Different protocols for cryobiopsy versus forceps biopsy in diagnosis of patients with endobronchial tumors

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Abstract

Introduction: *Forceps biopsy is the standard procedure to obtain specimens in endobronchial lesions. New studies have proposed flexible cryoprobe as an accepted alternative method for this technique. Although diagnostic use of the cryobiopsy is confirmed in few studies, there is paucity of data with regard to an optimum protocol for this method since one of the main considerations in cryobiopsy is the freezing time.* ***Objectives:*** *To evaluate diagnostic yield and safety of endobronchial biopsies using the flexible cryoprobe. Moreover, different freezing times were assessed to propose an optimized protocol for this diagnostic modality.* ***Patients and Methods:*** *For each patient with a confirmed intrabronchial lesion, diagnostic value of forceps biopsy, cryobiopsy in three seconds, cryobiopsy in five seconds and combined results of cryobiopsy in both timings were recorded.* ***Results:*** *A total of 60 patients (39 males and 21 females; Mean age 56.7 ± 13.3) were included. Specimens that were obtained by cryobiopsy in five seconds were significantly larger than those of forceps biopsy and cryobiopsy in three seconds ($p < 0.001$).We showed that the achieved diagnostic yields for all three methods were not statistically different ($p > 0.05$). Simultaneous usage of samples produced in both cryobiopsies can significantly improve the diagnostic yield ($p = 0.02$). Statistical analysis showed that there were no significant differences in case of bleeding frequency among the three sampling methods.* ***Conclusions:*** *This study confirmed safety and feasibility of cryobiopsy. Additionally, combination of sampling with two different cold induction timings would significantly increase sensitivity of this emerging technique..*

Keywords: *Cold induction timing, Cryobiopsy, Endobronchial tumor, Forceps biopsy*

Rezumat

Diverse protocoale de criobiopsie versus biopsia cu pensă la pacienții cu tumori endobronșice

Introducere: *Biopsia cu pensa forceps este metoda standard de obținere a produselor patologice în leziunile endobronșice. Noi studii au propus utilizarea criosondei flexibile ca o metodă alternativă pentru această tehnică. Deși utilitatea diagnostică a criobiopsiei este confirmată în câteva studii, există puține date privind protocolul optim pentru această metodă, deoarece unul dintre principalele considerente în criobiopsie este timpul de înghețare.* ***Obiective:*** *Evaluarea randamentului diagnostic și siguranța biopsiilor endobronșice folosind criosonda flexibilă. Mai mult decât atât, diferiți timpi de congelare au fost evaluați pentru a obține un protocol optimizat al acestei metode diagnostice.* ***Pacienți și metodă:*** *Pentru fiecare pacient cu o leziune endobronșică confirmată au fost înregistrate valorile diagnostice ale biopsiei forceps, criobiopsiei în trei secunde, criobiopsiei în cinci secunde și rezultatele combinate ale criobiopsiei în ambii timpi.* ***Rezultate:*** *Un total de 60 de pacienți, (39 de bărbați și 21 de femei; Vârsta medie=56,7 ± 13.3) au fost incluși. Probele obținute prin criobiopsie în cinci secunde au fost semnificativ mai mari decât cele obținute prin biopsie foceps sau criobiopsie în trei secunde ($p < 0.001$). Randamentele diagnostice obținute prin cele trei metode nu au fost semnificativ diferite ($p > 0.05$). Utilizarea simultană a produselor obținute în amândouă tipurile de criobiopsii poate îmbunătăți semnificativ randamentul diagnostic ($p = 0.02$). Analiza statistică nu a arătat nici o diferență semnificativă în ceea ce privește frecvența sângerarii între cele trei metode de recoltare.* ***Concluzii:*** *Acest studiu confirmă siguranța și fezabilitatea criobiopsiei. Adițional, combinarea recoltării prin doi timpi diferiți de inducție a frigului crește semnificativ sensibilitatea acestei tehnici în curs de dezvoltare.*

Cuvinte-cheie: *timpul de inducere a frigului, criobiopsie, tumoră endobronșică, biopsie forceps*

Introduction

Diagnostic bronchoscopy with endobronchial forceps biopsy is mainly applied in patients with suspected lung mass and visible endobronchial changes. Histopathological cell type and staging of intrabronchial lesions are needed for suitable diagnosis and proper management of lung cancer patients[1,2]. Although other laboratory methods as well as imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) have been developed, forceps biopsy with the aid of the flexible bronchoscope is still the diagnostic method of choice in cases of endobronchial lesions[3].

Diagnostic accuracy of forceps biopsy was reported from 50% to 70%. The accuracy is dependent on the indication, size and location of the lesions[4-7]. In this diagnostic modality, larger tissue samples are mandatory for increasing diagnosis quality. This aspect might confine the routine and successful utilization of this method. To overcome this limitation, new studies have proposed flexible cryoprobe as a novel technique to obtain adequate specimens. This method is primarily used for cryoextraction of malignant airway stenosis and was introduced as an alternative method for mechanical tumor debulking[8,9]. As cryorecanalization procedures provide samples that are larger in size with less mechanical damage, the technique has been employed in the biopsy of endobronchial lesions. Although diagnostic use of the method is confirmed in few studies, there is a paucity of data with regard to an

optimum protocol for this method. One of the main considerations in cryobiopsy is the freezing time. The aim of the present study was to evaluate the diagnostic yield and safety of endobronchial biopsies using the flexible cryoprobe. Moreover, different freezing times were assessed to propose an optimized protocol for this diagnostic modality.

Patients and methods

Patients

Our inclusion criteria required all patients to be older than 20 years of age and have a satisfactory respiratory function (oxygen saturation > 85% with no oxygen supplementation). Patients with respiratory failure, hypoxia or respiratory distress, a positive history of cardiac or hemodynamic instability and patients with any significant abnormality of coagulation studies were excluded from the study. Included patients had to have a previously confirmed endobronchial tumor. The presence of intrabronchial lesions were confirmed with previous bronchoscopy or CT-Scan. After a detailed explanation of the study to the patients, a signed informed consent was obtained from all patients. The entire study protocol was reviewed and accepted in ethics committee for human research of Shahid Beheshti University of Medical Sciences.

After receiving simple sedation (with Propofol, Midazolam, and Morphine) regular bronchoscopy (Olympus Corp Hamburg Germany) was performed. Continuous monitoring of oxygen saturation, heart rate, and blood pressure was performed during entire procedure.

Procedure

For the Forceps biopsy, six specimens were gathered with 21-C forceps and were maintained in a formaldehyde container. We used a flexible probe (90 cm in length and 2.3 mm in diameter) for cryobiopsy (ERBE Medizintechnik GmbLh, Tubingen, Germany). The cryoprobe had higher freezing capacity, higher stability to traction, and larger surface area than conventional probes for cryotherapy and used nitrous oxide which induces a temperature of -89.5° C at the tip of the probe. The probe was inserted into the lesions and sample was picked up. To assess diagnostic yield of cryobiopsy in different protocols, two timings were utilized. Cryobiopsy specimens were obtained within three or five seconds of action time and were placed in separate containers. To minimize confounding effect of the sampling method order, patients were randomly assigned to the order of the three techniques. Hence, for each patient, diagnostic value of forceps biopsy, cryobiopsy in three seconds, cryobiopsy in five seconds and combined results of cryobiopsy in both timings in diagnosis of an endobronchial lesion were recorded.

All the samples were transferred to the pathology ward and examined by a single pathologist. The pathologist was blinded to the study protocol and specimen's data. For each patient, a final diagnosis was made on the basis of the results of the pathology, past medical history, physical examination, laboratory findings and imaging studies.

Complications and safety

In our study, hemorrhage was defined as the main potential complication of both procedures. Hemorrhages were classified into four groups: no hemorrhage (I), hemorrhage which was controlled by normal saline (II), hemorrhage that was controlled by diluted adrenaline (III) and hemorrhage that needed argon plasma coagulator (APC) utilization (IV). We also assessed the quality of the samples according to pathologist's reports.

Statistical analysis

Quantitative variables were presented with mean ± standard deviation and qualitative variables were presented with frequency and percentages. Specimen size that were obtained from each sampling method were compared using Friedman's Two-way Analysis of Variance by Ranks and Wilcoxon signed-rank test. To determine the diagnostic yield of the three sampling methods, the pathologic results were compared with final diagnosis. Cochran's Q test was used to compare diagnostic accuracies. A p value of less than 0.05 was considered to be statistically significant.

Results

Over a period of two years (between April 2009 and June 2011), a total of 60 patients with an established endobronchial lesion referred to National Research Institute for Tuberculosis and Lung Disease (NRITLD) in Tehran, Iran were recruited (39 male and 21 female). Mean age of the study patients was 56.77±13.3 (range 27 to 76) years. Of all study population, final diagnosis was made in 57 cases. Among these subjects, 54 cases were diagnosed with a malignant lesion. Of the three remaining patients, in one case, another sampling session was needed for definite pathological diagnosis (*squamous cell carcinoma*) and two did not follow the study.

Specimen size

In case of specimen's size of tissue samples, Friedman's Two Way Analysis of Variance by Ranks showed specimens that were obtained by cryobiopsy in five seconds (median=1.6 cm, range=0.9-2.0 cm) were significantly ($p < 0.001$) larger than those of forceps biopsy (median=0.5 cm, range=0.1-1.2 cm) and cryobiopsy in three seconds (median=0.8 cm, range=0.4-1.7 cm). Moreover, two by two comparisons of the methods using Wilcoxon signed-rank test showed that both cryobiopsy techniques provided significantly larger specimens than forceps biopsy ($p < 0.001$ for both cryobiopsy techniques compared to forceps biopsy). Additionally, the cryobiopsy in five seconds also produced significantly larger tissue samples in comparison with its three seconds counterpart ($p < 0.001$, Figure 1).

Diagnosis accuracy

Conventional forceps biopsy could establish diagnosis in 40 patients (66.7%). Moreover, evaluation of the accuracy results revealed justifiable efficacy of the new cryobiopsy techniques in both timings. Our results showed that cryobiopsy in both proposed methods led to the final diagnosis in an acceptable proportion of the patients (48 patients; 80%, for cryobiopsy in three seconds and 46 patients;

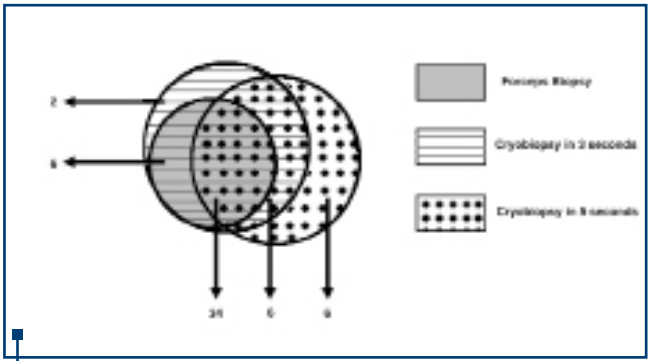

Figure 1. Overlap between the biopsy sampling methods. As it is evident in middle of the figure, in 34 cases, all three suggested methods could confirm diagnosis

76.6% for cryobiopsy in five seconds). Cochran's Q test showed that the achieved diagnostic yields for all three methods were not statistically different (p > 0.05).

Nonetheless, in this study, pathologic evaluation of both cryobiopsy specimens resulted in correct diagnosis in 54 patients (90%). Our statistical analysis also showed that simultaneous usage of samples produced in both cryobiopsies can improve the diagnostic yield significantly (Cochran's Q test;p = 0.02, Figure 2).

Complications

In both techniques there were no considerable respiratory complications such as pneumothorax or respiratory distress requiring mechanical ventilation. In case of bleeding, as the main potential complication of the procedures, no patient developed hypovolemic shock or needed blood transfusion. None of our patients were on anticoagulation medications. Moreover, all patients had been advised to stop their antiplatelet agents 7 days prior to the bronchoscopic procedures after consultation to their cardiologists. Our results showed that bleeding complications occurred in 8 cases (13.3%). These were regarded as class I bleeding in 2 cases (3.3%), class II bleeding in 4 cases (6.7%), class III bleeding in 1 case (1.7%) and one case of class IV (1.7%). Statistical analysis showed that there were no significant differences in case of bleeding type among the three sampling methods (Cochrane's Q test; p > 0.05).

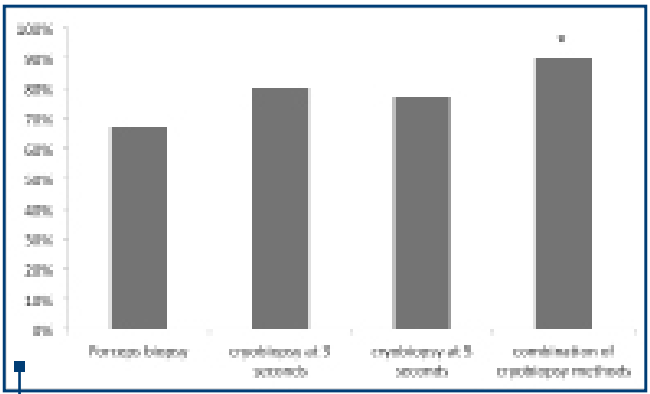

Figure 2. Comparison between sensitivity of four biopsy methods. As marked with star, combination of both cryobiopsy methods resulted in significant (p < 0.05) increase in sensitivity

Tissue sample quality

In cryobiopsy in three seconds, one necrotic tissue sample was reported. Moreover, in two cases only blood clots were reported. Noted artifacts were not observed in tissue samples of forces biopsy. Though, there was no statistically significant difference between tissue damage in forceps biopsy and the two cryobiopsy methods (Cochrane's Q test; p > 0.05).

Discussion

Forceps biopsy as standard method for evaluation of endobronchial lesions has a limited diagnostic yield. Previous studies reported that the sensitivity of forceps biopsy in patients with an established endobronchial lesion is about 74% [2]. Sensitivity of this diagnostic modality may be increased with utilization of combinatory cytologic methods such as brushing, washing, and use of needle, but these modifications are associated with a significant increase in procedural time and costs.This study demonstrates feasibility and safety of obtaining transbronchial lung biopsies using flexible cryoprobes in a selected population of patients. Moreover we compared two different protocols for this newly proposed method.

In our study, among the evaluated patients, in 54 (48 malignant) cases a definite diagnosis could be made. Sensitivity of forceps biopsy, three and five seconds cryobiopsy showed to be 67%, 80% and 76.7%, respectively.These sensitivities are comparable to those of previous reports. Schumann and colleagues[10] showed that the overall diagnostic yield of cryobiopsy in three seconds is 89.5%. This is while that study used a single cryobiopsy timing (three seconds). To the best of our knowledge there is no report regarding comparison of different protocols for cryobiopsy. Indeed, former studies just reported the efficacy and safety of cryobiopsy in patients with visible endoluminal tumor lesions. Our findings imply that both cryobiopsy techniques that were evaluated in the current report have an accepted diagnostic efficacy as compared to conventional forceps biopsy. Furthermore, our results propose that combination of two cryobiopsy methods might increase the diagnostic sensitivity by as much as 90% which is statistically higher than each single method. We believe that using only one sampling method for diagnosis of endobronchial lesions might lead to false negative results and might mislead management strategies. Increased diagnostic yield of tissue diagnosis in combination sampling is a well-documented issue in previous reports[2,11].

Tissue size analysis showed an advantage for both cryobiopsy techniques over conventional forceps biopsy. Our results demonstrate that cryo technique provides not only larger but also qualitatively better specimens. Less mechanical damage in cryo method might reflect tissue structure preservation since during this sampling procedure, the cryoprobe only needs to gently touch the tumor lesion. This is while forceps biopsy needs squeezing of the target tissue. Moreover, hemostatic effects of clot induction in the tissue, results in an artifact free specimen. Our results are in accordance with these explanations. We showed cryobiopsy in five seconds had a significantly larger mean tissue area indicating the higher quality of cryobiopsy specimens. Interestingly, although the specimen size for cryobiopsy in five seconds

was larger than the obtained sample from three seconds (1.6 cm vs. 0.8 cm), but as it is evident in Figure 2, sensitivity of cryobiopsy in three seconds appears to be higher. This finding might be attributed to repeated sampling from a single target area. Although we randomly changed the sequence of forceps biopsy and cryobiopsy, each three seconds cryobiopsy sampling was followed by a five seconds sampling and this sequence remains constant in all patients. Generally, there is no doubt that a larger specimen and better tissue quality leads to better results. But in our study, it seems the mechanical damage from multiple samplings from a small area influenced the results.

Recent studies shows that tissue samples with cryobiopsy have good quality without morphological artifact[10,12]. These studies confirmed that cryobiopsy could be useful for histological examination of human tumors without morphological artifacts associated with immersion-fixation followed by dehydration.

Although complications in bronchoscopy are rare, vasovagal reactions, post bronchoscopic fever, cardiac arrhythmia, hemorrhage, bronchospasm, pneumonia, pneumothorax, and death were reported as expected complications. Hemorrhage is the most frequent complication during bronchoscopic interventions[13]. Our results showed that the overall complications of the new biopsy methods are assumed to be generally low. In previous reports for conventional forceps biopsy, the bleeding rates vary from 1 major complication (an endobronchial visible carcinoma) to 11 potentially life-threatening hemorrhages after the procedure[10]. To overcome this frequent complication of conventional forceps biopsy, several methods have been proposed. Hot-biopsy is a successful alternative that reduces the bleeding complications in endobronchial sampling. It has been stated that hot biopsy with forceps shows a significant decrease in bleeding score, but a negative impact on the pathological samples is a major concern in this method[14]. In the present study we showed cryobiopsy not only decreases bleeding complications but also provides a better sample quality. Our findings are in accordance with previous reports. In a similar study conducted by Aktas and colleagues[15], cryoprobe biopsies were more successful than forceps biopsies in diagnosis of lung cancer, although that study used a single and long freezing time (20 seconds) for evaluation of cryobiopsy technique. Recently, a large multicenter study also showed advantage of cryobiopsy technique over the conventional forceps sampling[16]. In that study, unlike the present report, patients were randomly assigned to either techniques and both techniques were not applied for a single patient. The results of that multicenter report showed a significant improvement of diagnostic yield in cryo technique. This is while that report failed to show any superiority of cryobiopsy in reducing significant bleeding complications.

Our study has several potential limitations. Firstly we have not categorized endobronchial tumors according to their histopathological properties. A subgroup analysis considering tumor properties might result in various diagnostic accuracies among specific tumor types. Moreover, tumor location might exert a far-reaching influence on diagnostic accuracy of bronchoscopy with forceps or cryobiopsy. A new study with a large sample size and diversity of intraluminal lesions might answer these questions.

In conclusion this study showed that cryobiopsy is a safe technique with a diagnostic yield, which is comparable to that of conventional forceps biopsy. A larger specimen size and better tissue quality along with a low incidence of bleeding in cryobiopsy makes this method an acceptable alternative to forceps biopsy. We also showed that combination of sampling with two different cold induction timings would significantly increase sensitivity of this emerging technique. ■

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