UNIVERSITY OF MEDICINE AND PHARMACY
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PhD

THESIS

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

ABSTRACT

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TABLE OF CONTENTS

KEYWORDS

Extrapulmonary tuberculosis, osteoarticular tuberculosis, bone, joint

STAGE OF KNOWLEDGE

Tuberculosis (TB), endemic in yearsmals from the Paleolithic, long before affecting humans, still remains a major universal concern and health problem worldwide at the beginning of the XXIst century, being the second most frequent infectious disease after malaria and the most common cause of death due to an infectious disease worldwide, despite the remarkable progresses realized in the last decades concerning the screening, monitoring and therapeutic strategies [Steele și Ranney 1958; Morris et al. 2002; Mousa 2007; Trecarichi et al. 2012; Tseng et al. 2014; Cakir et al. 2014].

Extrapulmonary TB (EPTB), defined as any bacteriologically confirmed or clinically diagnosed case of TB involving organs or anatomical sites other than the lungs (pleura, lymph nodes, gastrointestinal tract, genitourinary tract, skin, osteoarticular system, or meninges), and more common in Asian and African countries follows the same trend as TB in general, with a “revival” in the last decades in developed countries like western European countries and USA, from 7.6 % of all TB cases in the 1960s to 20–40 % in recent studies and especially also among ethnic minorities, women, blacks, and HIV-positive TB patients [Jutte et al. 2004; Yang et al. 2004; Peto et al. 2009; Gunal et al. 2011; WHO 2015; ECDC 2017].

Osteoarticular tuberculosis (OATB) defines any inflammatory process determined by Mycobacterium tuberculosis (Mt) localized in bones, joins or both structures.

Tuberculosis (TB) still represents one of the major causes of skeletal infection in many parts of the world [Vallejo et al. 1995; Raviglione et al. 1995].

OATB is a rare form of TB. The limits of the incidence variation range among all TB cases are 1-6% [Petersdorf et al 1983; Al Saleh et al. 1988; Garrido et al. 1988; Dyearsel et al. 1994; González-Gay et al. 1999; Morris et al. 2002; Ruiz et al. 2003; Malaviya și Kotwal 2003; Yoon et al. 2004; Jutte et al. 2004; Shah și Splain 2005; Sandher et al. 2007; Hershkovitz et al. 2008; Vanhoenacker et al. 2009; Hong et al. 2010; Ali et al. 2012; Houston și Macallan 2014; Chen și Chen 2014].

The risk factors can be grouped depending on their area of intervention in three categories: general factors, factors encountered mostly in developed countries/areas and factors encountered mostly in underdeveloped countries/areas.

Data in the literature concerning the concerning the relationship of TB osteoarticular involvement and the patients’ gender are controversial.

Bone and joint TB is encountered in any age group [Wilcox și Laufer 1994; Vallejo et al. 1995].

The initial TB lesion is either primary or reactivated dormant visceral focus of infection.

The pathways through which TB spreads from the initial outbreak to osteoarticular structures are, in order of frequency, the following [Muradali et al. 1993; Heycock și Noble 1961; Wright et al. 1996; Abdelwahab et al. 1998; Dhillon și Tuli 2001; Tuli 2002; Sharma și Mohan 2004; Talbot et al. 2007; Kritski și de Melo 2007; Pleșea și Enache 2008; Shrestha et al. 2010]:

- Blood vessels pathway – the most frequently encountered. It is paucibacillary.
- Lymphatic pathway - less commonly encountered.
- Direct spread from a contiguous lesion.
- Rare paths:
- Direct inoculation of the Mt into the site
- Bone and joints accidental or operative trauma

TB infection of the bones and joints has some general features: it is chronic, slowly progressive and destructive, often resulting in walking difficulties and disability [Lesić et al. 2010].

In joint involvement, the clinical picture is more flagrant and obvious in adults than in children [Watts și Lifeso 1996].

The clinical myearsfestations are divided in two main groups: constitutional symptoms (accompanying systemic symptoms) and local symptoms.

There are no specific radiographic features that are pathognomonic of tuberculosis of bones or joints and, in the advanced stages, mimics other osteoarticular lesions.

CT demonstrates abnormalities earlier than plain radiography and is best because allows the evaluation of the osseous or joint involvement degree [Shrestha et al. 2010; Garg și Somvanshi 2011].

Magnetic resonance imaging (with gadolinium enhancement) is the modality of choice for early detection of joint TB even its early findings are nonspecific and may demonstrate intraosseous involvement earlier than the other imaging modalities in osteomyelitis.

PERSONAL CONTRIBUTION

MATERIAL AND METHODS

The study group consisted of 54 cases admitted in surgical clinics and especially Orthopedic clinic of Emergency County Hospital from Craiova, Romyearsa, between 1990 and 2015 and diagnosed in the Clinical Laboratory and Pathology Departments with TB inflammatory lesions of the osteoarticular system (OAS) structures. The group was part of a larger series of 841 patients proved as presenting tuberculous lesions in the Pathology Department in the above mentioned period of time.

The studied materials were from the following data sources: (a) Accompanying notes of tissue specimens coming from operation theaters; (b) Histological records, paraffin blocks and Hematoxylin Eosin (H-E) stained slides of each case from the Department of Pathology's archives. Besides the original H-E slides of each case, serial sections were cut from paraffin blocks and either stained with Ziehl-Neelsen staining for acid-fast bacilli or immunomarked for Mycobacterium tuberculosis (Mt) in order to confirm the etiology.

The study was of retrospective type, and contained admitted, diagnosed and registered cases within the Department’s Archives between 1990 and 2015.

Database files were created for data processing in which all the parameters were that were taken into consideration for the study were assigned.

The assessed parameters were grouped in two studies:

- Clinical study including: General involvement of OAS; Temporal distribution of cases; Clinic of origin; Gender; Age; Suspicion of the etiological diagnosis at admission,
- Morphological study, focused on lesions’ location in OAS, the assessment, on routine stained samples of: granuloma cellularity, presence and type of necrosis, presence of fibrosis and the degree of differentiation of the granulomatous lesions and clarification of atypical lesions or caseous necrosis as dominating aspect of the lesion but with a non-specific granulomatous reaction around.

The human biological material was represented by biopsy specimens that were fixed in 10% formaline which was buffered at neutral pH and then paraffin embedded.

Besides the original H-E slides of each case, serial sections were cut from paraffin blocks and either stained with Ziehl-Neelsen staining for acid-fast bacilli or immunomarked for Mycobacterium tuberculosis (Mt) in order to confirm the etiology.

Histopathological aspects were selected with a CX31 Olympus microscope using the X4 magnification eyepiece. For image acquisition we used optical planapocromate corrected objectives with magnification of X4, X10, X20 and X40. The most significant images were acquired using a Olympus ColorViewII digital camera, saved directly on the computer and processed using the specialized image analysis software analySIS Pro and the FotoCanvas Lite v1.1 module of the ACDSee 4.0 software.

For some parameters, be they clinical or morphological (temporal distribution, age, location, diagnostic suspicion following the clinical examination, granuloma cellularity, necrosis, fibrosis, granuloma differentiation), the need for an accurate assessment of the tuberculous inflammatory process required the development of allocation criteria of cases that generated stratification scales of cases according to each criterion.

For numerical parameters the following statistical indicators were calculated: Lowest value (VMIN); Highest value (VMAX); Mean value (AV); Standard deviation (STDEV). For graphical representation, VMAX, VMIN, AV + STDEV and AV - STDEV were used. "$\chi 2$" correlation test was used to compare distribution of parameters divided into classes using stratification scales.

CLINICAL STUDY

In our series, the patients with **bony lesions** was most often male, aged either under 44 years or over 65 years, suspected of TB in less than half of cases and, when not suspected, the diagnosis was most of the time oriented toward tumor formation (Table 1).

The profile of patient with **lesions of the joints** was a little bit different. The patient was also male in the majority of cases but mature adult (aged between 45 and 64 years). The TB suspicion was present in almost half of the cases but, when not, the diagnosis is mainly oriented toward an inflammatory process (Table 1).

Finally, the patients with **mixed bone and joints lesions** were females in one third of the cases, young (less than 44 years old) in one quarter of the cases. They had also the highest clinical suspicion at admission (almost two thirds of the cases) and, when not, the diagnosis was oriented towards an inflammatory disease (Table 1).

Table 1: Clinical profiles of patients from the study groups

Parameter	Tissue involved		
	Bone	**Joints**	**Bone and Joints**
Gender	68% Men	**75%** Men	**1/3 Women**
Age	42% < 44 years 31% – > 65 years	65% – 45-64 years	**26% – <44 years**
Clinical suspicion	Yes – 42% No – 63% **Neoplasia**	Yes – 45% No – 63% Inflammation	**Yes** – **60%** No – **Inflammation**

MORPHOLOGICAL STUDY

In patients with **bony lesions** from our study group the trunk bones, like ribs and coxal bone, often from the left side, were more frequently involved.

The recognition of TB etiology didn't need (except one case) the use of special staining techniques. Although the great majority of granulomatous reactions had

giant Langhans cells in the inflammatory cellular population, almost 40% of the granulomatous reactions were poorly differentiated (type III – Hyporeactive) or even disorganized (type IV – Areactive), with basophilic or suppurative necrosis respectively. These morphological features together with the presence of lesion extension in the neighboring tissues could plead for higher aggressiveness of MT and superinfection respectively. However, the presence of fibrosis in more than 40% of cases is an argument for lesion stabilization (Table 2).

Table 2: Morphological profiles of patients from the study groups

Parameter	Tissue involved		
	Bone	**Joints**	**Bone and Joints**
Location/ Body Segment	58% – **Trunk**	60% – **Lower Limb**	53% – Lower Limb
Location/ Sagital Plane	58% Left Side	60% Left Side	40% **Right Side**
Location/ Tissue	Rib / Coxal bone/ Radius	Knee /Hip / Fist	Knee+Femur+Shin /Spine
Cellularity	84% Granuloma with E+CGL	**20% Epithelioid Granuloma**	>90 Granuloma with E+CGL
Necrosis	38% Basophilic+Supurative	**15% Absent**	1/3 – Basophilic+Supurative
Fibrosis	**42% Present**	25% Present	1/3 – Present
Granuloma Differentiation	38% III Hipo+IV A	**20% for Hip**	1/3 – III Hipo+IV A
Extension	25% of cases	**Rare**	26% Adjacent muscles
Identification	Was not needed	30% of cases	**40% of cases**

The profile of patient with **lesions of the joints** was a little bit different. Lower limb and especially knee and hip joints on the left side were predominantly affected.

Rarely extended, the TB lesions had a significant percentage of granulomas with epithelioid cells only an without necrosis, meaning well differentiated (Hyperplastic – type Ia) granulomas, that pleaded for a recent and active conflict, fact confirmed also by the lower percentage of fibrosis.

Unlike the bony lesions, joint lesions required special techniques of Mt identification in one third of cases (Table 2).

Finally, for the patients with **mixed bone and joints lesions**, the lesions involved most often the right lower limb, particularly the knee joint and the neighboring bones and the spine. Almost all granuloma contained giant Langhans cells.

In one third of the cases the necrosis was basophilic or suppurative, meaning a significant percentage of poorly differentiated or disorganized granulomatous reaction. The inflammatory process was also extended in the neighboring tissues other than bone and joint in an important number of cases (one quarter) although one third of the cases had stabilizing fibrotic reaction. The poorly differentiated aspect of the lesions and their extension required in a very significant percentage of cases (40%) the use of special techniques in order to elucidate the mycobacterial etiology (Table 2).

Our attempt of comparing our results with literature was a very difficult task first of all because our study was the only one that separated the cases depending on the type of OAS tissue involved. Then, apart from few generally accepted facts like variable male predominance and most frequent involvement of the spine and then of the weight bearing joints, the literature reveals a wide range of information.

Thus, there are variable trends in the temporal evolution and different trends of the age distribution.

Clinical suspicion is only stated not analysed in detail. Appart from studies dedicated to only one of the tissues that are part of the OAS, in the other studies it is not always specified which and how much of these tissues (bone, joint or both) is involved.

It seems that some details offered by the histopathological examination do not raise the curiosity of the investigators although our study showed that these details could define differences between the different ways in which tuberculosis can affect OAS tissue components.

Excepting the indian study of Ramanathan et al [Ramanathan et al. 1999] dedicated to lymph node TB, we have not found any other study focused on the analysis of morphological aspects of the conflict between Mt and the tissue involved.

As we observed in our previous studies [Popescu et al. 2014, 2015], acest conflict poate prezenta pe de o parte o serie de profiluri morfologice particulare în funcție de țesutul afectat. this conflict could present on one hand particular morphological profiles depending on the tissue involved. On the other hand, the morphological picture could provide clues about Mt aggressiveness, quality of host reaction or temporal evolution of the lesions (the propensity for extension for instance), information that should not be overlooked or underestimated.

CONCLUSIONS

Our study led to the emergence of the following conclusions:

The incidence of the tuberculous process has evolved differently within the three study groups with tuberculous lesions of the OAS.Thus, if in the mixed bone and joint forms, the temporal evolution had an oscilating trend with a general ascending tendency towards the last part of the interval, for the localized forms of the bony lesions the temporal evolutuon was a constantly descending one.

There were also differences between gender, age and the clinics in which the patients were admitted. Thus, bony and joint lesions predominated in men but the first ones had a bimodal age distribution – adolescent and young adult on one hand and elderly on the other hand while the latter were predominant in mature adults. Mixed lesions, however, have significantly affected woemn and young patients.

The suspicion of ehiological diagnosis after the clinical examination was generally high, the highest mark being found within the group with mixed lesions. When TB was not suspected, the diagnosis was oriented towards neoplasia in the bony lesions group and towards an inflammatory process in the other two groups.

The granulomatous reaction was poorly differentiated or even disorganized, with fibrosis being found in a significant number of cases from the bony and the mixed lesions groups while in the group with only joint lesions there were many hyperreactive incipient lesions observed with only epithelioid cells and absent necrosis.

The extent of the lesions to the neighboring tissues of the OAS was observed especially within the groups of bony and mixed lesions.

The morphological picture was not a problem when establishing a pathological diagnosis for the bony lesions group but it hat to be further evaluated through special techniques in the other two groups in a significant number of cases.

OATB represents still a challenge for the health care systems because, especially in nonendemic areas, the diagnosis is delayed and this delay in diagnosis could lead to major complications as deformities and neurological damages.

Tuberculosis of the musculoskeletal system is a spectrum of dynamic clinical morphological pictures in close relation with the OAS tissue involved, the mycobacterial aggressiveness or the host immune status which is still partly known, described and understood especially in the field of morphological changes.

Although the number of cases is small in the three subgroups we defined within our series, in each tissue belonging to OAS, some particularities of the clinical morphological profile of the conflict between Mt and that tissue.

The disease is well known as an invalidating one. Therefore, in order to improve the therapeutic outcome, further detailed and integrative analysis of both clinical and morphological aspects is required so the suspicion of diagnosis at the admission becomes as soon as possible certitude.

We come again and insist with the idea of designing a unifying and complete way of presenting and reporting such cases on one hand and, on the other hand to share this valuable information as much as possible.

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