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Bronchial lavage in patients with negative BK tuberculosis directly

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Abstract
Introduction*: The bacteriological confirmation isthe diagnostical basis of tuberculosis. Bronchoscopy has been found to be useful in diagnosing suspected TB pulmonary suspects who do not have sputum or negative BK directly.*
Material and methods: *Prospective study in 167 tuberculosis patients, who during the hospitalization have resulted negative in the examination of BK sputum directly. In all patients, fiberopticbronchoscopic examination was performed accompanied by examination of selective bronchial lavage for BK directly and culture as well as the diagnostic result of the procedure and the type of material were evaluated.*
Results: *In cases thatbronchial lavage examination for direct BK was performed, the positivity rate was 40.4%. Out of 165 patients who were examined sputum for BK directly after performing FBS, the positivity rate was 53.9%. Out of 166 patients who were culture tested with bronchial lavage culture for BK during FBS, the positivity rate was 70.5%. Out of 166 patients, who were cultureexamined with sputum for BK before performing FBS, in hospitalization, the positivity rate was 12.7%. Out of 165 patients, who were examined with sputum culture for BK after performing FBS, the positivity was 61.2%. In 27 (16.2%) cases of patients taking part in the study, no positivity for BK was found in any of the procedures, of which in 5 cases the diagnosis was determined on the basis of histological examination.*
Conclusions:*Fibroceptive bronchoscopy with direct bronchial lavage examination for BK and culture for Mycobacterium tuberculosis has an important role in determing the diagnosis.*
It is a procedure that is recommended for daily pulmonary tuberculosis diagnosis, in cases when no patient can produce sputum spontaneously or when the examination of the sputum directly is negative.
Keywords: *fiberoptic bronchoscopy, pulmonary tuberculosis, bronchial lavage, direct BK, BK culture*

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I. Introduction

TB diagnosis relies on anamnesis, epidemiological characteristics, and bacteriological data. Although the positivity of the mycobacterial culture is the "gold standard" for diagnosis, the initial approach to the diagnosis of pulmonary tuberculosis (PTB) is the detection of BK bacilli directly in smears in sputum samples. However, the doctor should make a decision for patients who result in sputum BK directly negative or cannot produce sputum. Standard options are waiting for culture test results, molecular examinations, radiological follow-up, or empiric antitubercular treatment. In this study we aim to assess the bacteriological diagnostic efficiency of fiberoptic bronchoscopy in patients suspected of having tuberculosis, who are BK directly negative in sputum or unable to produce sputum. Bronchoscopy may be needed to obtain material samples for examination as well as for endobronchial evaluation.

The examing and finding of BK is of particular importance for the diagnosis of tuberculosis and the assessment of the results of the treatment and its epidemiological situation. (1, 2) BKs can be seen under direct microscopy of the sample being examined. In addition, the sample can be examined after the material has been cultivated in special feeding grounds for the growth and development of BKs. In cases when the result of the usual BK examination is negative, it is necessary to extent the search in the bronchoalveolar lavage. (3, 4, 5) Usually, for BKsto be seen during direct microscopy, 1ml of sputumshouldcontain about 5 thousand BK. In case 1ml sputum contains 1000-10000 BK, BK positivity is found in 40-50% of cases; when the BK concentration is <1000 BK the positivity is found in 4% of cases (2). Feeding groundscan be liquid or solid and the assessment of results is done after 4-6-8 weeks. Recently, new examination methods have greatly shortened the assessmentinterval of BK examination results. (1, 6)

Purpose

The study aims to assess the procedures and their correlations regarding the detection of Mycobacterium tuberculosis in patients suspected of having tuberculosis, who are directly negative BK in sputum or cannot produce sputum andbronchonchiallavage has been applied.

II. Material And Methodology

The paper is an observational,timeperspective study. 167 adult patients were past of the study who were admitted to the University Hospital "ShefqetNdroqi" during the period January 1, 2011 to December 31, 2015 with various patient diagnoses for pulmonary pathology or who were suspected of having pulmonary Tb with directly negative BK sputumand whose final diagnosis resulted in pulmonary TB. In all patients, fiberopticbronchoscopic examination was performed, accompanied by selective bronchial lavagedirectly for BK and culture. Bronchial lavage was performed in the lesioned areas, if present.

Fiberoptic bronchoscopy was performed in the Bronchoscopy Cabinet at the University Hospital "ShefqetNdroqi" Olympus BF IT 180- 6.0 mm flexible bronchoscopevideo. Prior to the procedure, patients underwent local anesthesia lidocaine 2% -20 ml as well as 3-5 mg mgi/vein. According to the criteria,bronchial lavage was performed, at the localization where radiological changes were previously found. Samples were sent to the microbiology laboratory for direct BK examination and culture for BK.

Criteria of inclusion were (1) age at least 18 years, (2) BK directly negative in 2 or more samples of sputum or absence, and (3) FBS was performed due to suspicion prior to initiation of anti-TB treatment. Exclusion criteria were (1) BK directly positive, (2) patients with unfinished diagnoses due to loss of observation. Statistical analysis was performed using SPSS17 and Medcalc. The classification of samples from adult patients with pulmonary TB examined for diagnosis was:

a direct-positive and culture-positive
b direct -negative and culture-positive
d direct -positive and culture-negative

From this classification, the contribution for TB diagnosis is calculated:

$$\frac{b}{a + b + d} \times 100$$

III. Results

In cases when bronchial lavage examination for directly BK was performed, the positivity was 40.4%. In 165 patients who were examined sputum for BK directly after performing FBS, the positivity was 53.9%. In 166 patients who were performed bronchial lavage culture for BK during FBS, the positivity was 70.5%. In 166 patients, who were examined with sputum culture for BK before performing FBS, in hospitalization, the positivity was 12.7%. In 165 patients, who were examined with sputum culture for BK after performing FBS, the positivity was 61.2%.

Tab. 1 Results of BK examination according to the type of sample

Examination	patients	negative	%	positive	%
Direct BK of bronchial lavage	166	99	59.6	67	40.4
Direct BK of sputum after FBS	165	76	46.1	89	53.9
Culture BK bronchial lavage	166	49	29.5	117	70.5
Culture BK before fbs	166	145	87.3	21	12.7
Culture BK of sputum after fbs	165	64	38.8	101	61.2

Figure 2 shows the results of the directly BK examinations and in culture and the type of sample examined.

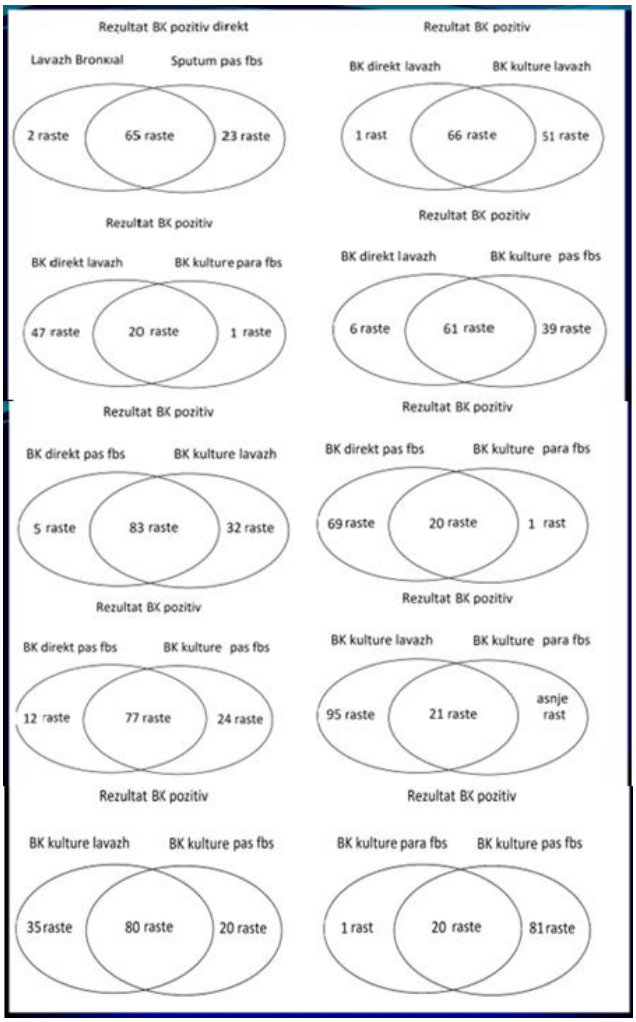

Figure 2 Results of direct and culture BK examination according to the examined sample

BK directly in lavage and directly in the material collected after fbs results in the same positivity in 65 cases, while it has positive result in 23 cases of sputum examination collected after fbs, which have resulted negative in BK directly in lavage. In addition, there are twopositive cases in the examination of bronchial lavage directly for BK that have resulted negative in the sputum collected after FBS. The contribution of sputum collected after FBS for BK in the diagnosis on direct examination of lavage results to be 25.5%

BK directly in lavage and BK culture in bronchial lavage results the same positivity of 66 cases, while there was positive result in 51 cases of BK culture examination of bronchial lavage, which have resulted negative in BK directly in lavage. Also,a case resultedpositive in the examination of bronchial lavage directly for BK which has resulted negative in BK culture of lavage. The contribution of culture to BK in diagnosis with direct examination results to be 43.2%:

BK directly in lavage and BK culture before fbs results positive in both examinations in 20 cases, while there is a positive result in 1 case of BK culture examination before fbs, which have resulted negative in BK directly in lavage. There were also 47 positive cases in the examination of bronchial lavage directly for BK, which were negative in BK culture before fbs. The contribution of culture to BK in diagnosis with direct examination results to be 1.47%:

BK directly in lavage and BK culture after fbs results in both examinations same positivity in 61 cases, while there is a positive result in 67 cases of BK culture examination after fbs, which have resulted negative in BK directly in lavage. There were also 6 positive cases in the examination of bronchial lavage directly for BK, which have resulted negative in BK culture after fbs. The contribution of culture to BK after fbs in diagnosis with direct examination in lavage turns out to be 36.8%.

BK directly after fbs and BK culture in lavage results same positivity in both examinations in 83 cases, whereas there is a positive result in 88 cases of BK culture lavage examination, which have resulted negative in BK directly after fbs. There were also 5 positive cases in the examination of sputum after direct fbs for BK,

which have resulted negative in BK lavage culture. The contribution of culture to BK in diagnosis with direct examination results to be 26.6%.

BK directly after fbs and BK culture before fbs results same positivity in both examinations in 20 cases, while there is a positive result in 1 case of BK culture examination before fbs, which has resulted negative in BK directly after fbs. There werealso 69 positive cases in the examination of sputum after direct fbs for BK, which have resulted negative in BK culture before fbs. Contribution of culture for BK before fbs in diagnosis with direct examination after fbsresults to be 1.1%.

BK directly after fbs and BK culture after fbs results same positivity in 77 cases, while there is a positive result in 24 cases of BK culture examination after fbs, which has resulted negative in BK directly after fbs. There were also 12 positive cases in the examination of sputum after fbs directly for BK, which have resulted negative in BK culture after fbs. Contribution of culture for BK after fbs in diagnosis with direct examination after fbsresults to be 21.24%.

BK culture lavage and BK culture before fbs results same positivity in both examinations in 21 cases, while there is no positive result in any case of BK culture examination before fbs, which has resulted negative in BK lavage culture. There were also 95 positive cases in the examination of lavage culture for BK, which have been negative in BK culture before fbs. The contribution of culture for BK lavage in diagnosis with BK culture examination before fbs turns out to be 81.9%.

BK culture lavage and BK culture after fbs results same positivity in both examinations in 80 cases, while there is a positive result in 20 cases of BK culture examination after fbs, which has resulted negative in BK lavage culture. There were also 35 positive cases in the examination of lavage culture for BK, which have been negative in BK culture before fbs. Contribution of culture for BK culture after fbs in diagnosis with BK examination lavage culture results to be 14.8%.

BK culture before fbs and BK culture after fbs results same positivity in both examinations in 20 cases, while there is a positive result in 81 cases of BK culture examination after fbs, which have resulted negative in BK culture before fbs. There was also 1 positive case in the culture examination for BK before fbs, which resulted to be negative in BK culture after fbs. The contribution of culture for BK after fbs in the diagnosis with BK culture examination before fbs turns out to be 79.4%.

In 27 (16.2%) cases of patients included in the study, no positivity for BK was found in any of the procedures. For these cases the diagnosis was based;in 3 patients from the histological result of the biopsy material - tuberculous granuloma; in 2 patients the tuberculous diagnosis was supported based on the histological result - epithelioid granuloma; and in one of these patients' histological examination resulted in nonspecific inflammation. In 22 (13.2%) cases not confirmed bacteriologically or histologically, the diagnosis was based on anamnestic, clinical-radiological data, and was confirmed by the progress and results of antituberculous treatment.

IV. Discussion

The initial diagnostic approach for suspected cases of pulmonary tuberculosis is to demonstrate Mycobacterium tuberculosis on direct examination of the sputum. In most tuberculosis centers, even after careful examing, there are positive bacteriological results from sputum in about 16 to 50% and a large proportion of the results remain negative and given the fact that the clinical profile and radiological lesions were consistent with the diagnosis of pulmonary tuberculosis. (7) Early diagnosis of pulmonary tuberculosis prevents disease progression, morbidity, disease spread, and permanent damage with fibrotic changes. Sputum culture for BK takes a long time and reliable serological test is not yet available. In such situation bronchoscopy is choosen for the rapid diagnosis of tuberculosis in cases of BK directly negative. The fiberoptic bronchoscope with bronchial lavage examination for BK directly, including culture for Mycobacterium tuberculosis, plays an important role in establishing the diagnosis. This is in cases when BK directly has been shown to be negative repeatedly and sputum examination is absent or sputum induction has failed.

Unfortunately, the diagnosis of active PTB is often delayed because less than half of these patients have BK directly positive in sputum (8), and isolation of Mycobacterium tuberculosis (MTB) requires a long time. Direct BK examination (at least two or more samples) is important for the rapid diagnosis of PTB, but direct sputum examination for BK has poor sensitivity (30-70%), despite high specificity (98-99%). Mycobacterial cultures are more sensitive than BK directly (80-85%), but culture results usually require 3-8 weeks. (9)

The use of fbs for the diagnosis of TB has been demonstrated in numerous studies. (10, 11, 12) It was found to be diagnostic between 11% and 96%; (10, 11, 12) being exclusively diagnostic in 2% to 52% of cases; (10, 11, 12) and providing immediate diagnosis in 4% to 79% of cases (10, 11, 12). This wide range of values may be due to the fact that these studies are from different geographical areas where the prevalence of TB is different.

In a retrospective study of 41 patients without sputum or with negative sputum for AFB, Danek and his colleagues reported that the Fbs result for TB diagnosis was in 39 of 41 (95%) patients. This led to TB diagnosis of 19 of 41 (46%) cases. (13)

In a larger retrospective study of 144 patients with pulmonary TB from Ip and co-workers, 125 patients were diagnosed with Fbsso about 87% of cases. (10)

In a prospective study of 222 patients suspected of having pulmonary TB and who were negative BK sputum from de Graciaandco., 20 patients underwent Fbs and the overall result for TB diagnosing withFbs was 16 out of 20 cases (80%).

(14) Willcox and co. prospectively studied 275 patients suspected of having TB but that sputum BK was negative and 89 patients in this study were diagnosed with TB. Fbs led to the diagnosis of TB in 60 out of 89 (67%) patients. (15)

Studies with higher diagnostic data in bronchoscopy have been retrospective, but in prospective studies, the sensitivity of bronchoscopy is on average 77%. Among the various bronchoscopic samples such as bronchial lavage, brushing, bronchial biopsy, transbronchial biopsy and bronchoalveolar lavage, which have been used in studies, it has been found that bronchial brushing does not provide any added advantage for TB diagnosis, but this can only be said in retrospective. (16)

Transbronchial biopsy culture for TB has been found to be of little use and this was not seen to be a source of material for positive culture in any patient. (17) The diagnostic result of bronchoscopy with bronchoalveolar lavage for tuberculosis mycobacteria is higher compared with bronchial lavage. (14) One explanation for this observation is the lower concentration of lidocaine in bronchoalveolar lavage compared to bronchial lavage. Local anestheticsubstances such as tetracycline and lidocaine used in bronchoscopy inhibit the growth of BK and other bacteria. (18) When 2 ml of 2% lidocaine is used, bronchial aspiration may contain up to 1% lidocaine, but most BKs are inhibited at this concentration. Because a large amount of saline solution is used for bronchoalveolar lavage, the concentration of local aspirated anesthetic is probably lower in the bronchoalveolar lavage compared to that of bronchial lavage. This phenomenon may explain the diversity of culture results from bronchial lavages in different studies depending on the amount of lidocaine used.

The importance of fbs is in determing the diagnosis of patients who are suspected of having pulmonary TB based on abnormalities in thoracic radiography but who are negative sputum or who do not have sputum. The alternative in such cases is to treat patients empirically with antituberculous drugs, but such an approach cannot be defended in today's era given the side effects of these medicamnets. In addition, a lack of knowledge about the body's sensitivity model can lead to therapy failure if the disease is caused by multidrug-resistant tuberculosis. Fbs provide immediate diagnosis in a significant proportion of TB suspects (positive sputum for BK, samples with transbronchial biopsy reveals caseous granulomas with or without BK, or malignant), reducing the time of final diagnosis. According to one study the total achievement of bronchoscopy in the diagnosis of BK negative pulmonary tuberculosis was 83.33% (50/60); bronchoscopy was the only diagnostic method in 66% of cases (40/60) with bronchial lavage being the only diagnostic method in 48.33%. Bronchial lavage with BK directly and with histopathological data of caseating granuloma has made possible the immediate diagnosis in 48.33% (29/60) patients.

(4) Fibrobronchoscopy provides material for early diagnosis e.g., bronchial lavage for direct microscopy and transbronchial biopsy for histopathological study. In the Bachh study (4) bronchial lavage in BK directly was positive in 35% of patients; all of these have been confirmed by positive culture. Danek and Bower (13) and Purohit and co. (19) demonstrated TB bacilli in 34% and 42%, respectively, while in the study by Kulpatiand co. (7) 40were % positive.

In the Bachh study (4), bronchial lavage culture was positive for TB bacilli in only 65% of cases, similar to that reported by Kulpatiand co. (7), Kvaleand co. (20) where there was an increase in TB bacillus in only one-third of patients suspected of having tuberculosis. Kato and co. (21) reported that high lidocaine concentrations had an inhibitory effect on mycobacterial growth. In the culture of biopsy material in Wallace and co.studies. (22), Funahashiand Co. (23) and Danek and Bower (13) bacilli increased to 20%, 60%, and 41%, but this was not the only diagnostic data in each of the studies and did not affect the diagnostic contribution of other methods. Kulpatiand co. (7) reported that exclusively positive diagnostic results were provided by prebronchoscopic sputum culture and bronchoscopic aspiration at 5% and 10%, respectively, while in the Bachh study (4) these results were 16.7 and 16.7%, respectively. Wallace and co.(22)dheand Bower (13) had reported 95% positive culture of samples taken from flexible fibrooptic bronchoscopy and therefore negative culture is strong evidence against the diagnosis of tuberculosis.

Post-bronchoscopy sputum examinations provide additional confirmation in cases of direct pulmonary tuberculosis BK directly negative in sputum. In various previous studies flexible fiberoptic bronchoscopy in combination with transbronchial lung biopsy has led to early diagnosis in 60% to 85% of BK pulmonary tuberculosis directly negative. (4)

According to (24) in bronchial lavage with fbs in direct examination 13 (23%) patients tested positive for Mycobacterium tuberculosis. Twenty-eight (50%) patients tested positive for culture. Histopathological results confirmed tuberculosis in 8 out of 20 patients, in whom mucosal biopsy was performed, four of seven with transbronchial biopsy, two-thirds with aspiration agobiopsy. From bronchoscopic procedures early diagnosis is performed in 27 (48.21%) patients. The authors conclude that fiberoptic bronchoscopy has an important role in diagnosing patients suspected of having tuberculosis, who are BK directly negative or could not extract sputum. It is useful and necessary in selected cases. (24)

The diagnostic ability achieved by the culture was significantly higher in the combination of samples after the first and second bronchial lavage compared to the first bronchial lavage. (Total cases: 163 (94%) compared to 141 (81%), p <0.001; cases with sputum BK directly negative: 86 (91%) vs 73 (77%), p <0.001; cases with lack of experimentation: 77 (98%) vs 68 (86%), p = 0.004). (25) (25) Taking an additional sample of bronchial lavage may be a useful alternative to consider TB daily diagnosis in patients with direct-negative BK sputum or who may not be able to examine sputum samples. (25)

If bronchoscopy is performed because it is a procedure that induces coughing, additional sputum samples for BK directly and culture should be collected after the procedure to increase diagnostic efficiency.

V. Conclusions

The fiberoptic bronchoscope with examination of the bronchial lavage for BK directly, including culture for Mycobacterium tuberculosis, has an important role in determinig the diagnosis.

Bronchial bronchoscopy with bronchial lavage is a procedure that is recommended to be applied for pulmonary tuberculosis diagnosis when no patient can produce sputum spontaneously or when the examination of the sputum directly is negative.

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