**UNIVERSITY OF MEDICINE AND PHARMACY OF**

**CRAIOVA**

**THE DOCTORAL SCHOOL**

## ABSTRACT

# STUDY OF OSTEOPOROSIS BY USING OF BIOMARKERS

PhD COORDINATOR:

Prof. univ. dr. Liliana NOVAC

PhD STUDENT:

PAVEL OANA ROXANA

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# CONTENT




**KEY WORDS:** osteoporosis, markers of bone turnover, fracture risk, DEXA

## INTRODUCTION:

Bone is a dynamic tissue which undergoes continuous remodeling through two opposing processes, new bone formation and old bone resorption,respectively. Under normal conditions, the formation and bone resorption are coupled, so that the amount of bone resorbed is equal to the amount of newly formed bone.

When degradation and bone formation are not balanced (when more bone is destroyed than formed) occurs the diminished of bone mass.

Consequently, osteoporosis (a disease whose name is derived from Latin and means "porous bone") is defined as a systemic disease of the skeleton characterized by: low bone mass, deterioration of the microarchitecture of bone tissue, increase the fragility of bone and exaggerating the risk of fracture.

Osteoporosis is generally recognized as an important public health problem due to significant morbidity and costs associated with her complications, namely hip, spine, forearm and other segments of the skeleton fractures. Osteoporosis is a disease completely asymptomatic until they lead to a fracture. In other words, its clinical manifestations are those that get complicated fractures.It is one of the few situations in medical pathology, in which case the disease begins with its main clinical complication and so this situation makes it difficult, but not impossible, early diagnosis.

Epidemiological studies show that at least half the people with osteoporotic fractures is from people with osteopenia (low bone density) which comprise a larger segment of the population than those with osteoporosis.

In everyday practice, there is an urgent need for clinicians to have a model that estimates the absolute risk of fracture patients because their decisions about whether to treat or not patients with osteoporotic drugs must rely on the effectiveness of these drugs, but also on the risk of fracture.

In all instances of estrogen deficiency appears to increase bone remodeling, resulting alteration of bone microarchitecture and bone loss. This is more evident after menopause.

Dosage of makers of bone turnover is useful in demonstrating the dynamic bone metabolism.

Serial dosages of bone markers can be used to monitor response to treatment.

Clinical studies have shown the correlation of the markers of bone formation and resorption with the rate of bone loss.

Bone markers can be regarded as a risk factor for fast bone loss in postmenopausal women.

## CURRENT STATE OF KNOWLEDGE:

**Chapter I -** ***"The epidemiological standards for screening of osteoporosis"*** shows the definition and classification of osteoporosis, recent data about the incidence and screening of osteoporosis as well as its impact on quality of life.

**Chapter II -** ***"The pathogenesis of osteoporosis"*** review the structure and bone formation, the main pathogenic mechanisms of osteoporosis and the main factors involved in osteoporosis.

**Chapter III -** ***"Biochemical markers of bone turnover"*** presents data on markers of bone formation and resorption, the main factors involved in their biovariability, and recent data about the clinical aspects related to bone markers.

**Chapter IV** ***"Diagnosis and treatment of osteoporosis"*** reviews the main methods of screening and diagnosis of osteoporosis,the assessment of osteoporotic fragility fracture risk, and recent data about the therapeutic means used.

## PERSONAL CONTRIBUTIONS:

### Chapter I. AIMS AND OBJECTIVES OF THE STUDY

**The aim** of this study is to evaluate the role of markers of bone turnover in the diagnosis of osteoporosis, and their involvement in monitoring the effectiveness of antiosteoporotic treatment.

**Specific objectives** that we intend to achieve through this research are:

- Evaluation of markers of bone turnover in patients under study
- Evaluation of bone mineral density ,T-score and the risk of fracture in patients with postmenopausal osteoporosis
- Histopathological evaluation of changes in bone turnover associated with postmenopausal osteoporosis
- Evaluation of correlations between serum markers of bone turnover and age, BMD, T-score,and initial fracture risk
- Evaluation of variation in serum levels of markers of bone turnover after 6 months / 1 year of antiosteoporotic treatment in the patients studied

- Evaluation of correlation between age, menopausal age, number of years since menopause and changes in bone markers at 6 and 12 months of treatment
- Evaluation variation of T-score, BMD and fracture risk at 1 year of antiosteoporotic treatment
- Evaluation of correlations between the change in BMD, T-score, risk for fractures and changes in bone markers after 12 months of treatment
- Evaluation of bone markers, BMD, T-score, risk for fracture, differentiated according to the treatment used, to 12 months of antiosteoporotic treatment
- Evaluation of correlations between initial serum levels of bone markers and answer of BMD ,T-score and risk of fracture to antiosteoporotic treatment
- Evaluation of correlation between the variation in BMD, T-score, risk for fractures and changes in bone markers after 12 months of antiosteoporotic treatment.

## Chapter II. MATERIALS AND METHODS

The cases studied were selected by ambulatory of Endocrinology and hospitalized in Endocrinology Clinic of Emergency County Hospital of Craiova in the period 2011- 2015.

During this period mentioned, patients were entered in the study by signing an informed consent, subject to an investigation protocol setting quantifiable set of parameters, specific tracking of cases.

For etiologic diagnosis were used **clinical** (history, physical examination) and **laboratory criteria**.

Research **bone metabolism** was performed by studying the biochemical markers of bone turnover: serum osteocalcin and CrossLaps, which were assessed in the laboratory of biochemistry of County Hospital Emergency Craiova, on serum taken from patients hospitalized in the Endocrinology Clinic of Craiova during National Osteoporosis Program.

Measurement of plasma levels of osteocalcin was realisated by electro-chemiluminescence immunoassay(ECLIA).

Evaluation of **bone mineral density** in the detection of osteoporosis was performed by dual-energy X-ray absorptiometry(DEXA) ,who is considered the "gold standard" for diagnosis of osteoporosis.

The diagnosis of osteopenia and osteoporosis is determined by the criteria recommended by the World Health Organization on the basis of T score:

- Normal BMD: T-score ± 1DS;
- Osteopenia: T-score between -1 and -2,5DS;

- Osteoporosis: T-score under -2,5DS;
- Severe osteoporosis: T-score under -2,5DS in the presence of a fracture.

Assessment of **fracture risk** was done by the method FRAX.

**Histopathological study** was carried out on fragments of bone harvested after surgery on head and femoral neck specially treated and stained with hematoxylin- eosin and Goldner tricromic green light Szekely.

**Data processing** were used Microsoft Excel (Microsoft Corp., Redmond, WA, USA) with XLSTAT suite for MS Excel (Addinsoft SARL, Paris, France) and IBM SPSS Statistics 20.0 software (IBM Corporation, Armonk, NY , DOOR).

Parameters measured for subjects in this study were stored in Excel files.

Secondary processing of data, calculating fundamental statistical parameters, mean and standard deviation, of their report - the coefficient of variation and their graphic representation was made with Excel by using controls Pivot Tables, Functions, Statistical, Chart and Data Analysis Module. To achieve data normality tests (Shapiro-Wilks and Anderson-Darling) and Student test and ANOVA were used XLSTAT command module.

## Chapter III. RESULTS

### *CHARACTERISTICS OF THE STUDY GROUP*

Patients were distributed according to the criterion în menopause in:

• 75 women with osteoporosis and physiological menopause, aged 52-79 years, of which:
  - 8 patients with early physiological menopause
  - 67 patients with physiological menopause

• 22 women with surgically menopausal , aged 45-80 years, of which:
  - 6 patients with surgical menopause installed until the age of 40 years
  - 16 patients with surgical menopause installed over the age of 40 years.

### *STATISTICAL ANALYSIS OF THE STUDY GROUP*

Analysis of cases studied, history and clinical examination allowed us to distinguish the data sought in the study. Data processing was accomplished through statistical analysis performed using statistical indicators applied to the studied cases. Determination of serum osteocalcin and β-original CrossLaps in all patients studied showed appropriate values (or more) of postmenopausal period.

In all patients was calculated BMD and T-score by DEXA method, thus establishing the diagnosis of osteoporosis; and through the FRAX algorithm-risk of fragility fracture, vertebral or non-vertebral.

Histopathological study showed a significant reduction in the number of osteocytes and osteoclast, especially internal osteonals tabs, grooves widened Havers with poor vascularity and abundant microfractures lines at interhaversians level . Both at haversian and interhaversian systems have note bone demineralization processes.

Biostatistical analysis we highlighted the following:

- At patients with early menopause studied there is a weak correlation between bone markers and age,respectively the number of years since menopause, considering the first statistical threshold (*r> 0.3*).
- At patients with early menopause there is a correlation between the average osteocalcin and β-CrossLaps serum and risk of major fractures, vertebral or non-vertebral: *r = 0.474; r = 0.378* for osteocalcin serum and *r = 0.345, r = 0.245* for β-CrossLaps.
- There is an inverse correlation between BMI and the risk of osteoporotic fracture (*r = -0.299; p = 0.003* respectively *r = -0.356; p = 0*), ie an increased BMI is more associated with a lower risk of fracture, correlation stronger for those with early menopause.
- There is a direct correlation between fracture risk and the number of years since menopause (*r = 0.495 r = 0.591*, respectively).
- There is a strong correlation (*r = 0.795; p = 0.001* respectively *r = 0.859; p = 0*) between patient age and the risk of osteoporotic fractures, vertebral or non vertebral, stronger for those with early menopause.
- There is a strong correlation between BMD and T-score at baseline (*r = 0.906*) with little difference in favor menopausal patients over 40 years.

After setting all the data on all patients in the study was instituted antiosteoporotic treatment: 23 patients (16 patients with osteoporosis and naturally menopause and 7 patients with osteoporosis and surgical menopause) treatment with strontium ranelate, 14 patients (10 patients with osteoporosis and naturally menopause and 4 patients with osteoporosis and surgical menopause) - hormone replacement therapy and 60 patients (49 patients with osteoporosis and naturally menopause and 11 patients with osteoporosis and surgical menopause) - treatment with bisphosphonates. It should be noted that all patients received therapy with calcium supplements (800-1200 mg / day) and vitamin D (500-1000 IU / day).

At 6 months after initiation of treatment, all patients in the study was performed biochemical balance, with repeated dosages for serum osteocalcin and β-CrossLaps, revealing an average decrease of 29.75% for osteocalcin and 29.77% for β-CrossLaps to initial serum.

12 months after initiation of treatment, all patients in the study was performed biochemical balance, with repeated dosages for serum osteocalcin and β-CrossLaps, revealing an average decrease of 39.84% for osteocalcin and 39.31% for β-CrossLaps to initial serum.

For the subgroup of patients with early menopause, change in serum levels of markers of bone turnover after 12 months of treatment are strongly correlated (*r = 0.649; p = 0.012; r = 0.506*) with age of patients and with age of menopause (*r = 0.534; p = 0.049; r = 0.338*), with a discrete difference in favor for markers of bone formation.

After 12 months of treatment was determined again by DEXA measurement, BMD and T-score in all patients studied, revealing an average increase of 3.31% for T-score and 2.35% for BMD.

Based on these results, the biostatistical analysis revealed:

- The baseline serum values of markers of bone formation was correlate better with decreased risk of fractures than those of markers of bone resorption.
- There is an inverse correlation between decreased of serum levels of osteocalcin and β-CrossLaps at 12 months of treatment and increasing BMD (*r = -0.193; p = 0.05; r = -0.201; p = 0.048*) and T score (*r = -0.214; p = 0.035; r = -0.135; p = 0.042*).
- Changes in bone markers at 12 months of antiosteoporotic treatment was correlated with BMD (*r = 0.476, r = -0.256*) and the T-score (*r = 0.248, r = -0.142*) and with the changes in BMD and score T (*r = 0.362; r = 0.197*).
- There is a correlation between the variation of bone markers and decreased of risk of fracture (*r = -0.556; p = 0.039; r = -0.556; p = 0.039; r = 0.377; r = 0.376*) .This correlation is stronger for the subgroup of patients with early menopause.

## **CONCLUSIONS**:

***1) In recent decades, osteoporosis has become a public health problem worldwide, with an increasing incidence due to increasing life expectancy.***

***2) The bone loss age-related is asymptomatic and morbidity of osteoporosis is secondary of fragility fractures occurring.***

***3) Identification of postmenopausal women at high risk of fracture is a priority, which is particularly important for women in early menopause eligible for early intervention to maintain or increase bone mass and thus reduce the risk of fractures.***

***4) Determination of bone markers, including those of bone formation, as well as those of bone resorption, is useful in demonstrating the dynamic bone metabolism.***

***5) Since about 30% of patients with fragility fractures not have T-score of osteoporosis,there is a need for new diagnostic tools for identifying individuals with risk for fracture.***

***6) Dosage markers of bone turnover is effective in identifying those women with quicker bone loss and this is important because this group responds better to antiresorptive therapy.***

***7) There is a correlation between serum biomarkers and BMD during treatment with bisphosphonates, the association being stronger with advancing age.***

***8) Markers of bone turnover can be used to monitor the effectiveness of treatment before the changes in BMD should be evaluated.***

***9) The expected variation of their serum values may be used to determine the clinical efficacy of the treatment and increase patient compliance.***

***10) The use of association between markers of bone turnover and BMD improves the prediction of fracture risk in postmenopausal women, this risk is increased in those with low BMD and / or raised markers.***

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