Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(1): 91-96
doi: 10.52142/omujecm.39.1.19

The risk of tuberculosis bacilli seeding during mediastinoscopy in mediastinal tuberculosis: Is it clinically important?

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Received: 08.06.2021 • **Accepted/Published Online:** 12.06.2021 • **Final Version:** 01.01.2022

Abstract

Mediastinoscopy is the gold standard for diagnosis in the absence of parenchymal lesions in mediastinal tuberculosis lymphadenitis. During mediastinoscopy biopsy, tuberculous bacillus seeding into the mediastinum is a rare complication. This study aimed to test the safety of mediastinoscopy in terms of *Mycobacterium tuberculosis* seeding in the mediastinum by microbiologically evaluating mediastinal lavage samples taken before and after biopsy. Classical cervical mediastinoscopy was performed all of patients and who were reported as granulomatous inflammatory events results of histopathological examinations and who underwent mediastinal lavage before and after biopsy, were included in the study. All the lavage fluids were tested for AFB and subjected to *Mycobacterium tuberculosis* PCR DNA testing and standard tuberculosis culture. The patients were divided into two groups, Group 1: Necrotizing granulomatous inflammation, Group 2: non-necrotizing granulomatous inflammation. The microbiological tests of the patients in Group 1 were negative before biopsy. However, in two patients of Group 1, the results of cultures of lavage fluids that taken from after biopsy were positive for tuberculosis. In all patients in Group 2, all microbiological tests of the lavage fluids were negative (Power of the decision: 99.8%, with 5% error). All of patients in group 1, Antituberculosis treatment was initiated and continued for 6 months. There weren't seen any serius complications due to treatment and recurrence during the follow-up period. Mediastinoscopy can be used safely, with low morbidity and mortality rates and a high success rate for the diagnosis of mediastinal tuberculosis.

Keywords: tuberculosis, mediastinoscopy, biopsy, lavage, culture

1. Introduction

Lymph node tuberculosis is the most common form of extrapulmonary tuberculosis after pleural tuberculosis. Mediastinal lymph node tuberculosis (MLNT)accounts for 5% of tuberculosis lymphadenitis cases (1). In the absence of parenchymal lesions in mediastinal tuberculosis lymphadenitis, obtaining a diagnosis with microbiological examination of sputum and bronchoalveolar lavage fluids is very difficult. Mediastinoscopy is the 'gold standard' method for diagnosis, especially for non-cancerous mediastinal lymphadenopathies such as tuberculous and sarcoidosis (2-5).

Complications during and after mediastinoscopy are rare. Reported morbidity and mortality rates for mediastinoscopy are 0.08% and 0%, respectively. Severe bleeding, tracheobronchial laceration, esophageal perforation, recurrent and phrenic nerve paralysis, thoracic duct injury, mediastinitis, venous air embolism and tumor cell seeding in the mediastinum and along the incision line are major complications that can accompany mediastinoscopy (6). However, there is no available information about the seeding of tuberculosis bacilli in the mediastinum during mediastinoscopy and its effects on the treatment process of tuberculosis. Therefore, this study aimed to test the safety of mediastinoscopy in terms of *Mycobacterium tuberculosis* seeding by microbiologically evaluating mediastinal lavage samples taken before and after biopsy.

2. Materials and Methods

This study was approved by the Medical Research Ethics Committee and Informed consent was obtained from patients who participated in our study. Four hundred and fifty-three patients of our clinic who underwent mediastinoscopy between 2012 and 2019 were included in our study. Mediastinoscopy was performed on 573 patients who had lung cancer or mediastinal mass identified for cancer staging or diagnosis. For diagnostic purposes, mediastinoscopy was also performed on 135 patients who had mediastinal lymphadenopathy. Subsequently, all the patients were further evaluated with chest X-Ray, thorax tomography, bronchoscopy. In addition, positron emission tomography/computed tomography (PET-CT) was applied to patients with suspected malignancy.

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Inclusion criteria for patients were histopathological diagnosis of lymph node biopsy as granulomatous disease, underwent mediastinal lavage before and after biopsy during mediastinoscopy. Exclusion criteria for patients were underwent mediastinoscopy for the staging of lung cancer, underwent mediastinoscopy due to malignant mediastinal mass, granulomatous disease diagnosis by mediastinoscopical biopsy but did not have lavage applied before and after biopsy during mediastinoscopy. Ultimately, 88 patients having mediastinal, pathological sized lymphadenopathy (LAP) and whose results were reported as granulomatous inflammation by mediastinoscopy and who underwent mediastinal lavage before and after biopsy in mediastinoscopy were included in the study. The patients were divided to two groups according to the results of histopathological examination of their lymph node biopsies: Group 1: Necrotizing granulomatous inflammation and Group 2: non-necrotizing granulomatous inflammation.

All patients underwent classical cervical mediastinoscopy. Under general anesthesia, a 3 cm skin incision was performed in the supine position at 2 cm above the jugular notch, over the cervical midline. After the skin and subcutaneous and muscle layers were dissected, the mediastinum was entered from the anterior of the trachea. The subcarinal region was reached with blunt and sharp dissection along the midline without exploring the lymph node stations. Before their exploration with blunt and sharp dissection, 20 cc of serum physiological solution was delivered to the pretracheal area via a 14-gauge feeding tube through a mediastinoscope. This process was repeated three times. Finally, all the laving fluid in the mediastinum was aspirated. Following pre-biopsy, the right-left 2., right-left 4. and 7. mediastinal lymph node stations [upper paratracheal, lower paratracheal and subcarinal] were explored (Fig. 1, Table 1). Samples were taken from all lymph nodes by multiple punch biopsies. After the biopsy procedure, mediastinoscope was advanced into the subcarinal area. The mediastinum was lavage with 20 cc of serum physiological solution again. So, both before and after biopsy, the mediastinum was laved with 20 cc of serum physiological solution. These fluids were collected into different test tubes labelled ‘prebiopsy mediastinal lavage’ and ‘post biopsy mediastinal lavage’. The incision was then closed, and the procedure was terminated. All patients were awakened in the operating room and followed up in our clinic.

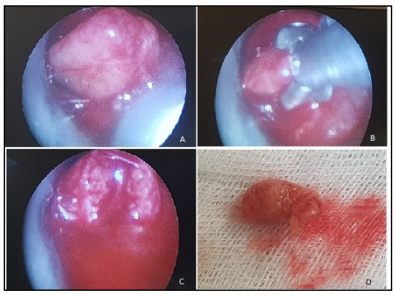

Fig. 1. Peroperative images of a patient whose tuberculosis culture of lavage fluid was positive

Table 1. Statistical results

Station number	Culture		Total n [%]
	Negative n [%]	Positive n [%]	
1	17 [100]	0 [0]	17 [100]
2	17 [89.5]	2 [10.5]	19 [100]
3	5 [100]	0 [0]	5 [100]
4	1 [100]	0 [0]	1 [100]
5	2 [100]	0 [0]	2 [100]
Total	42 [95.5]	2 [4.5]	44 [100]

2.1. Microbiological analysis

The mediastinal lavage specimens taken before and after biopsy were sent to the Medical Microbiology Laboratory. Specimens were sterilized by using sodium hydroxide [4% NaOH]. After concentration by centrifugation at 3000 g for 15 minutes, the sediment was re-suspended in 1.5 mL of 0.5 M phosphate buffer [pH 6.8] and inoculated onto Lowenstein-Jensen [LJ] medium and MGIT-7H9 broth supplemented with oleic acid-albumin-dextrose-catalase [OADC] and PANTA [Becton Dickinson]. The test tubes containing the inoculum and growth media were then incubated in Lowenstein-Jensen [LJ] medium at 37°C in a MGIT 960 unit [Becton-Dickinson and Company, Sparks MD, USA]. *Mycobacterium tuberculosis* complex strains grown on MGIT medium were tested for first-line anti-tuberculosis drugs in MGIT 960.

The Erlich, Ziehl and Neelsen staining method was applied to specimens that were then examined under a light microscope at 1000× magnification.

An XpertMLNTB/RIF device was used for the molecular identification of the *Mycobacterium tuberculosis* is complex. The testing was performed on samples by using version four cartridges, according to the recommendations of manufacturer. The Xpert assay sample reagent, which contains NaOH, and isopropanol was added within the ratio of 1 to 3 to the tubes to kill any mycobacteria present and to liquify the sample. The suspension was vigorously shaken and allowed to sit for 15 min before being shaken again and allowed to sit for another five min. Finally, 2 mL of the suspension was pipetted into anXpert assay cartridge and inserted into the GeneXpert unit for PCR testing. The measurements and analyses were conducted automatically, and reports were generated with Gene XpertDx software [Version 4.0].

2.2. Statistical analyses

In this study, descriptive statistical methods [percentage, frequency, mean] and the Chi-square test were used to analyze the data in the SPSS software program (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) For statistical calculations, each lymph node station was identified as an area and the level for statistical significance was set at $P < 0.05$.

3. Results

The general characteristics of patients are shown in Table 2. Sixty-one patients were female and 27 were male. [Group 1: Necrotizing granulomatous inflammation [n:44] and Group 2: non-necrotizing granulomatous inflammation [n:44].

Table 2. General features all of patients

General features	Group 1[n:43]	Group 2[n:45]
Age	53,52 [81-23]	50,34[75-25]
Sex		
Female	27	34
Male	16	11
Symptoms		
Cough	35	32
Sputum	20	12
Hemoptysis	2	0
Dispnea	6	10
Night sweats	12	5
Weight loss	10	10
No symptoms	8	12
AFB, tuberculous and common cultures		
in sputum	32	35
Negative	0	0
Positive	11	9
Notperformed		
Bronchoscopy		
EBL +	0	0
EBL –	17	24
Notperformed	26	21
Tuberculosis culture in BAL		
Negative	17	24
Positive	0	0
Lymph nodes that were biopsied		
Station 2nd		
Right	17	15
Left	7	4
Station 4th		
Right	35	39
Left	6	7
Station 7th	7	7
ML before biopsy		
PCR tbcDNA		
Negative	43	44
Positive	0	0
AFB		
Negative	43	44
Positive	0	0
Tbc culture		
Negative	43	44
Positive	0	0
ML after biopsy		
PCR tbc DNA		
Negative	43	44
Positive	0	0
AFB		
Negative	42	44
Positive	1	0
Tbc culture		
Negative	42	44
Positive	1	0

AFB: Acid-resistant bacteria EBL: Endobronchial lavage, BAL: Bronchoalveolar lavage, ML: Mediastinal lavage, PCR- tbc DNA: Polymerase Chain Reaction- tuberculous deoxyribonucleic acid, Tbc: Tuberculous

The mean age was 51.93 years with a range of 23 -81 years and the most common symptom was coughing. Twenty patients had no symptoms. Mediastinoscopy was performed on them due to the presence of pathological lymph nodes which were detected coincidentally. All patients had multiple mediastinal LAPs but none of them had endobronchial lesions. Additional radiological findings are shown in Fig. 2. All the patients were discharged at the first day after operation. There were no complications, except for hoarseness experienced in two patients which was resolved quickly.

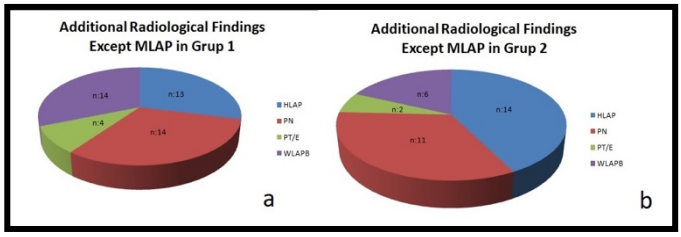

Fig. 2. a, b- Additionally radiological findings in Group 1(a) and in Grup 2 (b)

The most sampled lymph node station was the lower right paratracheal station [4R] and the least sampled station was the upper left paratrachealstation [2L]. A mean 1.9 [range 1-5] lymph node stations were sampled (Fig. 3). In 14 patients, pathological lymph nodes were detected in the subcarinal area, and all of them were sampled.

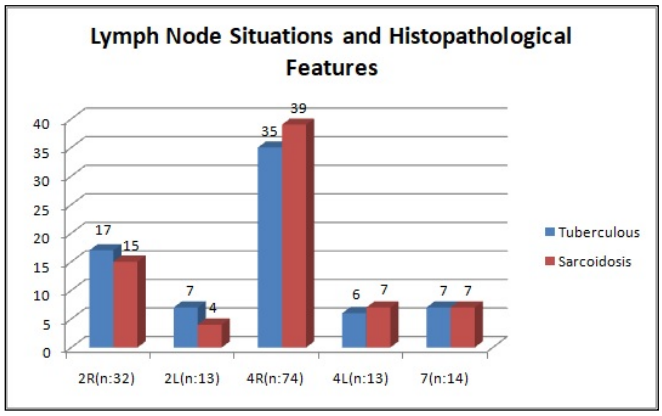

Fig. 3. Lymph node situations and histopathological features

Acid-fast *bacilli* (AFB) and *Mycobacterium tuberculosis* Polymerase Chain Reaction- tuberculous deoxyribonucleic acid (PCR DNA) testing and tuberculosis culturing were performed on all lavage fluids collected before and after biopsy. In all patients in Group 2, all microbiological tests were negative for all lavage fluids collected both before and after biopsy, and the pre-biopsy tests of the patients in Group 1 were all negative. However, the tuberculosis cultures of the post-biopsy lavage fluids of two patients in group 1 were positive (Fig. 4 a, b).

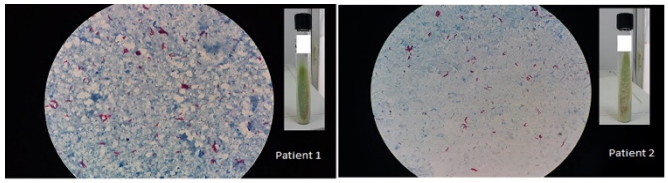

Fig. 4. a, b- Microbiological images of patients whose tuberculosis cultures of lavage fluids were positive: First patient (a), second patients (b)

Increasing the number of stations and / or the width of the dissection area did not have a significant statistical effect on the positive or negative expression of cultures. In addition, the rate of positive results for the tuberculosis culturing of mediastinal lavage fluids was 4.5%. The power of the decision was 99.8%, with a 5% margin of error, based on the power of the test and the sample size.

All of patients in group 1, antituberculosis treatment was

initiated. The patients were given on INH 300 mg [5 mg/kg], rifampicin 600 mg10mg/kg], ethambutol 1500 mg[20mg/kg], and pyrazinamide 2 g [25 mg/kg] for four months. Treatment was completed with INH and rifampicin for the next two months. All patients were followed up during the treatment period and for one year afterwards. There weren't seen any serius complications due to treatment. No recurrence was observed in any patient during the follow-up period.

4. Discussion

The most common form of tuberculosis after parenchymal tuberculosis is mediastinal tuberculosis. Mediastinal tuberculosis is mostly seen in children. MLNT without parenchymal lesion is a rare condition in adults. In Patients of our cohort, isolate mediastinal lymphadenopathy was detected in 46% of non-necrotizing granulomatous inflammation.; this rate was 25% in patients diagnosed with tuberculosis (7, 8). In our study, in both groups 1 and 2 the most common secondary pathologies were hilar LAP and parenchymal nodules (Figs. 2 and 3).

Mediastinal tuberculosis occurs more frequently in females than males, as same as the situation in our cohort of patients. Most patients are asymptomatic or present with non-specific symptoms. Coughing is the most common symptom in symptomatic patients. The most common symptoms in our patients were cough and sputum (Table 2). The probable cause of cough without parenchymal lesion is the irritation of the bronchi by mediastinal lymph nodes.

The most frequently affected lymph nodes in MLNT are the upper mediastinal and right hilar lymph nodes (9, 10). In our patients, the most frequently affected lymph nodes were the lower right paratracheal lymph nodes. The most affected lymph nodes after mediastinal lymph nodes were the hilar LAPs. Multiple biopsies were performed on all the pathological lymph nodes. The most frequently affected lymph nodes in both Groups 1 and 2 were the lower right paratracheal lymph nodes The least affected lymph nodes in both groups were the left upper paratracheal and subcarinal lymph nodes.

In some patients, bacilli from lymph nodes that are invading the bronchus can be detected in bronchoscopic lavage and / or sputum examinations. Our study was planned based on that information. In some patients with MLNT, bronchoalveolar lavage (BAL) can be found positive about tuberculosis due to mediastinal lymph node/nodes that invade bronchus in microbiological examination (11). This situation suggests that mediastinal lavages may be positive about tuberculosis after the mediastinoscopy of patients with MLNT.

The main purpose of this study was to determine the effects on prognosis of the seeding of the mediastinum with *M. tuberculosis bacilli* during biopsy procedures. In our study, tuberculosis cultures of mediastinal lavage fluids after biopsy were positive for only two patients. Those two patients did not show any differences from other patients in terms of response to treatment, complications, and recurrence during the follow-up period of two year.

Direct microscopy and culture are the 'gold standard' methods for the diagnosis of infections due to mycobacteria. The easiest and quickest method of diagnosis is ARB via the Ehrlich-Ziehl-Nielsen staining method. However, to show bacteria with direct microscopy, the material must contain at least 5000 to 10000 bacteria per mL [8]. In a study of newly diagnosed tuberculosis patients, it was reported that smear positivity could be shown in 50% to 80% of patients. Culturing is 500 times more sensitive than direct microscopy. However, the deficiency mentioned earlier in relation to the direct microscopic examination of tuberculosis bacilli and / or the amount of time consumed in culturing them prompts the use of molecular methods (12).

PCR is becoming used more widely because it has higher sensitivity and specificity rates and a wider identification spectrum than other molecular methods. It provides several advantages over culturing, including confirmation of the presence of *M. tuberculosis* within 1 to 3 days, compared to 2 to 6 weeks for culture techniques (13). The rapid diagnosis of tuberculosis with sensitivity like that of culturing has been reported for fresh specimens such as sputum and aspirated fluid by using PCR. Moreover, the sensitivity of PCR was 56.7%, the specificity was 100%, and the general efficiency of the test was 96.4% in a study conducted with endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TTAB) and endoscopic ultrasound-guided fine needle aspiration (EBUS-FNA) patients (14).

In our study, we used the three methods for the examination of mediastinal lavage fluids. Direct microbiological examination and PCR were used for the rapid and reliable detection of bacteria. In addition, tuberculosis culture, which is the 'gold standard' diagnostic method, was applied to mediastinal lavage fluids of all patients to prevent any false negative tests (15). Also in our study, while tuberculosis was diagnosed by pathological examination, mediastinal lavage examinations were performed microbiologically. We did not expect that other, atypical mycobacterial bacteria could be among the etiological agents. Because there was no additional immunodeficiency in our patients, that meant to be a low incidental of isolated mediastinal atypical mycobacterial infections in our patient groups. In addition to, successful results were obtained for all patients with anti-tuberculosis therapy. All these results collectively indicate that M. tuberculosis was the only factor directly impacting on the health status of the studied cohort.

A lot of complications, such as severe bleeding, mediastinitis, and tumor cell seeding in the mediastinum and along the incision line etc., can be seen during

mediastinoscopy (16, 17). However, no information was found in the literature regarding the seeding of tuberculosis bacilli during mediastinoscopy and its effects on follow-up treatment. In our study, the post-biopsy mediastinal lavage tuberculous cultures of two patients were positive. Our results showed that the seeding of tuberculosis bacilli in the mediastinum can occur during mediastinoscopy in patients with mediastinal tuberculosis.

The most likely explanation is the direct seeding of tuberculous bacilli into the mediastinum during biopsy. In these patients, biopsies were taken from only two stations which did not include the subcarinal area. These results suggest that the increased dissection and number of biopsies during mediastinoscopy are not predisposing factors for the seeding of bacilli in the mediastinum. In other words, our results showed that mediastinoscopy is a safe method that minimizes bacillus seeding of the mediastinum in MLNT. Statistically, according to the power and sample size of the applied test, the power of the decision was 99.8%, with a 5% margin of error. In addition, no difference was observed between the two patients' positive cultures of M. tuberculosis and others in terms of treatment period, response to treatment and recurrence during the follow-up period.

Especially in recent years, the new, non-invasive methods such as EUS-FNA and EBUS-TBNA have become frequently used in the diagnosis of mediastinal diseases. However, the diagnostic value of these methods in malignant diseases is higher than non-cancerous, mediastinal lymphadenopathies such as tuberculosis. In addition, with these methods, difficulties of exploration of the lymph nodes which are smaller than pathological dimensions and / or around the great vessels can lead to false negative results. This can also lead to delays in the initiation of treatment. For this reason, the diagnostic value of mediastinoscopy increases further, especially in non-cancer, mediastinal pathologies (6, 18).

This study has some limitations. First, because this study was prospective clinical study, mediastinal lavage fluids samples were obtained all of patients who performed mediastinoscopy and had not malign diagnosis. Patients whose pathology results were not reported as tuberculosis were excluded from the study. Secondly, since the follow-up of patients diagnosed with tuberculosis was carried out by tuberculosis dispensaries, the surgical follow-up of the patients could only be made by phone calls. Patients who could not be reached by phone during the follow-up period were also excluded from the study.

The authors of the presents study suggest that during mediastinoscopical biopsy, tuberculous bacillus seeding into the mediastinum was a rare complication. Furthermore, this situation had no effect on both the prognosis and treatment response. Therefore, mediastinoscopy remains the 'gold standard' diagnostic method in that it can be used safely, with a high diagnostic success rate and low morbidity and mortality, in the diagnosis of mediastinal tuberculosis.

Conflict of interest

The authors declare that they have no conflicts of interest in relation to this study.

Acknowledgments

The authors thank Dr. Gregory T. Sullivan for proofreading an earlier version of this manuscript.

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