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Research article

β-crosslaps in knee osteoarthritis – assessment and rehabilitation

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Abstract: The bone turnover is important for the progression of osteoarthritis. The C-terminal telopeptide of type I collagen, β-CrossLaps (β-CTx) is considered as the first-choice bone resorption markers. Today, the biochemical markers have been associated with pain and disease severity in knee osteoarthritis (KOA) patients.
We studied the association between β-CTx and functional status in KOA patients, pre- and postrehabilitation program. The primary goals of our study are both to establish the correlation between the serum level of β-CTx and other parameters of clinical and functional status in KOA patients and to compare the CTX-I values before and after rehabilitation program. We respected the actual international management recommendations for KOA.
A total of 130 patients were randomly allocated into two distinct groups: Study Group (SG = 90 patients) performed 10 sessions of complete rehabilitation program (magnetodiaflux, transcutaneous nerve stimulation, ultrasound and low intensity laser treatment, kinetic measures), and Control Group (CG = 40 patients) performed 10 sessions of kinetic program. Both programs were performed daily, 5 days/week, 2 weeks. Evaluation of patients was carried out in two stages - initial (T1) and after 3 months (T2).
Comparing the initial values with the final ones for β-CTx, we noticed a high significant difference between initial and final values only in SG patients. For the both T1 and T2 moments, there was no a statistically significant difference between β-CTx values from the two groups. Analyzing the differences between the values of clinical parameters (Six Minutes Walking Test, Timed Up-and-Go test, walk cadence and Symmetry index in walk) obtained between the patients from the two groups, we noticed that, for the T2 moment, there was a statistically significant difference between studied parameters, except walk cadence.
In the assessment of global KOA patient status (clinical and functional status in accordance with bone-cartilage interface dynamics), β-CTx has significant value and could be used for monitoring the individualized rehabilitation program.

Keywords: β-CrossLaps, knee, osteoarthritis, rehabilitation program

Introduction

The concerns of the medical world in the last decades regarding joint diseases have been focused on the musculoskeletal disorders, complex conditions that affect muscles, bones, joints and the spine. Arthritic diseases of synovial joints are some of the most common the musculoskeletal disorders [1].

Osteoarthritis (OA) is the most common form of arthritis, and a major cause of disability, morbidity and impaired quality of life [2]. In the Global Burden of Disease 2010 Study, hip and knee OA was ranked as the 11th highest contributor to global disability [2]. The 2015 Global Burden of Disease Study reports that OA is the most notable noncommunicable disease with total disability-adjusted life-years (DALYs) rising by 35% between 1990 and 2015 [3].

The prevalence of OA, has doubled in the last two decades, currently affecting a total global population of 500 million individuals [4].

This chronic rheumatic disease characterized by pain and declining physical function is a major cause of disability worldwide in elderly people and has a significant socioeconomic impact [5].

OA is defined by progressive and excessive cartilage degradation, bone remodeling (deterioration of subchondral bone with abnormal bone growth and sclerosis), osteophyte formation, and synovial inflammation [6, 7] leading to pain, stiffness, swelling, and loss of normal joint function [8].

OA is a highly heterogeneous disease characterized by pathology involving the whole joint [8],

with structural and functional changes of tissues around the joints [5], in which the first step involves molecular changes. Subsequent to these alterations, structural impairments manifest predominantly as cartilage degradation, accompanied by synovial inflammation, subchondral bone restructuring, and modifications in ligament and meniscal integrity [4].

The etiology of OA is different when referring to primary and secondary OA. For several years, great efforts have been invested in the study of risk factors. Besides age and sex, which seem to be the major ones, there are others to be mentioned, i.e. genetics, ethnicity diet, obesity, metabolic dysfunction, injury, muscle weakness, malalignment, and bone metabolism contribute to the onset and progression of OA. Despite all efforts, the prevalence of OA has been continuously rising [1].

OA may not only occur as a primary form, but also as secondary form, depending on the identifiable cause. The focus of recent research was on early identification of the pathology [9]. Osteoarthritis (OA) is correlated with initial bone loss resulting from heightened bone remodeling, succeeded by gradual turnover leading to the densification of the subchondral plate and eventual total cartilage depletion [10]. Bone turnover is hypothesized to hold a crucial role in the etiology of osteoarthritis (OA) [11], with its concomitant heightened vascularity considered indispensable for the advancement of OA [10].

In the early stages of OA, bone remodeling is elevated and may cause alterations in joint shape and abnormal mechanical loading that predispose to progressive cartilage loss. The bone matrix formation and degradation by osteoblasts and osteoclasts are disturbed. Bellido et.al. detected an increased number of osteoclasts with reduced trabecular thickness and increased bone loss as well as lower modulus in their experimental research [12].

In the late stage of OA, subchondral bone becomes sclerotic. Bone formation and osteoblastic activity are dominant in this phase a higher bone density and volume. The stiffness of subchondral bone is low, accompanied by decreased mineralisation [13].

Furthermore, there exists a partial interconnection between cartilage and bone metabolism, notably regarding subchondral bone turnover and its interplay with articular cartilage. This observation implies that biomarkers associated with osteoarthritis (OA) may originate from various anatomical compartments, underlining the perspective of OA as a disease involving the entire joint [14].

In this respect, Mobasheri and Henrotin studied OA biomarkers to diagnose, predict or assess the patient physical condition [1].

In 2006, Bauer et al. proposed the ''BIPED'' biomarker classification (Burden of Disease Investigative, Prognostic, Efficacy of Intervention and Diagnostic) [15]. This classification was subsequently revised and, now, we have a comprehensive array of biomarkers associated with osteoarthritis (OA), and ongoing initiatives are directed towards leveraging certain markers for the detection of subclinical and/or subacute inflammation.
Bone and joint destruction can be quantified by analyzing biomarkers levels in serum, so it is an imminent need for reliable biomarkers in the OA field. In the past three decades, biochemical markers encompass the entirety of the joint, including bone, cartilage and connective tissue biochemical turnovers, inflammation were analyzed in diverse studies [16, 17]. The bone turnover is important for the progression of OA, as increased bone resorption markers, most likely reflecting systemic skeletal changes [18].
β-CrossLaps (β-CTx) are the C-terminal telopeptide of type I collagen, the chief element (~90%) of the protein matrix of bone. Type I collagen telopeptide fragments, C-telopeptide crosslaps of type I collagen (CTX-1), and C-terminal telopeptide of type I collagen (1CTP) are currently considered as the most sensitive markers of bone resorption and are released from bone type I collagen by different enzymatic pathways. CTX-1 is generated by cathepsin K, which is the key osteoclastic enzyme for systemic bone resorption. 1CTP is generated by matrixmetalloproteases, the activity of which plays an important role in the collagen degradation [19]. Of note, β-CTx is considered as the first-choice bone resorption markers varied by gender and age. In a longitudinal study of patients with symptomatic osteoarthritis, Huebner et al. observed that disease progression (defined by features of joint space narrow and osteophyte) was associated with a level alpha CTX-I [20].
The knee, hip, and hand joints represent the most frequently affected appendicular joints in osteoarthritis (OA) [8]. The knee joint is an organ constituted of several highly specialized tissues organized in a very precise manner to facilitate proper joint function [4]. Therefore, failure in a single component joint structural integrity can compromise knee function. Knee osteoarthritis (KOA) is one of the most common OA and accounts for 10% of the worldwide population, making it an important public health problem. Its prevalence is high as 40% in adults between the ages of 70 and 75, and remains a principal contributor to pain and disability in elderly populations [21]. Variable clinical features, and biochemical/genetic characteristics suggest that multiple phenotypes and endotypes of KOA exist [7]. Classical, KOA patient assessment is based on clinical and functional evaluations of pain, joint stiffness and limitations in physical function as well as radiographic assessment of osteophytes, bone sclerosis and joint-space narrowing, using the Kellgren and Lawrence grading scale. The radiographic exam is focused on anatomic derangements of the disease [22] and its molecular pathogenesis is unapproachable. Today, the biochemical markers have been established due to their association with KOA pain and KOA disease severity [6]. Changes in the biochemical markers for bone and cartilage in knee osteoarthritis (KOA) may reflect changes in tissue turnover induced by interventions.
In the specialized literature, there is a dearth of articles that definitively specify the correlations between biomarkers, particularly β-CrossLaps (β-CTx), and the clinical-functional outcomes of a comprehensive rehabilitation program applied to patients with osteoarthritis, regardless of the affected joint. In 2016, Pascarelli et al. investigated the impact of mud-bath therapy as an adjunct to standard treatment on pain and function among patients with knee osteoarthritis. They noted a significant elevation in CTX-II levels exclusively, potentially attributable to an augmentation of cartilage turnover induced by thermal stress [23,64].
Seven years ago, Loeser et al. assessed the effects of a kinetic program combined with dietary interventions on a variety of biochemical markers among patients with knee osteoarthritis, failing to yield definitive outcomes [24].

In 2017, Mobasheri et al. did not document any trials investigating the correlation between biomarkers and rehabilitation programs within their comprehensive review of biomarkers in osteoarthritis [25].

In 2020, Antonioli et al. explored the association between the anti-inflammatory effects of factors derived from a rehabilitation program and their potential role in suppressing cytokine induction through the inhibition of HMGB-1 (high mobility group box 1 protein) [26].

Taking into consideration that a disease starts when detected by the best marker we develop to define it [22] we studied the association between Beta-crosslaps (the C-terminal telopeptide of type I collagen) and functional status in KOA patients, pre- and postrehabilitation program. So, the aim of our study is both to establish the correlation between the serum level of CTX-I and other parameters of clinical and functional status in KOA patients and to compare the CTX-I values before and after rehabilitation program. We respected the actual international management recommendations for KOA [27].

PATIENTS AND METHODS

Ethical approval. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practices. Before being included in the study, the details of the present research were explained to the KOA patients. Written informed consent was obtained from each patient. The protocol was approved by the local independent ethics committee (approval no. nr. 138/07.12.2020).

Study Design

Our prospective randomized study was conducted in Departments of Physical Medicine and Rehabilitation, Filantropia Hospital, Craiova from May 2022 to November 2023.

A total of 150 patients with KOA were enrolled in this study, with a 1:2 ratio between the treatment groups. Randomization was made by an indipendent physician. After initial assessment, a total of 130 patients were randomly divided into two groups: Study Group (SG = 90 patients) performed 10 sessions of complete rehabilitation program, and Control Group (CG = 40 patients) performed 10 sessions of kinetic program. Both programs were performed daily, 5 days/week, 2 weeks (Figure 1. Diagram of our study). Complete rehabilitation program contained electroptherapy measures and standard kinetic measures.

All patients included in the study performed physical exercises aimed at maintaining and restoring joint parameters and gait patterns throughout the entire period of hospitalization.

In order to assess the variation of the CTX-1 (β-CTx) biomarker in patients with knee osteoarthritis enrolled in a rehabilitation program, it was deemed necessary for this program to incorporate electrotherapy measures. Due to their multifaceted effects on joint structures, electrotherapy procedures have a pluripotent impact. The inclusion criteria taken into account when designing the groups were:

- patients older than 50 years diagnosed with KOA according to ACR criteria [8];
- at least 5 years of disease progression;
- absence of knee injuries at least 6 months before;
- absence of major disturbances in the frontal plane alignment of the knee;
- patients with other co-morbidities, but well controlled, like: arterial hypertension, dysplipemia, venous insufficiency and mellitus diabetes type II; a history of a symptomatic or complicated upper gastro-intestinal ulcer; for these conditions, patients were administered appropriate medication (antihypertensives, lipid-lowering agents, venous trophic agents, oral antidiabetics, proton pump inhibitors).
- compliance with physical exercise during the healthcare program.

Patients will be excluded in the presence of unstable medical conditions preventing the patient from participating in the rehabilitation programs, history of knee replacement and osteoporosis, neurological or any other conditions affecting strength or function of lower limbs.

Evaluation of patients was carried out in two stages - initial (T1) and after 3 months (T2) - during which an in-hospital program of rehabilitation and out-patient home-training were conducted.

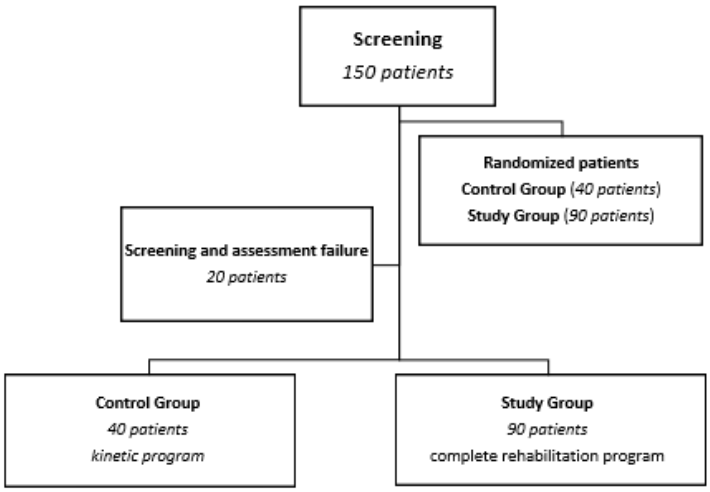

Figure 1. Diagram of our study

Patients assessment

The each study visit consisted of a clinical and functional assessment, vital signs measurement and laboratory testing.

After careful anamnesis (age, comorbid diseases), the *clinical assessment* included: general physical examination (body mass index – BMI); musculoskeletal and neurological examination, including balance and gait.

During the examination, we conducted *standard laboratory tests* (screening, inflammation tests – fibrinogen and C reactive protein, and *imagistic examination* of the knees (radiologic and MRI exams). The Kellgren and Lawrence Radiographic KOA (KL) scoring is often measured in grades 0 to 4 based on spatial narrowing and structural bone modifications. The KL grading was interpreted by a physician based on recent weight-bearing, anterior-posterior X-rays of the tibiofemoral joint for both knees without knowledge of the clinical conditions.

β-CTx (the C-terminal telopeptide of type I collagen – CTX1) measuring.

Serum CTX-1 (β-CTx) was determined by beta-CrossLaps (β-CrossLaps) Roche Elecsys. β-CrossLaps analysis was performed at the Craiova Emergency Clinical Hospital-Clinical Chemistry Department using a Roche automatic electrochemiluminescent immunoassay analyzer COBASe601 (Roche Diagnostics, GmbH, Mannheim, Germany).

The Roche β-CrossLaps/serum assay is a 2-site immunometric (sandwich) assay using electrochemiluminescence detection (ECLIA). Patient specimen, biotinylated monoclonal β-CrossLaps-specific antibody labeled with ruthenium react to form a complex. Streptavidin-coated microparticles act as the solid phase to which the complex binds. Voltage is applied to the electrode, inducing a chemiluminescent emission from the ruthenium, which is then measured against a calibration curve to determine the amount of β-CrossLaps in the patient specimen.

This assay is specific for crosslinked isomerized type I collagen fragments, independent of the nature of the crosslink. The assay specificity is guaranteed through the use of 2 monoclonal antibodies, each recognizing linear β-8AA octapeptides (EKAHD-BETA-GGR). The assay, therefore, quantifies all type I collagen degradation fragments that contain the isomerized octapeptide beta-8AA twice (β-CTx) (package insert: Elecsys β-CrossLaps/serum. Roche DIAGNOSTICS).

This method has been standardized against reference standards precisely defined by weighing out synthetic peptide. Every Elecsys β-CrossLaps/serum reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys β-CrossLaps CalSet. For quality control we used Elecsys PreciControl Varia 1 and 2. The patients serum, calibrators, and controls were at ambient temperature (20-25°C) before β-CrossLaps measurement.

Blood samples were collected as fasting, in the morning, using vacutainers without anticoagulant (Becton Dickinson, USA) according to the recommendations for β-CrossLaps analysis. Without an anticoagulant blood was drawn and left to clot at room temperature for 30 minutes. The blood samples were centrifuged for 10 minutes at 3000 rpm to obtain serum. The serum was separated from the clot and aliquoted in Eppendorf tubes at -80C until β-CrossLaps analysis.

Beta-CrossLaps Reference ranges are based on age and sex (Men: 30-51 yrs: ≤0.584 ng/ml; 51-70 yrs: ≤0.704 ng/ml; >70 yrs: ≤0.854 ng/ml. Women: premenopause ≤ 0.573 ng/ml; postmenopause: ≤ 1.008 ng/ml). Lower detection limit: 0.01 ng/mL (the lowest measurable analyte level that can be distinguished from zero) and measuring range**:** 0.010-6.00 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.010 ng/mL and values above the measuring range are reported as > 6.00 ng/Ml [28].

Functional evaluation. To measure the degree of functional impairment and to identify the severity of disease in our patients we included:

- the VAS - Visual Analogue Scale (from 0 to 10, 0 = absence of pain and 10 = maximum pain score, other values between 0 and 10 are directly proportional to the intensity of pain, depending on the individual pain threshold);
- the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and the Lequesne Functional Index were used for determining the effect the disease has in performing daily living activities. WOMAC contains 24 specific questions divided into three domains: pain (P-WOMAC; 5 items), stiffness (S-WOMAC; 2 items) and physical function (PF-WOMAC; 17 items). A score of 0 corresponds to maximal functional status, while high scores nearing 96 signify a minimal status, indicative of significant disruption in day-to-day tasks [29].

  For the Lequesne Index (a 10-item questionnaire) 0 is indicative of less functional impairment or maximum functional status (pain, maximum walking distance, and daily activities) while 24 is the minimum, the worst outcomes. Lower limb dysfunction is indicated from 0 (none), 4 (mild), 5-7 (moderate), 8-10 (severe), 11-13 (very severe and more than 14 is characteristic for extremely severe limiting and dysfunction [30].

  Physical performance-based measures were assessed by gait analysis. We used a wireless system - BTS G-WALK (BTS Bioengineering Corp., Italy) / BTS G-SENSOR 2 consisting of an inertial sensor composed by a tri-axial accelerometer, a magnetic sensor, and a tri-axial gyroscope, that worn by the patient allows a functional gait analysis [31].

  This system can be connected with a force platform (P-WALK), where the gait information is measured through pressure sensors and ground reaction force sensors (GRF) which measure the force exerted by the patient's feet on the floor when he/she walks. These parameters were: Timed Up-and-Go (TUG) test, Symmetry index, Six Minutes Walking Test (6 MWT) - "walking distance" (m) and „average cadence" (steps/min).

  The objectives of rehabilitation program were: pain management, inflammatory process control, correction of the abnormal gait scheme, restoration the lower limb functionality, preserving and increasing quality of life.

The components of applied rehabilitation program for patients were: education and diet recommendations, medication for each co-morbidity and anti-inflammatory drugs, physiotherapy (magnetodiaflux, transcutaneous nerve stimulation, ultrasound and low intensity laser treatment, only in SG) and kinetic exercises (daily exercises for motor control and gait).

Kinetic program included: stretching of calf muscles and hamstrings (daily, 5 sets of 6 seconds for each muscle group), exercises for improving quadriceps and medius gluteus strength (daily, in antigravity position for each muscle, 2 sets, 10 repetitions/ set, 2 minutes rest between sets), gait coordination (front and back cross over stepping, tandem walking, toe out gait modification, 3 days per week, 30 – 40 minutes / session). During the walking scheme, with slight variants, we used a cane for weight unloading.

Physiotherapy procedures were selected due to their analgesic effects and their ability to restore the functional status of the joint components, aspects supported by current studies and recommendations [32]. None of the procedures exhibit adverse effects, with benefits primarily focused on reducing disability in individuals with knee OA when compared to placebo in randomized controlled trials [33, 34].

Statistical analysis

In the stratification of numerical variables based on categorical variables, we utilized the mean value for comparisons. Descriptive statistics were presented as mean ± standard deviation (SD), and statistical differences between means were assessed using the parametric test Student t. Prior to the analysis, data normality was examined using the Kolmogorov–Smirnov and Shapiro–Wilk tests. All samples included in this research showed normal distribution [35].

To establish the relationships between various numerical variables, we employed Pearson's correlation coefficient (r), a widely recognized measure in statistical analysis. The correlation coefficient (r) is bounded between 1 and -1, with positive values indicating a direct correlation—when one variable increases, the other also increases—and negative values signifying an inverse correlation, where an increase in one variable corresponds to a decrease in the other. The strength of the correlation spectrum spans from no correlation (r=0) to perfect correlation (r=1 or r=-1). To facilitate interpretation, we categorized the correlation values empirically into five classes: 0-0.2 for no correlation, 0.2-0.4 for weak correlation, 0.4-0.6 for moderate correlation, 0.6-0.8 for high correlation, and 0.8-1 for almost perfect correlation.

Data logging and descriptive statistics were performed using Excel (Microsoft, USA), while inferential statistical analyses were conducted using Matlab (Mathworks, USA).

RESULTS.

More than 65% of the patients were women in both groups (Table 1), without significant difference from the sex repartition (the chi-square statistic is 1.5444 and the p-value is 0.213965 > 0.05).

The urban / rural ratio was relative identically, in SG 47:43 (1.09) and in CG 21:19 (1.10), but without significant difference (the chi-square statistic is 0.0009 and the p-value is 0.976652 > 0.05), which means there is no influence of the area of residence on the studied condition (Table 1).

The mean age of the both groups almost similar (66.6 years old for SG, 69.3 years old for CG), only three years difference between mean values. The most patients fell into the seventh and the eighth decades, in both groups (Table 1).

Weight distribution and BMI were skewed towards higher values, with weights skewed towards obesity grade 1 and (Figure 2). This parameter (BMI) had a significant correlation with gender, female were characterized by a high BMI to male.

Table 1. Demographic data of studied patients

Demographic parameters		Study Group (SG = 90 patients)			Control Group (CG = 40 patients)		
		Min	Max	Mean (SD)	Min	Max	Mean (SD)
BMI (Kg/m^2)		26.37	41.62	31.85 ± 3.43	24.39	43.51	32 ± 4.5
Age		45	84	66.6 ± 7.8	52	88	69.3 ± 8.1
		40 – 49 y = 1 (1%) 50- 59 y = 13 (14%) 60 – 69 y = 42 (46%) 70 – 79 y = 29 (32%) 80 – 89 y = 5 (5%)			40 – 49 y = 1 (2.5%) 50- 59 y = 4 (10%) 60 – 69 y = 14 (35%) 70 – 79 y = 17 (42.5%) 80 – 89 y = 4 (10%)		
Sex	Female	70 females (77%)			27 females (67%)		
	Male	20 males (2%)			13 males (33%)		
Residence (own place)	Urban	47 patients (52%)			21 patients (52%)		
	Rural	43 patients (48%)			19 patients (48%)		

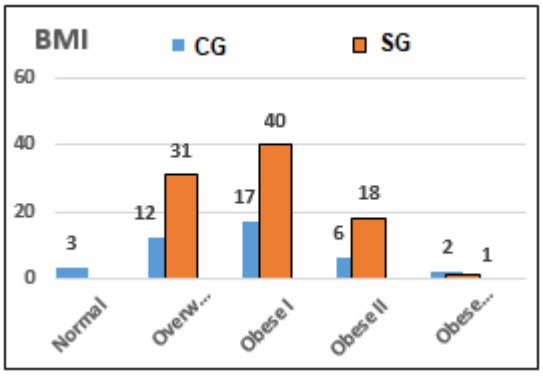

Figure 2.
BMI distribution in both groups.

Over half (51 %) of all patients had physically demanding job, with the involvement of lower limbs.

Among the comorbidities identified, it was observed that there are 31% of SG patients and 32% of CG patients with Diabetes mellitus, 44% of SG patients and 42% of CG patients with dyslipidemia and 32% of SG patients and 39% of CG patients with venous insufficiency. These aspects are in accordance with average age of our patients.

The mean values for all biochemical and inflammatory tests were included in normal interval. This aspects permitted to applied rehabilitation program in safe conditions.

We prescribed rehabilitation program adapted with individual clinical and functional status.

We presented the obtained results separately for women and men because the studied biochemical marker (β-CrossLaps) has different reference values, and its variation pattern is influenced by the pathogenic level of subchondral bone.

β-CrossLaps assessment

Comparing the initial values with the final ones for β-CrossLaps, we noticed a high significant difference between initial and final values only for the patients in the SG group. For the comparison between the SG group and the CG group we found that there was no statistically significant difference among β-CrossLaps values for both T1 and T2 moments.

We specify that the determination of this biomarker was conducted in the morning, following laboratory guidelines. Physiotherapy sessions took place after breakfast, between 10 - 12 a.m.
For patients in the SG group, electrotherapy was performed in the afternoon, between 4 - 6 p.m. The schedule of the rehabilitation program did not influence the method of β-CrossLaps collection.

Table 2. Studied parameters values in all patients.

Parameter		β-CrossLaps	6MWT	TUG	WALK CADENCE
Study Group Mean + SD	T1	0.5 ± 0.2	340.8 ± 61.4	20.4 ± 4.5	93.6 ± 12.1
	T2	0.3 ± 0.2	405.9 ± 58.6	17.7 ± 3.8	101.8 ± 11.4
Control Group Mean + SD	T1	0.6 ± 0.3	316.4 ± 44.6	24.4 ± 4.6	92.6 ± 13.4
	T2	0.5 ± 0.2	330.1 ± 37.2	23.7 ± 4.9	94.9 ± 13.2
p / Student's t test Final vs Initial SG		**HS = 0.000**	**HS = 0.000**	**HS = 0.000**	**HS = 0.000**
p / Student's t test Final vs Initial CG		0.153	0.222	**HS = 0.000**	0.471
p / Student's t test Initial SG vs CG		0.051	0.141	0.445	0.674
p / Student's t test final SG vs CG		0.479	**S = 0.012**	**HS = 0.000**	0.806
Parameter		SYMMETRY INDEX %	VAS	WOMAC	LEQUESNE INDEX
Study Group Mean + SD	T1	89.8 ± 8.5	6.7 ± 1	64.3 ± 7.9	8.1 ± 2.4
	T2	93.9 ± 6.3	5 ± 0.8	56.6 ± 6.5	6.6 ± 1.8
Control Group Mean + SD	T1	91.6 ± 5.6	6.6 ± 0.8	59 ± 5.4	11.4 ± 1.9
	T2	92.4 ± 6	6.7 ± 0.7	55.7 ± 5	11 ± 1.4
p / Student's t test Final vs Initial SG		**HS = 0.000**	**HS = 0.000**	**HS = 0.000**	**HS = 0.000**
p / Student's t test Final vs Initial CG		0.645	0.986	**HS = 0.000**	**HS = 0.000**
p / Student's t test Initial SG vs CG		0.099	0.469	0.062	0.291
p / Student's t test final SG vs CG		**S = 0.030**	0.476	**0.000**	**HS = 0.000**
Study Group (SG) = 90 patients, Control Group (CG) = 40 patients 6 MWT (meters) = Six Minutes Walking Test; TUG (seconds) = Timed Up-and-Go					

Clinical assessment
Analyzing the differences between the values of clinical parameters (6MWT, TUG, walk cadence and Symmetry index) obtained between the patients from the two groups, we noticed that, for the T2 moment, there was a statistically significant difference between studied parameters, except walk cadence.
Comparing the initial values with the final ones, we obtained the following results:

- Six Minutes Walking Test, Walk cadence or average cadence (steps/min) and Symmetry index – only for SG patients there were significant differences between the initial and final values, although the final value improved compared to the initial one for both groups;– only for SG patients there were significant differences between the initial and final values, being bigger at the end;
- Timed Up-and-Go test - for both groups there was significant differences between the initial and final values, being smaller at the end.

Functional assessment

Analyzing the differences between the values obtained between the patients from the two groups, we noticed that, for the both moments T1 and T2, there was statistically significant difference for WOMAC and Lequesne Index parameters. Probably, complete rehabilitation program, with gait control, had a real impact on the functional status.

Comparing the initial VAS values with the final ones, we obtained a significantly difference (p Student's t test < 0.05) only for SG. For both groups there was significant differences between the initial and final WOMAC and Lequesne Index values.

Table 3. Studied parameters values in female patients.

Parameter		β-CrossLaps	6MWT	TUG	WALK CADENCE
Study Group Mean + SD	T1	0.39 ± 0.21	361.8 ± 48.7	20.6 ± 3.9	95.3 ± 14
	T2	0.30 ± 0.17	411.8 ± 45.6	18.3 ± 3.7	100.5 ± 12.8
Control Group Mean + SD	T1	0.46 ± 0.15	322.6 ± 35.3	23.1 ± 3.7	92.2 ± 11.8
	T2	0.46 ± 0.13	331.5 ± 29.8	23 ± 4	91.1 ± 12.4
p / Student's t test Final vs Initial SG		0.174	**0.002**	0.068	0.200
p / Student's t test Final vs Initial CG		0.996	0.487	0.944	0.811
p / Student's t test Initial SG vs CG		0.216	**0.012**	0.073	0.506
p / Student's t test final SG vs CG		**0.004**	**0.000**	**0.002**	**0.039**
Parameter		SYMMETRY INDEX %	VAS	WOMAC	LEQUESNE INDEX
Study Group Mean + SD	T1	86.3 ± 11.3	6.5 ± 1.1	63.9 ± 8.4	7.8 ± 2.4
	T2	90.8 ± 10.3	4.8 ± 0.9	56.5 ± 6.9	6.3 ± 1.8
Control Group Mean + SD	T1	93.1 ± 5.6	6.4 ± 0.6	59.5 ± 5.2	11.8 ± 1.4
	T2	90.4 ± 5.6	6.5 ± 0.7	57.4 ± 4.7	11.2 ± 1.4
p / Student's t test Final vs Initial SG		0.191	**0.000**	**0.004**	**0.046**
p / Student's t test Final vs Initial CG		0.251	0.802	0.301	0.190
p / Student's t test Initial SG vs CG		**0.031**	0.591	0.076	**0.000**
p / Student's t test final SG vs CG		0.883	**0.000**	0.623	**0.000**

Study Group (SG) = 90 patients, Control Group (CG) = 40 patients
6 MWT (meters) = Six Minutes Walking Test; TUG (seconds) = Timed Up-and-Go

Analyzing the differences between the values obtained between the female and male patients from the two groups, we noticed that, for the both moments T1 and T2, there was no statistically significant difference.

In female patients (Table 3), we determined statistically significant difference between SG and CG in final assessment, after rehabilitation program and home-training, for all parameters with two exceptions – Symmetry index and WOMAC. Initial, there was a statistically significant difference for 6MWT, Symmetry Index and Lequesne Index between SG and CG. Moreover, the values for 6 MWT and functional parameters in SG had statistically significant difference between initial and final moments.

In male patients (Table 4), we determined statistically high significant difference between SG and CG in final assessment, after rehabilitation program and home-training, for all parameters, with one exception - VAS. Initial, there was a statistically significant difference for TUG, WOMAC and Lequesne Index between SG and CG. It must be emphasized that all studied parameters had statistically significant difference between initial and final moments in SG. We can explain these results due to clinical and functional consequences of complete rehabilitation program.

Table 4. Studied parameters values in male patients.

Parameter		β-CrossLaps	6MWT	TUG	WALK CADENCE
Study Group Mean + SD	T1	0.48 ± 0.23	334.8 ± 63.5	20.3 ± 4.8	93.2 ± 11.5
	T2	0.30 ± 0.15	404.6 ± 61.9	17.5 ± 3.7	102.1 ± 11
Control Group Mean + SD	T1	0.6 ± 0.3	313.4 ± 48.7	25.1 ± 4.8	92.7 ± 14.2
	T2	0.54 ± 0.23	329.3 ± 41.1	24 ± 5.2	93.2 ± 13.7
p / Student's t test Final vs Initial SG		**0.000**	**0.000**	**0.000**	**0.000**
p / Student's t test Final vs Initial CG		0.446	0.200	0.463	0.890
p / Student's t test Initial SG vs CG		0.072	0.082	**0.000**	0.897
p / Student's t test final SG vs CG		**0.000**	**0.000**	**0.000**	**0.002**
Parameter		SYMMETRY INDEX %	VAS	WOMAC	LEQUESNE INDEX
Study Group Mean + SD	T1	92.8 ± 7.3	6.7 ± 0.9	64.4 ± 7.8	8.1 ± 2.4
	T2	94.8 ± 4.3	5.1 ± 0.8	56.6 ± 6.4	6.7 ± 1.7
Control Group Mean + SD	T1	92.4 ± 5.6	6.7 ± 0.9	58.6 ± 5.5	11.09 ± 1.9
	T2	90.4 ± 6	6.9 ± 0.6	54.8 ± 4.9	10.8 ± 1.3
p / Student's t test Final vs Initial SG		**0.000**	**0.000**	**0.000**	**0.000**
p / Student's t test Final vs Initial CG		0.235	0.485	**0.010**	0.662
p / Student's t test Initial SG vs CG		0.279	0.746	**0.000**	**0.000**
p / Student's t test final SG vs CG		**0.002**	**0.000**	0.152	**0.000**

Study Group (SG) = 90 patients, Control Group (CG) = 40 patients
6 MWT (meters) = Six Minutes Walking Test; TUG (seconds) = Timed Up-and-Go

We conducted a correlation analysis of the βCross-Laps parameter with the other parameters for each group at the two evaluation time points. The obtained values (Table 5) suggest a lack of significant correlation between βCross-Laps and the other parameters. The explanation lies in the fact that the functional status of patients with knee osteoarthritis (KOA) is influenced by multiple clinical factors and the pain parameter (VAS) is the result of the involvement of all knee structures.

The same result was obtained for the subgroup of male patients (Table 6).

For the women studied (Table 7), we observed a moderate and high correlation between βCross-Laps and functional parameters defining the gait pattern (TUG, Symmetry index and walk cadence). This finding confirms the sex-dependent nature of βCross-Laps values, as well as the presence of a more dynamic bone turnover in women compared to men.

Table 5. Correlations of parameters values in all patients (Pearson's correlation coefficient).

	βCross Laps 1	**βCross Laps 2**	**6MWT 1**	**6MWT 2**	**TUG 1**	**TUG 2**	**SI 1**	**SI 2**	**WC 1**		**WC 2**	**VAS 1**	**VAS 2**	**W 1**	**W 2**	**IL 1**	**IL 2**
Study																	
βCross Laps 1	**1.0**	**0.7**	**-0.3**	**-0.3**	0.0	-0.1	-0.1	0.0	-0.1		-0.1	0.0	0.1	-0.1	-0.1	0.0	0.0
βCross Laps 2		**1.0**	-0.1	**-0.2**	0.1	0.0	0.0	-0.1	-0.1		**-0.2**	0.0	0.1	-0.1	-0.1	-0.1	-0.1
Control																	
βCross Laps 1	**1.0**	**0.9**	-0.1	0.0	**-0.2**	**-0.2**	0.0	0.1	0.0		-0.1	0.0	0.1	-0.1	**-0.3**	**-0.2**	-0.1
βCross Laps 2		**1.0**	0.0	0.1	**-0.2**	**-0.3**	0.0	0.1	0.0		-0.1	-0.1	0.1	**-0.2**	**-0.2**	**-0.2**	-0.1

6 MWT (meters) = Six Minutes Walking Test; TUG (seconds) = Timed Up-and-Go, SI = Symmetry Index in walk, WC = walk cadence, W = WOMAC scale, IL = INDEX LEQUESNE

Table 6. Correlations of parameters values in male patients (Pearson's correlation coefficient) .

	βCross Laps 1	**βCross Laps 2**	**6MWT 1**	**6MWT 2**	**TUG 1**	**TUG 2**	**SI 1**	**SI 2**	**WC 1**	**WC 2**	**VAS 1**	**VAS 2**	**W 1**	**W 2**	**IL 1**	**IL 2**
Study																
βCross Laps 1	**1.0**	**0.7**	**-0.3**	**-0.3**	-0.2	-0.2	0.0	-0.1	0.1	0.2	-0.1	0.0	-0.1	-0.1	0.0	0.0
βCross Laps 2		**1.0**	-0.1	-0.2	-0.1	-0.1	0.0	-0.1	**0.3**	0.2	-0.2	0.0	-0.2	-0.2	-0.2	-0.2
Control																
βCross Laps 1	**1.0**	**0.9**	-0.2	0.0	-0.2	-0.2	-0.1	0.1	0.1	-0.1	-0.1	0.2	-0.1	-0.3	-0.2	-0.1
βCross Laps 2		**1.0**	-0.1	0.1	-0.3	-0.3	-0.1	0.0	0.1	-0.1	-0.1	0.1	-0.2	-0.2	-0.2	-0.2

6 MWT (meters) = Six Minutes Walking Test; TUG (seconds) = Timed Up-and-Go, SI = Symmetry Index in walk, WC = walk cadence, W = WOMAC scale, IL = INDEX LEQUESNE

Table 7. Correlations of parameters values in female patients (Pearson's correlation coefficient).

	βCross Laps 1	βCross Laps 2	6MWT 1	6MWT 2	TUG 1	TUG 2	SI 1	SI 2	WC 1	WC 2	VAS 1	VAS 2	W 1	W 2	IL 1	IL 2
Study																
βCross Laps 1	1.0	1.0	-0.1	-0.1	**0.6**	**0.6**	**-0.5**	**-0.5**	**-0.5**	**-0.4**	0.2	0.3	0.0	-0.1	0.0	-0.1
βCross Laps 2		1.0	-0.2	**-0.3**	**0.7**	**0.7**	**-0.5**	**-0.5**	**-0.7**	**-0.5**	**0.3**	**0.4**	0.2	0.1	0.1	0.1
Control																
βCross Laps 1	1.0	0.9	**0.4**	0.2	-0.2	-0.3	0.0	0.2	-0.3	-0.3	-0.2	**-0.4**	**-0.4**	-0.2	-0.1	0.0
βCross Laps 2		1.0	0.2	0.2	-0.1	-0.2	0.1	0.3	-0.1	-0.2	-0.1	-0.2	-0.3	-0.1	0.1	0.2

6 MWT (meters) = Six Minutes Walking Test; TUG (seconds) = Timed Up-and-Go, SI = Symmetry Index in walk, WC = walk cadence, W = WOMAC scale, IL = INDEX LEQUESNE

DISCUSSIONS

In our study, we described and monitored clinical, lab (β-CTx / β-CrossLaps) and functional parameters of KOA patients during functional recovery.

The average age of studied patients was 66.6 years in SG and 69.3 years in CG. This result is similar to the one specified in the literature that more than 50% of persons aged >65 years suffer from some forms of arthritis [36].

In both patients group, women were dominant. Worldwide, sex differences exist in the incidence rates of KOA [37].

The most of patients were overweight and obese class I, in both groups (Figure 2). A parameter with significant mechanical impact upon lower limb joints, BMI can be reflected a painful and disable knee in our patients.

We didn't referred to primary or secondary KOA because in a recent study [38] it was argued that dividing OA into primary and secondary subsets is not useful since "all OA is secondary" and that any attempt to subset OA had to take into account the fact that OA is largely a condition that is influenced by the joint's response to mechanical stress.

In the most recent years, the medical research effort has made to elucidate elucidate the heterogeneity inherent in osteoarthritis (OA) which presents novel opportunities for advancing therapeutic development and elucidating the pathogenesis of this multifaceted condition. The identification of distinct OA phenotypes and endotypes holds promise for informing prognosis and directing therapeutic interventions, thereby offering the potential to enhance patient care outcomes [39].

In 2016, Dell'Isola et al. identifed six main sets of variables proposing the existence of six phenotypes: 1) chronic pain in which central mechanisms (central sensitisation) are prominent; 2) inflammatory (high levels of inflammatory biomarkers); 3) metabolic syndrome (high prevalence of obesity, diabetes and other metabolic disturbances); 4) bone and cartilage metabolism (alteration in local tissue metabolism); 5) mechanical overload characterised primarily by varus malalignment and medial compartment disease; 6) minimal joint disease characterised as minor clinical symptoms with slow progression over time [40].

In 2019, taking into considaraion the sensitization measures as assessed with quantitative sensory testing and psychosocial factors, Wallis et.al. distinguished four phenotypes characterized mainly on the presence of signs of sensitization [41].

With the update of OA definition, a new conception called "Bone-driven OA subtype" was put forward, suggesting that abnormal bone metabolism plays a key role in OA development [42].

This OA subtype may be included in the fourth phenotypes mentioned by Dell'Isola et al. We performed our research starting from this "Bone-driven OA subtype". When we began the study, we took into consideration the recommendation of international forum and the conclusions of Chen et. al. according to which the relationship between bone turnover biomarkers and OA is still being discussed [43].

The International Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine recommend β-CrossLaps to be used as a reference bone resorption marker and measured by standardized assays in observational and intervention studies [44].

The original aspect of our clinical study it represents the β-CrossLaps level in the monitoring the rehabilitation program in KOA patients.

The β-CrossLaps test helps in determining if a patient has an increased or decreased rate of bone turnover or to the diagnosis of a medical condition that is associated with increased bone turnover. It is used to detect response to bone formation and anti-resorptive therapies for bone disease and helps to determine if the receiving drug dose is effective or not.

It is recognized in recent years that OA affects all structures within the joint [45]. Due to its dynamic nature, both cartilage and subchondral bone are susceptible to abnormal external mechanical stress and internal biochemical or morphological changes. These structures lose the function of absorbing biomechanical forces in OA. Ten years ago, Yuan et.al. described the bone-cartilage interface as a complex functional unit and biocomposite at the centre of joint function and disease in which the individual components interact. This region between the deep layers of articular cartilage and the underlying subchondral bone is the space where biomechanical and biological processes take place [46]. In addition, cartilage and bone metabolism may be partly linked, particularly subchondral bone turnover and its interaction with articular cartilage [14]. The alterations of either tissue will modulate the properties and function of the other component. Mediators produced from both tissues may pass from one structure to another, affecting the local homeostasis. So, the interface of osteoarthritic and healthy joint is not an impermeable barrier to soluble molecules. Bone and joint destruction can be quantified by analyzing biomarkers levels in serum.

Numerous candidate biomarkers for diagnosis, monitoring, and prognosis have been reported to be associated with the primary pathogenic mechanisms of osteoarthritis (OA) [47].

Under conditions of increased bone resorption, type I collagen breakdown by different enzymatic pathways [19] leads to N and C-telopeptides. Two fragments result from the C-telopeptide end: ICTP (cross-linked carboxyterminal telopeptide of type I collagen) and CTx (C-terminal cross-linked telopeptide of type I collagen) [9]. These sensitive markers of bone resorption are released from bone type I collagen. CTx has a clinical relevance. The aging of bones transforms the alpha form of aspartic acid, which is part of CTx, into the beta form (β- CTx).

These isomerized telopeptides are specific for the degradation of type I collagen that predominates in bone. β – CTx (β-CrossLaps), the chief element (~90%) of the protein matrix of bone, occurs in the early stages of type I collagen degradation; therefore it is a specific and stable marker of bone resorption [48].

β-CTx is released into the blood during bone resorption and is excreted mainly by the kidneys.

Serum CTX-1 (β-CTx) is influenced by renal function; it also shows significant diurnal variability with a peak in the early morning and a nadir in the afternoon, and food intake leads to a decrease in the level. Therefore, we performed all sample collection in a fasting state in the morning. The food intake substantially decreases the levels of CTX-1 [49].

In our study, the level of CTX-1 (β-CTx) was not increased, unlike other studies [50]. A possible explanation of our result is the fact that studied KOA patients had no early stage of OA. It the early stages of KOA, bone remodelling and subchondral bone loss are elevated, so the level of CTX-1 is increased. In our research, most patients were diagnosed with moderate (80 patients) and severe (40 patients) KOA, in accordance with Kellgren and Lawrence Radiographic KOA (KL) scoring, based on spatial narrowing and structural bone modifications. These late stages of KOA are associated with thickening of subcondral sclerotic bone [46].

β-CTx is considered as the first-choice bone resorption markers varied by gender and age. Gender-specific disparities in biomarker profiles should be considered when assessing these markers in relation to the structural characteristics of OA [6].

In our study, the mean value for β-CrossLaps was within normal limits for both groups and at the two assessment points, regardless of sex or age group. In contrast to the findings of existing studies, which mention that women with KOA increased their β-CTx levels in middle age compared with men [43].

We found that the mean value for women in both groups was lower than that for men (see Tables 3 and 4). A possible explanation could be that our patients age is over 60 years, and the study included patients without other types of bone pathologies (osteoporosis, osteopenia, Paget's disease). It is also demonstrated that bone turnover is important for the progression of OA, as increased bone resorption markers, most likely reflecting systemic skeletal changes [43]. At T2 moment, the mean value for β-CrossLaps was lower for the SG, with improvement of the parameter observed for both women and men. Since such an evolution of β-CrossLaps was not observed in the CG, it can be inferred that the implementation of a comprehensive rehabilitation program yields favorable effects. Both electrotherapy procedures and kinetic measures, through their direct effects on periarticular muscle groups and vascular structures, contribute to an improvement in local metabolism. It can be considered that the amelioration of clinical and functional status in patients with KOA following the rehabilitation program positively influences the diffusion processes at the level of bone-cartilage interface.

In the future, rehabilitation program may be considered one of the strategies for treatment of OA, targeting angiogenesis, neurogenesis and subchondral bone remodelling to decrease the molecular interaction [46]. In this way, with technological advancements, new approaches and therapies are emerging to aid OA patients [51].

Similar to the role of CTX-1 as specific and sensitive biomarker of bone resorption capable of promptly indicating the response to therapy for postmenopausal osteoporosis this marker can serve as a reference for the effectiveness of the rehabilitation program in patients with KOA.

Today, it is important to use the best marker for mention the disease starts. The detection of KOA has traditionally relied upon consideration of a patient's medical history, physical examination, and radiographic (x-ray) images, without taking into consideration molecular pathogenesis [22].

Therefore the opportunity to intervene early in the disease process, as practiced for the treatment of silent early phases of other diseases (osteoporosis and heart disease) [52] is lost.

Molecular biomarkers have the potential to address this requirement by offering a molecular perspective on joint symptoms or by identifying tissue abnormalities within the joint during the early, asymptomatic stages of the disease progression. Kraus et.al. mentioned in 2022 that biomarker discovery could be informed by idiopathic, joint injury-related and even rare of OA [22].

In the next years, a common core set of OA biomarkers should be determined.

In our study, we monitored clinical and functional parameters commonly used in the medical community for patients with KOA. The rehabilitation program implemented resulted not only in a favorable modification of the β-CrossLaps biomarker but also in an improvement in clinical-functional status, as evidenced by the favorable evolution of the parameters (Table 2, 3 and 4), with values consistent with international data. The 6MWT assesses the aerobic capacity and long-distance walking activity, endurance and dynamic balance when changing directions during the walking activity. In our study, the difference between T2 and T1 values was 65 m for SG and 14 m for CG. The SG patients was higher than a substantial MCID of 50 m have been estimated for the test in a sample of community-dwelling older adults with mobility dysfunction [53].

We considered that TUG provides correct information for stability in walking, balance moving from sitting to standing, and gait course changes without using compensatory strategies. For the MCID, our results was 1.2 for SG patients and 0.7 for CG patients. The value for gait retraining patients respect a recommended reduction of 0.8---1.4 s in OA research [54].

In particular, we studied one global gait parameter – walk cadence (number of half-steps in one minute) and Symmetry Index. This index represents the subject's ability to accelerate the centre of mass in a similar way during the cycle of the right and left steps. The more the index approaches the value 100, the more symmetry there is during the path. Generally, non-pathological subjects show an index greater than 90. Our patients had nearly 90 value for Symmetry Index before rehabilitation program; after, in T2 moments, the mean value was 93.9 for SG patients and 92.4 for CG patients.

A pain reduction of 1.75 cm on the scale is the recommended MCID minimal clinically important difference in OA research. We obtained 1.8 in SG patients [55].

WOMAC is a self-report questionnaire designed to assess the problems experienced by individuals with lower limb osteoarthritis in the past 72 ours. An improvement greater than or equal to 12% from baseline is the recommended MCID in OA research [56]. Our SG patients had improved the functional status (WOMAC scale) and MCID was 11.9%.

The MCID for the Lequesne Index is still not established in knee OA research [44].

Our results sustain the conclusions of other studies about rehabilitation program effect in KOA.

Clinical application of physiotherapy improves blood circulation, provides anti-inflammatory and analgesic effects, and assists in the alleviation of symptoms [57, 58]. The potential mechanisms of pain relief by physiotherapy measures are due to the stimulus of tissue metabolism and modulation of the inflammatory process. We used electrical stimulation, electromagnetic therapy, laser therapy, and therapeutic ultrasound. Physical therapists commonly employ these physiotherapy interventions in the rehabilitation of patients with knee osteoarthritis (KOA).

Transcutaneous electrostimulation (TENS) may not have any side effects. In 2009, Rutjes et.al. proved the benefits of TENS in KOA patients. Their studied KOA patients had an improvement in their pain status and physical function [33]. In our study, TENS was applied not alone, so we could not considered that effects were not produced only by the application of this physical procedure. Today, OARSI guidelines presents a low quality of evidence for TENS in all patients with osteoarthritis [31].

After educational and neuromuscular exercise program in KOA patients it obtained decreasing use of analgesics, decreasing pain and increasing function [59].

LIMITS

Three limitations should be addressed.

First, in our study we determined only the CTX-1 (β-CTx) serum level, one of the bone biomarkers turnover. CTX-II, considered a biochemical marker of cartilage turnover [60] isn't studied. For optimal research of bone-cartilage interface is ideal to determine the both markers (CTX-1 and CTX-II). Furthermore, incorporating CTX-1 into a prediction nomogram, alongside other biomarkers, for the clinical-functional status evolution of patients with knee osteoarthritis (KOA), both pre- and post-rehabilitation, would have constituted an optimal monitoring tool.

Second, CTX-I was associated with disease progression and the serum level is modified with anti-resorptive treatment in OA [61]. In our study we didn't establised the correlation between β-CrossLaps and disease progression, defined by radiographic (x-ray) images (joint space narrow and osteophyte). It would have been beneficial to establish correlations between the serum level of β-CTx and the severity of knee osteoarthritis (KOA), as defined according to radiological classification. Furthemore, we didn't recommended any anti-resorptive bone drugs.

Finally, we didn't assessed our patients for one or more parameters that characterize any KOA phenotypes. It is known that patients could classify for one or more phenotypes at the same time.

FUTURE RESEARCH DIRECTIONS

In the future, we should measure biomarkers in blood or urine and in accordance with this phenotyping set [62] we'll decide the rehabilitation program.

Moreover, we aim to approach knee osteoarthritis (KOA) as a molecular disorder characterized by the interplay of numerous molecules [63]. Accordingly, we will comprehensively evaluate the patient with KOA, categorizing them into one of the four types within the pre-KOA molecular classification: early KOA, progressive KOA, and end-stage KOA. If the patient exhibits a progressive form of the disease, they will be classified into one of the four subtypes: cartilage degradation-driven, bone remodeling-driven, inflammation-driven, and pain-driven, based on the predominant pathophysiological features. Subsequently, a tailored rehabilitation program will be devised, and appropriate medication prescribed. By determining specific biomarkers for each subtype (e.g., β-CTx for the bone remodeling-driven subtype), either independently or as part of a nomogram, the real effect of the program on knee structures will be monitored.

Clinical Implications. This novel approach to managing patients with KOA is expected to yield optimal outcomes for the patient's clinical-functional status. Validating the most significant biomarkers involved in the molecular pathogenesis of KOA allows for early diagnosis and a kinetic prophylaxis program for the patient, thus preserving the function of the intermediate pivot in lower limb biomechanics for patients with KOA.

CONCLUSIONS

The C-terminal telopeptide of type I collagen, CTX1 or β-CrossLaps (β-CTx) can be considered an inexpensive additional OA biomarker used to diagnose and assess the KOA patient, in clinical research.

Rehabilitation program in KOA patients has a complete effect not only on functional status but also joint structures. The serum β-CTx level is possibly a marker of rehabilitation derived structural modification in KOA.

In the assessment of global KOA patient status (clinical and functional status in accordance with bone-cartilage interface dynamics), β-CTx has significant value and could be used for monitoring the individualized rehabilitation program.

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