in HIV patients published by CHOUAID *et al.* [34] concluded that the cost-effectiveness of the laboratory method chosen was dependent on the reference diagnostic strategy of each centre. In centres using BAL, PCR IS was found to be a more costly strategy due to a low prevalence of PCP. In contrast, when the reference strategy is to perform IS first and then BAL, where IS yields a negative result, the implementation of PCR is economically justified.

The effectiveness of routine analysis of IS in HIV patients for TB and PCP was studied by KVALE *et al.* [35]. The authors conducted a prospective study on 1,171 HIV patients who were analysed for TB and PCP by using IS. Staining for PCP included Giemsa, methamine silver or direct immunofluorescence. Only two cases of TB and one case of PCP (false-positive) were discovered at the beginning of the study. However, during the follow-up, three additional cases of PCP (one in the control group) and two of TB were discovered. It was concluded that routine analysis of IS was not efficacious. WEHNER *et al.* [36]

showed that the decision to use this technique should be based upon whether it will improve the yield of IS for diagnosing PCP. The prospective follow-up of 22 patients who were denied IS due to a low clinical suspicion of PCP revealed that there were no cases of PCP among them. Findings suggesting a diagnosis other than PCP included purulent sputum production, wheezing and radiographs with evidence of focal infiltrates, pleural infusion or adenopathy. The conclusion given by the authors stated that controlled utilisation of IS targets, in terms of selecting appropriate patients for PCP evaluation, would save money and avoid exposure of personnel to infectious aerosols.

Highly active antiretroviral therapy era

There has been a sharp decrease in morbidity from opportunistic infections since highly active antiretroviral therapy (HAART) was introduced for routine use in 1996 [37]. During the HAART era, the incidence of PCP reported by the Centres for Diseases Control and Prevention decreased by 21.5% per yr, which represents 3 per 100 person-yrs in 1998 in comparison to 10 per 100 person-yrs in 1995 [38]. As previously shown in several studies, prophylaxis against PCP could be stopped after there had been an increase in the CD4 cell counts and almost no PCP infections occurred later [39, 40]. This demonstration of epidemiological changes should be taken into consideration when deciding on the most appropriate technique for diagnosing pulmonary diseases in HIV patients. Given the low prevalence of PCP today, IS should not be cost-effective.

Conclusion

In conclusion, the limitations of IS in HIV patients include the time required for obtaining sputum and the need for a skilled team. While IS is considered safe and noninvasive for the patient, as previously shown in asthmatic patients, the risk of exposure of personnel to aerosolised TB has to be considered. IS is sensitive and specific for diagnosing PCP in HIV patients and depends upon the laboratory method employed. Cost-effectiveness depends on the prevalence in the population. Since the prevalence of PCP has decreased sharply since HAART, and given that other pathogens or nonmicrobial pathology may be responsible for pulmonary disease, this review proposes that BAL should be used as the first procedure for diagnosing pulmonary diseases in HIV patients. Although PCR may improve the yield of IS, this technique is expensive, time consuming, not widely available and its results continue to be contradictory.

The methods for diagnosing *Pneumocystis carinii* pneumonia in human immunodeficiency patients have undergone considerable improvement over recent years. It may be time to conduct fresh studies to compare expectorating sputum, induced sputum and bronchoscopy for diagnosing in *Pneumocystis carinii* pneumonia in human immunodeficiency virus patients.

Acknowledgements. The authors would like to thank E. Eshkol for her editorial assistance.

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