

## The histopathological study of osteoporosis

FL. MARCU<sup>1)</sup>, FL. BOGDAN<sup>2)</sup>, GABRIELA MUȚIU<sup>3)</sup>, L. LAZĂR<sup>1)</sup>

<sup>1)</sup>*Department of Neurology–Psychiatry–HydroPhysioKinetotherapy,  
Faculty of Medicine and Pharmacy, University of Oradea*

<sup>2)</sup>*Research Centre for Microscopic Morphology and Immunology,  
University of Medicine and Pharmacy of Craiova*

<sup>3)</sup>*Preclinical Department II,  
Faculty of Medicine and Pharmacy, University of Oradea*

### Abstract

The osteoporosis is characterized by the imbalance between the activity of the osteoblasts, the bone forming cells, and the osteoclasts, the cells that resorb the bone tissue, imbalance that favors the osteoclasts. As a conclusion, in the case of osteoporosis, for the same volume, the bone is less compact and more fragile. The objective of our study is to make a histological evaluation of the different elements of the bone tissue in many 47 bone samples: 27 bone fragments were collected from the head and the femoral head of patients who required hip arthroplasty and 20 bone fragments were collected from the vertebral body of dead patients. The results of our study emphasized the thinned trabeculae of the bone that lost continuity, the preferential resorption of the horizontal trabeculae, the consecutive trabecular anisotropy and the reduction of the trabecular connectivity with enlarged areolae and the adipose degeneration of the marrow. One notices in the osteoporosis a reduction of the trabecular network connectivity directly proportional with the stage of the illness; thus, we determined a strong reduction of the trabecular connectivity in advanced osteoporosis stages. The growth aspects of the medular adiposity, associated with the intratrabecular connectivity concurs to highlight the functional connection between bone and marrow. The diminution of the medullar cellularity together with its enrichment in fat cells has negative outcomes on the bone.

**Keywords:** osteoporosis, bone trabeculae, areolar spaces, marrow degeneration.

### Introduction

The osteoporosis, the most frequent metabolic bone disease, became a public health problem on one hand because of the increase of the average life span followed consecutively, by the increase of the disease incidence and on the other hand because of the complications due to disease, osteoporotic fractures, that determine the individual's life alteration followed by the increase of the morbidity indices, that leads to high material and social costs; this disease is characterized by low bone mass and architectural deterioration of the bone tissue together with the subsequent increase of the bone fragility and fracture predisposition [1]. The decrease of the bone mass is the result of an imbalance occurred between the processes that control the accretion and the preservation of the skeletal mass and is not accompanied by a significant decrease of the relation between the mineral and the organic phase, nor by anomalies of the bone or mineral matrix [2].

Lately, the importance given to these matter has grown significantly because of the mutations occurred in population structure, that result in the increase of the humans' life span. Thus, the data provided by the *World Health Organization* shows that if in 1960 the number of the persons over 60-year-old was approximate 250 millions, in 2020 it will reach 1 billion [3].

The bone tissue, the hardest tissue in organism, is a form of connective tissue [4] and represents 20% of the mass of the adult organism [5]. The bone tissue holds

five main functions: support, locomotion, protection, hematopoiesis and calcium phosphate [6]. The bone tissue is not static, the bone is characterized by a continuous inner restructuring in order to adapt to the requirements of the various moments in the life of the organism he is part of. The preexisting tissue is gradually replaced by new tissue, with a speed that decreases with age, the micro-fissures are restored, the endurance of the skeleton and the mineral homeostasis are adjusted [7]. The bone tissue is the main structural component of the bones, in the structure of which other connective tissues that include hematopoietic tissue, adipose tissue, blood vessels and nerves take part. The bones represent the organs of the human skeleton; they are living organs subjected to continuous modifications/changes and who respond to internal and external stimuli by means of turnover.

The understanding of the process of the bone mass loss, observed in elder people, in the context of osteoporosis, continues to represent a challenging subject in all aspects. The analysis of the bone architecture in osteoporosis has a particular importance for the understanding of certain clinic manifestations and of certain complications because it is known that the structural changes affect the quality of the bone.

In order to better seize these changes, we conducted a histomorphological research of the changes of the bone tissue architecture in osteoporosis and the examination of these structural elements in bone tissue dynamics.

## ☒ Materials and Methods

The material for the histopathological research/study is represented by 47 fragments of human bone collected at the Orthopedic Clinic of the Emergency Municipal Hospital Timișoara and at the Institute of Legal Medicine Timișoara during 2005–2009. Due to the fact that the tissular alterations and cellular injuries are more pronounced and visible at femoral and vertebral level, we collected fragments of bone tissue at head and at femoral head level from 27 patients (20 females and seven males) aged between 50 and 76-years, who required hip arthroplasty, as well as fragments of bone tissue collected from the vertebral body of 20 deceased patients (14 females and six males) aged between 66 and 74 years.

The smaller number of the collected vertebral fragments is because they were harder to obtain.

We used, within our research/study, for harvesting, that is essential to establish the histopathological diagnosis, as well as for sampling, well sharpened special tools, because of the solid texture of the bone tissue. The purpose was to obtain equally consistent sections in order to avoid the decalcification differences; these sections were made by using a pendulum saw.

The obtained material was subjected for two weeks to a fixation operation in a 10% formalin fixation solution obtained from the commercial formalin (aqueous 40% formaldehyde solution). In order to neutralize the formic acid, formed spontaneously in concentrated formic solutions, 10% calcium bicarbonate solution was added.

After the sampling, the fragments were washed in tap water for 24 hours to clean the tissues of formalin; subsequently, in order to treat the bone fragments with paraffin (wax), they were subjected to a decalcification process. For the latter, increasing quantities (2%, 5%,

and 10%) of trichloroacetic acid were used for a period of 180 days; the trichloroacetic acid solutions were replaced with new ones every time it was necessary.

Afterwards, in order to apply the paraffin (wax), the bone fragments were processed according to classical histological technique; the technique involved the following operation periods: dehydration, clarification, paraffin coating, inclusion, sectioning of the block, adhesion of the sections to the lamellae and their drying, staining of the sections, drying and storing of the histological material.

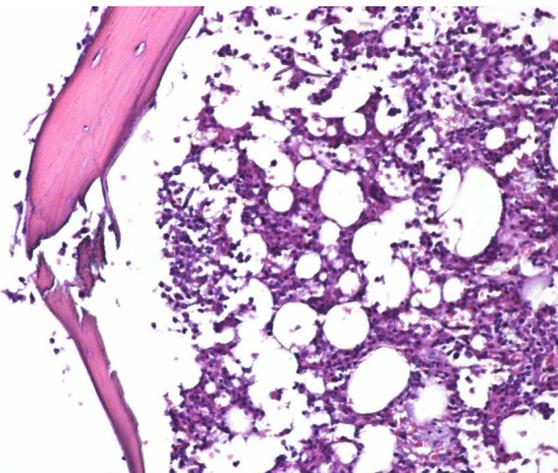
For the staining of the histological material, we used, in our research, two staining methods intensely used within our country and worldwide: the Hematoxylin and Eosin staining method; it uses successively, for the whole study, a basic nuclear stain, the hemalaun (hematein + potassium alauun) and a cytoplasmic acid staining agent, the Eosin; actually the hematein is the oxidized form of the Hematoxylin, a natural compound of vegetal origin.

The Masson trichrome staining protocol to highlight the collagen fibers. This staining method uses the following colorant agents: Ferric Hematoxylin, Acid Fuchsin and Aniline Blue.

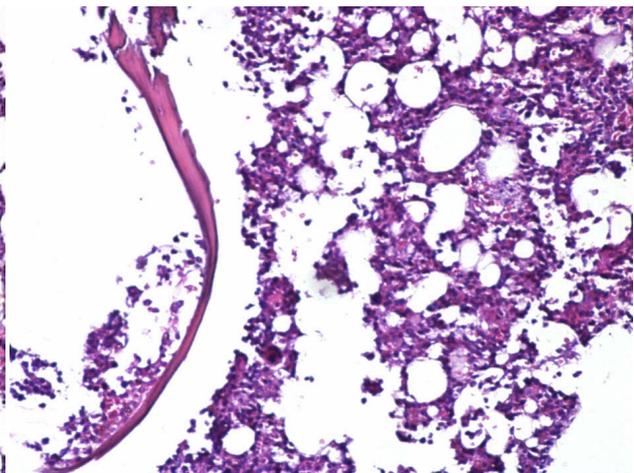
## ☒ Results

At the examination of the bone sections, done with an optical microscope, we noticed the thinned bone trabeculae that lost continuity, aspect emphasized in the images below (Figures 1 and 2).

Trabecular fracture at this level (Figure 2) is probably recent, the two-trabecular ends being separated by a narrow space. In the mean time, the surface available for reshaping increases by the occurrence of the two-trabecular extremities. As the disease advances, the distance may grow because of the continuous bone resorption.



**Figure 1** – Contracted and perforated bone trabecula of a post menopausal 63-year-old woman, with microfractural lesions by bone resorption, on a background of marked decrease of BMD (HE stain,  $\times 100$ ).



**Figure 2** – The loss of trabecular bone accompanied by the marked reduction of the bone matrix of a 67-year-old man, here and there trabecular perforations occur, on a background of trabecular connectivity diminution (HE stain,  $\times 100$ ).

Another aspect emphasized during the histomorpho-

logical examination (Figures 3–5) is the preferential

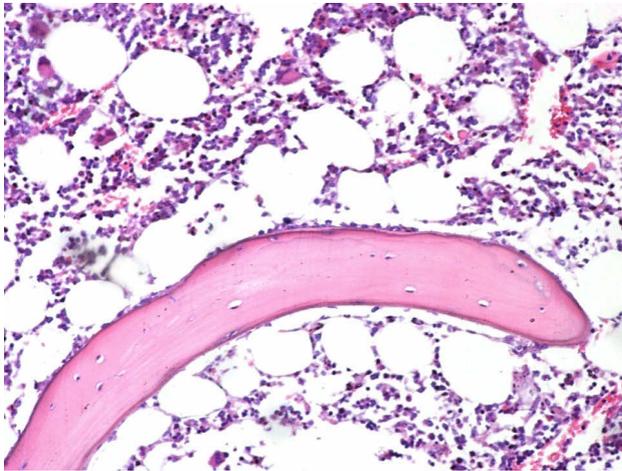
resorption of the horizontal trabeculae, this being accompanied, in many cases, by the compensating thickness of the vertical trabeculae. We noticed the different aspect of the vertical trabeculae that are thickened compared to the horizontal ones that are obviously thinned and present microfissures. The result of the thickening of the vertical trabeculae is the trabecular anisotropy (Figure 4) that ensures an increased resistance on the main direction of the mechanical strain of the bone but not on the directions where the strain is infrequent, case in which their fracture may occur.

We also noticed an irregularity of the thickness of the remaining trabeculae (Figures 3 and 4) probably still compensatory, because of the attempt to replace the decrease of the bone resistance, produced by the resorption of some connecting trabeculae.

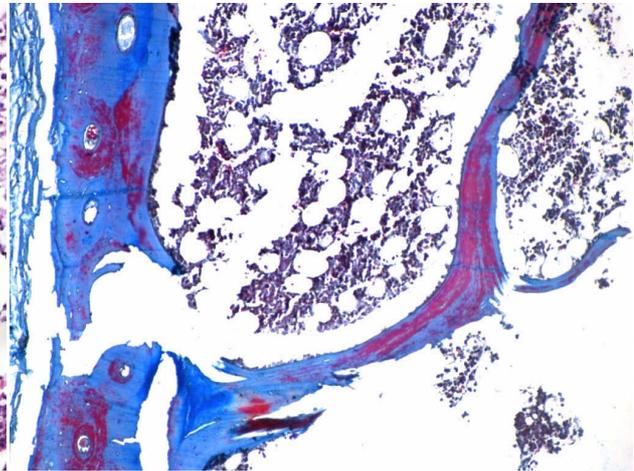
One of the consequences of the decrease of the connectivity of the trabecular network and of their

thickening is the increase of the areolar spaces. We were able to determine on our material an adipose degeneration tendency of the hematogenous marrow within the enlarged areolar spaces and incompletely septated because of the lysis of the walls. The cancellous bone decrease is associated with the increase of the adipose marrow–hematogenous marrow relation in the areolae. The result is the decrease of the enlarged areolae trabecular connectivity and the adipose degeneration of the marrow, aspect shown at the microscopical examination in the figures below (Figures 6 and 7).

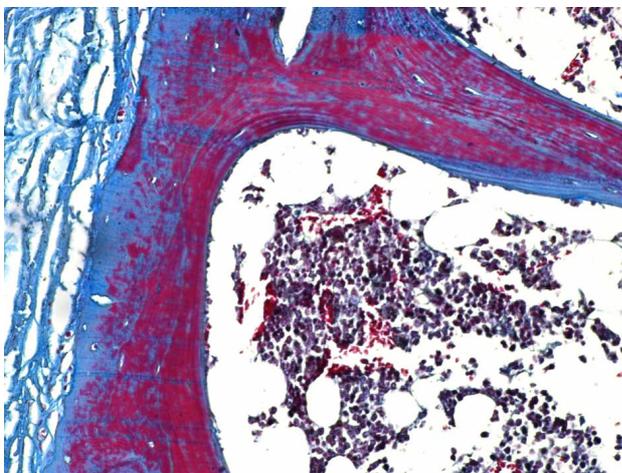
We noticed, in certain sections, the presence of some areolar spaces with reduced myeloid content, as well as cases of necrobiosis of the myeloid tissue (Figures 6 and 7), that can be explained on the base of the modifications within the blood perfusion at osteoporotic bone level.



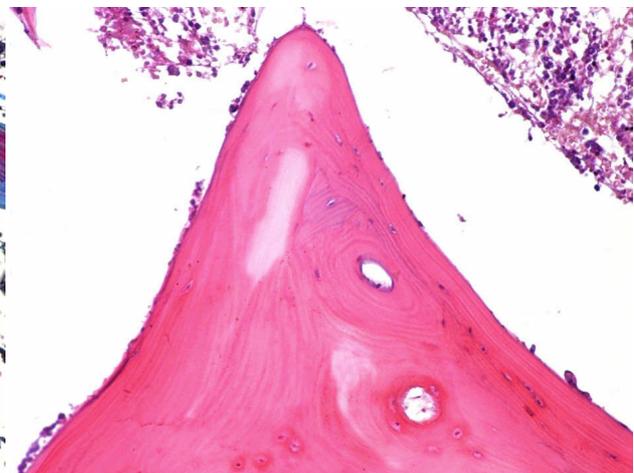
**Figure 3** – Thinned bone trabecula of a 70-year-old woman, with the decrease of the trabecular connectivity, with the rod-like aspect, the most probable horizontal type of trabecula; discreet regeneration aspects on the convex area (HE stain,  $\times 200$ ).



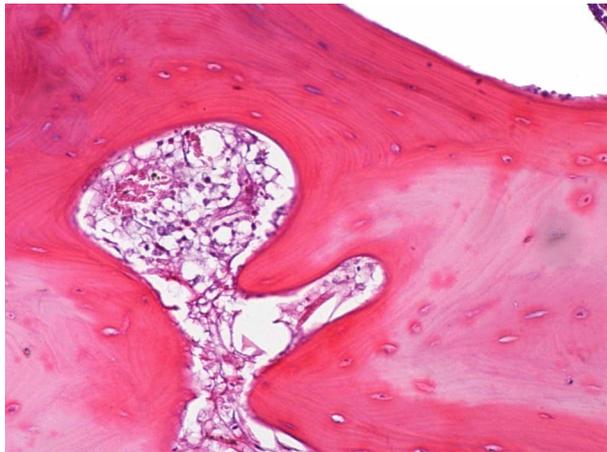
**Figure 4** – Aspect of the osteoporotic bone with trabecular anisotropy in a 50-year-old man with thickened vertical trabeculae and thinned horizontal ones presenting microfissures; simultaneously, one notices an enlargement of the medullary spaces with the trabeculation of the bone cortical during the accelerated bone loss period (Masson's trichrome stain,  $\times 200$ ).



**Figure 5** – Trabecular disconnection tendency and endosteal resorption in a 52-year-old woman (Masson's trichrome stain,  $\times 200$ ).



**Figure 6** – Necrobiosis and retraction of the myeloid in the bone areolae, much enlarged, in a 72-year-old woman (HE stain,  $\times 200$ ).



**Figure 7 – Regenerated cancellous bone with a necrobiosis aspect of the myeloid tissue in a 55-year-old woman (HE stain,  $\times 200$ ).**

## Discussion

In osteoporosis, the contraction or even the loss of some trabeculae, because of the negative result of the reshaping, is followed by the compensatory thickening of other trabeculae in order to meet the mechanical strain. This may be produced because of the fact that through the resorption of some trabeculae the mechanical load will operate only on the remaining trabeculae that, in the attempt to resist to the new increased mechanical strain, will adaptively thicken. The thickening will occur consequently to the accentuation of the forces that operate on that section. Even when a trabecula is not entirely broken, and is only connected to the entire trabecular system, its functional importance is significantly diminished.

In advanced stages of resorption, the bone trabeculae are thinned, reduced to a thickness of only a few bone lamellae. These trabeculae, although more fragile because of excessive osteolysis, represent an aspect less severe in osteoporosis compared to the diminution of the trabecular number occurred after the complete resorption. This happens, because as Frost points out [8], the decrease of the trabecular number with aging represents an irreversible aspect of the bone loss, while the remaining trabeculae, even thinned, can amplify their resistance by means of a thickening accomplished in the wake of reshaping with a positive result concerning the osteoformation. On the other hand, it has been demonstrated [9] that in the case of a very reduced trabecular thickness, even a resorption cavity that is not oversized can penetrate the thickness of that trabecula, generating an increased tension around it.

The maintenance of the connectivity of the trabecular system is of high importance to the bone resistance. The loss of the elements of the horizontal trabeculae determines the growth in length of the unsustained vertical trabeculae that strongly influences their bending resistance. The resistance of the vertical trabeculae varies inversely proportional with the square of the distance where they are not enforced by the presence of the horizontal trabeculae [10]. The maintenance of the horizontal trabeculae has a profound

influence on the resistance of the vertical trabeculae, without significant modifications of the bone mass [11]. The diminution of the resistance of the cancellous bone by trabecular loss can be explained with the help of Euler's principle: the resistance of a bar during a vertical load is inversely proportional with the square of its whole length. This, considering the trabeculae as bars, can also be applied in the case of the trabecular resistance. One notices that when losing, by bone resorption, two of the horizontal trabeculae, for instance, the length of the vertical trabecula (unsustained by horizontal support) increases three times, that which according to Euler's principle, will decrease the resistance of the section nine times.

The results of our research are similar to the results of other specialty studies [12]. This means that one noticed in the majority of the examined sections, the decrease of the trabecular connectivity as well as the occurrence of trabecular perforations, phenomena that become more pregnant with aging.

The bone marrow and the bone present a tight functional interdependence; the modifications occurring at the bone marrow level, with the decrease of the cellularity paralleled by an increase of the adiposity at medullar level are to be expected. The increase of the adiposity is produced because of the diminution of the differentiation of the osteoblasts within the stromal cells precursor in favor of the adipocytary differentiation. Our results concur with other specialty studies [13] that also show the increase of the height of the adipose cells at bone marrow level in osteoporosis. Another cause that can be ascribed to the increase of the medullar adiposity is linked to the vascularization of the bone. In order to optimally perform its functions, the bone marrow, as well as the bone, is dependent on an adequate blood supply. Following a set of research at femoral level, Griffith JF *et al.* [14] pointed out that, in osteoporosis, a reduction of the blood flux in the bone may take place; this being reduced in the subjects diagnosed with this affection compared to the subjects having normal values of the mineral bone density and even suffering from osteopenia. The same author showed the importance of the connection between bone tissue blood perfusion and that of the bone marrow, a decrease of the blood supply at medullar level being followed by the increase of the adipose content of the marrow. This can be explained by the fact that the marrow shows a tight functional dependency; the marrow cells being the precursors of the cells that will take part/participate in the reshaping process, their alteration having consequences on the reshaping process.

Other researchers [15] propose to explain the structural modifications occurred in osteoporosis by means of the prism of an ischemic mechanism, that is, the diminution of the blood flux being in tight connection with the vascular degeneration and the atherosclerosis occurring with aging.

## Conclusions

In osteoporosis are numerous sequels that lost any contact with the global trabecular architecture. By this

disconnection, the sequel trabeculae became inefficient from a functional point of view because they cannot share the forces transmitted to the bone. The existence of such trabecular reminiscences, although provide bone mass, do not contribute to ensure the bone resistance because of the alteration of the bone architecture.

In the areas of trabecular bone resorption the thinning of these trabeculae takes place, fact that determines the compensatory modifications of the diameter of certain residual trabeculae, in an attempt to supply the loss of bone material. This fact creates trabecular anisotropy with the increase of the resistance on the main strain directions but not on other directions where the functional strain rarely takes place, thus facilitating their fracture when the strain is operates from these collateral directions.

The areolar spaces are extended, delimited by incomplete bone septa, fact that can be explained by the decrease of the connectivity of the trabecular system that will lead to the joining of the areolae by the osteolysis of certain walls. In the same time with the increase of the areolae, modifications of their content were noticed, most of them consisting in the modification of the cellularity of the marrow with a degenerating adipose tendency.

The increase aspects of medullar adiposity, associated with the decrease of the inter-trabecular connectivity contributes to emphasize the functional connection between the marrow and the bone. The diminution of the medullar cellularity with its enrichment in adipose cells has negative consequences on the bone. The increase of the adiposity can be explained by the decrease of the differentiation of the osteoblasts from precursor stromal cells in favor of adipocytary differentiation.

## References

- [1] LAZĂR L, MARCU F, *Osteoporoza. Geriatrie și recuperare specifică*, Ed. Universității Oradea, 2009, 76–90.
- [2] Krane MS, Holik FM, *Osteoporoza, boli metabolice. Principiile medicinei interne*, Harrison, Ed. Internațională, vol. 2, 2003, 2475–2481.
- [3] BĂLĂNESCU A, *Osteoporoza – prezent și perspective*, Congresul Mondial al Fundației Internaționale de Osteoporoză, Lisabona, mai 2002.
- [4] DICULESCU I, ONICESCU D, *Histologie medicală*, vol. I, Ed. Medicală, București, 1987.
- [5] DAY J, *Bone quality: the mechanical effect of micro-architecture and matrix properties*, Optima Grafische Comunicatie, 2005, 11–18, [http://publishing.eur.nl/ir/repub/asset/6761/050608\\_Judd%20Day.pdf](http://publishing.eur.nl/ir/repub/asset/6761/050608_Judd%20Day.pdf).
- [6] IANCULESCU A, MOGOȘ G, *Compendiu de anatomie și fiziologie a omului*, Ed. Științifică, București, 1972.
- [7] STOICA E, IGNAT L, *Materiale compozite bioactive pentru reconstrucție osoasă. Materiale compozite sustenabile pentru îmbunătățirea calității vieții*, Ed. PIM, Iași, 2006, 52–121.
- [8] FROST HM, *On the trabecular “thickness”-number problem*, J Bone Miner Res, 1999, 14(11):1816–1821.
- [9] PARFITT AM, *High bone turnover is intrinsically harmful: two paths to a similar conclusion. The Parfitt view*, J Bone Miner Res, 2002, 17(8):1558–1559; author reply 1560.
- [10] ALEXANDER C, *Idiopathic osteoporosis: an evolutionary dys-adaptation?*, Ann Rheum Dis, 2001, 60(6):554–558.
- [11] MARCUS R, FELDMAN D, NELSON D, ROSEN C, *Osteoporosis*, 3<sup>rd</sup> edition, Elsevier, 2007, 27–81.
- [12] REHMAN MT, HOYLAND JA, DENTON J, FREEMONT AJ, *Histomorphometric classification of postmenopausal osteoporosis: implications for the management of osteoporosis*, J Clin Pathol, 1995, 48(3):229–235.
- [13] COMPSTON JE, *Bone marrow and bone: a functional unit*, J Endocrinol, 2002, 173(3):387–394.
- [14] GRIFFITH JF, YEUNG DK, TSANG PH, CHOI KC, KWOK TC, AHUJA AT, LEUNG KS, LEUNG PC, *Compromised bone marrow perfusion in osteoporosis*, J Bone Miner Res, 2008, 23(7):1068–1075.
- [15] CHEN WT, SHIH TT, CHEN RC, LO SY, CHOU CT, LEE JM, TU HY, *Vertebral bone marrow perfusion evaluated with dynamic contrast-enhanced MR imaging: significance of aging and sex*, Radiology, 2001, 220(1):213–218.

### Corresponding author

Florin Marcu, Assistant Professor, Department of Neurology–Psychiatry–HydroPhysioKinetotherapy, Faculty of Medicine and Pharmacy, University of Oradea, 10 1 December Square, 410068 Oradea, Romania; Phone +40745–883 047, e-mail: mfmihai27@yahoo.com

Received: November 9<sup>th</sup>, 2010

Accepted: December 15<sup>th</sup>, 2010