CONTRIBUTIONS TO THE STUDY OF THE CLINICAL-MORPHOLOGICAL PROFILE OF EXTRAPULMONARY TUBERCULOSIS

PhD THESIS SUMMARY

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Tuberculosis of the Female Reproductive System

Osteoarticular Tuberculosis

Tuberculosis of Soft Tissues

Tuberculosis of the Skin

CELLULARITY OF THE GRANULOMA

NECROSIS

FIBROSIS

DEGREE OF DIFFERENTIATION

EVOlUTION OF THE TUBERCULOUS PROCESS

ATYPICAL LESIONS
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GENERAL PROFILE OF AL TBCEP

LOCATION PROFILES

Lymph Node Location
Pleural Location
Location within the CAS
Digestive Location
Location within the Urinary System
Location within the AGM
Location within the AGF
Osteoarticular Location
Location within Soft Tissues
Location within the Skin

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ABBREVIATIONS

AD
AGF
AGM
Ami
AU
BK
BT
CAS
CE
CG/CGM/CGL
CH GEN
CH PED
CH TOR
CUER
C.V.
DERM
GIN
GL
HEM
HIV
I.I.
L-A-M
MED
MT
NC
OMF
OMS/WHO
ORL

Digestive Tract
Female Reproductive System
Male Reproductive System
Mycolic Acid(s)
Urinay Apparatus
Koch Bacillus
Tuberculous Bacillus(i)
Upper Respiratory Pathways
Epitheliuid Cell(s)
Giant Cell(s)/Multinucleated Giant Cell(s)/Langhans Giant Cell(s)
General Surgery Clinic
Pediatric Surgery Clinic
Thoracic Surgery Clinic
External Urinary Pathways
Variation Coefficient (%)
Dermatology Clinic
Obstetrics and Gynecology Clinic
Lymph Nodes
Hematology Clinic
Human Immunodeficiency Virus
Confidence Interval (95%)
Lipoarabinomanan
Internal Medicine Clinic
Mycobacterium tuberculosis
Caseous Necrosis
Oral and Maxillofacial Surgery Clinic
World Health Organisation
Otorhinolaringology Clinic
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>ORT</td>
<td>Orthopedic Surgery Clinic</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>PL</td>
<td>Pleura</td>
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<td>PMN</td>
<td>Polymorphonuclear Leukocytes</td>
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<td>SL</td>
<td>Sulfolipid</td>
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<td>SIDA</td>
<td>Acquired Immune Deficiency Syndrome</td>
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<td>SNC</td>
<td>Central Nervous System</td>
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<td>SOA</td>
<td>Osteoarticular System</td>
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<td>STDEV</td>
<td>Standard Deviation</td>
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<td>TBC</td>
<td>Tuberculosis</td>
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<td>TBCAD</td>
<td>Tuberculosis of the Digestive Tract</td>
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<td>TBCCAS</td>
<td>Tuberculosis of the Upper Respiratory Pathways</td>
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<td>TBCEP</td>
<td>Extrapulmonary Tuberculosis</td>
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<tr>
<td>TBCGg</td>
<td>Lymph Node Tuberculosis</td>
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<td>TBCGF</td>
<td>Tuberculosis of the Female Reproductive System</td>
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<td>TBCGM</td>
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<td>TBCOA</td>
<td>Osteoarticular Tuberculosis</td>
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<td>TBCP</td>
<td>Pulmonary Tuberculosis</td>
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<td>TEG</td>
<td>Skin</td>
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<td>TM</td>
<td>Soft Tissues</td>
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<tr>
<td>UROL</td>
<td>Urology Clinic</td>
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<tr>
<td>USA/SUA</td>
<td>United States of America</td>
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<td>VMAX</td>
<td>Maximum Age</td>
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<td>VMIN</td>
<td>Minimum Age</td>
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<td>VMEDIE</td>
<td>Mean Age</td>
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Although it seems outdated (but not for phthisiologists or pathologists) to talk about tuberculosis (or phthisis) in the third millennium, the subject I chose for my PhD Thesis is: “Contributions to the study of the clinico-morphological profile of extrapulmonary tuberculosis”.

During daily work, starting from '94-'95, I noticed an increase of cases in which the surgical samples revealed diagnoses which, in terms of pathology, were compatible with tuberculosis and we were happy: It was not cancer! With proper anti-tuberculous treatment, the patient was cured, life expectancy and subsequent reintegration were complete!

Later, questions began to arise: why so many cases with tuberculosis? Why did the pathological diagnosis of tuberculosis on surgical samples excision parts cease to be a surprise (even enjoyable) for us and for the operator clinician? Is it really tuberculosis? How to improve our relationship with the phthisiologist so that these patients diagnosed with extrapulmonary tuberculosis would receive adequate curative anti-tuberculous treatment as soon as possible?

Assessed in 1993 by the World Health Organization (WHO / WHO) as a "global emergency" [Didilescu and Marica 1998], tuberculosis reaches in Romania the highest values recorded in Europe, resulting in great economic and social implications for it affects mainly the population at the peak of the professional activity.

We must not forget to show that a World Health Organization report stated that despite expansion and relatively bleak prospects, "Tuberculosis still remains the most neglected health problem worldwide".

To delineate more clearly the problem of tuberculosis worldwide, we must emphasize the special hazard represented by the association with AIDS which raises the problem of a very negative interconditioning, as well as the multiple drug resistance which transforms this disease into an incurable one.

It should be noted that the foreground of the current system for reporting the cases brought by the WHO (1998) is represented by the cases with positive microscopic examination of sputum and classifies tuberculosis in pulmonary and extrapulmonary.

Based on these considerations, the purpose of the present study is to make a review of extrapulmonary tuberculosis cases histopathologically diagnosed during a period of 16 years (between 1990 and 2006) in the Pathology Laboratory of the Emergency County Hospital in Craiova.

Taking into account the finding that romanian literature mentions very few data concerning the morphological study of extrapulmonary tuberculosis, I decided to address this issue. Another argument also supporting my choice is that the efficiency and rapidity of the etiological diagnosis is directly linked to justified hopes for new therapeutic approaches of tuberculosis.

Thus, the present study is attempted to be a modest contribution to the understanding of this formidable disease.

Samples from necropsy were not included in this study, thus limiting the extent of the study group to surgical excision samples. The problem should not be ignored and may be subject of future research in this area.
**BACKGROUND**

Tuberculosis (TB) is a disease that has affected man ever since the first historical evidence of its existence. Although TB was assessed in 1993 by the WHO as a "global emergency", paleopathology studies showed that tuberculous disease was an important cause of death in humans at least for the past 5000 years [Wilbur and Buikstra 2006]. TB is an infectious disease which is spread through the air by droplets dispersed as nuclear aerosol containing *Mycobacterium tuberculosis* (MT), from hosts coughing or sneezing. *Mycobacterium tuberculosis* is an aerobic intracellular pathogen with binding affinity for oxygen-rich lung tissue. If not treated, each person with active TB infects 10-15 other people. The bacillus may be transmitted by inhalation, ingestion or, rarely, by direct implantation. *In utero* transplacental fetal infection is also a possibility. Worldwide, TB is a disease that is “a dynamic equilibrium between the human body and MT” [Raja 2004, Kuhn and Askin 1990, Bates and Stead 1993].

While in industrialized countries, with a better health system, it is assumed that TB is kept under control, developing countries should prepare for a disaster. In undeveloped countries, TB remains a public emergency as long as the incidence remains high, even after introduction of vaccine and drug therapy [Murray et al. 1990]. In developing countries, HIV epidemic has had a devastating effect on global TB control, especially among those aged between 20 and 35 years.

Usually, BT enters the body by inhalation. They disseminate from the initial site of pulmonary infection through blood or lymphatic circulation to other parts of the body. Gastrointestinal tract is more resistant to infection than the lung, as demonstrated both in animal studies and by rarity of tuberculous enteritis in individuals who swallow large numbers of bacilli at once together with the sputum. Transplacental infection of the fetus is rare, when the mother has miliary tuberculosis. The fetus is surprisingly resistant to infection and can remain without lesions even in severe placental involvement [Raja 2004, Kuhn and Askin 1990]. MT pathogenicity is linked to its ability to avoid the destructive effect of M and to induce delayed hypersensitivity (DTH).

Inhaled mycobacteria enters alveolar macrophages (M) after which many series of complex reactions are triggered which can be grouped into four types of evolution [Dannenberg 1994; Schluger 2005]: the complete elimination of BK (host's initial response is complete, efficient in killing all bacilli and the patient will never develop tuberculosis); immediate progression towards clinical active disease (BK multiply and grow immediately after infection, causing the clinical disease known as primary tuberculosis); sequestration of BK within a granuloma for a long time (or forever - BK can go to a dormant state without causing disease at all, so that the patient has what is called a latent infection, which is manifested only as a positive tuberculin skin test, either until reactivation of latent infection by the collapse of the granuloma – the changing of the immune status leads to latent BK development, thus giving rise to clinical disease known as reactivated tuberculosis. The later usually appears only in conditions of altered immunity).

Granuloma is the main feature of the initial host immune response to TB and many efforts were made to understand the formation and its role in immune defense against MT [Saunders and Cooper 2000, Mohan et al. 2001].

From the morphological point of view, tuberculous granuloma is an epithelioid granuloma, in terms of cell kinetics it represents a high turnover granuloma, characterized by a high rate of recruitment and the local division of M, which compensates for the diminished life and high cell death rate within the lesion and, in terms of immunological dependence of lesions, it is an immunological granuloma in which cell-mediated immunity and the delayed hypersensitivity are involved in the accumulation and differentiation of mononuclear phagocytes [Spector 1969 and 1974, Ryan and Spector 1970; Warren 1976, Williams and Williams 1983].
MT can affect any organ or tissue in the body, however, its most common location is the lung [Caminero 2003, Farge 1992, Dannenberg and Tomashefki 1988, Murray and Nadel 1988]. The classical definition of extrapulmonary tuberculosis (TBCEP) is tuberculous involvement of an organ outside the lung [Sharma and Mohan 2004]. However, when there is an obvious extrapulmonary location in a patient with pulmonary tuberculosis (TBCP), these patients are included in the group of pulmonary tuberculosis according to WHO rules [Maher et al. 1997]. Extrapulmonary involvement can occur with or without tuberculous involvement of the lung and includes: Disseminating Disease and Bacteraemia, Pleural Disease, Intrathoracic Lymphatic Disease.

TBCEP is also an important issue whose clinical importance should not be overlooked [Caminero 2003; World Health Organization 1994; Iscman 2000, Dutt and Stead 1999, Fanning 1999]. Tuberculosis in other organs than the lung has been observed for centuries but was not recognized. As patients with TBCEP rarely have positive smears, it is generally accepted that the potential contagion of this type is negligible and thus it was never a priority of national programs to control TB [Caminero 2003; World Health Organization 1994].

TBCEP incidence rate varies in different countries because it is closely correlated with the prevalence of TB in a given region [Fuentes and Caminero 2006]. In the era before the HIV pandemic, studies on immunocompetent adults had shown that TBEP represented approximately 15-20% of of TB cases [Mohan and Sharma 2001; American Thoracic Society 2000; Fanning 1999; Reported tuberculosis in the United States 2000; Medical Research Council Cardiothoracic Epidemiology Group 1992; Snider and Roper 1992; Pitchenik et al. 1988; Report from the Medical Research Council Tuberculosis and Chest Diseases Unit 1987; Weir and Thornton 1985].

The number of reported TBCEP cases has increased in both industrialized and developing countries, particularly in regions with increased HIV prevalence and in areas served by hospitals with qualified personnel and adequate material resources for the diagnosis of tuberculosis in different organs and systems [Fuentes and Caminero 2006]. In the past, TBCEP was a children’s disease which decreased with age and peaked again in third age individuals.

In the post-AIDS era, tuberculosis with multiple determinations predominates among the elderly and people with immune deficiency, particularly those infected with both HIV and M. tuberculosis [Barbu 1977; Chaissen et al. 1987; Pitchenik et al. 1988; Kim et al. 1990]. Sex ratio is much lower in cases with extrapulmonary determination than those with positive sputum smears. The explanation for this difference and its programmatic implications require investigation and further studies.

TBCEP diagnosis, especially the one located in deeper and less accessible regions is very difficult. The slightest delay in diagnosis is in pleural disease, and the greatest is in skeletal disease, probably because the tissue is less accessible and the symptoms are more subtle [Marini 1988].

Between 20% to 50% of patients with TBCEP are discovered at autopsy, unlike TBCP cases which are discovered after an autopsy in only 5% of the cases [Rieder et al. 1990; Edlin 1978; Chastonay and Gardiol 1987]. These figures may be increased in the elderly and patients with HIV.

Various forms of TBCEP have some characteristics that distinguish them as a group from TBCP: their etiology, the way MT reaches various organs or tissues, the pathogenic response, the survival possibilities of bacilli in various organs, the symptoms and performance of diagnostic methods. Therefore, a correct approach of patients suspected of having TBCEP requires a good knowledge of the differences between this entity and TBCP.
PERSONAL CONTRIBUTION

MATERIAL AND METHODS

The basis of this PhD thesis was made up of a batch of 462 patients hospitalized in the Surgical Clinics of the Emergency County Hospital of Craiova from 1990 to 2006 whose histopathological diagnosis of extrapulmonary tuberculosis was established in the Pathology and Cytology Department of the same hospital.

Selected cases were divided into ten groups according to location of tuberculous process, namely: Group I: cases with Lymph Node location; Group II: cases with Pleural location; Group III: cases with localization in the Upper Respiratory Pathways; Group IV: cases with localization in the Digestive System; Group V: cases with localization in the Urinary Tract; Group VI: cases with localization in the Male Genitalia; Group VII: cases with localization in the Female Genitalia; Group VIII: Cases with localization in the Osteoarticular System; Group IX: cases with localization in Soft Tissues; Group X: cases with localization in the Skin.

The following data sources were used for the study: medical records of patients included in the study (clinical observation charts, surgery protocols, registers of histopathological diagnosis); histopathological slides of each case; paraffin blocks and archived materials from the Archive of the Pathology Department of the Emergency County Hospital Craiova.

Our study was conducted in two main directions: a retrospective analysis that targeted hospitalized and diagnosed cases between 1990 and 2002 (cases registered in the documents of the Laboratory prior to the commencement of the study) and a prospective analysis, which included the new cases during the preparation of the thesis, between 2003 and 2006.

The studied parameters were divided into two major categories: clinical parameters (temporal distribution of cases, the department from which the biopsy material came, gender, age, location of the tuberculous inflammatory process according to apparatus or system, suspicion of etiologic diagnosis after the clinical examination) and morphological parameters (location of tuberculous inflammatory process within each apparatus/system, inflammatory granuloma cellularity, presence of necrosis, presence of fibrosis, type of granuloma depending on morphology - Degree of differentiation of granuloma -, evolution of the tuberculous process - The degree of extension -, presence of atypical granulomas). Each of these parameters were individually examined in relation to the location of the tuberculous inflammatory process.

Analysis of data was structured into two studies: a clinical study and a morphological study. For the morphological study both conventional staining techniques (HE and van Gieson) and specific immunohistochemical techniques were used. Antibodies used to identify inflammatory cell subpopulations (B lymphocytes, T lymphocytes and macrophages) were monoclonal ones and were produced by Dako. For each antibody the dilution applied was the one specified in the individual staining protocol.

In 57 cases, in which the HP diagnosis was uncertain, on one hand due to the presence of large areas of necrosis and, on the other hand, to some features of atypical granuloma, PCR was performed as a fast and accurate method to determine the presence of Mycobacteria in biological samples. The blocks were processed at Forschungszentrum Borstel, Germany.

Histopathological aspects were selected using an Olympus CX31 microscope using the eyepiece with magnification of 4. To purchase images we used optical corrected planapocromate objectives with magnification of X4, X10, X20 and X40.

The most significant images were taken with an Olympus DP12 digital camcorder and were recorded directly into a computer using the analyzeSIS Pro software and further analyzed using the analyzeSIS Pro software and the FotoCanvas Lite v1.1 module in the ACDSee 4.0 software pack.
The data were processed using the Microsoft Excel module of the Microsoft Office 2003 Professional software package.

For some parameters, be they clinical or morphological, the need for an accurate assessment of the tuberculous inflammatory process required the development of criteria for allocation of cases which generated scales for stratification of cases according to each criterion.

For numerical parameters the following statistical indicators were calculated: the lowest value (VMIN); The highest value (Vmax); mean value (VMEDIE); standard deviation (STDEV); coefficient of variation (CV - %); confidence interval (II - 95%).

**CLINICAL STUDY**

Shaping a true and accurate situation of extrapulmonary forms of TB is difficult because detection of patients with extrapulmonary lesions is influenced by a multitude of factors acting divergently. Therefore, the literature shows a great variability of data on locations of extrapulmonary TB.

Although there are countless TBCEP related references, systematic studies dedicated to pathogenic parameters and/or especially epidemiological ones, clinical and morphological, evaluating a palette of the most comprehensive ones and taking into account patient groups large enough to have statistical validity, are very few. Also, there are few studies that establish a dynamic time analysis of the development of different descriptive parameters of the involvement of extrapulmonary tissue structures by the tuberculous process.

The overwhelming majority of samples were obtained in the surgical clinics of our hospital (including the Dermatology Clinic), particularly in General Surgery clinics which cover lesions in several organs and systems. Some tissue fragments came only from the department addressing that particular structures within a certain apparatus or system. However, none of the cases with TB of the skin were biopsied, as it possibly would have been expected, in the Plastic Surgery Clinic, and half of the biopsies of female genital structures came from a General Surgery clinic (Table 1).

**Table 1: Distribution according to the origin of the surgical/biopsy sample**

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<td>17</td>
</tr>
<tr>
<td>LEZ</td>
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<td></td>
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<td>1</td>
<td>3</td>
<td>4</td>
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<td></td>
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<td></td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
<td>27</td>
<td>9</td>
<td>104</td>
<td>17</td>
<td>80</td>
<td>79</td>
<td>33</td>
<td>27</td>
<td>3</td>
<td>73</td>
<td>6</td>
<td></td>
<td>4</td>
<td></td>
<td>462</td>
</tr>
</tbody>
</table>

**Total** | 27 | 9 | 104 | 17 | 80 | 79 | 33 | 27 | 3 | 73 | 6 | 4 | 462 |
The statistical weight of the various extrapulmonary TB lesions in the geographical area covered by our hospital unit (Dolj county) falls within the values found in comparable studies in terms of number of cases, geographical and temporal coverage, but represent only a small proportion of cases recorded in the same period in Dolj county, stressing however the importance of large medical units with diversified and modern investigative possibilities in detecting TBCEP.

The time-related analysis of the tuberculous process revealed, starting from 1997, a reduction in the number of cases detected incidentally in hospital, or requiring surgery for therapeutic purposes, for the benefit of organized screening in stages of disease that allow only medical intervention therapy.

In terms of location, the lymph node was the structure with the most frequent microscopic identification of tuberculous inflammation, half of the cases included in this study showing such impaired tissue structures. This was followed at a distance, with a percentage between 9% and 10%, by male genital organs and then the digestive system. A damage higher than 5% was also encountered in the components of the upper airways, organs of the urinary system, structures within the osteoarticular system and soft tissues. The comparison of our data with similar studies in the literature was quite difficult because the criteria for including cases in the study groups were often different from one study to another (Table 2).

Table 2: Comparison of statistical weight of TBCEP location according to type of tissue/apparatus

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tissue/Apparatus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Lymph nodes</td>
<td>50,6</td>
<td>52,9</td>
<td>36,5</td>
<td>18,2</td>
<td>20,2</td>
<td>39,8</td>
<td>43,2</td>
<td>42,5</td>
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<td>Male reproductive system</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary system</td>
<td>7,1</td>
<td>14,2</td>
<td>4,5</td>
<td>4,5</td>
<td>18,2</td>
<td>16,9</td>
<td>5,0</td>
<td>4,4</td>
</tr>
<tr>
<td>Female reproductive system</td>
<td>2,6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digestive tract</td>
<td>9,3</td>
<td>6,2*</td>
<td>3,5</td>
<td>7,4</td>
<td>2*</td>
<td>6,1**</td>
<td>6,3**</td>
<td></td>
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<tr>
<td>Upper airways</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteoarticular system</td>
<td>6,3</td>
<td>11</td>
<td>13,1</td>
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<td>8,1</td>
<td>10,9</td>
<td>10,4</td>
<td></td>
</tr>
<tr>
<td>Soft tissues</td>
<td>6</td>
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<td></td>
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<td></td>
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<tr>
<td>Skin</td>
<td>2,4</td>
<td>3,4</td>
<td>2</td>
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<td></td>
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<tr>
<td>Pleura</td>
<td>1,7</td>
<td>41,2</td>
<td>35,6</td>
<td>33,8</td>
<td>18,5</td>
<td>18,5</td>
<td>16,6</td>
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<tr>
<td>SNC</td>
<td>-</td>
<td>3,2</td>
<td>2,4</td>
<td>2,3</td>
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<tr>
<td>Others</td>
<td>-</td>
<td>12,5</td>
<td>15</td>
<td>5,8***</td>
<td>4,8</td>
<td>10,3</td>
<td>11</td>
<td>13,4</td>
</tr>
<tr>
<td><strong>Total number of cases</strong></td>
<td>462</td>
<td>499</td>
<td>1283</td>
<td>312</td>
<td>622</td>
<td>5675</td>
<td>3029</td>
<td>2697</td>
</tr>
</tbody>
</table>

* Abdomen; ** Peritoneum only; *** Including Miliary TBC

Thus, in our study, there were few cases with pleural location because they are usually detected in medical clinics and directed to specialized pneumology clinics, the cases with CNS lesions were not included in the study, cases with reproductive and urinary involvement were assessed separately and not combined as in most studies and, within the digestive system both its segments and peritoneum were included.

There was a slight male predominance in terms of inflammatory process due to extrapulmonary tuberculosis, which was demonstrated by the the ratio between the number of cases of each of the two genders which was just over "1". This is similar to most studies in the literature taken for comparison (Table 3).
Table 3: Comparison with the literature

<table>
<thead>
<tr>
<th>Study</th>
<th>Interval</th>
<th>Total</th>
<th>M</th>
<th>F</th>
<th>M/F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Craiova</td>
<td>1990 - 2006</td>
<td>462</td>
<td>52.8 (244)</td>
<td>47.2 (218)</td>
<td>1.1</td>
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<tr>
<td>Yoon et al. 2004</td>
<td>1997 - 1999</td>
<td>312</td>
<td>149</td>
<td>163</td>
<td>0.91</td>
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<tr>
<td>Ong et al. 2004</td>
<td>1991 - 2000</td>
<td>301</td>
<td>51.5 (155)</td>
<td>48.5 (146)</td>
<td>1.06</td>
</tr>
<tr>
<td>Pheme et al. 2005</td>
<td>1991 – 2000</td>
<td>622</td>
<td>54 (335)</td>
<td>46 (287)</td>
<td>1.16</td>
</tr>
<tr>
<td>Chan-Yeung et al. 2002</td>
<td>1996</td>
<td>1283</td>
<td>56 (719)</td>
<td>44 (564)</td>
<td>1.27</td>
</tr>
<tr>
<td>Forssbohm et al. 2008</td>
<td>1996-2000</td>
<td>5675</td>
<td>48.2 (2739)</td>
<td>51.8 (2936)</td>
<td>0.93</td>
</tr>
<tr>
<td>Kipp et al. 2008</td>
<td>1993 - 2006</td>
<td>1366</td>
<td>53.7 (733)</td>
<td>46.3 (633)</td>
<td>1.15</td>
</tr>
</tbody>
</table>

Extrapulmonary tuberculosis inflammatory processes were encountered from the earliest age to very advanced ages. Mean age was still quite high, almost 40 years, because the most affected periods were those of young and mature adult but there was a significant impairment in young and very young ages, which meant that almost 60% of cases were encountered by the age of 44 (Chart 1).

<table>
<thead>
<tr>
<th>Study</th>
<th>Age Group</th>
<th>Child</th>
<th>Adolescent</th>
<th>Young Adult</th>
<th>Mature Adult</th>
<th>Elderly</th>
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<tr>
<td>CRAIOVA</td>
<td>60</td>
<td>72</td>
<td>132</td>
<td>130</td>
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<tr>
<td>ONG*</td>
<td>27</td>
<td>127</td>
<td>84</td>
<td>63</td>
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<tr>
<td>KIPP</td>
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<td>112</td>
<td>459</td>
<td>334</td>
<td>399</td>
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<tr>
<td>FORSSBOHM</td>
<td>373</td>
<td>486</td>
<td>1684</td>
<td>1446</td>
<td>1686</td>
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</tr>
</tbody>
</table>

Chart 1: Comparison of statistical weight of age groups

Differences in the impairment of different age groups and periods between our study and the studies of Forssbohm et al. and Kipp et al. could be due, on one hand, to the increased life expectancy in industrialized developed countries leading to the increase of the percentage of elderly individuals within the population, which become with increasing age a more susceptible target for TBCEP infection and, on the other hand, to poorer socio-economical conditions and an underdeveloped system of specific and specialized medical care seen in our country, leading to impairment of young and very young ages.

The statistical weight of suspected TB diagnosis did not exceed 30%, except skin lesions. Within the entire group, the value was 17.5% (Chart 2).
Chart 2: Statistical weight of suspected TBC EP diagnosis in different locations

The highest values for percentage of suspicion were recorded for digestive lesions (27.9%) and osteoarticular lesions (27.6%). One might conclude that the degree of suspicion has been influenced to some extent by the availability of direct physical examination of the lesions, but the assumption is somewhat contradicted by the small percentage of diagnostic suspicion of lymph node lesions, of which a very significant percentage were located within the lymph nodes accessible to physical examination. In cases in which the bacillary etiology was not suspected, the diagnosis that accompanied the surgical sample sent for histopathological examination varied widely, from suspected inflammatory process to suspected cancer.

MORPHOLOGICAL STUDY

Cellularity of Granuloma. The main types of cells within the tuberculous granuloma are: potentially phagocytic cells and immune cells. Except for skin and urinary tract, granulomas with giant Langhans cells (GLC) together with early epithelioid cell (EC) granulomas dominated the morphology of the majority of cases - 75% and over 75% of cases - in all locations, betraying, as shown before, an ongoing conflict between MT and inflammatory and immune defense systems of patients (Chart 3).
Chart 3: Statistical weight of types of granulomas according to type of effector cells

Figure 1: E and CGL Granulomas showing subcapsular confluence within a lymph node, with lymph-cells distributed mainly at the periphery a) HE stain; b) CD 68 + within the granuloma; c) weak CD 45 + within the granuloma; d) CD20 – within the granuloma and CD20 + in its periphery
Also, except in the upper airways location, the percentage of patients with early lesions did not exceed 14% in either location. Finally, with the above mentioned exceptions (AGF and CAS), together with the SOA group, lesions with PMNs present were encountered in over 10% of cases in each group, which could mean a reduced defense capacity in these patients.

**Necrosis.** Cases in which we did not identify the presence of necrosis were very few (10% of cases), over half of them being part of the group with lymph node lesions. Generally, the most common morphological appearance of the necrotic debris was the classic one, microgranular acidophilic/eosinophilic. Early necrosis was frequently seen in CAS and skin lesions and was absent in pleural lesions and those of the AU (Chart 4).

<table>
<thead>
<tr>
<th>Apparatus</th>
<th>Acidophilic Necrosis</th>
<th>Eosinophilic Necrosis</th>
<th>Basophilic Necrosis</th>
<th>Caseous Necrosis</th>
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<tr>
<td>AU</td>
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<td>GL</td>
<td>12</td>
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<td>39</td>
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<tr>
<td>TM</td>
<td>12</td>
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<td>7</td>
<td>2</td>
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<tr>
<td>AGF</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>0</td>
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<tr>
<td>TEG</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>AD</td>
<td>15</td>
<td>6</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>AGM</td>
<td>19</td>
<td>4</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>PL</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CAS</td>
<td>25</td>
<td>9</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

*Chart 4: Statistical weight of necrosis types within lesions of each apparatus/system*

Acidophilic necrosis dominated pleural lesions and then those of the female genitalia, osteo-articular lesions and male genital lesions. Basophilic necrosis was present in a significant number of cases, within female genital lesions and lymph nodes. Non-caseous necrosis was absent from the lesions of the CAS and the AGF, but instead, it was present in over 1/3 of cases with lesions of the skin and lesions and the AU.
Figure 2: Morphological types of Caseous Necrosis: (a) Classical appearance; (b) Fine granular Eosinophilic N.; (c) Macrogranular Basophilic N.; (d) Basophilic necrosis (left) and Eosinophilic N. (right); (e) Non-caseous N.

Fibrosis. As indirect indicator for the degree of aggressiveness of tuberculous infection, fibrosis was seen in almost one quarter of patients (Chart 5).

Chart 5: Statistical weight of Fibrosis within different locations of TBCEP
Degree of differentiation. We applied a granulomatous lesions classification system according to their organization, proposed but only for lymph node lesions by Ramanathan et al. in 1999 in order to determine the degree of intervention efficiency of the inflammatory cell complex.

Well-differentiated granulomas include hyperplastic granulomas (Ia and Ib) and reactive granulomas (II) and were found in almost two thirds of the cases. Profiles of the statistical weights for the five types of granuloma defined by the degree of organization for each location varied widely (Chart 6).

After grouping locations according to the presence of morphological or functional similarities I noticed that pleural location represented a separate group, with reactivation of old stabilized tuberculosis foci, which is supported by the predominance of the classical type of granuloma accompanied by fibrosis.

Then, the morphological profile of lymph node lesions covered the entire range of tuberculous granuloma ranging from hyperplasia to non-reactive (IV) lesions, with a high degree of severity, supported by the higher proportion of reactive and hiporeactive granulomas (III).

In the CAS and AD lesions, both entrance gates for MT in the body, and having a common segment (oropharyngeal), the former showed predominantly early lesions and the later advanced, aggressive lesions, prone to enlargement.

Injuries of the uro-genital complex were usually (more than 70% of cases in each of the systems) of extensive and destructive type, with a specarding the urinary tract where over one third of injuries showed secondary bacterial infection.

Tissues responsible for support/protection had lesions that covered the whole spectrum of morphological aspects of granulomas. Overall, the destructive nature of granulomas is significantly reduced starting from osteoarticular structures to the skin. However, in the case of the skin there is a somewhat polarized situation: on one hand we see early lesions, and on the other complicated lesions, with secondary bacterial infection.
Analysis of morphological profiles of granulomatous lesions also taking into account their organization could lead to the assumption that some tissues, such as those of AU and lymph node tissue would have a greater susceptibility to aggressive, extensive and destructive forms of TB infection as indicated by the presence of non-reactive and hiporeactive granulomas. Meanwhile, there are tissues such as the ones of the AGM, pleura and CAS, in which the granulomatous reaction is usually of well differentiated type. This may mean that these tissues have a better local responsiveness to bacillary aggression.

**Evolution of the TB process.** Any tuberculous process, irrespective of the hosting tissue structure, goes through several stages, which can have multiple pathways of evolution.

*Figure 3: Synopsis of cellular changes in TBC granuloma TBC*

Red Arrows – Foamy cells; Blue Arrows – Apoptotic cells; Orange Arrows – Early necrosis; Yellow arrows – Perilesional fibrosis

*Figure 4: Evolution of caseous necrosis: (a) Granuloma with CE, some foamy and some apoptotic; Early necrosis (b, c), Organised necrosis (d) and Extensive necrosis (e)*
Thus, tuberculous lesions may evolve to stabilization-recovery, may move towards stabilization, dormant state, reactivation and recurrence and finally, they can show an extensive development which in turn has two variants, namely local extension - step by step, and distant extension.

Usually, granulomatous inflammation was located within one segment of the apparatus/system that was analyzed. However, in nearly one fifth of the cases the lesions have spread to many segments of the apparatus/system or even beyond, with four of the locations studied, namely AGF, AGM, skin and pleura, being locations where at least one quarter of the cases showed extensive damage to more than one constituent segment (Chart 7).

There were a series of particular forms of local extensions, depending on the hosting apparatus/system of tuberculous lesion. Such a form of local extension was the formation of fistulous channels, present in 8 of the 264 cases studied, 7 of them reaching and opening outside the skin and the 8th being a cecal-epiploic fistula.
Another particular form was perforation, seen in three cases with AD lesions. Finally, another form was extension within the fatty tissue around the parenchymatous organ, a situation encountered in one case where the initial lesions were within the renal parenchyma.

**Atypical lesions.** Just over 10% of the studied cases had peculiar lesional features (extended and/or non-caseous necrosis, abnormal cellular architecture and polymorphism) for which histopathological examination was insufficient for elucidating the etiologic diagnosis. They were elucidated by PCR technique which revealed mycobacterial DNA in all cases. In two thirds of them, the materials that were examined were tissue fragments with morphologically atypical granulomas, the remaining third being tissues with mostly necrotic debris. Most atypical lesions (over one quarter of cases) were found in the skin and soft tissues (Chart 8).

![Chart 8: Statistical weight of atypical lesions within each apparatus/system](chart8)

Also, the results from the PCR investigation (the absence of mycobacterial DNA) represented an exclusion criterion for a group of 15 patients who had either large foci of liquefaction necrosis or nonspecific atypical granulomatous reactions, who were not included in the final group.
CLINICO-MORPHOLOGICAL PROFILE OF TBCEP

General Profile of TBCEP

Clinical features. Extrapulmonary impairment had an oscillating evolution with a general downward trend of number of cases, which remained, after the 1996 peak incidence, under 30 cases per year.

It is interesting to mention that the material sent for histopathological examination came, in the vast majority of cases, from clinics with surgical profile, especially those to which patients usually address for changes in superficial lymph node groups, accessible to clinical examination.

To these, the Department of Urology was added which, by covering the pathology of the urinary and male genital apparatus, has admitted the largest number of tuberculosis patients with extrapulmonary location other than the lymph node.

Bacillary extrapulmonary processes affected men and women almost equally, with a slight propensity in men, and were seen at all ages, but with an obvious concentration in the young and mature adults.

Suspected etiologic diagnosis on clinical examination was very reduced, considering that many of the lesions, mostly located in the lymph node groups were superficial and accessible to clinical examination. If we do not take into account the cases with lymph node location in which the diagnosis of suspicion was a very general one, being labelled as “Adenopathy/Poliadenopathy”, clinical examination focused the diagnosis mostly towards the suspicion of tumoral proliferation, either benign or malignant, and only then towards an inflammatory reaction.

Morphological features. Overall, the efector cellular inflammatory complex was dominated by specialized cells in both pathogen phagocytosis and processing of antigen, but the PMN component should also not be neglected; this component having a dual meaning in terms of diagnosis namely, one on one hand, signalling the deficit of defense reactions against bacillary aggression, and on the other hand, the changing of the morphological appearance of inflammatory process that can lead to errors in suspicioning the cause that led to the injuries.

The almost constant presence of irreversible tissue deterioration and especially the significant proportion of the macrogranular, basophilic non-strutural aspects of necrosis areas highlight the aggressive nature of BK strains involved but, as I outlined above, all this within an overall low reactivity of patients.

Cumulating the evaluation of the spectra of effector cells present within the inflammatory nucleus, with the assessment of the extent and appearance of tissue destructions, it seems that the overall appearance of granulomatos lesions (also called the degree of differentiation), with a significant share occupied by the non-reactive and hiporeactive morphological type, argues for the existence of an increased severity of bacillary aggression in patients included in the study.

The absence of the fibrillogenic isolation process of the conflict areas and the limiting of their expansion in most cases is another argument which emphasizes the gravity of extrapulmonary involvement in the study group.

The severity of lesions in the studied group is strengthened by the extension to more than one component segment of an apparatus/system in a significant percentage of cases.

Last but not least, there is a number of cases that can not be neglected where both composition and cellular architecture of the cellular complex, and also the appearance of tissue destructions, have deviated from the classical morphological pattern, requiring further investigation to specify the etiology, supporting the severity of the conflict between the pathogen and the hosting tissues.
**Location Profile**

**Lymph Node Location**

**Clinical features.** In the case of the lymph node location, which was the most frequent, the incidence of tuberculous process showed a decreasing trend starting from 1998, with a comeback after 2004, but below the values seen in the beginning of the time period.

Overall, the biopsy material came from the clinics in which clinical examination could identify the presence of superficially located lymph nodes and the suspected etiologic diagnosis on clinical examination was very low, below the average for the entire group, paradoxically, when most lesions that were examined were accessible to clinical examination and when there was a larger share of the group of lesions within the studied group. Women were more frequently affected at all ages, but especially at young ages.

**Morphological features.** Bacillary aggression was stationed in the vast majority of cases within a single lymph node group, usually superficial, but the deep locations of the cephalic extremity should not be neglected.

Granulomatous reaction was, in half the cases, of poorly differentiated or disorganized type, with predominance of CE and CGL but also PMN as well as a more basophilic noncaseous necrosis, with reduced perifocal collagenous fibrillary reaction, but with very few cases showing a difficult bacillary diagnosis.

**Pleural Location**

**Clinical features.** In the case of the pleural location, which was the least frequent one, cases were registered during the last period of the study and came only from the clinic specialized in the surgical approach of the pleura, with a significant suspicion diagnosis, above the average for the whole group, perhaps because of symptoms that led rather to an infectious inflammatory process. Almost all patients were adult males, with lesions most commonly located on the right side and extended along the whole pleural cavity.

**Morphological features.** Granulomatous reaction was only of well differentiated type but with necrosis, mostly acidophilic, and fibrosis present in all cases, and only one case in which bacterial overgrowth, translated by non-structural necrosis, masked the tuberculous inflammation.

**Location within the CAS**

**Clinical features.** Within the upper airways, the incidence of tubercular process had an oscillating evolution with a downward trend since 1995, patients were mostly adult men, with an average age around 44 years and admitted only in the ORL clinic. Suspicion of bacillary etiology was low, the clinical appearance usually suggesting a neoplastic proliferation.

**Morphological features.** Granulomatous reaction was identified, with equal proportions, only within the pharynx and larynx. It revealed virtually only a well differentiated pattern, but at first, which is emphasized by the highest percentage of lesions with epithelioid granulomas, it also revealed the presence of necrosis also in early stages and the absence of fibrosis.

** Digestive Location**

**Clinical features.** The incidence of tuberculous process in the segments of the digestive tract also revealed a highly oscillating evolution, with an overall decreasing trend, but a comeback by the end of the interval, with patients, especially young men, usually being admitted in general surgery clinics for adults and children.

One of the highest rates of suspicion of bacillary etiology was registered – more than 25% of cases – the clinical examination suggesting a neoplastic proliferation in more than half of non-tuberculous cases, or an inflammatory processes.
**Morphological features.** Most lesions were located within subdiaphragmatic segments of the digestive tract; the colon is more frequently affected than the ileum, with salivary glands being the most frequent involvement of all glands within the digestive tract.

The tuberculous process showed some degree of expansion in more than one segment of the digestive tract, but also with atypical types of extension - perforation and fistula - signs of aggressive destructive lesions. Granulomatous reaction displayed the whole range of morphological aspects but with a significant proportion of hiporeactive and disorganized granulomas, with a CE and CGL dominated cellularity, but also occasional PMNs, and mostly acidophilic necrosis. Collagenous fibrillary reaction was also reported. There were rare atypical lesions, mostly atypical aspects of the granulomatous cell complex.

**Location within the Urinary System**

**Clinical features.** The incidence of tuberculosis within the component segments of the AU showed a downward trend until the complete absence of cases in 2002. Patients, men and women equally, usually adults and older adults, were mostly hospitalized in the Urology Clinic and the etiologic diagnosis was rarely suspected, symptoms usually suggesting an urinary obstruction or tumoral proliferation.

**Morphological features.** The inflammatory process most frequently affected the renal parenchyma, the lesions had mostly intraparenchimatous location, with a significant proportion being placed simultaneously in the parenchyma and the intrarenal urinary pathways, without any predilection for one kidney.

The presence of hydronephrosis suggested old stenosed scar lesions in the extrarenal urinary pathways, and thus, that the current process is a reactivation generally dominated by destructive lesions, mostly extending from the kidney to the intrarenal urinary pathways but sometimes, also to the extrarenal urinary pathways, especially the homolateral ureter.

Granulomatous reaction was, in more than half of the cases, of hiporeactive or disorganized type, with a notable presence of PMNs within the inflammatory complex, with destructive lesions, often with unfavourable-looking aspect, basophilia or non-structural non-caseos necrosis and quite frequent fibrosis usually representing scar sequelae of old injuries.

Atypical aspects were rarely encountered and were mostly large areas of necrosis with an important non-caseos component.

**Location within the AGM**

**Clinical features.** Tuberculous lesions in the AGM segments showed an oscillating evolution with a decreasing trend since 1996, but with a comeback during the last part of the study. Patients were mostly hospitalized in the Urology Clinic and were usually adults and older adults, with the average age (around 55 years) reaching the highest value in the entire group.

The suspicion of the etiology on clinical examination had the lowest rate of the entire batch, suggesting rather a inflammatory process or a neoplastic proliferation.

**Morphological features.** The epididymis was the most affected segment but often, the lesions were stationed at the same time within the testis, with the prostate being an isolated location in most cases.

Granulomatous reaction was generally of well differentiated type, with CE and CGL and necrosis but also with early or disorganized hyperplastic aspects, with neutrophils present in a significant number of cases. Fibrosis, present in one quarter of cases, was the hallmark of an effective reaction of defense mechanisms. However, the male reproductive system was one of the few systems in which atypical lesions, mostly atypical granulomatous complexes, reached the significant threshold of 20%.

**Location within the AGF**

**Clinical features.** The involvement of AGF segments was rarely seen in the first part of the time interval considered and disappeared in its second half.
Somewhat paradoxically, half of the patients, usually young women aged around 30 years, were hospitalized in the general surgery clinic.

There was a significant suspicion rate and the clinical examination at hospitalization, being most often suggestive of neoplastic proliferation.

**Morphological features.** The only segments that were affected were the fallopian tubes and the ovary, with quite a few bilateral involvements.

Granulomatous reaction was either well differentiated, with CE and CGL and acidophilic necrosis or hyporeactive, with basophilic necrosis. Atypical lesions were not found in this site and fibrosis was rarely encountered.

**Osteoarticular Location**

**Clinical features.** The incidence of osteoarticular tuberculosis also showed an oscillating evolution with multiannual intervals of presence but also of absence, with an overall decreasing trend but with a dramatic resurgence, with the highest number of cases per year during the last year of the studied period.

In most cases the patients were adult and elderly males, and naturally, they were hospitalized in clinics addressing the osteoarticular pathology. Suspected etiologic diagnosis on clinical examination was significant (over one quarter of the cases) with symptoms also suggesting either chronic inflammatory processes or tumors.

**Morphological features.** Tubercular inflammation was located most commonly within the long bones of the extremities but also the epiphysial and diaphyseal-epiphysial segments, followed by the joints, particularly the knee joint and the hand, with quite a lot of cases with simultaneous involvement of joint and adjacent bone.

Granulomatous reaction displayed all important morphological aspects, mostly the well differentiated type, but also the early hyperplastic one and the hyporeactive one alike. The cellular component was dominated by the CE and CGL but there were also cases with predominance of PMNs. Atypical lesions were rare. This location was characterized by the highest percentage of cases in which necrosis was absent, betraying the early lesions, but there were also quite a few cases with basophilic necrosis. Also, collagenous fibrillary reaction was often present.

**Location within Soft Tissues**

**Clinical features.** Tuberculous lesions showed an oscillating evolution with an increasing general trend. The location of lesions, mostly in the trunk (thoracic region), is responsible for the patients, mostly adult men, being hospitalized in surgical clinics, in particular general surgery and thoracic surgery clinics. The suspicion of the etiologic diagnosis on clinical examination was relatively high compared to other sites, with symptoms suggesting either an acute inflammation or a tumoral proliferation.

**Morphological features.** The granulomatous reaction, generally of localized type, usually presented either a poorly differentiated pattern with necrosis, basophilia, or a disorganized one, with PMNs dominating the cell complex and non-structural necrosis. Atypical lesions showed the highest incidence, being, most of the times, changes in granuloma morphology. However, the perilesional fibrillogenetic process was observed in one third of the cases.

**Location within the Skin**

**Clinical features.** Tuberculous lesions was rare, with oscillating annual evolution and a general linear trend. Patients, mostly adult and older females, were hospitalized in the dermatology clinic but also in the general surgery clinic, over half of cases raising suspicion of tuberculosis, the rest of the cases having symptoms suggestive of an acute bacterial infection.

**Morphological features.** Lesions were usually located within the cephalic extremity and chest wall, showing quite often local extension as a particular form of fistula.
Granulomatous response revealed polarized aspects: the most frequent one was the well differentiated type, even with early necrosis, but also the disorganized appearance, with cellular component dominated by PMNs and non-structural necrosis. In many cases showing the latter morphological picture, atypy was present, requiring further investigations in order to clarify the etiology.

**CONCLUSIONS**

After analyzing the results from both studies, some conclusions have emerged that may have relevance and applicability in the future approach of extrapulmonary locations of tuberculosis in medical practice:

1. The extrapulmonary location of tuberculosis has been a difficult diagnostic problem, as attested by the wide range of terms, from syndrome to the most variate forms of cancer, and of diagnoses accompanying the surgical resection samples. The low concordance index for etiological diagnosis in the cases that were studied requires a review of the clinician’s attitude, especially the surgeon’s one, with orientation to specific investigation of any suspicion of tuberculosis, regardless of the mask that it would hide under.

2. In the case of extrapulmonary TB, it is the histopathological examination that diagnoses or raises the suspicion of tuberculosis, triggering the whole chain of further exploration designed to confirm or refute the diagnosis, with immunohistochemical assay and especially DNA or RNA amplification being by far the most important ones, decisive in the case of atypical histopathological aspects.

3. The lack of consistent data in the literature made it difficult to report and compare our results, none of the larger studies identified covering the full panel of clinical evaluation parameters that we have established in our study or attempting a parameter analysis the way we did.

4. Overall, extrapulmonary sites proved to be difficult to diagnose clinically. They were the site of either early lesions that have betrayed the reactivation of old either pulmonary or local lesions, or also active lesions, destructive, not infrequently with local extension within the affected apparatus or system, and atypical morphological aspects that betrayed an altered, low or inefficient reaction of the host.

5. Individual profiles, within the apparatus or tissue system, exhibited a great variability both of the clinical component (especially the relationship with the gender and age of the patients but also the relationship between location and tuberculous and its clinical expression) and in terms of morphologic component, the main indicator of the status of the conflict between the agent and each type of apparatus or system.

6. This paper, which we can say it is the first of its kind in our scientific literature and among the very few in the foreign literature in terms of the complexity of approach of the clinical and morphological profile of the conflict between BK and extrapulmonary tissues, may be a reference point but also a starting point in addressing the location of extrapulmonary tuberculosis in a clinical and morphological portray of the disease, because of the particular image conferred by the origin of the cases included in the study, that is the availability of full investigation offered by a modern hospital even if the study group was not a quantitatively significant one in the context of the phenomenon being recorded in our geographical area.

7. It is essential for physicians and clinicians to know and refresh their knowledge about the various manifestations of TB sites, so they can recognize and diagnose this curable disease before performing definitive surgery in order to protect patients from
inappropriate application of therapy. Thus, surgery should be limited to the diagnosis or treatment of life-threatening complications.

8. The severe nature of extrapulmonary tuberculosis which is due on one hand to the frequent late diagnosis (extrapulmonary location is not usually suspected as the detection of specific inflammation in inaccessible areas of the body is difficult) and, on the other hand, to the complicated evolution of the disease, leads to a compulsory multidisciplinary approach of the patient with a team in which, along with the clinician and microbiologist, the pathologist should always be present.


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