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Research Article

White Blood Cells Can Predict the Level of Osteoclasts in Patients with Femur Fracture

Yonatan Crispel[1,2*], Maytal Schwartzman[2], Rami Sasson[2], Eli Peled[2,3]

[1]Department of Hematology, Thrombosis and Hemostasis Unit, Rambam Health Care Campus, Haifa, Israel

[2]Rappaport Family Faculty of Medicine, Technion, Israel Institute of Technology, Haifa, Israel

[3]Orthopedic Division, Rambam Health Care Campus, Haifa, Israel

***Corresponding Author:** Yonatan Crispel, Department of Hematology, Hematological Institute, Rambam Health Care Campus, Hospital of Haifa, Haifa, Israel Rambam Medical Center, POB 9602, Haifa 31096, Israel

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Abstract

Background: Bone is a mineralized and elastic connective tissue which consist of four types of cells: osteoblasts, bone lining cells, osteocytes, and osteoclasts. Bone tissue is an active organ that undergoes continuous remodeling process in which it is resorbed by osteoclasts and newly formed by osteoblasts, a process, which is necessary for fracture healing and skeleton adaptation to mechanical use, as well as for calcium homeostasis. Osteoclasts are differentiated from precursors of the monocyte. It is already known that under chronic inflammatory conditions, an increase in inflammatory cytokine levels induces pathological osteoclast differentiation and initiating bone resorption, which can explain the relationship between osteoclast levels and white blood cells. **Methods:** 1. Histomorphometry: Histologic sections were prepared from 5-6 sites along the bones and stained with hematoxylin and eosin for quantitative evaluation of osteoclasts and osteoblast. Complete blood count was collected. **Result:** From the results significant negative correlation found between osteoclasts number and monocytes level (Figure 1). Significant Positive correlation found between the osteoclasts number and the leukocytes (granulocytes) level (Figure 2). **Conclusions:** The correlation found between the monocytes blood level to osteoclasts number was statistically significant, therefore may indicate that the activity of macrophages and osteoclasts in the bone can be predicted by the monocytes and granulocytes level in the blood. This can lay the foundation for using a simple blood test as an indication for bone resorption

Keywords: Osteoblasts; Bone Lining Cells; Osteocytes; Osteoclasts; Chronic Inflammatory Conditions.

Introduction

Bone is a mineralized and elastic connective tissue which consist of four types of cells: osteoblasts, bone lining cells, osteocytes, and osteoclasts. The bone has important functions in the body, such as locomotion, mechanical support to the muscles and joints, protection of vital organs, storage of minerals and harboring of bone marrow. The bone tissue is an active organ that undergoes continuous remodeling process in which it is resorbed by osteoclasts and newly formed by osteoblasts, a process, which is necessary for fracture healing and skeleton adaptation to mechanical use, as well as for calcium homeostasis. Osteocytes are also involved in this bone remodeling and act as mechanosensors of this process. The function of the bone lining cells is not well clear, but they seem to play an important role in coupling bone resorption to bone formation [1]. The synthesis of bone matrix by osteoblasts occurs in two main steps: In the first step, the osteoblasts secrete collagen proteins, which form the organic matrix. Thereafter, mineralization of bone matrix takes place. The mature osteoblasts can undergo apoptosis or become osteocytes or bone lining cells [1]. Osteocytes, which comprise 90–95% of the total bone cells, have a lifespan of up to 25 years. Bone remodeling cycle can be divided into three main phases: (1) bone resorption by osteoclasts, (2) transition from resorption to new bone formation, and (3) bone formation by osteoblasts. This process requires coordination of osteoclasts, osteoblasts, osteocytes, and bone

lining cells. Moreover, it depends on the action of several local and systemic factors including hormones, cytokines, chemokines, and biomechanical stimulation. Under normal remodeling, osteoclasts are differentiated from precursors of the monocyte/macrophage lineage originated from bone marrow HSCs. However, it is already known that under chronic inflammatory conditions, an increase in inflammatory cytokine levels induces pathological osteoclast differentiation and initiating bone resorption. TNFα (tumors necrosis factor) is critical for the generation of osteoclasts from human monocytes has various implications since TNFα synthesis in monocytes is also induced by pro-inflammatory cytokines or by bacterial products. Several researches had shown that stimulating bone marrow monocytes with combination of TNFα and interleukin 6 (IL-6) can induce differentiation of osteoclasts. Hence bacterial infections induce TNFα synthesis which lead to bone resorption [3-5].

Research Objective

1. Study the relations and correlations between inflammation and infection to osteoclastogenesis and bone loss. 2. Study the effect of inflammation and infection on osteoclasts and monocytes levels.

3. Study the correlation between osteoclasts level to calcium level in blood and bone density.

Material and Methods

The local Institutional Review Board approved the study before it began, and all participating patients signed an informed consent form. Surgeons from the orthopedic department at Rambam hospital took bones from the thigh. After sawing the femurs and soaking them in acid, the tissues were trimmed. The prepared cassettes were cut with a microtome, and the slides were stained with H&E (Hematoxylin & Eosin). This process was performed in the pathology department at Rambam hospital. To count the number of osteoclasts, we used an Image Analyzer mounted on a light microscope and connected to a computer. Histomorphometry: Histologic sections were prepared from 5-6 sites along the bones, fixed in 0.05 mmol/L saline phosphate buffer containing 4% formaldehyde. Paraffin-embedded specimens were longitudinally sectioned (6 μm), and stained with hematoxylin and eosin for quantitative evaluation of osteoclasts and osteoblast [7]. An Image analyzer (Trichip RGB video camera; Sony, Tokyo, Japan) installed on a light microscope (Zeiss, Jena, Germany) and attached to a computer equipped with a frame grabber was used to analyze the extension of positively stained tissue [8,9]. Images were captured, digitized, and displayed on a high-resolution colored monitor. The ten most intensely stained fields were analyzed at a power lens of 40×10. Images were loaded on screen buffers having a resolution of 760×570 pixels and measured in standardized frames (62993 μm2). Patients' blood tests results were collected. We made correlation between the number of osteoclasts we counted from the samples and the results of blood tests (biochemistry and hematology). Statistical Analysis: Physiological data was analyzed by using SAS 6.12, utilizing two-way ANOVA. Histomorphometry data was analyzed using SPSS 6.0 (Chicago, IL). Comparison between cases use student T-test, P values < 0.05 were considered significant.

Results

To test the correlation between infection and inflammatory conditions to differentiation of monocytes to bone-resorbing osteoclasts, we compared the levels of monocytes and leukocytes to osteoclasts number in people who faced fatal femur fracture and went under hip replacement surgery. All of them were diagnosed with an inflammation process. We assume that the inflammation either caused the fracture or was a result of it. The images were divided into 4 groups based on patients' age. The number of osteoclasts were counted on each image. The osteoclasts found in the images are pointed with arrows. Selected images are shown in (figures 1A - 4D). We checked the correlations between osteoclasts number counted on the images to monocytes (macrophages), leukocytes (granulocytes) and calcium levels from blood tests. The results average of each age group is shown in (Table 1).

Average Osteoclast	WBC	Calcium mg/dl	Monocytes X10^3
19.33	14	8.7	6.1
20	15	8.7	6
19.79	14.2	8.1	6.18
25	15.5	8.0	5.8

Table 1: Osteoclast Compared to Blood Sample

The ratio of osteoclasts to monocytes (Figure 1): demonstrate negative relationship between macrophages and osteoclasts: the higher the level of osteoclasts, the lower the level of macrophages, significantly p<0.005 SD = 2.66.

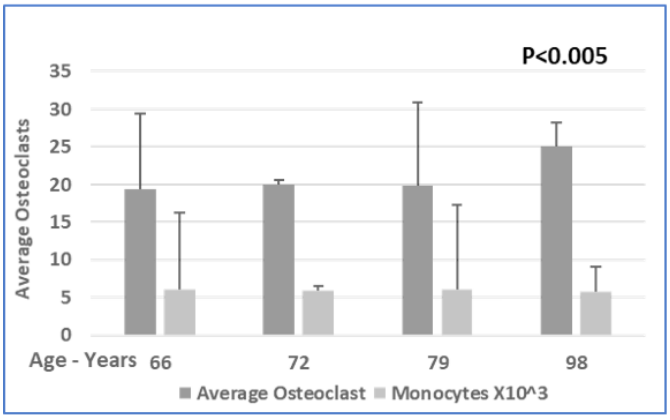

Figure 1: The ratio of osteoclasts to monocytes

Osteoclasts compared WBC granulocytes (Figure 2): demonstrate a positive correlation between the level of osteoclasts and the level of granulocytes. The higher the level of osteoclasts, the higher the level of granulocytes p<0.001 SD = 0.699.

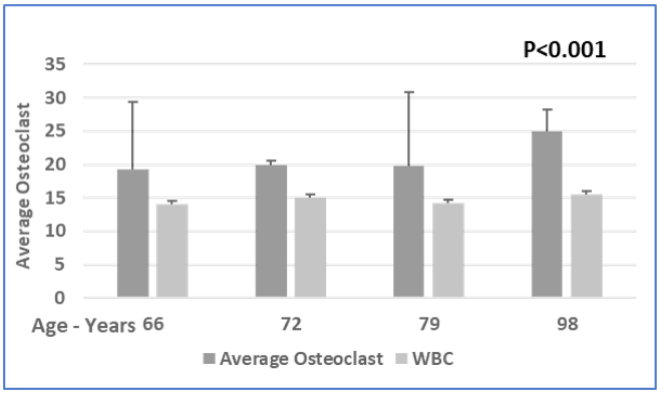

Figure 2: The ratio of Osteoclasts to WBC. G

Osteoclasts compared Calcium (Figure 3): Blood calcium levels were at the lower limit of normal and slightly below normal in all patients regardless of age. Osteoclast levels were significantly higher than calcium levels p<0.005 SD = 0.377.

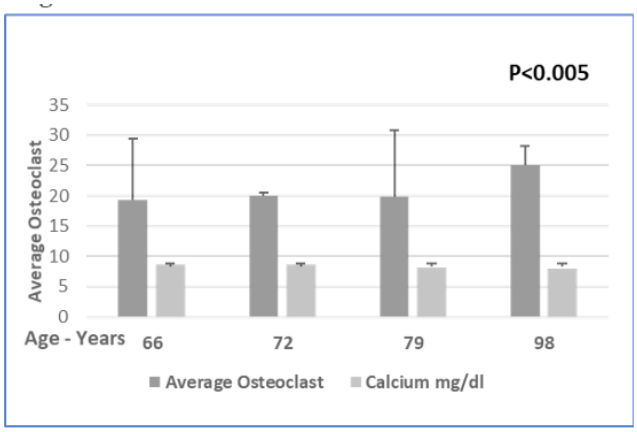

Figure 3: The ratio of Osteoclasts to Calcium

(Figure 4): demonstrate general blood samples Compered to Osteoclast (Table 1). A significant positive correlation was found between osteoclast levels and age; as age increased, osteoclast levels also increased p<0.005. A significant negative correlation reveled between age and calcium level as age increases, calcium levels decrease p<0.005.

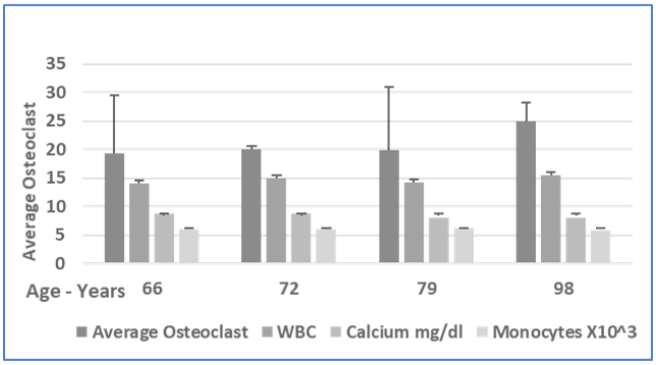

Figure 4: The ratio of Osteoclasts to Osteoclast

Discussion

In this study, the results show that osteoclast levels observed in patients with hip fracture can be predicted through a peripheral blood test. A significant negative correlation was found between osteoclast levels and the level of non-granulated white blood cells, mainly monocytes (macrophages) (Figure 1).

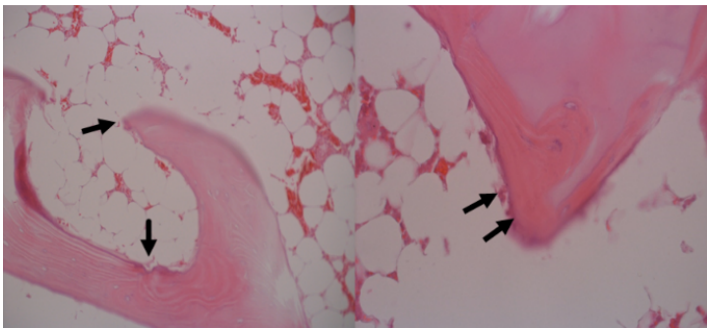

Figure 1: Histologial section of femur, A. Age of the patient's is-66 years

In addition, it was found that the non-granulated white cells, mainly neutrophils, were in a significant positive correlation with the level of osteoclasts, which were observed in the hip joint in patients after femur fracture of various ages (Figure 2).

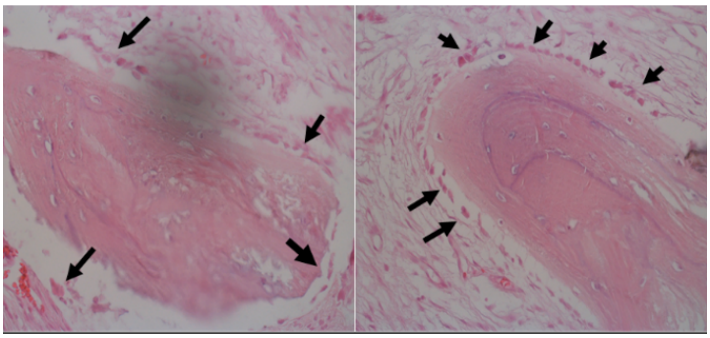

Figure 2: Histologial section of femur, B. Age of the patient's is-72 years

We also found that blood calcium levels were low in all patients who underwent surgery for a hip fracture. Despite the expectation that an increase in osteoclasts would increase calcium levels, there was still a significant negative correlation between osteoclast levels and calcium levels (Figure 3).

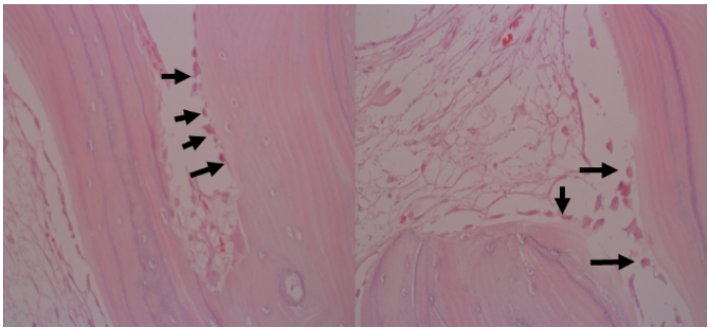

Figure 3: Histologial section of femur, C. Age of the patient's is-79 years

A significant positive correlation was found between osteoclast levels and age; as age increased, osteoclast levels also increased (Figure 4). A significant negative correlation reveled between age and calcium level as age increases, calcium levels decrease (Table 1).

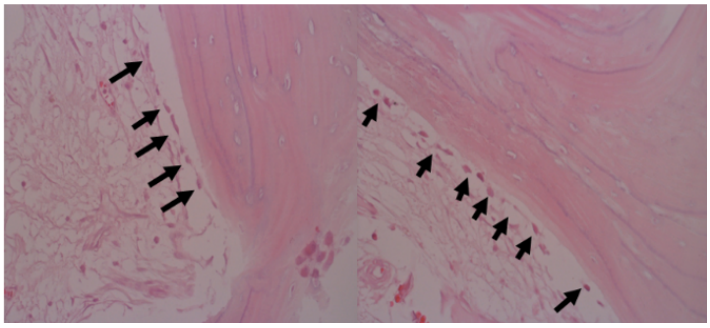

Figure 4: Histologial section of femur, D. Age of the patient's is-98 years

Studies that discuss the construction and remodeling of bone show very broad control of hormonal and molecular processes that act on bone tissue. The formation, proliferation, differentiation, and activity of the bone tissue cells are controlled by local and systemic factors. The local factors include autocrine and paracrine molecules such as growth factors, cytokines, and prostaglandins produced by the bone cells besides factors of the bone matrix that are released during bone resorption. The systemic factors which are important to the bone homeostasis include parathyroid hormone (PTH), calcitonin, 1, 25-dihydroxyvitamin D3 (calcitriol), glucocorticoids, androgens, and estrogens. PTH interact with receptors on osteoblasts and on certain stromal cells that produce the osteoclastogenic factor RANKL. (RANKL is a crucial factor for osteoclastogenesis. When it binds to its receptor RANK in osteoclast precursors, osteoclast formation is induced). Estrogen plays crucial roles for bone tissue homeostasis; the decrease in estrogen level at menopause is the main cause of bone loss and osteoporosis. The mechanisms by which estrogen act on bone tissue are still studied. RANKL is also expressed by activated T lymphocytes. These cells can directly trigger osteoclastogenesis and are probably pivotal to the joint destruction seen in rheumatoid arthritis [1]. Under normal remodeling, osteoclasts are differentiated from precursors of the monocyte/macrophage lineage originated from bone marrow HSCs. However, it is already known that under chronic inflammatory conditions, an increase in inflammatory cytokine levels induces pathological osteoclast differentiation and initiating bone resorption. The signaling induced by many of the inflammatory cytokines produced during infection, including tumors necrosis factor (TNFα), and interleukins, share much in common with RANKL. These factors enhance the osteoclasts differentiation in the presence of RANKL. The most intensively studied factor is TNFα, which has potent synergistic effects with RANKL. The fact that TNFα is critical for the generation of osteoclasts from human monocytes has various implications since TNFα synthesis in monocytes is also induced by pro-inflammatory cytokines or by bacterial products. Several researches had shown that stimulating bone marrow monocytes with combination of TNFα and interleukin 6 (IL-6) can induce differentiation of osteoclasts. Hence bacterial infections induce TNFα synthesis which lead to bone resorption [3-5]. Abnormal increase in osteoclast formation and activity leads to some bone diseases such as osteoporosis, where resorption exceeds formation causing decreased bone density and increased bone fractures of the hip joint with a high frequency in older people, but not only for instance in periodontitis, a disease of the periodontium caused by bacterial proliferation induces the migration of inflammatory cells. These cells produce chemical mediators such as IL-6 and RANKL that stimulate the migration of osteoclasts. Therefore, it can be assumed abnormal increased bone resorption occurs in the alveolar bone, contributing to the loss of the insertions of the

teeth and to the progression of periodontitis. As a result, it can be assumed that infections and inflammations in the bone cause an increase in white blood cells that stimulate the formation of osteoclasts, which increases bone resorption and reduces bone density and strength. This process increases the risk of fractures, especially in the femoral neck, where there is a load on a daily basis. We also demonstrated that when the osteoclast level is high, the monocyte level is low, this can indicate on negative feedback between them. We assume that the high level of osteoclasts causes an effect that decrease the monocytes numbers trying to downregulate the activated osteoclasts. Regardless the number of osteoclasts the calcium level in the blood remains always on the lower range or even slightly below the minimum normal level. This indicates that there is a separate mechanism that maintains the calcium level in the blood at a low level although calcium is release from the resorbed bone. In summary - the correlation found between the monocytes blood level to osteoclasts number was statistically significant, therefore may indicate that the activity of osteoclasts in the bone can be predicted by the monocytes and Granulocyte's level in the blood. This can lay the foundation for using a simple blood test as an indication for bone resorption. To support our findings, we recommend extending the research scope by [1] increasing the number of participants, [2] reviewing the patients' data of bone density. We also suggest analyzing the correlation between monocytes level, leukocyte level and bone density of a large database of random people, hypothesizing that high leukocyte (Granulocytes) together with low monocyte levels will go along with low bone density.

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