"OVIDIUS" UNIVERSITY OF CONSTANTA
**DOCTORAL SCHOOL OF MEDICINE**
FIELD MEDICINE
ACADEMIC YEAR 2024

# DOCTORAL THESIS

Scientifical Supervisor: **Univ. Prof. Valeriu Ardeleanu, MD, PhD**

PhD Candidate: **Nicoleta-Bianca Tudorachi**

**CONSTANȚA**

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# STUDY OF ANATOMIC AND IMAGING CHARACTERISTICS IN RELATION TO THE RISK FACTORS FOR THE PROGRESSION OF KNEE OSTEOARTHRITIS

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# I. CURRENT STATE OF KNOWLEDGE

# ABBREVIATIONS

| | |
|---|---|
| 8-oxo-dG | 8-oxo-2-deoxyguanosine |
| DNA | deoxyribonucleic acid |
| ANOVA | analysis of variance |
| APTT | activated partial thromboplastin time |
| AUC | area under the curve |
| Ca | calcium |
| CAT | catalase |
| Cd | cadmium |
| $CeO_2$ | cerium oxide |
| Cl | chlorine |
| COMP | cartilage oligomeric matrix protein |
| Cr | chrome |
| Cu | copper |
| DICOM | digital imaging and communication in medicine |
| DCF | dihydrofluorescein |
| EDX | energy dispersive x-ray system |
| Fe | iron |
| $Fe_3O_4$ | iron oxide |
| FS | fat suppression |
| FSE | fast spin echo |
| GPx | glutathione peroxidase |
| GSH | reduced glutathione |
| GS-SG | glutathione disulfide |
| HDL-cholesterol | high-density lipoproteins |
| $H_2O_2$ | hydrogen peroxide |
| HO• | hydroxyl radical |
| HTA | high blood pressure (hypertension) |
| IL | interleukin |
| BMI | body mass index |
| K | potassium |
| KL | radiological Kellgren and Lawrence system for classification of osteoarthritis |
| KOA | knee osteoarthritis |
| CSF | cerebrospinal fluid |
| LDL-cholesterol | low-density lipoproteins |
| MDA | malondialdehyde |
| Mg | magnesium |
| mL | milliliter |
| MMP | matrix metalloproteinases |
| MPO | myeloperoxidase |
| Na | sodium |
| NAC | n-acetyl-cysteine |
| NADP | nicotinamide adenine dinucleotide phosphate |

| | |
|---|---|
| nm | nanometer |
| •NO | nitric oxide |
| $O_2^{\bullet-}$ | superoxide radical |
| $^1O_2$ | singlet oxygen |
| $^3O_2$ | triplet oxygen |
| $O_3$ | ozone |
| $ONOO^-$ | peroxynitrite anion |
| P | phosphorus |
| Pb | lead |
| PD | proton density |
| PQQ | pyrroloquinoline quinone |
| PRX | peroxiredoxins |
| RNS | reactive nitrogen species |
| RO• | alkoxide radical |
| ROC | receiver operating characteristic |
| ROO• | peroxyl radical |
| ROS | reactive oxygen species |
| Se | selenium |
| SEM | scanning electron microscopy |
| Si | silicon |
| SOD | superoxide dismutase |
| T | tesla |
| TAC | total antioxidant capacity |
| TNF-α | tumor necrosis factor-α |
| TR | repetition time |
| TRA | cross-sectional |
| TSE | Turbo spin eco |
| UV | ultraviolet radiation |
| UV-Vis | ultraviolet-visible |
| ESR | erythrocyte sedimentation rate |
| μM | micrometer |

Study of anatomic and imaging characteristics in relation to the risk factors for the progression of knee osteoarthritis

## Introduction

Knee osteoarthritis (KOA) is a public health concern and one of the leading causes of disability. It is a degenerative pathology that progressively affects the knee and causes symptoms such as pain, instability or functional impotence, with a negative impact on patients who, over time, need to readjust their professional and social life to the new conditions. The diagnosis can be made clinically and objectively using imaging techniques, the most commonly used being knee radiography. The pathology is multifactorial, and among the risk factors involved in its onset or progression include: gender, age, anatomical changes in the knee that may affect bone, meniscal, muscular, ligamentous structures and tendons and, implicitly, deviations in the lower limb alignment that affect biomechanics due to the change in the way forces are applied. Moreover, several other risk factors for KOA are imbalances in redox homeostasis or metabolic syndrome.

The pathophysiology of arthrosis is not fully understood to date, and it is therefore of paramount importance to identify and reduce or eliminate modifiable risk factors, particularly through individualized prevention strategies. The prevalence of KOA is increasing, estimated to be approximately 33.6% for Americans over 65 years of age, predominantly affecting females. However, the incidence is significantly increasing in the 20-49 age groups. In the current context of increasing incidence of obesity and knee arthroplasty, it is estimated that by 2030 the number of surgical procedures may increase by 276% (1,2) and it is approximated that in 2032, compared to 2012, the number of consultations could be 15.7% higher (3).

Through the scientific research that I have carried out as part of my Doctoral Thesis, I have therefore approached a topic of interest, and I have focused on studying the anatomic and imaging characteristics in relation to the risk factors involved in the progression of KOA.

# I. General Part

## 1. ANATOMY OF THE KNEE JOINT

In the first chapter I have covered comprehensively with the anatomy of the structures that make up the knee joint, including the bone and muscle, cartilaginous, meniscal, ligamentous, bursae, innervation, venous drainage and arterial irrigation components. I have also mentioned several anatomical variations and their importance. I have presented the anatomic changes in the knee joint where I have described the femoral trochlear dysplasia or the angle of the intertrochlear groove, the patellofemoral congruence, changes in the position of the patella alta and baja type, the distal femoral torsion and the varum and valgum deformity.

## 2. KNEE OSTHEOARTHRITIS

In the second chapter, I have reviewed a brief history of KOA, definition, epidemiology, pathophysiology, clinical and radiologic classification, risk factors, as well as biomarkers involved in KOA. Given that free radicals and antioxidants have important implications in the context of pathology, I have detailed aspects about certain species of free radicals and antioxidants.

The complex pathophysiology of KOA still requires further research and in this context, it is instrumental to identify and reduce modifiable risk factors as well as protective factors to help develop appropriate prevention strategies (4). Risk factors such as age, gender, weight, systemic inflammatory mediators, cellular processes and biochemical changes that may affect bone homeostasis, anatomical changes and local trauma (5), as well as low social and economic status (6) lead to the onset and slow disease progression. Degenerative changes also occur when degradation processes exceed the purification capacity of the body (7), specifically, these processes are related to oxidative stress.

Oxidative stress is an imbalance between the production and accumulation of reactive oxygen species (ROS) at the cellular level and the ability of cells to inactivate them (8). The resulting dysfunctions and the generation of cytotoxicity cause a decrease in the level of antioxidant enzymes in biological fluids and cartilage and an increase in the level of free radicals that affect proteins involved in structural resistance (9). The effects of free radicals on cartilage components involve promoting inflammation, chondrosenescence, reduces the ability of growth factors to stimulate the synthesis of proteoglycans and induces apoptosis (8). At the time of the onset of symptoms, imaging changes that can be moderate or even serious are often detectable (10).

The most common diagnosis of KOA is made by frontal and lateral knee radiographs, depending on the joint space narrowing, bone damage or osteophytes (11). The most widely used radiological classification to determine these changes is KL, described as early as 1957. The pathology can be categorized into the 4 grades of the classification as follows; the exception is grade 0 which means the absence of radiological changes in the knee joint - healthy knee (12):

- Grade I - doubtful joint space narrowing and possible osteophytic lipping;
- Grade II - definite osteophytes and possible joint space narrowing;
- Grade III - definite narrowing of joint space, large osteophytes and possible deformity of bone ends;
- Grade IV - marked narrowing of joint space, severe sclerosis and large osteophytes (13).

## 3. THE ROLE OF IMAGING IN THE DIAGNOSIS OF KNEE OSTEOARTHRITIS

Imaging has an essential contribution in the diagnosis and monitoring of many pathologies and brings important information including in musculoskeletal conditions. Conventional radiography is the first-line test used to diagnose KOA. It is a cost-effective methodology, with the requisite equipment and trained personnel widely accessible, and the exposure time is relatively brief (14). Magnetic resonance imaging is the imaging method of choice in case of suspicion of knee lesions. It allows to evaluate the joint in various planes and sequences and unlike conventional radiography it offers the possibility of detecting early changes in arthrosis, but the examination is not so easily accessible (15). The acquisition time is longer, the costs are high and the investigation is contraindicated in patients with certain types of implants or stents.

The advantages of this method are that it provides detailed information on all the anatomical elements and the changes that may occur at their level, it is non-irradiating and non-invasive, but it is not routinely requested, only when there is suspicion of damage to various joint components such as ligaments or menisci.

Ultrasonography is a non-irradiating and non-invasive investigation method based on ultrasound that provides real-time information. It is widely available and as it has no contraindications, it can be repeated if necessary. However, the examination is operator-dependent and requires special high-resolution probes (16). It is often used for musculoskeletal inflammatory conditions and less often for degenerative conditions. It has specificity and sensitivity for morphological determinations of anatomical structures (17).

# II. PERSONAL CONTRIBUTION

## II 1. Justification of the Choice of PhD Topic, General Research Purpose and Objectives

Arthrosis is one of the leading causes of functional impotence worldwide, and particularly affects adults over the age of 40, both men and women, with a predilection towards women.
The impact of the disease on the physical and mental condition of patients and especially its prevalence is alarming, and its prevalence is expected to increase in the coming years. General and individual risk factors alter bone homeostasis and predispose to musculoskeletal pathology.
Although KOA has been objectified since ancient times, its pathophysiology is still being studied, and hospitalizations do not show promising results for the clinical status of patients. In this context, there is a need to identify and reduce potentially modifiable risk factors and in particular to identify protective factors. The achievement of these objectives is contingent upon the implementation of personalized prevention strategies. In order to identify the risk factors, it is essential to conduct a comprehensive medical history and engage in interdisciplinary collaboration.
In terms of therapeutic conduct, it is emphasized that this should be individualized in accordance with the specific phenotypes associated with KOA.
As a young specialist and doctoral student, I have found this to be a significant challenge, particularly given that the initial consultation tends to focus on the presenting symptoms, with a brief medical history that includes questions designed to facilitate the early detection of risk factors that can be addressed or avoided.
Accordingly, addressing KOA in accordance with phenotypic classifications and corroboration with imaging examinations and other paraclinical investigations may prove beneficial in establishing individualized treatments.

## II 2. General Criteria for Inclusion and Exclusion of Patients Selected for Clinical Trials Conducted

Clinical studies were conducted based on the approval no. 10/A757 issued by the "Dr. Iacob Czihac" Military Emergency Clinical Hospital, Iași, Romania Ethics Committee. The conducted studies were registred in the World Health Organization's international trials database (WHO) ClinicalTrials.gov; on the approval no. NCT05749601, under a unique protocol: ID:10/A575, under the title: "Risk Factors Involved in Knee Osteoartrhtitis". The retrospective clinical study in which I initially included 373 patients of "Dr. Iacob Czihac" Military Emergency Clinical Hospital (SMUIS) Iași, Romania, selected by applying the inclusion and exclusion criteria, in accordance with the diagram CONSORT ("Consolidated Standards of Reporting Trials").
*General criteria for inclusion in the study:* patients with uni- or bilateral knee pain who underwent diagnostic antero-posterior radiographs to establish the diagnosis of KOA; recording in the consultation chart of the parameters suggested for follow-up, patients who underwent uni- or bilateral magnetic resonance imaging, patients who underwent arthroscopic procedures for meniscal lesions of degenerative cause and in whom synovial fluid was collected in an amount of at least 1 mL.
*General exclusion criteria:* patients who have not met the inclusion criteria. Patients with post-traumatic meniscus lesions, those who have been previously given intra-articular injectable treatment or who had undergone ligamentoplasty surgical procedures, meniscectomies, fractures

of the lower limb or pelvis, arthroplasties; inflammatory rheumatic diseases - rheumatoid arthritis, gout, neoplastic diseases.

*I have structured my Doctoral Thesis into 4 research directions, as follows:*

*Clinical Study I* - Correlational Study of Gonarthrosis in Relation to Parameters in the Composition of the Metabolic Syndrome and the Background as Risk Factors.

85 patients were eligible after applying the inclusion and exclusion criteria for this part of the study.

*Clinical Study II*-Analysis of the Influence of Knee Anatomy on Gonarthrosis.

43 patients were selected for analysis after applying the inclusion and exclusion criteria.

*Clinical Study III*- Correlational Analysis between Radiologically Assessed Grades of Gonarthrosis and the Concentration of Reactive Species in the Synovial Fluid in Relation to its Rheological Characteristics and Systemic Inflammation.

According to the selection criteria, 28 patients were enrolled in this part of the study.

*Clinical study IV*-Synovial Fluid Analysis Using Scanning Electron Microscopy and Energy-Dispersive X-ray System (SEM-EDX)

There were 28 samples eligible for this chapter.

# Chapter 4.

## CORRELATIONAL STUDY OF KNEE OSTEOARTHRITIS IN RELATION TO PARAMETERS IN THE COMPOSITION OF THE METABOLIC SYNDROME AND THE BACKGROUND AS RISK FACTORS - CLINICAL STUDY I

The association between KOA and the elements of the metabolic syndrome such as obesity, hyperglycemia, dyslipidemia, HBP is frequently highlighted, leading over time the onset and slow progression of the disease (18). The aim of my study was to evaluate the association of KOA with the components of the metabolic syndrome represented by obesity, dyslipidemia, hyperglycemia, as well as the association with the background. The results of an ordinal logistic regression model that includes all the variables analyzed are presented in Table I. By comparison, keeping all other variables constant, the change with one unit of BMI increases the risk of KOA by 14%, and an increase with one unit of glucose increases the risk of illness by 1.7% (Table II).

**Table I.** Share of the increase in the risk of disease associated with different parameters.

| Parameter | IMC | LDL | Triglycerides | Glycemia |
|---|---|---|---|---|
| The risk of pathology (%) | 14,00 | 1,02 | 0,8 | 1,7 |

However, LDL and triglycerides do not influence the incidence and severity of KOA in the applied model, considering a level of significance of 5%, with a statistical *p-value* higher than 0.05.
I have evaluated a binomial logistic regression model for patients with bilateral disease, keeping male gender and the urban background as references, subsequently finding that only the background was statistically significant ($p < 0.05$) (Table II).
Keeping all other variables constant, it was revealed that patients from rural areas ($n = 20$) were associated with a higher risk of developing severe degrees of KOA compared to patients from urban areas ($n = 27$), with a ratio of 5.18.

**Table II.** Binomial logistic regression model for bilateral disease.

| Parameters | Odd ratio (OD) | Confidence interval 2,5% | Confidence interval 97,5% | *p* value |
|---|---|---|---|---|
| Sex (M) | 1.212 | 0,272 | 5.431 | 0,797 |
| The environment of origin (URBAN) | 0,194 | 0,043 | 0,743 | 0,022 |
| IMC | 1.076 | 0,920 | 1.277 | 0,370 |
| LDL | 1.011 | 0,987 | 1.037 | 0,386 |
| Triglycerides | 0,997 | 0,983 | 1.011 | 0,720 |
| Glycemia | 1.002 | 0,979 | 1.023 | 0,888 |

Using the adopted model in which only the background is statistically significant variable, I have obtained an average AUC value of 0.72, indicating acceptable discrimination (19) between the group with moderate KOA and the group with severe KOA.
The influence of the background in relation to the other parameters considered was performed using statistical studies, thus highlighting its specific contribution.

Through this study, I have highlighted the statistically significant influence of blood glucose values on KOA, the increase by one unit of blood glucose increasing the risk of disease by 1.7%, while each step of increasing the body mass index by one unit generated an increase in the risk of disease by approximately 14% of patients affected by KOA compared to healthy patients. By using the male gender as a reference, the parameters under review revealed a significant decrease of 0.281 in the disease rate.

In the binomial logistic regression model in which I included only patients with bilateral impairment, only the background was statistically significant. The rural background increased by 2.63 the rate of illness in different degrees of severity considering all patients in the target group of the clinical trial, thus, the risk of developing KOA was 5 times more severe for people in rural areas compared to those in urban areas.

In conclusion, KOA can be included in the metabolic phenotype, leading to an individualized treatment, with a focus on risk factors for a satisfactory clinical response, both for the physician and especially for the patient.

# Chapter 5.

## ANALYSIS OF THE INFLUENCE OF KNEE ANATOMY ON KNEE OSTEOARTHRITIS – CLINICAL STUDY II

Arthrosis initially affects the patellofemoral joint, and most studies correlate the variations in the morphological determinations of the bone components in the knee joint with patellofemoral arthrosis (20-22). I have chosen as an approach the assessment of the influence of the morphological bone variations in the knee on the radiological severity of the tibiofemoral compartment osteoarthritis, finding at international level a single study (23) to address the problem of KOA from this perspective. Therefore, the purpose of this chapter was to assess the influence of patellofemoral morphology in the severity of tibiofemoral compartment KOA, under the influence of highly predictable risk factors for knee pathology such as gender and age.
Patients were grouped according to the KL classification and the morphological analysis was performed according to gender and age.
The variables were the distal femoral torsion angle, the intercondylar (intertrochlear) groove angle and the patellofemoral congruence angle.
The distal femoral torsion angle was statistically significant correlated with the patellofemoral congruence ($p<0.034$), while age was positively correlated with patellofemoral congruence ($p<0.026$) – Fig. 1 and negatively correlated with intertrochlear groove angle ($p<0.057$).

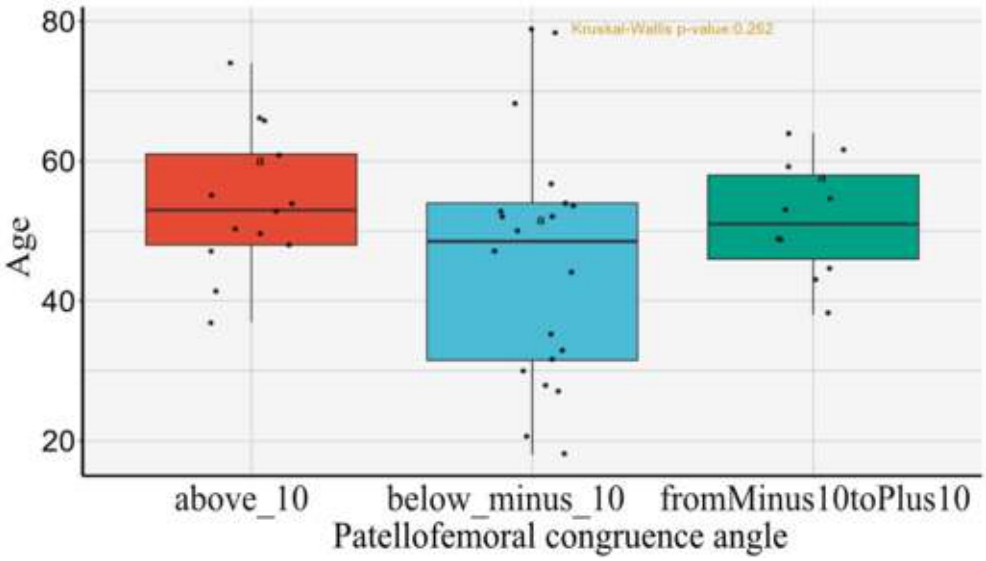



Fig. 1 Age distribution in relation to patellofemoral congruence

Analyses of distal femoral torsion and intertrochlear groove angle did not provide significant details either by age, gender or KL classification; these observations may suggest that the values of the 3 angles are independent, and their combination would not bring any additional insight, except for the distal femoral torsion angle and patellofemoral congruence that showed statistically significant correlations.

However, the statistically significant influence between the severity of the pathology and the age of the patients was objectified ($p<10^{-6}$).

From the study I have conducted, I have found that the only interdependent variables were the patellofemoral congruence and the distal femoral torsion angle, and the statistically significant results were the correlation between age and patellofemoral congruence.
None of the 3 parameters analyzed showed statistically significant correlations with the gender or severity of KOA, the only one of the variables analyzed that showed significant influence on the severity of KOA was age. In relation to the increasing prevalence of KOA, I have found that there are few studies to assess the influence of the morphology of the bone components of the knee for correction in order to prevent the onset of this pathology, the studies analyzed objectified heterogeneous results.
Considering the results obtained, in conjunction with those in the literature, I consider it appropriate to analyze the pathology also from these perspectives, with the potential inclusion of morphological variations in the phenotypic classification of KOA.

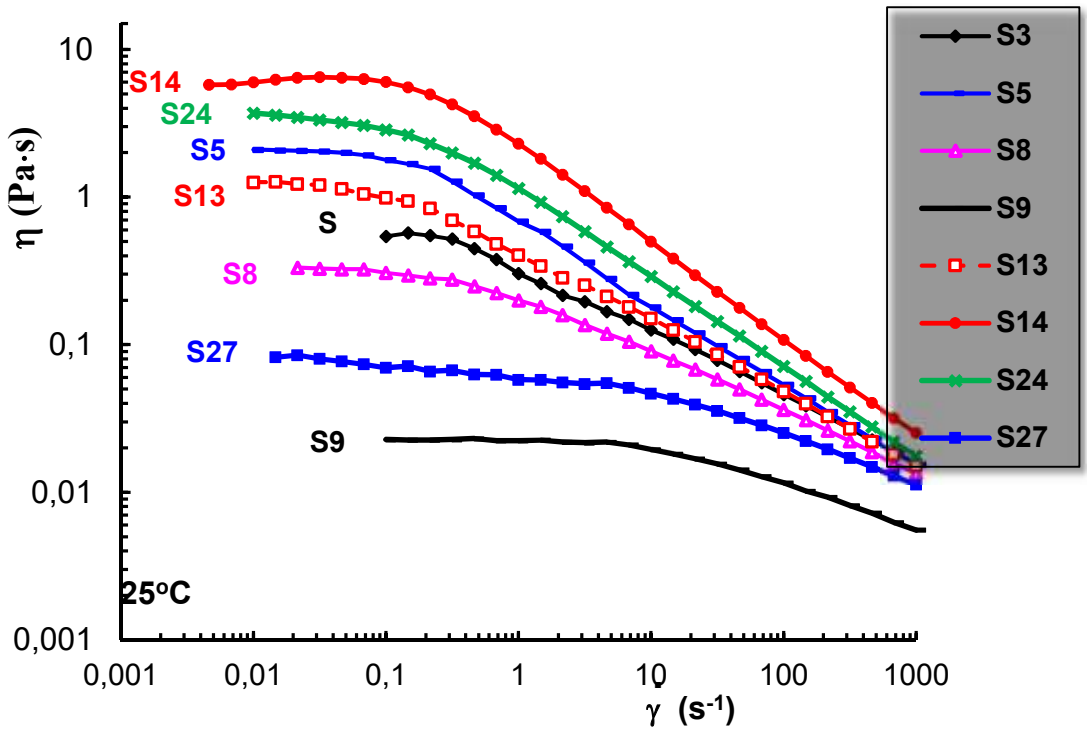
# Chapter 6

## CORRELATIONAL ANALYSIS BETWEEN RADIOLOGICALLY ASSESSED GRADES OF KNEE OSTEOARTHRITIS AND THE CONCENTRATION OF REACTIVE SPECIES IN THE SYNOVIAL FLUID IN RELATION TO ITS RHEOLOGICAL CHARACTERISTICS AND SYSTEMIC INFLAMMATION – CLINICAL STUDY III.

Knee osteoarthritis is a multifactorial chronic degenerative pathology with several phenotypes (24), the inflammatory being one of the most important (25,26), with the pathology being characterized by a low level of chronic inflammation (27). Knee osteoarthritis is also associated with changes in the viscoelastic properties of synovial fluid, caused by a decrease in the level of hyaluronic acid and increased amounts of free radicals, thereby reducing the ability to attenuate shock and inflammation (28). Oxidative stress and systemic inflammation are important factors that generate changes throughout the body, affecting the musculoskeletal system.
The aim of the study is to assess the influence of free radicals in the radiological severity of KOA and the rheological properties of synovial fluid in relation to systemic inflammation evaluated by HSV, CRP and fibrinogen and the following serum elements - total cholesterol, triglycerides, calcium, uric acid.

As shown in Figure 2, viscosity values vary with the degree of disease severity of patients.

a)



b)

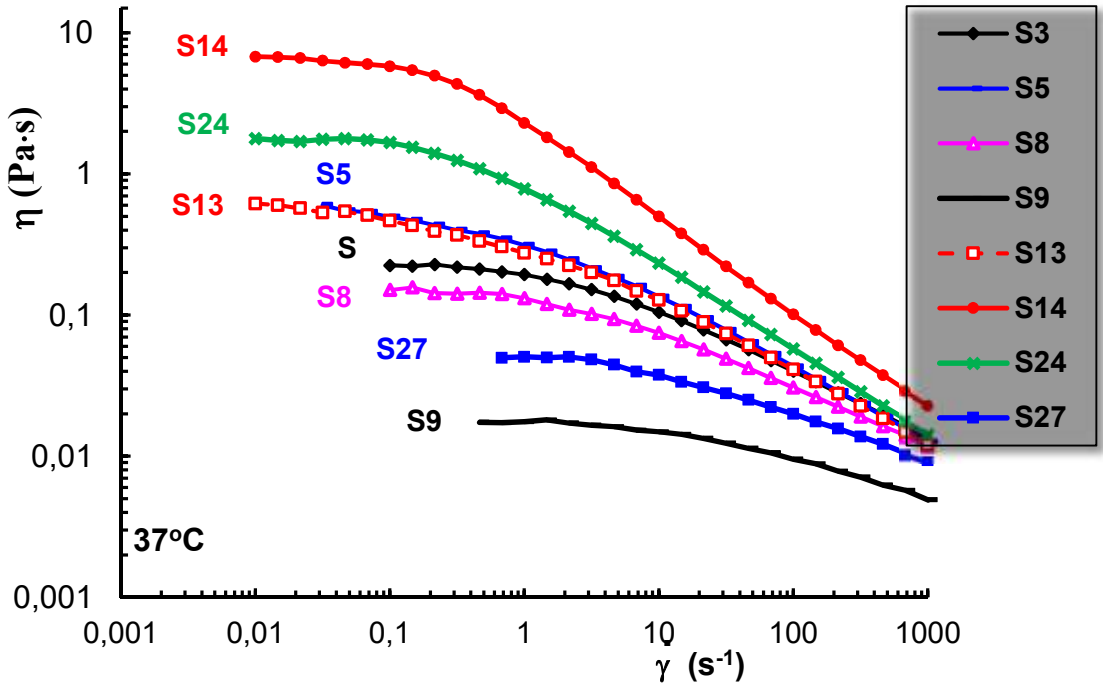



Fig. 2 Variation of viscosity with distinction for different degrees of severity of gonarthrosis for synovial fluid samples at different temperatures.
a). 25 °C; b). 37 °C.

In the research I have conducted there was no direct and clear correlation between KOA severity and viscosity. However, I have noted minimum values of the free radical mean ranging between 157.88 nM for grade II KOA and maximum values of 1,278.37 nM for grade III, with some heterogeneity.

Moreover, from the analysis of the viscosity of the synovial fluid at 37 °C in relation to free radicals and ESR, I have noticed increased values of these inpatients with grade I of the pathology. Although viscosity and ROS levels did not show significant variations between grades of KOA, the severity of the pathology seems to influence the combined effect of CRP levels and viscosity (Kruskal-Wallis p-value $<5.3x10^{-2}$).

The levels of glucose, APTT and the combined impact of uric acid and ESR showed differences according to the severity of the pathology, and the composite biomarker for oxidative stress and triglycerides showed suppression in higher degrees of severity (Kruskal-Wallis p-value $<5.53x10^{-2}$).

## Chapter 7. SYNOVIAL FLUID ANALYSIS USING SCANNING ELECTRON MICROSCOPY AND ENERGY-DISPERSIVE X-RAY SYSTEM (SEM-EDX) - CLINICAL STUDY IV

Scanning electron microscopy (SEM) scans electron bundles at nanometer resolutions and in combination with the energy-dispersive X-ray system (EDX) has an increased sensitivity for morphologic and chemical analysis of tissues as well as in the detection of nanoparticles that are used to improve the therapeutic performance of some chemotherapeutic agents (29, 30).In this latter study, I have aimed to highlight the synovial fluid elements using scanning electron microscopy and energy dispersive X-ray system and spectral analysis of the chemical elements of the detected crystals, depending on the radiologic severity of the KOA.

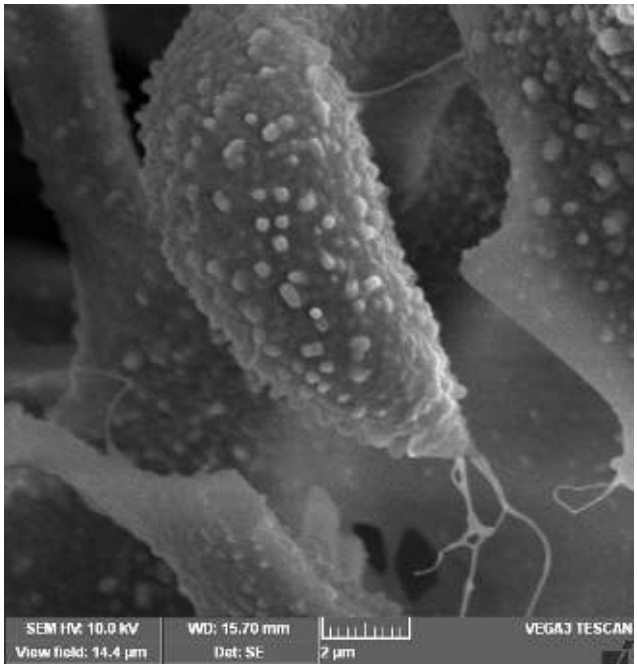



Fig. 3 Highlighting of crystals present in the synovial fluid

With the help of SEM, I have analyzed the synovial fluid of the patients included in the study, highlighting the presence of crystals and chemical elements. I have detected crystalline formations in all three stages of the disease, with the difference that in the case of a patient with first grade of severity, the size of the crystals was reduced, and their presence was limited. In grade II cases, the size of the crystals was larger and the frequency of their occurrence higher. For patients with grade III KOA, the frequency of crystals was high, with the most increased sizes.

Spectral analysis of the crystals indicated the presence of elements such as Se, K, Na, Cl, Ca, Si, Mg, S with a significant presence of Na, Cl, K and Ca. By spectral smear analysis I have revealed the presence of Ca, Mg and K for all the 28 patients studied, while the presence of Cu, P, Fe, Cl and heavy metals like Mn, Cr, Pb, Cd was noticed in a small number of patients. The heavy metals that I have detected by SEM analysis in the synovial fluid were Cd in 2 of the cases and lead in 6 cases, all patients having grade II or III KOA, except one patient with grade I KOA in whom lead was objectified.

The characteristics identified by means of SEM images and spectral analysis of the chemical elements of the crystals present at the level of the synovial fluid of the patients with KOA contribute with important information to the understanding of the pathophysiology and may play an essential role in determining the etiology of the arthrosis thus innovating new diagnostic techniques.

The elements analyzed were objectified in the highest concentrations in the advanced stages of KOA in the vast majority of cases, there being some peculiarities, where I have encountered slightly lower levels in some patients with the same advanced degree of pathology. These variations suggest the need for further research, including questionnaires that may target patients' diet and lifestyle. Moreover, it is important to understand the mechanisms by which these minerals and metals act on the musculoskeletal system and the pathways that induce inflammation in order to take preventive measures, include adjuvant supplements, antioxidants and personalized dietary plans, as most of the elements analyzed may have bidirectional effects (positive or negative) in the context of KOA.

# GENERAL CONCLUSIONS (SUMMARY)

Knee osteoarthritis is a multifactorial pathology that requires a multidisciplinary approach. One direction of its approach is prevention, by detecting potential risk factors and reducing or eliminating them, as well as establishing individualized treatment and highlighting potential new therapies. The initiation of individualized therapy taking into account phenotypic classifications of KOA along with other factors is instrumental. With the help of the results I have obtained, I have highlighted statistically significant correlations between the severity of KOA and the elements of the metabolic syndrome, thus contributing to the existing literature by strengthening the perspective of approaching KOA from the point of view of the metabolic phenotype, also particularizing the importance of the background.

With regard to the influence of anatomical variations of the bone components of the knee in the severity of KOA, none of the parameters analyzed showed statistically significant correlations with the severity of KOA or gender. The only interdependent variables were the patellofemoral congruence with the distal femoral torsion angle, and the statistically significant results were the correlation between age and the patellofemoral congruence. The evaluation of the influence of morphology on the radiologically quantified severity of tibiofemoral arthrosis is for the second time addressed in the literature and also there are few studies analyzing these effects, therapeutic proposals having mainly preventive aim, in order to correct morphological variations with trochlearoplasties.
In the relationship between severity of KOA, reactive species, viscosity and systemic inflammation, viscosity and free radical levels did not show significant variations between grades of KOA. The severity of the pathology seems to influence the combined assessment of viscosity and CRP. In the analysis of the viscosity of the synovial fluid at 37 °C, significant changes were also noted for oxidative stress and ESR.
Through the studies I have conducted, I have confirmed the classification of KOA in the metabolic and inflammatory phenotype, more studies are needed in order to integrate the morphological peculiarities in the phenotypic classifications.

In the evaluation of synovial fluid components using SEM-EDX, I have found that their presence, especially crystals but also higher concentrations of the present elements were objectively present in advanced stages of the pathology, with a few exceptions in which I have noted slightly lower levels in severe cases of KOA in patients with the same grade. The slightly heterogeneous results demonstrate the need for further research, which will lead to better understand the pathophysiology and to implement new preventive and therapeutic measures.

## ORIGINALITY OF THE DOCTORAL THESIS AND DEVELOPMENT DIRECTIONS

Knee osteoarthritis is a potentially disabling degenerative condition that has been studied internationally from a variety of perspectives. As a believer in the "better to prevent than to treat" principle, it was interesting for me to address the problem in terms of assessing potential risk factors for the severity of the condition assessed radiologically, with a view of correcting them at an early stage for preventive purposes. I aimed to assess the correlation of the radiologic severity of KOA with multiple parameters, the ones I targeted being the components of the metabolic syndrome, the background, the influence of the patellofemoral anatomy in the osteoarthritis of the tibiofemoral compartment, oxidative stress, systemic inflammation and the relationship with certain serum elements that were total cholesterol, triglycerides, calcium and uric acid.

The originality of the Thesis lies in the different perspective of the problematic approach, compared to the existing works. In my doctoral research, I approached for the first time in the Southeast European region the background as a risk factor. Moreover, with regard to the evaluation of the patellofemoral anatomy in the severity of KOA of the tibiofemoral compartment, the study I have conducted is the first in Romania and the second in the international literature researched in which this perspective is analyzed, most studies correlating the influence of anatomical variations with arthrosis of the patellofemoral compartment. Last but not least, the analysis of the synovial fluid and its characteristics using scanning electron microscopy and energy dispersive X-ray system and spectral analysis of the chemical elements of the detected crystals, in relation to the radiological severity of KOA is for the first time approached in this way in Romania. Most of the studies analyze by these targeted methods certain elements of the synovial fluid in the context of arthroplasties or arthropathies with crystals.

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