

INCREASED LEVEL OF C3 IN SYNOVIAL FLUID IN PATIENTS WITH ACTIVATED OSTEOARTHRITIS AS A DIAGNOSTIC AND THERAPEUTIC TARGET – PILOT GROUP OF PATIENTS FROM THE RHEUMATOLOGY DEPARTMENT AT UMHAT BURGAS

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Abstract: *The clinical course of osteoarthritis (OA) indicates the role of low-grade synovitis as the main driver of the degenerative process. Components of the extracellular matrix, fibronectin isoforms, and fragments of hyaluronic acid are presumed ligands for DAMPs (particularly TLR). Complement fractions bind to the corresponding receptors on the cell membranes of chondrocytes and synoviocytes through TLR. Does the complement cascade play a role only as a clearing system and/or a leading pathogenetic factor in activated OA? The aim of this study is to answer this question. The study included 50 patients with activated OA of the knee joint. We examined the levels of C3 and C4 complement fractions in the blood plasma and synovial fluid. We found that the values of these proteins in synovial fluid were on average 34.90% for C3 and 30.97% for C4 of their values in blood plasma, with a generally accepted norm of 10% for complement levels in a healthy joint. The observation regarding the strength of correlation between the above results and the radiological stage confirmed a stronger correlation of the obtained results in the earlier stages of the disease when the activity of the repair processes is more pronounced. In this way, we objectified the pathogenetic role of complement in the arthritic process, as well as its role in clearing the joint space from degradation products. The question arises whether pharmacological intervention to balance complement activation could represent a future therapeutic strategy in the treatment of OA and prevent its progression.*

Key words: *osteoarthritis, complement fractions, innate immune response, low-grade inflammation*

INTRODUCTION

The current understanding of osteoarthritis (OA) is that it is a heterogeneous, multifactorial, multidimensional, and polyorigin heterogeneous disease [1]. Patients with OA of the knee exhibit various phenotypes, defined as subtypes of OA, with different pathogenetic mechanisms, structural characteristics, clinical manifestations, and functional consequences. Within the scientific community, there are proposed pathogenetic definitions: “OA is a disease affecting mobile joints, characterized by cellular distress and degradation of the extracellular matrix, caused by micro- and macro-injuries, leading to the activation of non-adaptive repair reactions, including through the innate immune system’s inflammatory pathways” [2].

Due to the absence of systemic inflammation and neutrophils in synovial fluid, OA was previously described as a non-inflammatory disease. Today, it is believed that low-grade inflammation is a primary driver of the degenerative process in this condition [3]. The clinical course of the disease supports this fact. Quantitative determination of synovitis activity in osteoarthritic joints before the occurrence of irreversible structural damage is necessary, enabling

timely innovative therapeutic intervention similar to the paradigm applied in the early aggressive treatment of rheumatoid arthritis (RA) [4]. Recent research data support the hypothesis that low-grade inflammation in OA involves the engagement of toll-like receptors (TLR) and activation of the complement cascade through degradation products during joint destruction [5, 6, 7]. TLR7 polymorphism in the Chinese population determines susceptibility to knee OA, the severity of OA, and the risk of synovitis [8]. Cartilage fragments may act as neoantigens inducing aseptic inflammation [10].

Currently, there is a wealth of data on the leading role of innate immunity in OA [11, 12]. Activation of the innate immune response begins with the stimulation of cell membrane receptors recognizing molecules secreted by pathogens – PAMPs. In cases of cell and extracellular matrix damage, these receptors also recognize and activate DAMPs (damage-associated molecular patterns) [13]. In the context of degenerative processes in the joint, components of the extracellular matrix, fibronectin isoforms, and hyaluronic acid fragments are presumed DAMP ligands. Toll-like receptors (TLRs) are among the DAMPs [14, 15, 16]. The disruption of matrix ho-

meostasis during OA is an example of the activation of these receptors in chronic injury. TLRs 1-7 and 9 have been found in the synovium of OA patients in vitro studies [17, 18].

The humoral factors of the innate immune system include CRP (C-reactive protein) and the complement system. The three pathways of complement activation share a key stage – C3 convertase, which cleaves and activates C3, generating C3a and C3b (19, 20). The degradation products of C3 can serve as markers for complement system activation. Unlike the aforementioned C3 fragments, C3c does not bind to other structures such as pathogens, cell receptors, and plasma proteins, and it has a longer half-life. C3c is stable and reliable for assessing C3 levels in laboratory tests [21, 22]. Cell surface receptors for complement, called CRs, are found on many cells. Their function is to adhere to objects targeted for phagocytosis. Complement activation itself is triggered through C3R, and it also plays a role in the cell's oxygen metabolism, which is crucial for phagocytosis [23, 24]. The end result of complement activation is the lysis of target cells, opsonization, followed by the chemotaxis of macrophages and neutrophils, phagocytosis, and the transport of immune complexes to Kupffer cells in the liver and to the spleen for clearance [25]. Apart from its primary, evolutionarily determined role in the protection against pathogens, the complement system also plays a role in the removal of cellular debris and immune complexes, opsonization, and the stimulation of B and T lymphocytes [26]. Apoptotic chondrocytes, various components of the extracellular matrix, and their fragments in degenerative joints represent a distinct class of potent complement activators [27, 28, 29, 30, 31, 32].

The generally accepted norm for complement levels in synovial fluid is around 10% of plasma levels. Previous studies in osteoarthritis have shown higher levels compared to healthy individuals. The local production of complement fractions by various tissues is essential for tissue homeostasis. It has been shown that components of the complement system from C1 to C9 are produced in the synovium of human joints, with the exception of C8 and properdin [33], except for C8 and properdin [34]. Complement fractions bind to the respective receptors on the cell membranes of chondrocytes and synoviocytes through TLR [35]. The question of whether the complement cascade plays a role solely as a clearing system and/or as a leading pathogenetic factor in activated OA remains unanswered.

Goals and objectives of the pilot group study on oa

The hypothesis of this study is to find evidence for the direct involvement of the complement system in the pathogenesis of osteoarthritic disease.

The aim of the study is to measure the levels of complement fractions to establish their clinical significance in patients with activated osteoarthritis.

Objectives

Selection of patients with activated gonarthrosis according to ACR criteria (1991) and the absence of any other condition that could cause joint effusion.

Verification of the radiological stage using the Kellgren-Lawrence scale.

Utilization of ultrasonography to confirm synovial effusion and controlled arthrocentesis.

Examination of C3 and C4 complement fractions in blood plasma and synovial fluid.

Comparative analysis of their levels in both bodily fluids and the search for a correlational relationship.

MATERIALS

The study included 50 patients aged between 50 and 90 years who met the ACR criteria for knee osteoarthritis. All participants in the study signed an informed consent form. The scientific research was approved by the Ethics Committee of UMHAT Burgas AD (Minutes of the committee meeting dated January 23, 2019). The investigations were conducted in accordance with the requirements of Good Clinical Practice and adhered to the Helsinki Declaration on the rights of research subjects.

CRITERIA

Inclusion Criteria: The sole inclusion criterion was the presence of knee joint effusion (a sign of activated OA) in patients with osteoarthritic disease, regardless of the radiological stage according to Kellgren-Lawrence.

Exclusion Criteria:

- Comorbidity with other rheumatic diseases, including RA, psoriatic arthritis, spondyloarthritis, SLE, vasculitis, gout, fibromyalgia, and others.
- All contraindications for arthrocentesis.
- Severe decompensated accompanying illnesses; very low AN (antinuclear antibody) values.
- Immunocompromised patients.
- History of vasovagal shock, pregnancy, breast-feeding.

– History of systemic glucocorticoid treatment in the last 3 months or intra-articular depot glucocorticoid treatment in the last 6 months.

- Use of NSAIDs in the last 7 days.
- Patient refusal for arthrocentesis.

Prenalytical challenges are one of the main reasons why the complement system has not been well studied in the last 100 years. Complement proteins are temperature and time-sensitive. The instability of samples is the first problem for their analysis. Coagulation can also activate the complement since coagulation enzymes can cleave complement components into activating fragments. Therefore, complement is studied in plasma rather than serum. The viscosity of synovial fluid complicates the analysis. We added hyaluronidase to the joint fluid, and the subsequent results were adjusted for a dilution factor of 1.4 [39].

Based on the aforementioned preanalytical conditions and recommendations for sample collection and storage, we adopted our working protocol.

METHODS

Clinical Methods

– A venous blood sample of 3 ml was obtained through venipuncture using EDTA monovettes. After the separation of blood plasma, it was analyzed for the levels of C3 and C4 complement fractions.

– Synovial fluid obtained through arthrocentesis was also placed in EDTA monovettes. Subsequently, both samples were analyzed for the levels of C3 and C4.

Laboratory Methods

The obtained plasma and synovial fluid samples, as described above, were sent to a certified clinical laboratory. Plasma and synovial concentrations were then compared. The samples were processed at the certified “Lina” Laboratory in Burgas on a Cobas c501 analyzer, using Roche reagents and calibration for C3, C4 – c.f.a.c. Pr. C3 and C4 were analyzed using an immunoturbidimetric assay, a laboratory-standardized method (according to 36, 37).

Instrumental Methods

To verify the radiological stage according to the Kellgren-Lawrence scale in all study participants, conventional X-ray imaging was performed in an upright position in the “Schuss” position. Verification of synovial effusion in the knee joint was performed using ultrasonography, which was also used for controlled arthrocentesis when necessary.

Statistical Methods

The software used for statistical data processing was IBM SPSS Statistics 20.

The primary statistical method used in our work was the test of the statistical hypothesis for paired samples, known as the Student's t-test. The alpha level of significance used was 1% or 0.01, guaranteeing a 99% probability of the obtained results under the hypothesis we set.

RESULTS

Results for synovial C3 levels compared to plasma: The average percentage of C3 values in synovial fluid for the sample is 34.90% of the plasma levels. P-value = 0.0000 => We reject H_0 in favor of H_1 at a significance level of $\alpha = 1\%$ => With 99% confidence, we can say that the mean for the general population is greater than 10% (the norm according to the literature used) in patients with activated knee osteoarthritis (Tables 1 and 4).

Table 1. Average levels of C3 in synovial fluid versus plasma

One-Sample Statistics				
	N	Mean	Std. Deviation	Std. Error Mean
PercentC3	50	34.898334	18.3458908	2.5945008

Table 2. Average levels of C3 in synovial fluid versus plasma

One-Sample Test						
	Test Value = 10					
	t	df	Sig. (2-tailed)	Mean Difference	99% Confidence Interval of the Difference	
					Lower	Upper
PercentC3	9.597	49	.000	24.8983335	17.945196	31.851471

The results for synovial C4 levels compared to plasma are presented in Tables 11 and 12. The average percentage of synovial C4 levels for the sample is 30.97% of the plasma levels. P-value = 0.0000 => We reject H_0 in favor of H_1 at a significance level of $\alpha = 1\%$ => With 99% confidence, we can say that the mean for the general population is greater than 10% (the norm according to the literature used) in patients with activated knee osteoarthritis.

Table 3. Average levels of C4 in synovial fluid versus plasma

One-Sample Statistics				
	N	Mean	Std. Deviation	Std. Error Mean
PercentC4	50	30.972715	19.7838817	2.7978634

Table 4. Average levels of C4 in synovial fluid versus plasma

One-Sample Test						
	Test Value = 10					
	t	df	Sig. (2-tailed)	Mean Difference	99% Confidence Interval of the Difference	
					Lower	Upper
PercentC4	7.496	49	.000	20.9727146	13.474575	28.470854

After analyzing the obtained results in the examined group of 50 patients with activated knee osteoarthritis, it was found that the values of the studied proteins in the synovial fluid are on average 34.90% for C3 and 30.97% for C4 of their values in the blood plasma, with a generally accepted norm of 10% for complement levels in a healthy joint.

The present study quantitatively assesses the trapping of complement for cellular debris and fragments and, as a final stage of its activation, the clearance of this detritus from the joint space.

The observation regarding the strength of the correlation between the above-mentioned results and the radiological stage confirms a stronger correlation of the obtained results in the earlier stages of the disease when the activity of the repair processes is more pronounced.

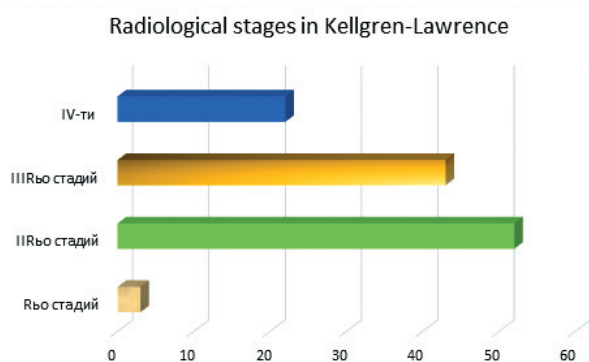


Fig. 1

CONCLUSIONS

The present scientific study objectifies the involvement of complement in the pathogenesis of activated osteoarthritic disease.

It supports the fact that in OA, complement activation occurs through the alternative pathway (higher levels of C3 in synovial fluid compared to C4).

It confirms the involvement of complement in the process of eliminating degradation products from the joint space in knee OA.

We found a stronger correlation between elevated clinical indicators and radiographic changes in the knee joint in the earlier stages of the disease when the activity of repair processes is more pronounced.

By objectifying the pathogenetic role of complement in the osteoarthritic process, the present study may provide direction for future therapeutic strategies.

POSSIBILITIES

Current therapies for osteoarthritis (OA) are typically aimed at pain relief, improving joint function, and patient education. Non-pharmacological methods,

such as all types of physical activity, weight reduction by more than 10%, and physical therapy, are beneficial for weight-bearing joints and the axial skeleton. The most commonly used treatments, including non-steroidal anti-inflammatory drugs (NSAIDs) – non-selective and COX-2 selective, non-opioid and opioid analgesics, SYSADOA, intra-articular injections with depot glucocorticoids, hyaluronic acid, and PRP, offer only symptomatic relief and the possibility of slowing structural damage in the early stages of the disease.

The development of medications for osteoarthritis lags behind that of inflammatory joint diseases. Disease-modifying agents are urgently needed. Various treatment targets for OA have been discussed for about 90 years. Despite the clear role of inflammation in OA, the use of systemic and/or intra-articular biological agents to inhibit TNF α and IL-1 β proved disappointing. In phase 3 clinical trials are: granulocyte-macrophage colony-stimulating factor antibody; Wnt inhibitor Lorecivint (SM04690, Biosplice) for intra-articular application; interleukin-1 inhibitor canakinumab (Ilaris, Novartis); synthetic transdermal cannabidiol; selective aggrecanase inhibitor; oral Cathepsin K inhibitor; anti-NGF (nerve growth factor) antibodies (Merck); cellular and gene therapies; Sprifermin, FGF-18 (fibroblast growth factor 18) for intra-articular application. Inhibiting TLR as a new therapeutic option in rheumatology is associated with the following issues: accelerated renal excretion, imprecise tissue distribution, degradation in endosomes (which could potentially be solved by pegylation). Skeletal stem cells - as a regenerative therapy, may be a promising method due to their potential to influence cartilage regeneration and potentially surpass the effectiveness of pain management drug groups.

The question arises whether pharmacological intervention to balance complement activation could represent a future therapeutic strategy for OA treatment and prevent its progression.

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