ORIGINAL PAPER

Morpho-physiological aspects of rapports between bone and marrow bone

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Abstract

Border between bone and marrow bone has many problems regards differentiation, cells topography, dynamic process of osteoclastogenesis. So, this study tries to present morphophysiological aspects of this border, using lots of bone fragments that include compact and spongious bone. We observed genesis process of trabecular bone near the chondroclast zone and the osteoblast between sinusoid capillars. Also, the development of lamellar bone and the rapports between this structures, new marrow cells and angiogenesis process that exist near the lamellar trabecular bone surface. We observed the relation between immune system and bone, because it exist some factors that involve the development of cells precursors of lymphocyte B. Using a special staining method we observed the process of angiogenesis, hematopoetic system and reticuline fibers.

Keywords: marrow bone, lymphocyte B, bone, osteoclast.

Introduction

The borders between elements of bone and marrow bone are unknown yet, in the present, even if we know the important role of bone in biomechanics and production of blood cells.

Regards border between bone and marrow bone exist some theoretic and practical problems like: coincidence of building and destruction phenomenon, induction of structures that are going to differentiation, topography of specialized cells and their rapports with bone structures, dynamic of fundamentals processes at bone marrow, especially osteoclastogenesis, dynamics of extra cell matrix during chondrogenesis and osteogenesis, mechanism of transformation of macrophage in osteoclast cell, communications signs between osteo- and hematopoetic systems, relation between immune system and osteomedular system, relation between process of angiogenesis, osteogenesis and hematopoetic process.

Objectives of our study are to present a morphophysiological and anatomic study regarding the rapports between osteo- and hematopoetic structures. For this study we go from few questions:

- Which are the mains structures of bone ?
- Different rapports between bone surface and marrow bone depend by geometric forms of bone or by bone structure ?
- Which is the co-operation between osteoblast and osteoclast during bone genesis and modeling process of bone ?
- How the topography of bone and marrow bone can be change ?

Material and methods

In this study we included 88 bone pieces, 35 pieces from axial skeleton and 53 pieces from lower limb skeleton. All these pieces are without of first or second disorders. For study the morpho-physiological aspects of bone trabecular structure we used 28 pieces of *calcaneum* that have been taken from 15 death subjects. Bone pieces have been included in formaline with 10% calcium carbonate, and then they have been treated for decalcification in 5% nitric acid and cold urea, that is change every day. The pieces of bone have been dehydrated and the have been included in paraffin blocks. Assessment of pieces has been made using Nikon microscope and for acquisition of images we used Lucia M soft.

The microanatomical study has been made using classic histological methods, but the news in this study is that we used few methods of staining that are not use usually. These were: Gömöri, Giemsa, Mac Mannus, from usual color we used van Gieson method.

Results

Microanatomical genesis aspects of rapports between osteo- and hematopoetic structures at fetus femur bone

Genesis of non-lamellar trabecular structures

On a series of sections at upper extremity of femur bone, we observed genesis and differentiation processes of non-lamellar trabecular structures. They could be observed near the side of chondroblast and near the

place where osteoblasts appear. On the frontal section we remark the spatial disposition and the non-lamellar trabecular structures differentiation, the chondrocytes apoptosis process nearby the angiogenesis and chondrolysis process and, also marrow bone differentiation process (Figure 1).

Blood vessel differentiation neighborhood the transforming zone of the cartilage is contemporary with the chondrocytes genesis nearby the vascular endothelium.

Basal membranes of sinusoids capillaries are separate by a space in which appear some osteoblasts. The chondroclastes are localized in vascular new formed "bight". The primordial non-lamellar trabecules are bordered by picofuxinofile collagen bands and by epithelial cells edge (Figures 2 and 3).

Genesis of lamellar trabecular structures

We considered that lamellar trabecular structures are the structures that precede non-lamellar elements and that go to a differentiation process in the presence of induction effect of mesenchymal osteoblast and osteoclast generator structure. The same mesenchyma is the generator of the hematoformation elements and it is osteoclastogenerator.

Series sections using van Gieson method and HE staining show to us in the 1/3 upper extremity of femur diaphisis bone, a process of volume growth in non-lamellar structures, due to presence of two process: osteoblastogenesis and collagenogenesis.

Environment of osteoblasts is rich in collagen material that goes in side of non-trabecular structures and so involves the development of plurilamellars structures (Figures 4–6).

On the sections provided from the superior epiphysis of femur fetus bone, we can observe the morphogenesis of the lamellar type of the trabecular structures, and osteoblasts localized on the endomedullar surface of the trabecul, which are face to face with thick collagen bands. Inside of each trabecul can be remark easily the presence of collagen lamellas punctuates with osteocytes (Figure 4).

The phase of the lamellar trabeculars forming evolution suppose the early differentiation of the hematoforming marrow structures, and topographic rapports establish with the lamellar trabecular structures, and also apparition of collagen layers and osteocytes in the area of the trabecular structures (Figure 5).

In the same sections it can observe the differentiation of the osteocytes, collagen stratification in the lamellar trabecular structures (Figure 6).

Consolidate of rapports between lamellar trabecular structures and hematogenerator structures

Using Gömöri method for a good observation of reticulins fibbers, we observed that in 1/3 lower femur epiphysis, exist some changes of lamellar trabecular structures near the contact side with hematogenerator structures. At this place the surface of trabeculars are not continuous (Figures 7 and 8).

We can observe islets of hematoformator marrow which contains reticulinic fibers in phase of differentiation, and non-differentiated cells of the blood series. Neighborhood the multilamellar trabeculs we observe the existence of reticulinic fibers wisps which are parallel between themselves, and in continuity rapports with the hematoforming structures (Figure 7).

In immediate vicinity of the bone trabeculs bordered by osteoblasts we observe medullar sinusoids dilated and fill with erythrocytes. Also we observed the marrow bone cells at the ring of trabeculars structures (Figure 8).

Microanatomical genesis analyze of rapports between osteo- and hematopoetic structures al coast number VII

On the series sections that were made at coast number VII we can identify cellular population from trabecular and hematogenerator structures. Here are strong relation between two systems, and also sides with angiogenesis processes in areas that are into the lamellar trabecular elements (Figure 9).

Discussions

Considerations regards genesis of bone structures

The bone is the place where two kinds of structures: osteo- and hematogenerator, can growth and can go to the way of differentiation. Relation between these two structures was made by the studies that showed the co-operation between osteoblast and osteoclast, first from mesenchymal origin and second from hematopoetic system.

Problems of osteogenesis are the questions for many studies. Intramembranous osteogenesis is the first moment and going on in all ontogenesis phases. It begins in extra cell matrix where the development of osteogenesis centre is around the vessels and also said that osteogenic cells can produce an angiogenic factor that can increase and activate the vascular invasion.

In our study, we showed that exist a relation between osteogenic cells and vascular system, because osteoblasts cells are near the blood vessels and non-lamellar trabeculars can be observed between mesenchymal cells.

So, the osteoblasts will go to differentiation process from mesenchymal cells. Lamellar trabeculars should be develop in right angles and result the areols in which the osteoblasts will be including in cell matrix and became osteocyte. Also, the mesenchyma that are on the bone surface has osteogenic proprieties during ontogenesis phases, and participate to produce the fibrovascular periost.

That is in relation with the studies of [1] that presented subperiostal aposition. Endochondral osteogenesis is present frequently in long bone but even here the first step is intramembranous process. The important aspect of this process is that the compact structure of diaphisis is from periost and so we can say that the intramembranous process is the origin.

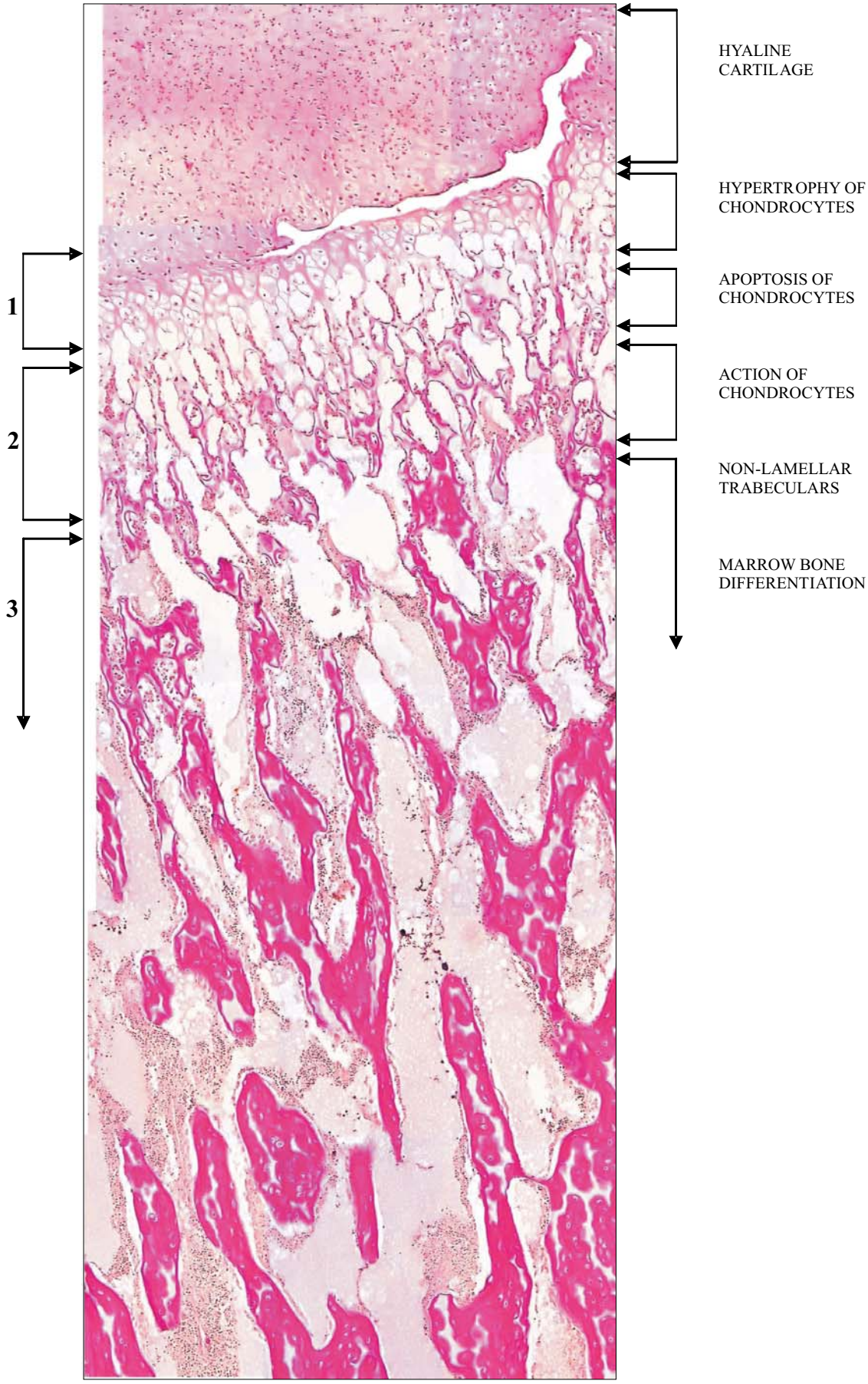

Figure 1 – ***Upper extremity of femur bone, frontal section. Remark of spatial disposition and differentiation of non-lamellar trabeculars during genesis: 1. Hypertrophy and apoptosis of chondrocytes; 2. Angiogenesis and chondrolyses; 3. Genesis of non-lamellar trabeculars; 4. Marrow bone differentiation. Van Gieson staining. Reconstruction using sequential images by ob. ×10***

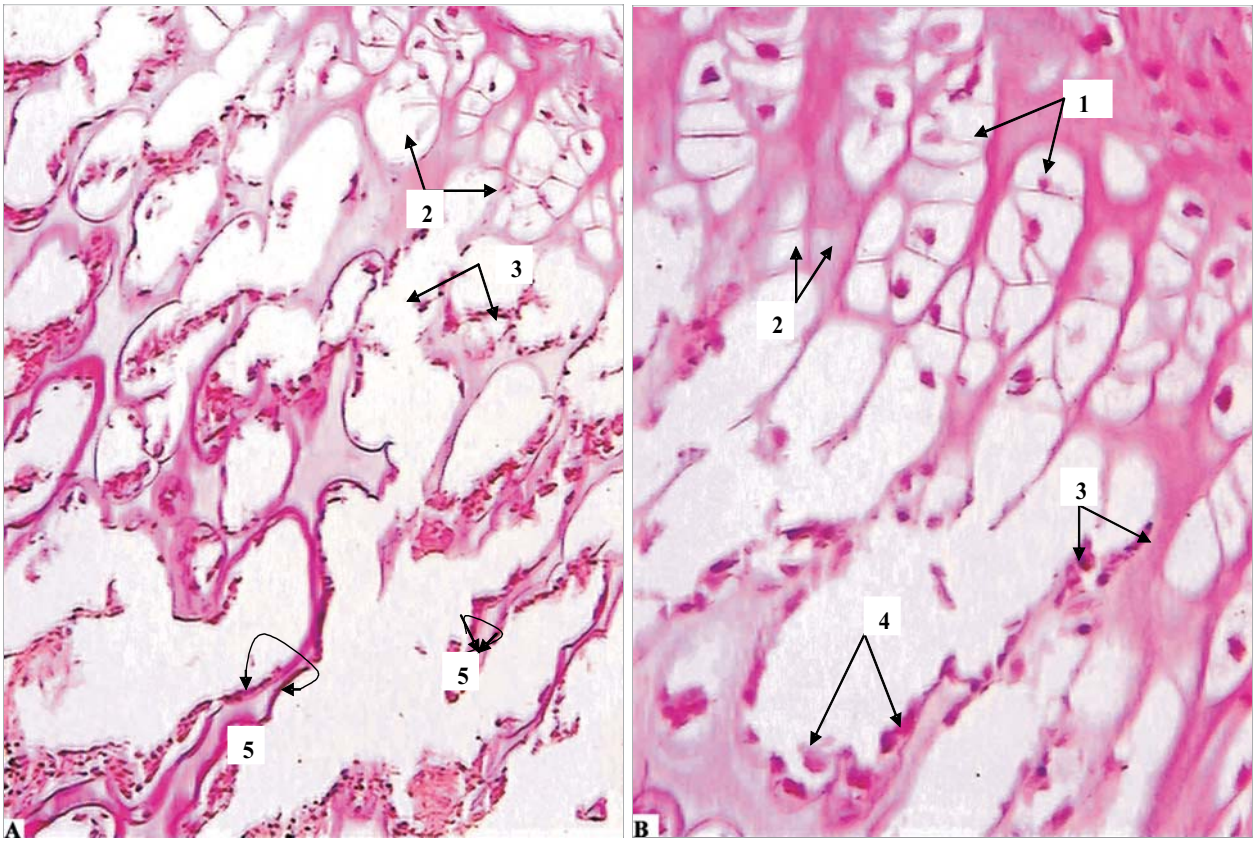

Figure 2 – ***Differentiation of blood vessels in the neighbor of the side where the change of cartilage is in the same time with chondrogenesis near the vascular endothelium. 1. Hypertrophic chondrocytes; 2.Chondrolyses; 3. Blood vessels endothelium 4. Chondroclasts (Van Gieson staining; oc. ×7; ob. ×20; ob. ×140)***

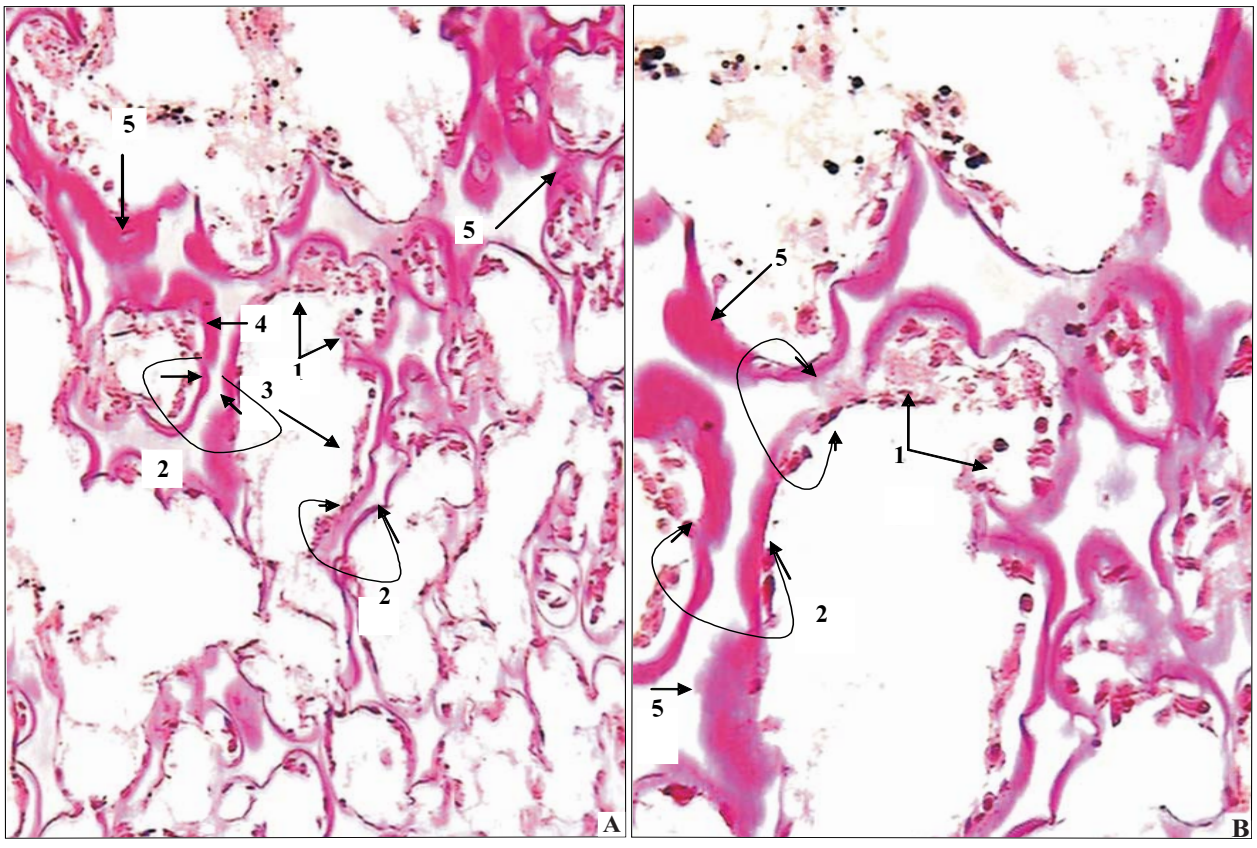

Figure 3 – ***Chondroclasts in the new vascular golfs. Non-lamellar trabeculars have around collagen fibers and epithelial cells. 1. Chondroclasts; 2. Non-lamellar trabeculars; 3. Epithelial cells in the space of vascular walls; 4. Collagen ; 5. Osteoblasts (Van Gieson staining; oc. ×7; ob. ×10; ob. ×70 – A)***

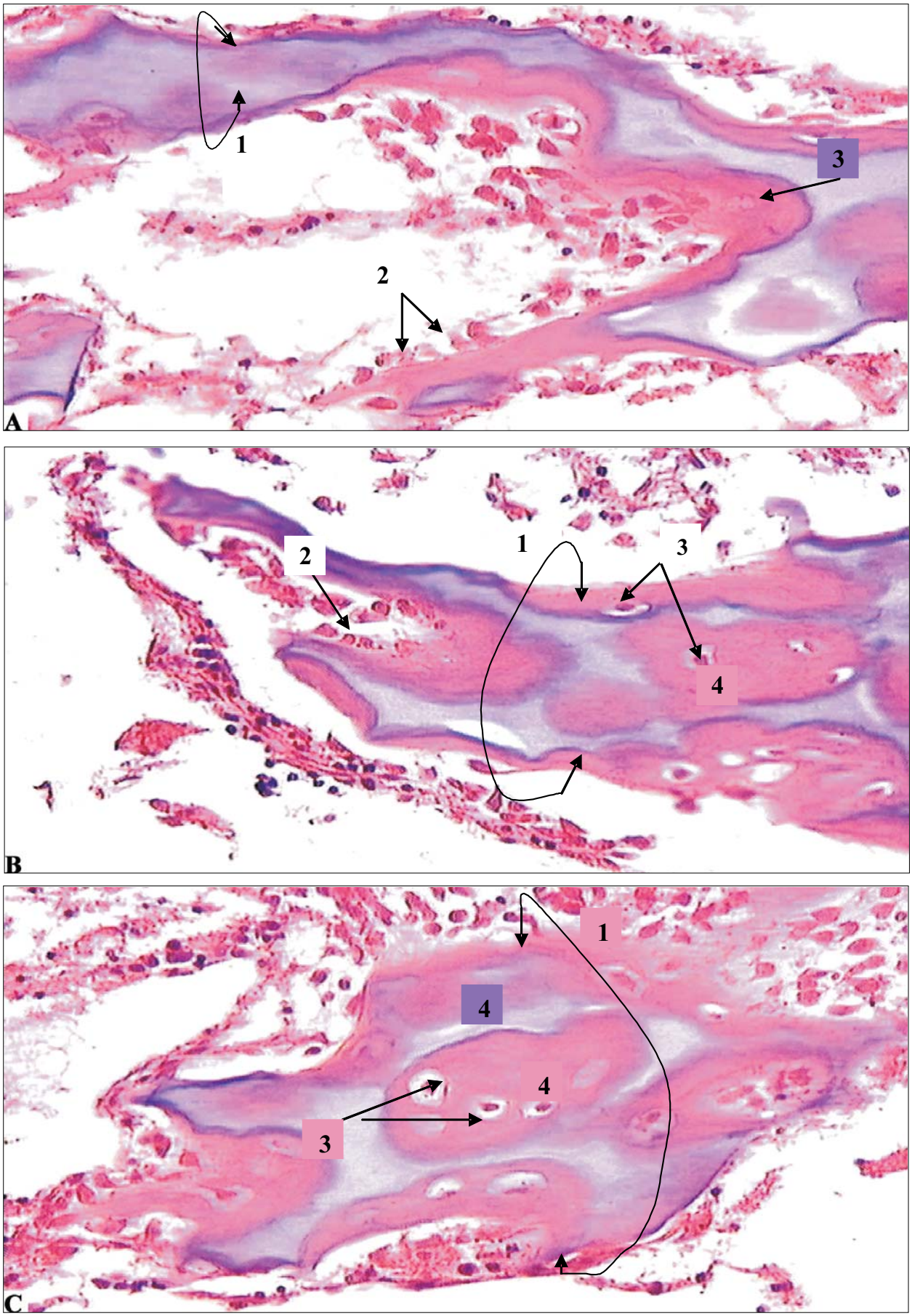

Figure 4 – ***Morphogenesis of lamellar trabeculars structures in upper extremity of femur bone. Osteoblasts that are at the surface of trabeculars structures and give rapports with bigger collagen fibers. Also we remark osteocytes. 1. Lamellar trabeculars structures during differentiation process; 2. Osteoblasts; 3. Osteocytes; 4. Extracellular matrix. (HE staining; oc. ×7; ob. ×20; ×140 – B)***

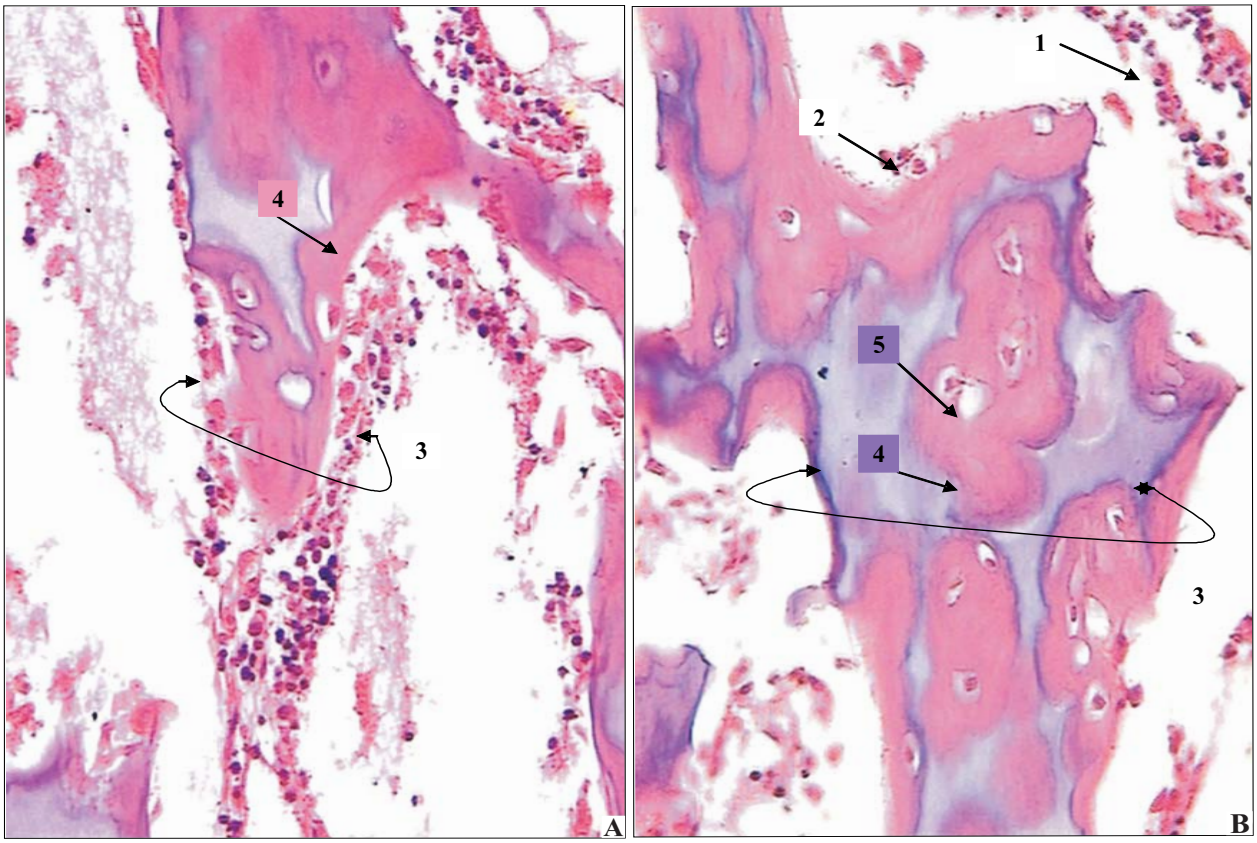

Figure 5 – ***Phases of development the lamellar trabeculars structures. Collagen fibers and osteocytes in the area of trabecular structures. 1. Marrow bone; 2. Osteoblasts around the trabecular structures; 3. Lamellar structures during differentiation process; 4. Collagen; 5. Osteocytes (HE staining; oc. ×7; ob. ×20; ob. ×140 – A, B)***

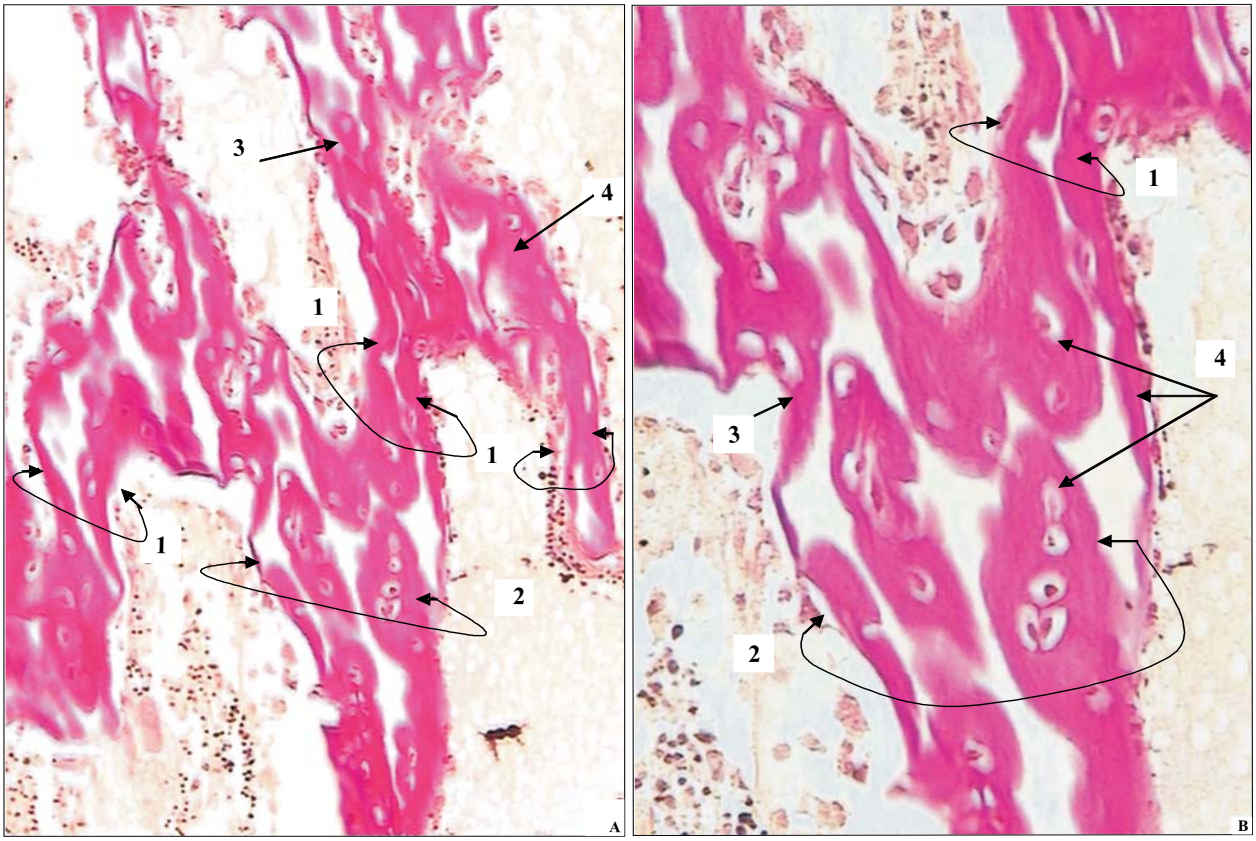

Figure 6 – ***Differentiation of osteocytes and collagen in lamellar trabecular structures at upper extremity of femur bone. 1. Bilamellar trabecular structures; 2. Trilamellar trabecular structures; 3. Osteocytes; 4. Collagen (Van Gieson staining; oc. ×7; ob. ×20; ob. ×140)***

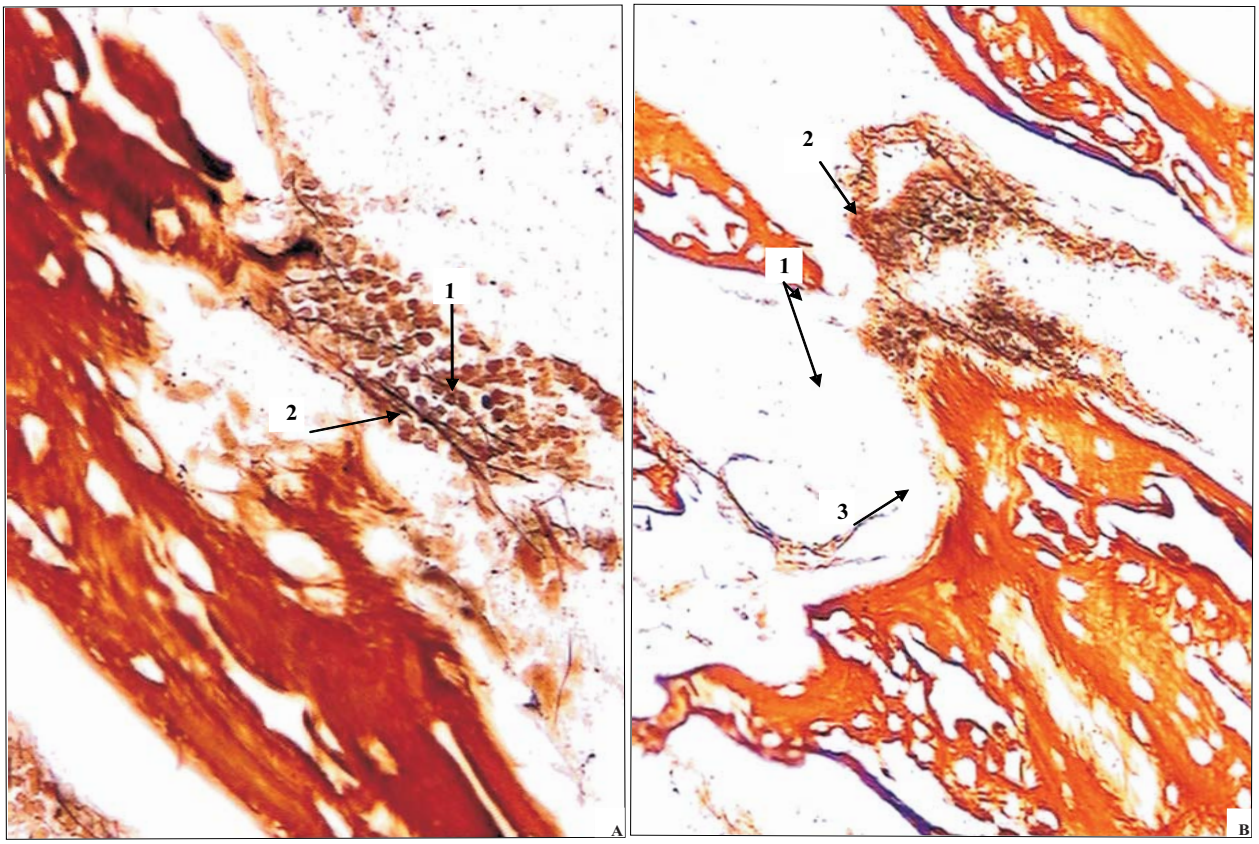

Figure 7 – ***Islands of haematoformator marrow that contain reticulin fibers during differentiation and non-differentiation cells of blood counts. In the neighbor of multilamellar trabecular structures we observed reticulin fibers that give rapports with haematoformator structure. 1. Haematoformator marrow; 2. Reticulin fibers; 3. Group of reticulin fibers (Gömöri staining; oc. ×7; ob. ×10; ob. ×70)***

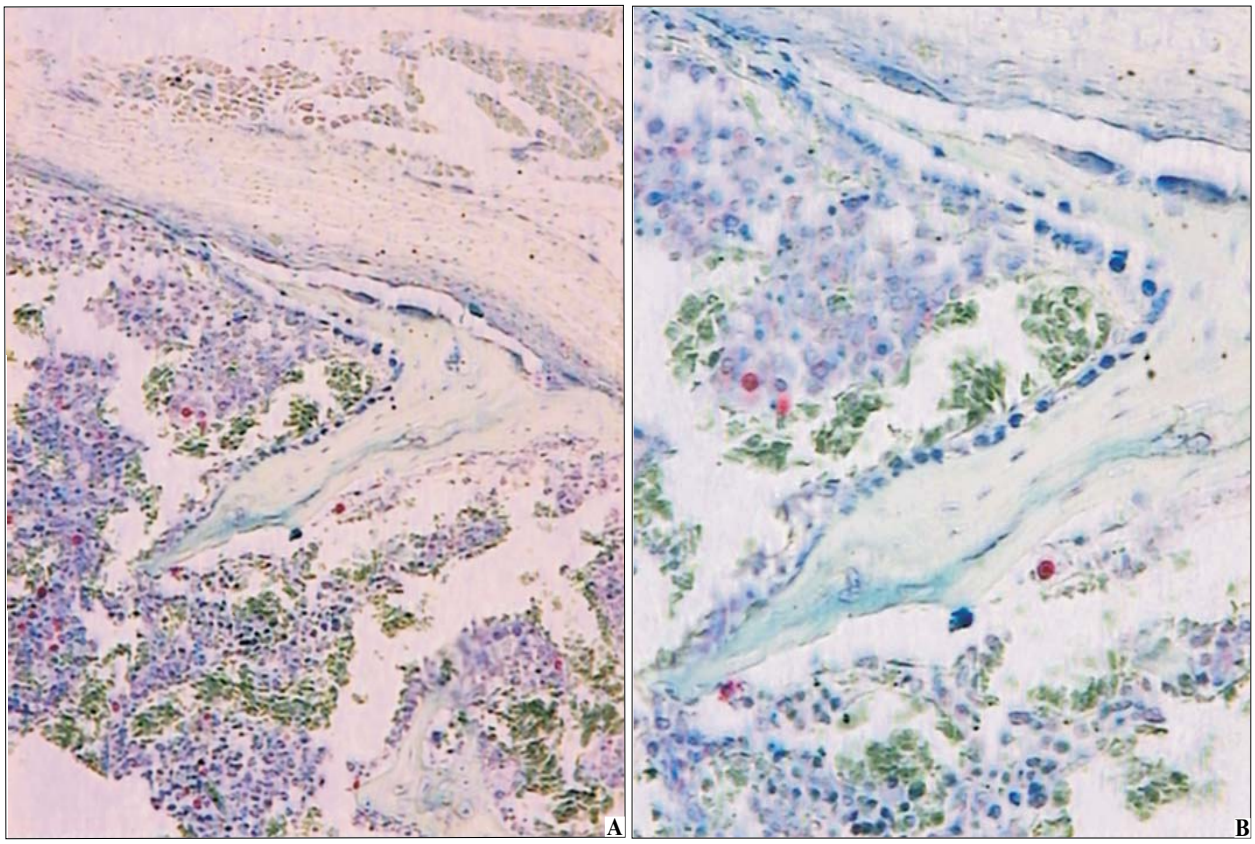

Figure 8 – ***Enlargements and dilatations of sinusoides at marrow level and the presence of red counts near the bone trabecular structures that have around osteoblast cells (H. Mac Mannus staining; ob. ×20; ob. ×140)***

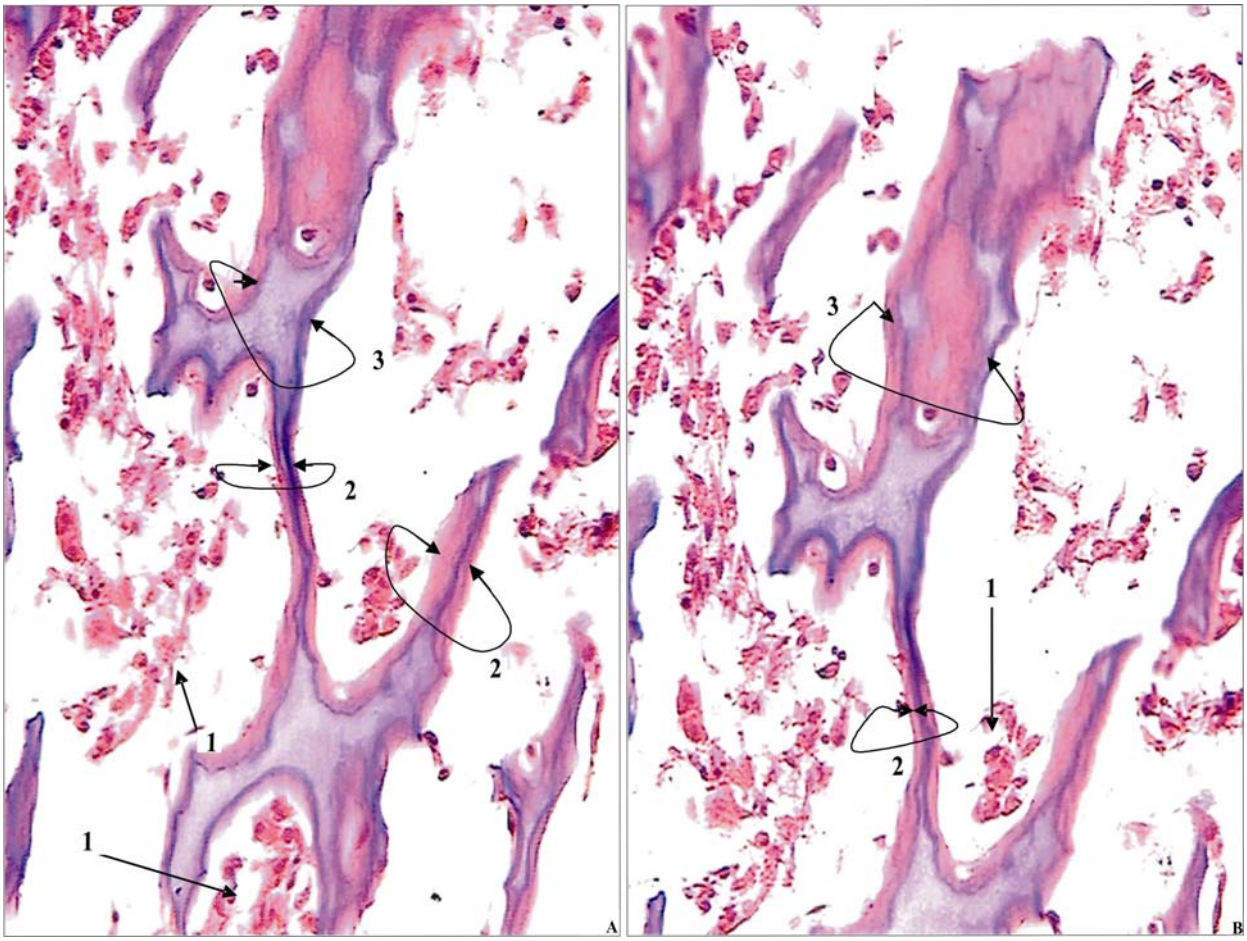

Figure 9 – ***In the same microscope field exist two phenomenons: chondrolyses and genesis of non-lamellar trabecular structures. 1. Chondroclasts; 2. First non-lamellar trabecular structures; 3. Second non-lamellar trabecular structures that include extracell matrix; 4.Osteoblasts (HE staining; oc. ×7; ob. ×20; ob. ×140)***

We must to relieve the role of extracellular matrix in produce of osteoprogenitors cells. That will be place in new bone tissue for participate to lamellar trabecular structures (Figure 3).

We must to relive also that real endochondral osteogenesis is only in cartilaginous models. This is in concordance with some studies that present "periostal reactions" during pathologic process at immature bone.

Considerations regard biomorphology of osteoclasts and osteoblasts

Osteoclast is a cell that comes from monocyte populations, in the presence of cytokine like ODP (RANK/OPGL/RANKL) from TNF alpha family. Osteoblasts are the cells from marrow bone stroma and they are important for osteoclast differentiation, in the present of stimulating factor M-CSF. Also is important the receptor that is on osteoclast surface [2–5].

During all life the process of bone remodeling is a cycle. This process is developing in focal units name basal multicell units (BMU) during 3–4 months [6, 7].

This process has four phases activation, resorption, change (reversal phase) and formation. The question is how macrophage can became osteoclast ?

The possible answer is that the presence of receptor for RANK. This receptor increases its activity during lymphocyte T (ly T) activation. But also the factors that involve the appearance of RANK receptor come from stromal cells. So we can say that osteoblast and stromal cells are important in osteoclasogenesis, because they participate to produce RANK receptor.

Co-operation between osteoclast and osteoblast

Relation between these two cells is regard produce of RANK/RANKL receptor [3]. That means a contact cell-cell and the presence of two proteins at cell membrane: RANK/RANKL that is activating by some factors and goes to differentiation of stromal cell to osteoclast.

The most important protein is OPG (osteoprotegerine) [8], which is a glycoprotein that can reduce osteoclastogenesis and so decrease the bone loses.

Interactions between immune system cell and bone

The first interaction exists if we think that they have the same precursor cells. We observed ly T and ly B and their role in modulation of bone metabolism. T-lymphocytes can stimulate or inhibit osteoclasto-genesis, but we showed that if ly T go out, is possible the process of differentiation of osteoclasts *in vitro.*

B-lymphocytes are more binding with bone cells and their precursors could induce functional osteoclast. Also, cytokine system RANK/RANKL/OPG has been discovered in both systems-bone and immune system [4].

This relationship between these systems has been showed by studies that describe the cells differentiation using the same cells support [9, 10].

Much more ly T can produce RANKL and macrophage colony stimulating factor (M–CSF) that are very important for osteoclast differentiation and activate the hematopoetic precursors of osteoclast [11].

Also, some studies said about negative role of ly T in osteoclastogenesis because of INF γ production. Born of ly B in marrow bone can show to us that linear osteoblsts cells at endostum level of bone and so we believe that stromal cells can produce some factors that increase ly B precursors. Regards these aspects we must to think to a molecule of vascular cell adhesive VCAM-like [12].

Angiogenesis and lamellar trabecular structures

Hypertrophic chondrocytes can produce vascular endothelial growth factor (VEGF). For these reason we can say that growth process of bone depend on vascular invasion, under the control of VEGF, and so is clearly that chondrogenesis is in connection with osteogenesis. Angiogenic factors by the fibroblastic growth factor family participate in the growth process.

Answers at the preliminaries questions

- There are specialized cells populations at the boundary bone-marrow, provided from the marrow;
- This cells activity depends on factors of the hemathopoethic medium [13];
- The cells of this zone presents RANK (TRANCE) receptors, which activated by specific factors befriend osteoclasts forming;
- In bone-marrow zone, there are envelope cells which interpose the connection between marrow cells, monocit-macrophag series and osteoclast forming;
- In the same time, at this zone level we observed an absence of the bone resorption process, in agree with Miller J [14];
- In this study, we observed the presence of the osteoclasts in the osteogenesis focus ring, before the tabular bone formation, which change the order of the fundamental structures of the bone which begun with osteoblasts;
- We observed the presence of the envelope cells also, at the periostal zone of the bone with straiten cavity, where favor the resorption bone process;
- The relationship angiogenesis–bone tissue shaping is proved by an intense angiogenesis process which is in process at the bone–marrow border;
- We observed, also the tabular bone shaping around the angiogenesis focus ring;
- The communication mechanisms between the bone cells and the hematoforming cells are realized through cells attached molecules.

Conclusions

Rapports between osteo- and hematopoetic structures are heterogenic and are involving in growth, differentiation and remodeling of immune, bone system and hematopoetic system.

In the osteomedular system is a morphogenic synergism, in which the connections between osteogenesis and angiogenesis could be developed.

We can begin to understand new aspects of organize these systems.

Disappear of trabecular structures at femur bone are made on large surface due to action of osteoclasts. We observed between trabecular structures and marrow bone clearly space.

At spongious bone like coast (in our study) we observed that the process of trabecular borne is possible due to mesenchymal contribution and osteoblasts action. Rapports between marrow bone and trabecular structures will be possible due to “cover cells”.

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