PhD THESIS

ABSTRACT

ASEPTIC NECROSIS OF THE FEMORAL HEAD
STATISTICAL, CLINICAL, HISTOLOGICAL
AND IMMUNOHISTOCHEMISTRY STUDY

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Introduction

The aseptic necrosis of the femoral head is a disease, which is caused by partial or total disruption of the blood flow to the femoral head. The lack of blood flow at this level results in the death of the bone marrow and bone cells followed by the collapse of the bone structure, events which finally lead to the destruction of the bone tissue, local pain and loss of functionality of the hip joint. Aseptic necrosis of the femoral head is an illness, which affects mostly young adults, in their 3rd or 5th decade. It is considered that this disease affects men 4 times more frequently than women. The severity of this disease resides in the fact that it affects mostly young adults, socially and professionally active and with a great life span, which are initially asymptomatic but will eventually need hip arthroplasty surgery until their 5th decade.

Nowadays, there are more than 16 different classification systems used to classify and describe aseptic necrosis of the femoral head, from which the classification system introduced by Ficat is the most commonly used. The Ficat classification system consists of five stages (0 to IV): the first three describe the events that occur before the collapse of the femoral head and the last two stages describe the post-collapse changes. Aseptic necrosis of the femoral head can be either primary (idiopathic) or secondary (traumatic or nontraumatic). Trauma, alcohol consumption, smoking and corticotherapy are frequently incriminated in the development of this disease, as secondary causes. Considering the above information and literature findings we proposed to study the macroscopic, microscopic and morphometric changes found in the femoral head of patients with aseptic necrosis and correlate this data with their biological status and clinical and paraclinical investigations.

Our study proposed to research the contribution of correlating clinical data with imaging, histological and immunohistochemistry findings, for early detection of the disease and for creating a better therapeutical approach according to the stage of the disease. We hope our study will bring new information regarding the histopathological mechanisms that lead to the appearance and development of aseptic necrosis of the femoral head that can be used to an early diagnosis increasing the success rate of conservatory treatment.

KEY WORDS: aseptic necrosis femoral head, pathogenesis, immunohistochemistry, CD68, morphometry
THE STAGE OF KNOWLEDGE

Chapter I

Bone tissue morphology

In the first chapter the structure and classification of the bone tissue are presented, starting from the classical view to the most recent findings in the field, concentrating especially on the morphological features of the femur, describing its architecture and histological characteristics. The main classification systems are described and the bone structure is presented thoroughly.

In this chapter we describe the main components in the bone structure, the major bone types (primary and secondary) and we also present a comparison between compact and spongious bone tissue.

Chapter II

Aseptic necrosis of the femoral head

In the second chapter we describe the history, etiology and risk factors incriminated in the development of aseptic necrosis of the femoral head. We reviewed recent epidemiologic data regarding the incidence and prevalence of the aseptic necrosis of the femoral head.

In this chapter we also reviewed the pathogenic theories meant to shed light on the events that initiates the illness. The etiology of the disease is multifactorial. There are many theories which have been proposed, most of them agreeing that the final event that marks the debut of the illness is the blood flow occlusion of the terminal vessels.

We described the most recent literature data regarding the clinical diagnosis, the imaging methods used in the diagnosis of the disease and the differential diagnosis theories. Also, we described the main classification systems used to stage the aseptic necrosis of the femoral head. In this chapter we covered the modern approaches in the treatment of the disease with an emphasis on early diagnosis in order to achieve an optimal result.
PERSONAL CONTRIBUTION
Chapter III
Clinical – statistical study

Objectives:
- Determining the number of patients diagnosed with aseptic necrosis of the femoral head and admitted in the Orthopedics Department of the Emergency County Hospital of Craiova between 2007 and 2011.
- Identifying the number and type of intervention that each patient underwent, the stage of the disease at the time of diagnosis, the reasons for admittance and the biological markers assessed in order to diagnose the disease.
- Finding the risk factors and the adjacent pathology associated with the development of aseptic necrosis of the femoral head.

Materials and Methods
In the study there were included 92 patients diagnosed with aseptic necrosis of the femoral head, admitted in the Orthopedics Department of the Emergency County Hospital of Craiova between 2007 and 2011. The data necessary for our study was obtained reviewing the patient charts of the 92 patients. The inclusion criteria was that the patients place of residence had to be Dolj county. The data was analyzed statistically in order to obtain correlations between clinical and paraclinical data of the patients diagnosed with this disease.

Results and discussion
We noticed an increase in the number of patients diagnosed with this disease, from 11 in 2007 to a number of 25 in 2011. Most of the patients included in our study (85.87%) were diagnosed in advanced stages of the disease, stages III and IV. More than half of the patients in our study (57.61%) were between the age of 30 and 50 years. The study group was composed of 68 male patients and 24 women.

The main risk factors involved were represented by smoking in 36.96% of the patients, chronic alcohol consumption in 20.65%, trauma was associated in 11.96% of the cases and corticotherapy was incriminated in 8.70% of the patients. For 29.35% of the cases, no risk factors were found, so we considered the disease to be idiopathic.
A percentage of 38.04% of the patients came from rural surroundings and 61.96% lived in the city. The percentage of people in urban surroundings is significantly higher than rural ones in the population of the county.

Most of the patients in the study diagnosed in early stages (I and II) benefited from surgery for salvaging the femoral head through decompression drilling and required an average 2 and a half days of hospital care. The patients in advanced stages (III and IV) didn’t benefit from other therapeutical methods than reconstruction of the femoral head, respectively hip arthroplasty and needed around 13 days of hospital care.

The results of the clinical and statistical study matched, for the most part, to similar research in this field. There were variables of our study to which we could not find any correspondence in literature (repartition of the patients according to their residence environment, the number of days of hospital care required by the therapeutic method used).

Chapter IV
Histological and immunohistochemistry study

The Objectives of the histological study are to describe and compare the morphology of the necrotic area to the adjacent viable tissue, to assess the changes of the fibrous tissue around the area of necrosis and to present the qualitative and quantitative changes in vascularization of the necrotic area.

The Objectives of the immunohistochemistry study were to observe the reaction of morphological components to the used antibodies and compare it in patients with different risk factors.

Materials and Methods

For the histological and immunohistochemistry study we used sections of femoral head and neck obtained from 26 patients diagnosed with aseptic necrosis of the femoral head during hip arthroplasty. The biological material was instilled in a fixing neutral 10% formaldehyde solution. The bone fragments were decalcified using trichloracetic acid and were then processed using classical histological technique for paraffin inclusion.
The histological samples were stained using hemtoxylin-eosin and trichromic staining (Goldner-Szeckeli). The antibodies used in our study were represented by osteoprotegerin, osteonectin, osteopoetin and CD68.

**Results and discussion**

We demonstrated that on bone tissue samples from the patients in our study we found multiple microscopic aspects, described in the literature as being characteristic for some pathogenic theories. More so, on samples from the same patient we found histological aspects described as common to more than one diseases incriminated in the development of aseptic necrosis of the femoral head.

A notable result of the immunohistochemistry study was the presence of positive immunostaining for CD68 in all patients included in our study, highlighting the presence of macrophage cells in the necrotic area, as well as in the adjacent areas of the necrotic area.

**Chapter V**

**Morphometric study**

**Objectives**

The main objective of this study was to underline the differences between the areas occupied by the bone trabeculae from the necrotic area compared to the bone trabeculae in viable tissue areas.

Another objective was to compare the rapport between the area of bone trabeculae inside the necrotized area and that of bone trabeculae, in the healthy tissue located in adjacent area, in patients with different risk factors.

**Materials and methods**

The studied group consisted of 16 patients diagnosed with aseptic necrosis of the femoral head between 2010 and 2011. They were admitted in the Orthopedic Department of the Emergency County Hospital of Craiova and underwent hip reconstructive surgery. The biological material was represented by bone fragments from the head and neck of the femur from patients that underwent hip arthroplasty.

The histologic samples were stained using hematoxylin eosin and trichromic Goldner-Szeckeli staining. Samples containing decalcified bone tissue were studied using an Nikon
Eclipse 55 i electronic optic microscope (Nikon, Apidrag, Romania) and photographed using a 5 megapixel CCD camera. In order to achieve the results of the morphometric study we chose images of the bone trabeculae from the necrotic areas and from viable adjacent tissue, from all patients in our study. The trabecular area was determined using the Image Pro Plus software from Media Cybernetics. The software used images of trabecular areas that were modified using Adobe Photoshop in order to differentiate between tissue elements on the samples.

Data regarding age, sex, risk factors, debut of the illness, stage of disease was obtained from patient charts for statistical purposes. Data processing was achieved using Microsoft Excel (Microsoft Corp, Redmond, WA, USA) together with XLSTAT suite for MS Excel (Addinsoft SARL, Paris, France). Because we compared small number groups we used the non parametric test Kruskal-Wallis. We used the ANOVA test to compare post hoc pairs (Tuckey HSD test).

**Results and Discussions**

The entire group was divided into 4 separate groups according to their risk factors: group I was composed of patients with trauma as a prime risk factor, group II had corticotherapy as a main risk factor, group III was characterized by chronic alcohol consumption and group IV had chronic alcohol and smoking as a prime risk factor. Microscopic images presented common elements in all 4 groups but also significant differences. These images varied within the same group and even the same patient according to the necrotic area that was studied.

All 4 groups presented similar morphologic changes inside the necrotic area represented by the replacement of healthy tissue with extensive areas of fibrosis, diminishing trabecular bone.
General Conclusions

Between 2007 and 2011, 92 patients were admitted with the diagnosis of aseptic necrosis of the femoral head, in the Orthopedic Department of the Emergency County Hospital of Craiova. We remarked an increase in the number of patients admitted yearly with this diagnosis from 11 patients in 2007 to 25 patients in 2011. Most of the patients in the study (85.67%) were diagnosed in advanced stages of the disease, stages III and IV.

The studied group consisted of 68 men and 24 women with a male to female ratio of 3:1. More than half of the patients in our study (57.61%) were between the age of 30 and 50 years.

The main risk factors involved were represented by smoking in 36.96% of the patients, chronic alcohol consumption in 20.65%, trauma was associated in 11.96% of the cases and corticotherapy was incriminated in 8.70% of the patients. For 29.35% of the cases, no risk factors were found, considering the disease to be idiopathic.

The morphologic aspects in the histological study varied from one patient to another and also the same patient presented different changes, according to the studied area.

In all patients included in the study, the trabecular areas inside the necrotic area were decreased in size compared to trabecular areas from healthy tissue in the same patient.

The remnant trabecular bone was characterized by the placement on their surface of hypertrophic osteoblasts, well highlighted, which denotes an activation of osteofroming cells which in the absence of oxygen and nutrients cannot carry on their function.

Inside the trabecular bone from the necrotic area we noticed small osteocytes with small channels, sometimes absent, with small and Hypercom nuclei. These histologic aspects denote a reduced metabolism of osteocytes and an almost absent activation of osteoforming cells.

Within the necrotized tissue we found collagen fibers with variable widths, with chaotic displacement, inhomogeneous aspect, with numerous fibroblastic cells and rare fragments of disintegrating bone.
The necrotic area highlighted arterioles with a thickened wall, atherosclerotic, few veins and rare capillaries. We observed overly developed collagen fibers which were compressing the blood vessels contributing in some manner to the decrease of vascularization of the bone tissue. These microscopic aspects plead to the ischemic pathogenic hypothesis of the aseptic necrosis of the femoral head because local hypoxia is the stimulating factor for the increase in fibroblast activity which leads to a high synthesis of collagen and a decrease in proteoglycans and glycosaminoglycan.

All 28 cases of the studied group in the immunohistochemistry study had positive immunostaining for CD68 and highlighted the presence of macrophage in the necrotic area and the healthy tissue adjacent areas, as well.

A high number of macrophages was present in patients that had corticotherapy as the main risk factor and the lowest number of macrophage was found in patients whose risk factor was trauma.

Osteoprotegerin had a very diminished activity which leads to the idea that aseptic necrosis of the femoral head patients present a high resorbing rate of the affected bone tissue, through the osteoclasts activity.

Osteopontin and osteonectin were highlighted especially in the extracellular level which may indicate an increase in the number of osteoblasts needed for bone tissue synthesis.

No significant statistical difference was discovered between the trabecular area from the necrotic area and the area of viable tissue, in patients from the morphometric study with different risk factors.
Selective references


