THE UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA

PHD THESIS

CLINICAL, RADIOLOGICAL AND EPIDEMIOLOGICAL CONSIDERATIONS IN BACTERIOLOGICALLY UNCONFIRMED PULMONARY TUBERCULOSIS

SCIENTIFIC COORDINATOR:
Prof. Assoc. Dr. CRISTIAN DIDILESCU
Senior Scientific Researcher I

PHD STUDENT:
CONSTANTIN DUTA

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Key words: tuberculosis, radiological modifications, bacteriological confirmation, induced sputum, bronchial lavage, tuberculin skin test, people exposed.
THE GENERAL PART

Introduction

Tuberculosis continues to remain a major public health issue in Romania, the level of TB incidence at present (99,7\,000 in 2009) placing 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. Pulmonary tuberculosis (TB) is the most common way of tracking 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%.

There is still a significant percentage of new cases of TB bacteriologically unconfirmed, the ones for which the alternative of whether implementing an anti TB treatment or not is pretty controversial. Due to an absence of a bacteriological confirmation, what contributes to give a good diagnosis is the anamnesis, the clinical and radiological examination, the patient’s epidemiological and biological data.

Chapter I contains data on tuberculosis etiology, classification of mycobacteria, the disease pathogen (M. Tuberculosis) along with microscopic morphology and biochemical structures. There are also shown the development conditions according to the environmental cultures and the pathogen’s resistance to physical and chemical agents.

Chapter II describes the procedures for collection, transport and storage of pathological products, essential for the diagnosis of the tuberculosis. There are illustrated general aspects in sputum collection and particular methods such as: induced sputum, bronchial lavage, bronchial aspirate and gastric lavage, and as for extrapulmonary TB: fragments of tissue, urine and pleural effusion.

Chapter III contains information about performing sputum smears and other pathological products, followed by examination under an optical and fluorescent microscope, also presenting the causes of failure of the microscopic examination.

Chapter IV shows how to conduct the examination by culture, focusing on the preparation of the spontaneously collected sputum by other special methods such as seeding according to environmental cultures, thermostatic incubating, growth control. What is also described is the process of results interpretation and evaluation along with the causes of failure of examination by culture.
Chapter V includes antibiotic susceptibility testing of M tuberculosis. It displays general aspects, direct and indirect anti-biogram, anti-biogram in liquid environment, anti-biogram by rapid methods.

Chapter VI deals with the epidemiology of tuberculosis and the following aspects: tuberculous infection sources, pathways and mechanisms of transmission, infection and tuberculosis disease, risk factors and environmental factors, socio-economic conditions, associated diseases.

Chapter VII pictures the key indicators in assessing tuberculosis endemy (incidence, prevalence, mortality, fatality, lethality) monitoring indicators of the NTP-(National Programme for Control of TB), indicators for TB diagnosis and TB treatment outcomes evaluation.

Chapter VIII important information about tuberculosis around the world and in Romania, such as: distribution of TB in the WHO regions, multidrug resistance, characteristics of the tuberculosis endemy evolution in Romania.

Chapter IX THE SPECIAL PART
MOTIVATION FOR CHOOSING THE THEME

For most patients with pulmonary TB in our country, a diagnosis can be established by sputum examination (microscopy and culture), highlighting the etiologic agent, M. tuberculosis. There is a percentage of 20-25% of the cases recorded, without bacteriological confirmation, the diagnosis being supported, according to The National TB Control Programme, by anamnestic, clinical-radiological and epidemiological data. I considered the approach of some clinical-radiological aspects connected with negative pulmonary TB to be necessary so that the results obtained should be useful in routine practice, when having to choose the best ways to approach patients with negative sputum at the bacteriological examination.

Moreover, the purpose of this study was to evaluate the contribution of special techniques to: 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. I have also evaluated the epidemiological impact of these patients on the people exposed in the pest hole of tuberculosis.

MATERIAL AND METHOD

Two studies were conducted:

The first one is a retrospective study conducted on 108 patients living in Valcea district hospitalized between 01.01.2006 and 31.12.2008, diagnosed with bacteriologically unconfirmed pulmonary tuberculosis, (code: A160), at Pneumatology Hospital ,, Constantin Anastasatu Mihaesti-Vilcea . The second one, a prospectiv study, was conducted between 01.01.2007-
31.12.2009 on a group of 98 patients hospitalized at the same hospital, diagnosed with pulmonary TB for whom, apart from the standard methods of collecting and processing of the pathological product (sputum), there were also performed some techniques to provoke cough and sputum (aerosol process) along with sputum collection methods in bronchial endoscopic examination (lavage and bronchial aspirate), in view of assessing the contribution of these methods to increase the confirmation of the disease etiology.

**OBJECTIVES**

1. **Evaluation** of data on reported cases of bacteriologically unconfirmed tuberculosis of the Retrospective study group.
2. **Peculiarities** of the bacteriological, radiological and biological investigation, seen in patients in the two groups studied.
3. **Monitoring** and evaluation of cases according to the technical standards, evolution of the outbreak of negative tuberculosis.
4. **Assessment** of results for cough and sputum challenge techniques (sputum induced by aerosol with saline 5%), along with sputum collection techniques (lavage with bronchial aspirate through FBS examination), applied to patients in the Prospective Group.

What was recorded from the patients’ clinical observation sheets and files for epidemiological investigations was as follows: patients data, symptoms and clinical signs at admission, duration of symptoms, associated diseases, radiological pulmonary test results at admission, biological tests, the treatment administered, side effects, the clinical, radiological and bacteriological evolution under treatment, evaluation of the treatment, results of radiological examinations performed on adults exposed, tuberculin skin test values (TST) for the children exposed in the pest hole.

- **Statistical processing was done with EPI-INFO computer program**, and the value of the reliability threshold: \( p<0.05 \), was considered statistically significant.

**Chapter X  RESULTS**

Of the retrospective group of 108 patients, 29 were women (27%) and 79 men, (73%), aged around 17-76 years old, the average age was of 46.48 years for women and 50.59 years for men. In what concerns the living environment, 70 patients (64.8%), were living in the countryside and 38, (35.1%) in the city, and concerning consumption of toxics, about 93% of men and 45% of women were smokers. Symptoms presented by patients in the group studied at admission were mostly cough with or without expectoration, in 85% of cases, followed by loss of appetite and of weight, (48%), chest pain, (29%), night sweats, (28%), asthenia, (21%), haemoptysis. 70% of the study group reported an average duration of symptoms between two and four weeks until hospitalization.

The most common diseases associated with tuberculosis (comorbidities) were: chronic hepatitis, gastric and duodenal ulcer, diabetes, neuro-psychiatric diseases, renal failure and chronic
alcoholism, seen in 32% of the patients in the study group. At hospitalization, the pulmonary radiological picture was polymorphic, with infiltrative- nodular type lesions, seen in 74% of cases, caseous-cavity lesions in 23% of cases, miliaria in 3% of cases and pleurisy associated to lung changes, in 4% of the cases. Cavity type lesions were met more frequently in patients with relapse and associated diseases. The distribution of lesions showed a predominant localization in the upper lobes, (93% of the patients), the right upper lobe being affected in greater proportion- 72% than the left upper lobe- 48% and radiological changes in the lower lobes were found in 3% of patients. The number of bacteriological tests conducted at admission, was met, according to NTP, in 32% of the patients, at the microscopic examination and in 19% of the patients, at the examination by culture.

Of the patients studied, 57 (53%) were treated with nonspecific antibiotics, before antituberculous therapy, among them 43 (74%) were male and 15 (26%) were women. In terms of TB treatment, 94% of patients received standardized treatment and only 6% received an individual scheme. Adverse reactions in patients in the study group were mostly minor side effects in 28 patients (26%) and in four cases they were considered major reactions. The categories of adverse reactions recorded in order of frequency are: Liver-29%, Gastro-intestinal-25%, Skin-16%, General-14%, Neuro-psychiatric-10%, Blood-6%. A favorable clinical evolution was recorded in 86% of treated patients, a radiological favorable evolution was seen in 88% of the patients and therapeutic success was recorded in 84.5% of patients.

Epidemiological impact of patients on the exposed people in the pest hole has been evaluated according to the results of tests carried out under 67 epidemiological surveys. Pulmonary radiological aspects common to the people exposed, domiciliary or non-domiciliary, with the patients enrolled in the research group were described as nodular infiltrative-type lesions (5% of patients), disabling fibrotic (9%) and normal aspects in 86% of cases. The interpretation of skin test (TST) showed that 10 children (37%) had a response < than 9 mm, five children (18%) had an induration diameter of 10-14 mm, 9 of them, (33%) had a response ≥ than 15 mm, (hierergia) and in three cases, (12%), the reaction was negative.

The prospectiv group included 98 patients: 36 women (37%) and 62 men (63%), ages 18-69, the average age being 46,4 years, while 54 patients (55%), were living in the countryside and 44 (35,1%) in the city. 74% of men were smokers and 71% were alcohol consumers and 33% of women smoked. Symptoms presented by patients: cough with or without expectoration, in 91% of cases, followed by loss of appetite and of weight, (66%), chest pain, (32%), night sweats, (71%), asthenia, (69%), haemoptysis (14%). 71% of the study group reported an average duration of symptoms between two and four weeks until hospitalization. The most common diseases associated with tuberculosis were, like the retrospective group: chronic hepatitis, ulcer, diabetes, neuro-psychiatric diseases, renal failure, seen in 32% of the patients.
The pulmonary radiological picture was: infiltrative-nodular type lesions, seen in 79% of cases, caseous-cavity lesions in 10% of cases, miliaria in 2% of cases and pleurisy associated to lung changes, in 2% of the cases. In 93% of the patients the distribution of lesions showed a predominant localization in the upper lobe. The bacteriological examination according to the standards, was performed on all the patients in the prospective group and nonspecific antibiotic treatment was applied to 91% of 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. There were 85 patients fibrochoscopically 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).

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. 92% of patients received standardized treatment and only 8% received an individual scheme and adverse reactions, ascended after treatment were noted in the following percentages: Liver-18%, Gastro-intestinal-27%, Skin-24%, General-13%, Neuro-psychiatric-13%, 5%-Blood.

After six months of treatment 88% of patients had favorable clinical evolution, 92% radiological favorable evolution, all patients had negative sputum examination site for BK, and therapeutic success was recorded in 74.2% of patients. The results of tests carried out under 87 epidemiological surveys was: pulmonary radiological aspects with nodular infiltrative-type lesions (5% of patients), disabling fibrotic (9%) and normal aspects in 86% of cases. Tuberculin skin test (TST) showed that 14 children (36%) had a response < than 9 mm, 11 children (28,9%) had an induration diameter of 10-14 mm, 6 of them, (15%) had a response ≥ than 15 mm, (hi perergia) and in seven cases , (18%), the reaction was negative.

Chapter XI DISCUSSION

Patients with obvious clinical and radiological signs of pulmonary TB but repeated negative sputum investigations are a common clinical problem in specialty practice. In these cases, the diagnosis is usually supported in the first stage, by clinical anamnestic and radiological pulmonary findings, but it is known that these have a high sensitivity and a low specificity.
In terms of demographic data, no statistically significant differences were found between the mean age of patients in the two groups (Test Kruskal-Wallis: $p=0.25$). The link between tuberculosis and increased consumption of alcohol and tobacco is well-known, the disease appearing more frequently in people who have these vicious habits. There was a statistically significant difference ($p = 0.001$) between male patients, consumers of toxic of the two study groups (93% in the first one and 74% in the second).

The symptoms of pulmonary TB in adults are generally, cough, with or without expectoration, along with the "syndrome of tuberculous impregnation" [2] [4] which installs insidiously, progressively. In the study, the most common symptoms were cough in 85% of cases, loss of appetite with weight loss in over 60% of cases, physical fatigue and night sweats.

There were no significant differences concerning the duration of symptoms prior to admission, the period between two and four weeks being recorded most frequently (65% of cases) in both groups. Pulmonary radiological changes were infiltrative, nodular type, located in the upper lobes, the frequency of these changes being similar, with no statistically significant differences between groups ($p=0,101$). Literature showing that the favorite location of tuberculosis in the lung is in the superior lobes and the nodular-infiltrative type probably expresses the specific alveolitis, processes most frequently met in the early stages of these illnesses.

In the Prospective Group, all patients were submitted to bacteriological investigations at admission, as recommended by NTP, compared to 55% of patients in the Retrospective Group, and from this point of view the differences between the two groups were statistically significant ($p=0,001$). In negative microscopic TB a diminished interest can be noticed concerning the indication of techniques for valid sputum samples’ generation and culture. In specialized literature it was 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.

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% to 33%. Lacking major adverse effects, the procedure is simple, cost effective and can 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. 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.

Before administering anti-TB treatment, some patients in the two groups followed a series of non-specific antibiotics treatment, in view of differentiating (in the absence of bacteriological argument, the presence of AFB in sputum), according to other criteria (clinical, radiological, biological), the patients suffering from TB from the ones having other infectious diseases or other cause, who can „mimic” the disease. The evaluation was performed in 53% of patients in the Retrospective Group and in 91% of patients in the Prospective Group, a statistically significant difference ($\chi^2$: 10.49, $p=0.001$).

Anti TB treatment of patients in the two groups was administered in both hospital and ambulatory, according to NTP, being adjusted according to patient type, antibiogram results (ABG), tolerance and adverse drug reactions, contraindications of associated diseases. Generally, anti TB treatment administered to patients under observation, was well tolerated, the main types of adverse reactions reported in the patients studied were similar, only some proportion of them was different, but without statistical significance.

Until recently, negative tuberculosis was not considered as having significant contribution to the secondary transmission of disease, the confirmation of the link between microscopy negative sources and the cases supposed to have been disordered by them, is pretty controversial. Carried out by means of genetic testing, it estimated that patients with negative TB can contribute with at least 17% to the disease’s incidence in the community. Investigations conducted on adult contacts with patients of the two groups have found six patients, who were declared and treated for pulmonary tuberculosis. Strong positive reactions of IDR (induration diameter greater than 15 mm) suggesting a possible infection with M. tuberculosis and an increased risk of development of lesions, were seen in nine (33%) of the children exposed about first group, and in 15% of the children about second study group. Of the children examined, two were diagnosed with tuberculosis. Children are more susceptible to tuberculosis and the risk of a progression of the infection in a severe disease is higher.

**Chapter XII  CONCLUSIONS**

- Pulmonary tuberculosis remains a major public health issue in Romania and the current percentage of bacteriologically unconfirmed cases (20-25%) shows that the results of the microbacteriological investigation are still insufficient;
- The current level of incidence for TB in Romania, (99,7‰) 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;
• Bacteriologically unconfirmed pulmonary tuberculosis was clinically expressed by cough with or without expectoration and the tuberculous impregnation syndrome, the duration of these events being of about 2–4 weeks;

• Infiltrative nodular pulmonary lesions located at the upper lobes were the ones that prevailed;

• 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 a diminished interest can be noticed concerning the indication of techniques for valid sputum samples generation and culture;

• 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;

• 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.

• Significant differences were found between the number of non-specific antibiotic treatments applied on patients of the two groups and the treatments administered to differentiate patients with tuberculosis from those with other infectious diseases.

• The main types of adverse reactions reported after treatment did not differ only by their proportion, being more frequent in those with associated diseases (diabetes, ulcer, hepatitis, alcoholism).

• Tuberculosis treatment outcomes (the assessment) were comparable between the two groups, no statistically significant differences being found;

• There were found statistically significant differences between the number of epidemiological investigations conducted in connection with the patients of the two groups (67 in first group and 87 in the second);

• The investigations carried out during the epidemiological survey led to the record of four of the contacts of pulmonary tuberculosis patients in the Prospective Group and two contacts of patients in the Retrospective Group.

• Detection of new cases of disease on the adults involved, of M. tuberculosis infection in children from the pest hole, draws attention upon the potential source of infection of this category of patients;
• 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;
• 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.

CURRICULUM VITAE

1. First Name: CONSTANTIN
2. Name: DUTA
3. Date and place of birth: 31 October 1960, Pietrari Commune, Vâlcea District
4. Nationality: Romanian
5. Marital status: Married, 1 children
6. University and postgraduate studies:
   – 1980 -1986 The Faculty of General Medicine Craiova,
   – 1986 -1989 - junior doctor at the Sanitary Department Valcea,
   – 1990 - general medicine physician medical services Mateesti, Valcea,
       Bucharest - Pneumatology specialty
   – 1994 - Specialist in Pneumatology at the Hospital „Constantin Anastasatu” - Mihaiesti, Valcea
   – 1998 - Senior Pneumatology at the Hospital „Constantin Anastasatu" -Mihaiesti, Valcea
7. Skills: bronchology, respiratory functional exploration,
8. Member of the professional associations:
   • SRP (Romanian Society of Pneumology)
   • SRP - Respiratory Pathophysiology,
   • The Romanian Society of Bronchology,
   • ERS (European Respiratory Society)
9. Languages: English and French
10. Currently: MD, Head of Medical Department at the Hospital „Constantin Anastasatu" Mihaiesti, Valcea.