Induced Sputum *versus* Bronchoalveolar Lavage in the Diagnosis of *Pneumocystis jiroveci* Pneumonia in Human Immunodeficiency Virus-Positive Patients

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Induced sputum is a useful technique for assessing airway inflammation, but its role in the diagnosis of lung disease in immunosuppressed patients needs further investigation. This study compared the use of induced sputum and BAL in the diagnosis of pneumocystosis, in HIV patients. From January 1, 2001, to December 30, 2002, HIV-positive patients older than 14 were evaluated at a hospital in Florianópolis, Santa Catarina, Brazil. Patients with respiratory symptoms for seven days or longer, with a normal or abnormal chest X-ray, and those without respiratory symptoms but with an abnormal chest X-ray, were included in the study. All patients were submitted to clinical, radiological and laboratory evaluation, after which induced sputum and bronchoscopy with bronchoalveolar lavage were carried out. The samples were subjected to the following techniques: Gram and Ziehl-Neelsen staining, quantitative culture growth for pyogenic bacteria, direct staining for fungi, culture growth for mycobacteria and fungi, and Grocott-Gomori staining for *Pneumocystis jiroveci*, as well as total and differential cell counts. The samples with *P. jiroveci* were selected, as well as the samples for which no etiologic agents were observed. Forty-five patients with a mean age of 34.6, 38 male and 40 Caucasian, comprised the subjects. Interstitial infiltrate was the most frequent radiological pattern (53.3%). The induced sputum sensitivity was 58.8%, specificity 81.8%, predictive positive value 90.9%, predictive negative value 39.1% and accuracy 64.4%, for the diagnosis of pneumocystosis, compared with BAL. Based on these data, induced sputum is a useful technique for the diagnosis of pneumocystosis in HIV patients.
Key-Words: Pneumocystosis, induced sputum, bronchoalveolar lavage.

It has been estimated that 65% of HIV-infected patients have pulmonary involvement in the first clinical presentation of AIDS and that about 80% of these patients will have some pulmonary manifestation during the course of the disease [1]. Pneumocystosis, caused by *Pneumocystis jiroveci*, is one of the most prevalent conditions [2].

Pneumocystosis may have an insidious or acute onset with dyspnea and a dry cough, accompanied by a chest X-ray with reticular or reticulonodular perihilar infiltrates. The physical examination is often normal, and about 10% of the cases present no evident radiological change [3].

Since *Pneumocystis* can not grow in artificial culture media, the diagnosis of pneumocystosis in sputum, bronchoalveolar lavage (BAL) or pulmonary tissue needs direct examination by microscopy [4].

The high rates of morbidity and mortality of pneumocystosis sufferers justify the specific treatment of infected patients [5-8].

There is still controversy regarding the best approach to lung disease and immunocompromised patients [9,10].

Pneumocystosis patients invariably have a greatly impaired gas exchange, which inhibits and, in most cases, contraindicates the performance of invasive diagnostic techniques like bronchoscopy. Sputum analysis is the least invasive method available [6]. For individuals unable to spontaneously produce sputum, the induced sputum technique can be used [11-13]. Its diagnostic role and its importance as a diagnostic tool in immunocompromised patients need better definition [10,14,15].

The aim of this study was to determine the utility of induced sputum in pneumocystosis diagnosis in HIV-positive patients, taking bronchoalveolar lavage as the gold standard.

Material and Methods

From January 1, 2001 to December 10, 2002 all HIV-positive patients, older than 14, admitted to Nereu Ramos Hospital (Florianopolis/SC/Brazil) were evaluated. Patients presenting respiratory symptoms for at least seven days, with or without radiological signs of pulmonary disease, were included in the study. Respiratory asymptomatic patients with alterations in their chest X-ray were also included.

The exclusion criteria included: a) prophylactic treatment for *P. jiroveci* pneumonia in the 30 days prior to the hospital admission date and/or more than seven days of empirical treatment for pneumocystosis; b) Cachexia and deterioration of general state, reflected in a Karnofsky rate of 20-30% [16]; c) alterations in consciousness (obnubilation and coma); d) partial oxygen pressure below 80 mmHg, receiving two liters per minute of supplementary oxygen via nasal catheter; e) a fall in FEV1 of 20% during sputum induction and/or signs and symptoms that contraindicate the procedure [17]; f) non effective sputum induction; and g) refusal to participate of the study, or non collaboration with diagnostic procedures.

All patients that fulfilled the inclusion criteria were invited to participate; they received information about the study and diagnostic techniques. All of them signed an information agreement. After this procedure, they were submitted to clinical and laboratory evaluation and registered on an inclusion form. Afterward, the sputum induction and the bronchoscopy with

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bronchoalveolar lavage were performed. The interval between each procedure was 24 hours.

The sputum induction was performed with a US-800 Air Standard® nebulizer, with a nebulization rate of 1 mL/min (± 0.2) and a manufacturer's specified particle size of 0.5 μm to 10 μm, and a Microlab 3.500® spirometer, according to procedures given in Efthimiadis et al. (hypertonic saline solution was inhaled for a fixed time and in progressively higher concentrations - 3%, 4% and 5%) [17]. The bronchoscopy with bronchoalveolar lavage was performed with an Olympus® instrument, with 2.6 mm diameter. The bronchoscopies were carried out according to Strausz [18].

Induced Sputum Processing

In the clinical analysis, laboratory smears were performed according to the Petrillo method [19].

The tests carried out were: acid-fast bacilli with Ziehl-Neelsen staining, potassium hydroxide (KOH) wet mount slides, Giemsa, Papanicolaou and Grocott-Gomori methenamine silver staining for *Pneumocystis jiroveci*, differential cytology, analysis for cytomegalic inclusions and parasites, and bacterioscopy with Gram staining for pyogenic bacteria. Also carried out were: Löwenstein-Jensen slants for mycobacteria, growth of fungi cultures on agar Sabouraud and quantitative culture growth for pyogenic bacteria on blood agar and MacConkey agar. Part of each sputum sample was sent to an anatomy/pathology laboratory for evaluation of cytopathological alterations using Papanicolaou staining.

The sample was considered satisfactory if it had less than ten squamous epithelial cells, more than 25 leukocytes per 100x microscopic field and the presence of alveolar macrophages [20]. Samples that did not have these characteristics were considered unsatisfactory and the sputum induction was repeated within 48 hours of the first procedure.

Bronchoalveolar Lavage Processing

The bronchoalveolar lavage smears were subjected to centrifugation for ten minutes at a speed of 2,500 rpm, and then stained using the same methods used for the induced sputum samples. A non-centrifuged portion was used to grow the same cultures listed above for the induced sputum samples.

The bronchoalveolar lavage samples were considered satisfactory if they had ciliated cells, alveolar macrophages and a maximum of 10% epithelial cells [21]. The bronchoalveolar lavage was repeated within 48 hours of the first procedure if the sample was unsatisfactory.

During this step of the study, the induced sputum and BAL samples from which *P. jiroveci* was recovered and those without microorganism growth were selected. The clinical and laboratorial data were summarized as percentage or average, as indicated.

To determine the sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the induced sputum, the bronchoalveolar lavage was taken as the gold standard.

This study was approved by the Human Research Ethics Committee.

Results

Sixty-eight patients fulfilled the inclusion criteria, from which eight were excluded, two for personal reasons, three for having resistant hypoxemia to oxygen supplementation and three for deterioration of external general state (Karnofsky performance score 20).

Of the 60 participants, 45 had *P. jiroveci* identified through induced sputum and/or BAL or had no etiological agent detected.

The mean age of the participants was 34.6 years (SD ± 7), 38 (84.4%) were men, and 40 (88.8%) Caucasians.

The most frequent radiographic presentation was an interstitial pattern (53.3%), and only four patients (8.8%) had a normal chest X-ray.

Table 1 shows the chest radiographic presentation.

The most frequent symptom was a dry cough (62.2%), and only one patient (2.2%) was totally respiratory asymptomatic. Eight patients (17.8%) had a productive cough.

Table 2 shows the distribution of participants according to symptomatology.

The pulmonary function tests showed that restrictive ventilatory defect was the most frequent (75.5%), and only five patients (11.1%) had a pulmonary function test within normal limits.

Table 3 shows the distribution of participants according to spirometry results.

Complications were not encountered during the sputum induction or bronchoscopy procedures.

The yield of sputum induction compared with the BAL for the diagnosis of *P. jiroveci* pneumonia showed a sensitivity of 58.8%, specificity of 81.8%, positive predictive value of 90.9%, negative predictive value of 39.1% and accuracy of 64.4%.

The sensitivity, specificity, predictive values and the accuracy of sputum induction are shown in Table 4.

Discussion

The results of this study reveal that induced sputum analysis is a simple procedure, without significant adverse effects, and with a good diagnostic yield for *P. jiroveci* pneumonia determination, when compared with bronchoalveolar lavage, in HIV-positive patients.

Although radiographic alterations are very frequent, a high number of etiological agents can result in respiratory symptoms, but without concomitant image alterations. Nodular or reticulonodular infiltrate is the typical finding in pneumocystosis, however, in as many as 10% of patients, the chest X-ray may be entirely normal. For this reason, patients presenting respiratory symptoms without concurrent radiographic alterations were included in the study [3].

Pathogen detection through bronchoalveolar lavage is significantly reduced in HIV-positive patients previously

Table 1. Distribution of participants according to radiographic presentation

Radiographic presentation	N	%
Interstitial	24	53.3
Alveolar	12	26.7
Mixed	3	6.7
Alveolar + Mediastinal adenomegaly	1	2.2
Interstitial + Pneumothorax	1	2.2
Normal	4	8.8
Total	45	100

Table 2. Distribution of participants according to symptomatology

Symptomatology	N	%
Dry cough	28	62.2
Productive cough	8	17.8
Dry cough + Dyspnea	6	13.3
Dry cough + Fever	2	4.4
Asymptomatic	1	2.2
Total	45	100

Table 3. Distribution of participants according to spirometry results

Spirometry test results	N	%
Mild restrictive ventilatory defect	22	48.9
Moderate restrictive ventilatory defect	6	13.3
Serious restrictive ventilatory defect	6	13.3
Moderate obstructive ventilatory defect with reduced FEV	6	13.3
Within normal limits	5	11.1
Total	45	100

Table 4. Yield of sputum induction compared with the bronchoalveolar lavage (BAL), for the diagnosis of *P. Jiroveci* pneumonia, in 45 HIV-positive patients

Induced sputum	Positive BAL	Negative BAL	Total
Positive	20	2	22
Negative	14	9	23
Total	34	11	45

Sensitivity = 58.8%; Specificity = 81.8%; Positive Predictive Value = 90.9%; Negative Predictive Value = 39.1%; Accuracy = 64.4%; Prevalence = 75.5%.

treated empirically. Studies have shown that procedure yields for non-treated patients are high (91%) when compared with patients previously treated empirically (64%) [22]. In the same way, prophylaxis to some conditions also affects the incidence of various microorganisms [23]. As the initial objective of this study was the evaluation of the yield of diagnostic techniques, and not the incidence of pulmonary infection, in this group of patients we opted for the exclusion of individuals undergoing empirical or prophylactic treatment for the studied condition, due to their direct influence on the yield of the techniques employed.

In this study, the hypertonic saline solution was inhaled for a fixed time and in progressively higher concentrations (3%, 4% and 5%), through an ultrasonic vaporizer. It is known that the use of techniques with different inhalation times and saline concentrations do not produce a difference in the cellularity and that the use of ultrasonic vaporizer improves the collection success [24]. Chuard et al. warn that it is not impossible that the hypertonic saline modifies the viability of some pathogens, among them *P. jiroveci* [25]. If there is this effect, *P. jiroveci* negative samples may have suffered this bias. Additional studies should be conducted to test this hypothesis.

The main criticisms of sputum analysis as a diagnostic method for pulmonary infections are concerned with the potential contamination of the sputum with material from upper airways. Such criticisms are not baseless, since approximately 45% of the samples sent to laboratories are contaminated by saliva [25]. Sputum induction does not improve the quality of the material, having similar yields to those of spontaneous expectoration, when adequate samples are selected [25]. In this study, this problem was greatly minimized with the association of mechanical techniques to remove saliva and microscopic selection of samples through the presence of macrophages, polymorphonuclear leukocytes and epithelial cells.

The most frequently observed symptom in this study was a dry cough. In this group of patients, sputum is frequently not spontaneously produced [26]. This data supports the importance of determining the yield of induced sputum in the diagnosis of pulmonary diseases in this specific group of patients, since this procedure allows the non-invasive harvest of material from lower airways in patients unable to produce expectoration spontaneously.

We have to consider, in the case of some patients, that the isolation of *P. jiroveci* from induced sputum or from bronchoalveolar lavage may indicate colonization and not infection, since histopathological criteria were not required for the diagnosis in this study. On investigating the frequency of colonization and infection in HIV-positive and HIV-negative patients, Nevez et al. demonstrated that *P. jiroveci* can colonize immunosuppressed patients for reasons other than HIV. However, in HIV-positive patients with respiratory symptomatology, this possibility is remote [27].

When we compare induced sputum with bronchoalveolar lavage, we compare samples which are anatomically equivalent

and, theoretically, with the same diagnostic capacity. Some studies have even shown that induced sputum analysis is better for the diagnosis of some specific etiological agents, such as *Mycobacterium tuberculosis* [28,29].

In this study, induced sputum showed a sensitivity of 58.5%, specificity of 81.8%, positive predictive value of 90.9%, negative predictive value of 39.1% and accuracy of 64.4%. Miller et al. prospectively compared the use of induced sputum with BAL for HIV-positive patients with acute respiratory disease and concluded that this technique has a low yield for *P. jiroveci* and other pathogens. In Miller's study, the induction was not effective in 28 patients and was inadequate in 29, leaving 85 patients for analysis. Of these, 82 had a positive diagnosis of pneumocystosis, induced sputum showing a sensitivity of 13%, which is lower than the sensitivity found in our study [30]. Weinberg et al. reported, for induced sputum, a yield of 40% in pneumocystosis diagnosis [31]. A sensitivity of 74% with a negative predictive value of 58% was reported by Ng et al., however not all patients with negative sputum were subjected to bronchofibroscopy [32]. Our findings agreed with those of Bigby et al., who found a sensitivity of 56% and Pitchenick who reported a positivity of 55% [33,34].

In this study, no complications were observed with the sputum induction procedure and all the harvests were effective. In a study on the effect of sputum induction on oxygen saturation and spirometry values carried out by Leigh et al., no patient or control had severe side effects that required procedure interruption [13].

Our results show that sputum induction is an effective technique for obtaining samples from the lower airway tract in this group of patients. It is shown to be particularly useful in populations with infrequent spontaneous sputum production and where traditionally invasive procedures for sample collection would need to be carried out.

Our findings demonstrate clearly that for pneumocystosis diagnosis, in comparison with the BAL, the best individual parameters of the sputum induction technique are the positive predictive value and the specificity. Both of these parameters were associated with others; indicating that this diagnostic strategy may be used to diminish the necessity for invasive procedures in an appreciable number of patients. It may be used as the first step in the investigation of *P. jiroveci* pneumonia in HIV-positive patients, invasive harvest techniques being necessary only for those patients with negative sputum or with a lack of response to initial treatment. Moreover, since this technique is easy to perform, and does not require expensive equipment, it represents an alternative to the initial diagnostic approach in places where access to invasive methods is not feasible.

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