# THE OPTIMIZATION OF BACTERIOLOGICAL INVESTIGATION IN THE CASES OF PULMONARY TUBERCULOSIS WITH NEGATIVE MICROSCOPIC EXAMINATION, FOR ACID- FAST BACILLI (AFB), OF SPONTANEOUS SPUTUM CULTURE

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***Keywords:*** *tuberculosis, bacteriological confirmation, induced sputum, bronchial lavage*

***Abstract:*** *Introduction. Tuberculosis continues to remain a major public health issue in Romania, the level of TB incidence at present placing our country among the first places in the European Union. Pulmonary tuberculosis (TB) is the most common way of localization of the disease in the adult patient (over 90%), the level of bacteriological confirmations of newly proven cases being, over the last few years (2006-2008), around 50%-70%. Aims. The evaluation of the input of special techniques for cough and expectoration induction (sputum induced by aerosol exposure with 5% of saline solution), along with methods of sputum culture (normal saline solution lavage and bronchial aspirate by fibrobronchoscopical examination.) Materials and methods. The object of study was a group of patients hospitalized with the diagnosis of pulmonary tuberculosis who, apart from the standard process of sputum culture and processing, received treatment by means of ultrasonic aero Venturi type face-mask, together with lavage and bronchial aspirate by endoscopic examination. The recorded issues were: the aspect of products taken, the results of bacteriological examinations carried out for negative sputum-smear for acid-fast bacilli (AFB), by means of microscopy and culture (C) of the products taken, adverse effects and necessary costs. Results. Bacteriological examinations of induced sputum proved to be valid through microscopic examination (M+) for seven patients and through culture (M-,C+) for nine patients, having a diagnostic sensitivity of 18,1%. Then, the examinations of bronchial lavage liquid confirmed 4 patients (4%) through microscopy and 8 patients (8%) through culture, with a diagnostic sensitivity of 14%. The methods used were considered to be accurate and valid, with minor adverse events, applicable to routine practice and also low cost. Conclusions. The results of this study prove the importance in implementing all the recommendations made by the Tuberculosis National Control Programme concerning sputum culture, in order to obtain the best results (bacteriological confirmations) in pulmonary tuberculosis.*

***Cuvinte cheie:*** *tuberculoza, confirmare bacteriologică, sputa indusă, lavajul bronşic*

***Rezumat:*** *Introducere. Tuberculoza continuă să rămână o problemă majoră de sănătate publică în România, nivelul incidenţei actuale (99,9‰) situând ţara noastră pe primul loc în Uniunea Europeană (UE). Tuberculoza pulmonară (TB) este cea mai frecventă localizare a bolii la pacientul adult (peste 90%), iar nivelul confirmărilor bacteriologice a cazurilor noi declarate a fost în ultimii ani (2006-2008) între 50-70%. Obiectiv. Evaluarea aportului tehnicilor speciale de provocare a tusei şi expectoraţiei (sputa indusă prin aerosolizare cu soluţie salină 5%), completate cu tehnici de recoltare a sputei (lavaj cu ser fiziologic şi aspiraţie bronşică prin examen fibrobronhoscopic), la creşterea procentului de confirmare bacteriologică la pacienţii cu TB pulmonară, la care examenul microscopic (M) pentru BK din sputa recoltată spontan, a fost negativ. Material şi metodă. S-a luat în studiu un lot de pacienţi internaţi cu diagnosticul de tuberculoză pulmonară, la care pe lângă aplicarea metodelor standard de recoltare şi prelucrare a produsului patologic (sputa), s-a efectuat tehnica de recoltare şi îmbunătăţire a produsului patologic prin aerosolizare ultrasonică pe masca Venturi, completată cu lavajul şi aspiraţia bronşică prin examen endoscopic. S-au consemnat: aspectul sputei recoltate spontan, rezultatele examenelor bacteriologice pentru BK efectuate prin microscopie şi cultură (C), reacţiile adverse apărute şi costurile necesare. Rezultate. Examenele bacteriologice ale sputei induse au arătat o confirmare prin examen microscopic (M+) la şapte pacienţi (7%) şi prin cultură ( M-; C+) la nouă pacienţi (9%), având o sensibilitate diagnostică de 18,1%, iar examenele lichidului de lavaj bronşic au confirmat patru bolnavi (4%) prin microscopie (M+) şi opt pacienţi (8%) prin cultură, cu sensibilitate diagnostică de 14%. Metodele aplicate au fost considerate sigure, cu reacţii adverse minore, posibil de aplicat în practica de rutină, fără costuri mari. Concluzii. Rezultatele studiului demonstrează importanţa aplicării tuturor recomandărilor Programului Naţional de Control al Tuberculozei (PNCT) privind recoltarea sputei, în vederea obţinerii celor mai bune rezultate (confirmări bacteriologice) în tuberculoza pulmonară.*

## INTRODUCTION

The current level of incidence for TB in Romania, (99,9‰)(8) places our country on the seventh place among the countries in the European Region of the World Health Organization (WHO) and on the first place in the EU. What can be noticed from the data analysis concerning the bacteriological confirmation of cases with pulmonary localization is the preservation of a low value for new cases and relapses both country level and local level, around 50%-70% confirmations[(8)]. There is still a significant percentage of new cases of TB bacteriologically unconfirmed, the ones for which the alternative

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Article received on 07.07.2010 and accepted for publication on 20.07.2010
ACTA MEDICA TRANSILVANICA September 2010; 2(3)207-209

of whether implementing an anti TB treatment or not raises enough dilemmas.

## PURPOSE OF THE STUDY

The purpose of this study is to evaluate the contribution of special techniques for: the generation and culture of valid samples of sputum (induced sputum and fibronchoscopic examination with aspirate after bronchial washing), the optimization of bacteriological investigation (rise of etiological confirmations), techniques used on patients suspect of pulmonary tuberculosis but still with negative microscopic examination for acid-fast bacilli (AFB), of spontaneous sputum culture.

## MATHERIAL AND METHOD

The study was conducted between 01.01.2007-31.12.2009 on a group of 98 patients hospitalized at Spitalul de Pneumoftiziologie in Mihaiesti, Valcea, diagnosed with pulmonary TB.

What was insisted upon was the application of standard methods of sputum culture and processing, according to PNCT[6]. as an accepted alternative of sputum culture, there were three samples of sputum used, (necessary for a bacteriological examination), daily, every 6-8 hours.

The procedure was repeated uninterruptedly for three days, after knowing the result of the previous examination, reaching to up to three check-ups (bacteriological examinations). What was used for the patients who could not expectorate and for the ones with three bacteriological examinations with negative sputum result, was the method of cough and expectoration induction, with 10 ml of hypertonic saline solution 5%, making use of an ultrasonic nebulizer and a Venturi type face-mask. This procedure was implemented after the technique had been explained, the patient had given the written agreement and the spirographic examination had been done.

The test was considered finished with a positive result when the patient managed to collect, al least 3ml of sputum.The fibronchoscopic examination completed with broncho alveolar lavage and ended with bronchial aspirate was applied to patients with negative microscopical examination for acid-fast bacilli (AFB) from the spontaneous sputum culture or after aerosol intake. The investigation was preceded by the patient's anamnesis, clinical examination, EKG examination and spirogram, accomplished only after the patient's written agreement, using local anesthesia with lidocaine, 1% and 2% and 20 ml of sterile saline solution for bronchial lavage. The sputum collected after the aerosol exposure and the bronchial lavage liquid were processed and bacteriologically investigated through microscopic examination and culture in view of identifying the etiologic agent, M. Tuberculosis.

## RESULTS

The study group included 98 patients: 36 women (37%) and 62 men (63%), ages 18-69, the average age being 46,4 years, (Table 1), 84% new cases recorded and 16% relapses, according to therapeutic history.

In what concerns the aspect of sputum collected at hospitalization, there were concluded the following: on one hand, in female patients mucous aspect was found in a 53% percentage, compared to 39% in male patients; on the other hand, muco-purulent aspect was more frequently met in male patients, 45%, compared to 31% in female patients.

For 70% of the patients, 44 male and 26 female, the results of the sputum bacteriological examination at hospitalization, were negative. The induced sputum was collected for 88% of the patients, three patients refused the test and seven patients interrupted the procedure because of an adverse reaction.

The results of the bacteriological examination of the sputum obtained after exposure to aerosols illustrated 16 cases of bacteriological confirmation, seven patients were recorded with positive examinations in microscopy and culture, while for nine patients the microscopic examination was negative and the culture one, positive (Figure1). There were 85 patients fibronchoscopically examined, out of which 55 men and 30 women, four patients refused the investigation, in two cases the patient was uncooperative and seven patients were confirmed positive through the previous method (induced sputum).

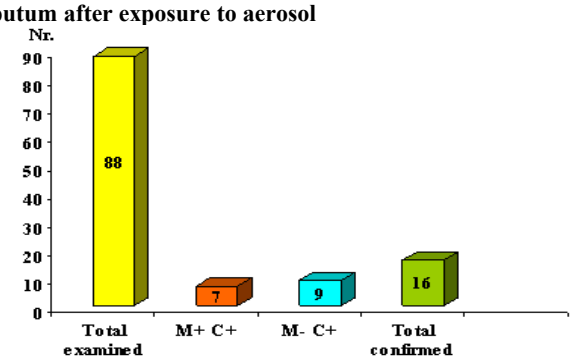
**Table no. 1. Age and sex distribution of patients**

| STUDY GROUP | | | | | | |
|---|---|---|---|---|---|---|
| Age (years) | Female | | Male | | TOTAL | |
| | frequency(no.)Absolute | Relative frequency(%) | frequency(no.)Absolute | Relative frequency(%) | frequency(no.)Absolute | Relative frequency(%) |
| < 30 | 5 | 14 | 6 | 10 | 11 | 11 |
| 31 – 40 | 12 | 34 | 12 | 19 | 24 | 25 |
| 41 – 50 | 9 | 25 | 17 | 27 | 26 | 26 |
| 51 – 60 | 7 | 19 | 16 | 26 | 23 | 24 |
| 61-70 | 3 | 8 | 11 | 18 | 14 | 14 |
| TOTAL | 36 | 100 | 62 | 100 | 98 | 100 |

**Table no. 2. Aspects of spontaneous sputum culture at hospitalization**

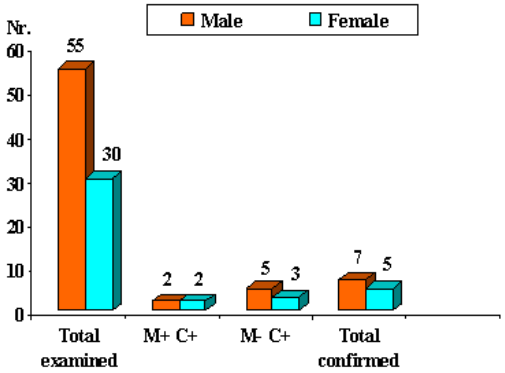
| STUDY GROUP | | | | |
|---|---|---|---|---|
| Sputum aspect | Female | | Male | |
| | Absolute frequency (no.) | Relative frequency (%) | Absolute frequency (no.) | Relative frequency (%) |
| Sero-mucous | 19 | 53 | 24 | 39 |
| Muco-purulent | 11 | 31 | 28 | 45 |
| Hemoptoic | 2 | 5 | 5 | 8 |
| Salivary | 4 | 11 | 5 | 8 |
| TOTAL | 36 | 100 | 62 | 100 |

**Figure no. 1. Result of bacteriological examination of sputum after exposure to aerosol**




The bacteriological examination carried out of the bronchial lavage liquid was registered positive for four patients both through microscopy and culture, as for eight patients it was negative at the microscopic examination and positive at the culture examination.( Figure 2).

**Figure no. 2. Results of bacteriological examination achieved out of bronchial aspirate**




By means of the two methods applied (induced sputum and bronchial lavage), there were 28 patients diagnosed with pulmonary TB, representing 28% of the patients, 18 patients were new cases (22% of the group) and 10 patients were part of the second category (66% of the relapses).

The adverse events encountered during aerosol exposure and fibronchoscopy were minor in the majority of cases (salty taste, throat irritation, cough, dysphagia or irreversible hoarseness) and the average cost for an endoscopic examination was estimated to 40,55 RON.

## DISCUSIONS

What is essential in pulmonary TB management for initiating, monitoring and evaluation of the treatment is the earliest bacteriological confirmation possible, the "golden standard" of laboratory investigation. In negative microscopic TB, where the diagnosis is based firstly on the clinic and radiologic(5) criteria a diminished interest can be noticed concerning the indication of techniques for valid sputum samples' generation and culture. In specialized literature Anderson et al(1), proved that the usage of induced sputum and bronchoscopy in the diagnosis of patients suspect of TB, but with negative microscopic examination, contributed to the improvement of the bacteriological confirmation percentage by 12% up to 19%.

In our country, modern methods of etiological confirmation of the disease (gene tests), could not be introduced in routine practice because of the expensive costs, the complexity of the procedure and last but not least, because of the insufficient funding for health system.

The quality of the collected product (sputum) is influenced by saline aerosols generated by the nebulizer through an effect of irritation and osmosis; in the study group sputum was obtained in 89% of the patients.

Conde et al(2) obtained sputum in 97 % of their patients and Peri et al(9) obtained sputum in 73 (89%) of the patients, 26 of whom had negative AFB smear and 47 were unable to expectorate before sputum induction.

The sensitivity gained by this method for supporting the diagnosis for pulmonary TB was of 18,1%, literature data showing values ranging from 20,5%(10) to 33%(7). Lacking major adverse effects, the procedure is simple, cost effective and cand be performed in the sputum collection chamber of any stationary.

Although bronchial endoscopy is considered to be an invasive technique and many doctors refuse to use it, in the study conducted there were examined 87% of the patients, only 4 of them refusing the procedure. Diagnostic sensitivity of 14% in the group under study is similar to results published in literature(1). The adverse reactions after the bronchoscopic investigation performed with local anesthesia were minor, that is why the method was considered to be safe, low cost and applicable to routine practice on condition that all indications and contraindications are strictly followed.

## CONCLUSIONS

Bacteriological examination of sputum is essential for a reliable diagnosis of pulmonary TB; therefore all PNCT recommendations concerning sputum culture in view of obtaining the best results at the laboratory examination are mandatory and need to be taken into consideration.

Sputum induced through saline aerosols and fibronchoscopic examination along with lavage and bronchial aspirate are two efficient culture methods for increasing the percentage of bacteriological confirmations, in the diagnosis of pulmonary TB.

The procedures are considered to be safe, they can be applied in routine practice, the adverse events are usually minor and the costs are reduced.

Assistance in cases of microscopy pulmonary TB, needs a higher degree of professional perseverance in the bacteriological investigation of casuistry which often generates a dilemma whether the process of active tuberculosis exists or not.

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