Sputum induction has long been established as the best available non-invasive method to evaluate airway inflammation. It was first used for diagnosing Pneumocystis carinii pneumonia (PCP) in patients infected with human immunodeficiency virus (HIV). By administering 5% hypertonic saline through an ultrasonic nebulizer for 10 or 20 minutes, sputum could be induced in the majority of patients with acquired immunodeficiency syndrome (AIDS). The method was later adapted for examining the inflammatory response in asthma. In recent years, sputum induction by hypertonic saline and its subsequent processing has been refined as a noninvasive research tool and, increasingly, as a clinical tool to provide important information about inflammatory events in the lower airways.

Induced sputum has been used for studying various illnesses, including asthma, chronic obstructive pulmonary disease (COPD), tuberculosis, PCP, cystic fibrosis, lung cancer and chronic cough. Asthma is a heterogeneous disorder characterized by chronic airway inflammation, hyperresponsiveness and remodeling. Being the hallmark of asthma, airway inflammation has become the most important target for therapeutic agents.

COMPARISONS AND ADVANTAGES OF THE TECHNIQUE

Sputum induction by inhalation of hypertonic saline is a simple, cost effective and safe procedure, and has several advantages over other techniques. It has a higher yield in comparison to spontaneous sputum, bronchoalveolar lavage (BAL), bronchial washing and gastric lavage. Bronchoscopy allows sampling of the cells, biomarkers and pharmacokinetic data by means of BAL and enables the biopsy of mucosal tissue. Although relatively safe, BAL is an invasive procedure and more expensive than sputum induction, and is not easily applicable on a large scale in clinical studies. Also, from a safety perspective, the procedure still entails some morbidity.

With sputum induction, samples can be obtained from the lower airways with minimal discomfort to the patient, and is thought to provide a better representative sample of several proximal airways. The technique also allows the distal parts to be sampled with prolonged induction, as evident from increased numbers of macrophages from the alveolar compartment. The technique can be used for the evaluation of inflammatory cells and eosinophils, of biomarkers such as: tumor necrosis factor (TNF), interleukin (IL), macrophage inflammatory protein-1 (MIP-1α), monocyte chemotactic factor-1 (MCP-1), metalloproteinases (MMP), transforming growth factor-β (TGF-β), platelet derived growth factor (PDGF) and also for studying local immunogenicity against inhaled drugs. It is widely used in asthma and COPD but also as a complementary tool to BAL both in the research and clinical monitoring of patients with interstitial lung disease.

The cells, recovered from spontaneous coughing can be used to study lung cancer, respiratory infections and in diagnosis of PCP in patients infected with HIV. In developing countries with a high prevalence of pulmonary tuberculosis, sputum induction can increase the diagnostic yield, resulting in better categorization of patients for treatment purposes. The method can also increase the case detection rate of smear negative pulmonary tuberculosis especially in areas where facilities for more invasive and expensive techniques are not available or viable.

The reproducibility of the method has been repeatedly questioned however, and the preparation of the obtained sputum samples for bio-analysis is labor intensive and demanding for technicians trained in the procedure. There have been only a few methodological studies that have examined the influence of various technical factors on the repeatability of sputum induction and collection, and as such, there is no ‘gold standard’ practice for this technique.
TECHNICAL ASPECTS

The technique of sputum induction consists of inhaling an aerosol of hypertonic saline with increasing concentrations of 3, 4, and 5% over different time-periods. Ultrasonic nebulizers are recommended for inducing sputum since other nebulizers do not usually have sufficient saline aerosol output. Spirometry is necessary to assess the baseline airway caliper and avoid excessive bronchoconstriction during saline inhalation. Spirometers are preferable to peak flow meters because of the greater sensitivity of measuring the dynamic lung volume after one second of forced expiration (FEV1) in detecting induced bronchoconstriction. Sputum induction requires a high degree of cooperation from the patient, and only an experienced technician under the supervision of an experienced physician should conduct the procedure.

The technique requires pretreatment with salbutamol, preferably 4 puffs of 100 μg via spacer or nebulizer and then post-bronchodilator spirometry, which serves as the pre-induction FEV1 baseline for safety monitoring. This pre-induction FEV1 should be greater than 65%.

Sputum will be induced for approximately 21 minutes divided into three, seven minute sessions of nebulization, followed by a three step cleansing procedure and a focused cough attempt. Throughout the process, regular spirometry is required for safety monitoring. In recognition of intra-subject variability between visits, guidelines have been provided for investigators to consider where subjects whose post-bronchodilator FEV1 may be below 65% at visits where sputum induction is to be performed. If the pre-induction post-bronchodilator FEV1 is greater than 60%, then sputum induction may be performed starting with 3% and increasing to 4% and then 5% hypertonic saline, if their FEV1 remains at least 90% of the pre-induction baseline before increasing to 4% and then 5% saline. With lower FEV1 values a more cautious approach is followed, starting with a lower concentration and a more gradual increase of the dose.

No medication holds are required for sputum induction unless the procedure is performed after other procedures that do require medication holds, for example bronchodilator reversibility. To assess for excessive bronchoconstriction during sputum induction, measurements of FEV1 will be taken at approximately seven minute intervals, or sooner if the subject becomes symptomatic. Pulse oximetry should be monitored in conjunction with FEV1, at the same seven minute intervals during the induction.

It is recommended that sputum be processed as soon as possible and within two hours, in order to ensure optimum cell counting and staining. The sample preparation can take up to two hours per sample and requires trained technicians for both the induction procedure and the sample handling/cell counting.

CASE STUDY

SGS undertook a study of the characteristics of healthy volunteers and asthma patients in sputum induction during proof of concept studies to assess its viability as a method. Healthy volunteers and asthma patients were recruited in a phase 1 unit designed for participants undertaking early phase respiratory studies, where sputum induction in the baseline screening was included. Sputum induction was performed according to the technique described above and basic demographics and spirometer results were collected. In some cases a second induction was performed on participants after 2-30 days.

The outcome parameter was an adequate sputum sample, which was defined as a weight of more than 0.4 g and visible plugs. Of the 150 eligible subjects, 102 volunteers (78 healthy non-smokers, 10 healthy smokers and 14 asthma patients) underwent sputum induction. The mean age was 40.3 ± 10.9 years and 84 % of the participants were male. Adequate sputum samples were obtained in 68 (67 %) subjects, 29 % in healthy, 75 % in smokers and 74 % in asthmatic patients. There were no differences in age, sex, BMI, vital signs between the groups with adequate and non-adequate samples. Reproducibility in 51 subjects tested twice was 86 % (intra subject difference in sputum weight -2.2 mg 95%CI 27.7). There were no major decreases in FEV1, and the procedure was well tolerated. It was concluded that the use of sputum induction in healthy volunteers and asthma patients is feasible and reproducible for the study of biomarkers in respiratory diseases.

The positive outcome is entirely due to the strict follow-up of procedures and repeated operational and laboratory staff training. Patients with a history of asthma and smoking are of course better candidates for this procedure, but the same strict procedures and training are necessary. There were no other parameters that could predict adequate sampling.

The application of this procedure has also been studied successfully by others in infants with different chronic airways diseases. Moreover sputum induction can also be safely and successfully performed in patients with severe, difficult-to-control asthma if a standardized protocol is used.

CONCLUSION

The identification of biomarkers that allow monitoring and optimization of drug development in lung disease therapy is one of the most ambitious goals in respiratory medicine. The induced sputum technique allows sampling of the airways in a noninvasive manner and as such offers a unique opportunity for the identification of biomarkers for potential clinical use in respiratory medicine. It is hoped that, in the future, induced sputum will provide clinicians with useful markers that can be used to perform more accurate and, ideally, more rapid development of drugs and for determination of disease phenotypes in many lung diseases, leading to precision medicine.

The hope is that the induced sputum technique will provide a simple and cost-effective tool for assessing the anti-inflammatory potential of new treatments, an approach that was precluded by previous techniques such as bronchial biopsy and bronchoalveolar lavage.
REFERENCES


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