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Expression of IL-1β Receptor Antagonist in Patients with Severe Osteoarthritis of the Knee–The Mechanism Limiting the Inflammation in Osteoarthritis

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Abstract

Introduction: Expression of interleukin-1β (IL-1β) in the synovial fluid of patients with osteoarthritis has been confirmed. The overall effect of this cytokine can be described as a chondrocyte-dependent cartilage destructive potential. The interleukin-1β receptor antagonist (IL-1Ra) blocks the interaction between IL-1β and its receptor on the cell surface. Human chondrocytes and synoviocytes produce IL-1Ra in a response to stimulation by IL-1β and IL-6. The local production of IL-1Ra in the joint has a protective effect against the catabolic action of IL-1β.

Material and Methods: Synovial fluid and blood serum were obtained from 31 patients undergoing knee replacement surgery because of osteoarthritis. For comparison, samples were obtained from another 30 patients during diagnostic or therapeutic arthroscopy performed because of knee injury. The samples were analyzed for IL-1β and IL-1Ra using enzyme-linked immunosorbent assay. The U Mann-Whitney test and Spearman's test, as well as receiver operating characteristic (ROC) were used for statistical analysis.

Results: Statistical analysis showed that the concentration of IL-1Ra in serum was significantly higher in the osteoarthritis group compared to the injury group (p=0.0001), and there was a positive correlation of IL-1Ra concentration in the serum and in the synovial fluid (correlation coefficient R=0.41; significance p=0.02). In the osteoarthritis group there was a very high negative correlation between the intensity of pain and the concentration of IL-1Ra in the synovial fluid (correlation coefficient R=-0.75; significance p<0.000001). Furthermore very high negative correlation was observed between the intensity of pain and level of this cytokine in serum (R=0.86; p<0.000001). For the measurements of IL-1Ra in serum the sensitivity of this test was 71.0%, specificity 70.0%, positive prediction of a result was 71.0%, negative prediction 70.0%, and the area under the ROC curve was 0.792.

Conclusion: Late stage osteoarthritis is characterized by a systemic inflammatory reaction with high concentration of IL-1Ra in serum, which is positively correlated with its concentration in the synovial fluid. Pain in patients with late stage osteoarthritis may be reduced due to the high concentration of anti-inflammatory IL-1Ra both in the synovial fluid and in blood serum. The measurement of IL-1Ra in serum is a reliable immunoassay test that determines the status of osteoarthritis.

Keywords: Osteoarthritis; Cytokines; Interleukins; Knee injuries

Abbreviations ELISA: Enzyme Linked Immunosorbent Assay; IL-1β: Interleukin-1β; IL-1Ra: Interleukin-1β Receptor Antagonoist; OA: Osteoarthritis–Study Group; INJ: Injury Reference Group; RA: Rheumatoid Arthritis; ROC: Receiver Operating Characteristic; WOMAC: Western Ontario and Mcmaster Universities Index of Osteoarthritis; VAS: Visual Analog Scale.

Introduction

Processes occurring within the joint in osteoarthritis (OA) still remain largely unexplained. An unknown initiating factor leads to an imbalance between anabolic and catabolic processes within the cartilage. One of the effects of increased catabolic changes is presence of products of extracellular matrix degradation in the synovial fluid. The joint microenvironment altered in this way stimulates the activity of chondrocytes, which attempt to rebuild the structure of the cartilage, and this leads to increased expression of proinflammatory cytokines as well as mediators with pleiotropic, modulating and anti-inflammatory action.

Interleukin 1, a cytokine with proven inflammatory action, has a catabolic potential in relation to the articular cartilage. Active form of this cytokine (IL-1β) acts on the cartilage through the receptor found on the surface of chondrocytes (IL-1R). When stimulated these cells produce other inflammatory and modulating cytokines, proteases and prostaglandins. The overall effect of IL-1β can be described as a chondrocyte-dependent cartilage destructive potential [1]. In addition, IL-1β inhibits the synthesis of proper forms of collagen in chondrocytes [2].

Concentration of IL-1β in the synovial fluid of patients with OA is much lower than in rheumatic patients, but still the expression of IL-1β in the synovium of patients even in the early stages of OA has been confirmed [3,4].

Animal model studies have shown that intraarticular administration of IL-1β reduces the amount of proteoglycans within the cartilage. On the other hand, blocking the biological function of IL-1β through

administering a soluble receptor antagonist slows down the progression of OA in animals [5]. Similar observations were also made in human patients with osteoarthritis of the knee [6].

The interleukin-1β receptor antagonist (IL-1Ra) is a member of the IL-1 family, it acts as a competitive inhibitor of IL-1R. IL-1Ra blocks the interaction between IL-1β and its receptor on the cell surface and thus inhibits the biological effects of IL-1β. The antagonist alone does not show any action on the cells. Human chondrocytes and synoviocytes produce a soluble form of the receptor antagonist (sIL-1Ra) in a response to stimulation by IL-1β and IL-6 [7]. The local production of sIL-1Ra in the joint has a protective effect against the catabolic action of IL-1β [8]. Clinical studies demonstrated that autologous IL-1Ra (Orthokine ®) given intraarticularly to patients with OA reduces pain and improves joint function [9,10]. As suggested by recent studies, severity of radiographic changes in knee OA is associated with agonistic activity of IL-1Ra in relation to interleukin-1β, however this relationship can also be affected by the receptor gene polymorphism [11].

The purpose of this study

Examine the serum and synovial fluid concentrations of cytokine IL-1β and its antagonist (IL-1Ra) in patients with advanced osteoarthritis of the knee and compare the concentrations of these cytokines with concentration in patients after knee injuries.

Study the correlation between the concentration of these cytokines in serum and the synovial fluid.

Determine the correlation of IL-1β and IL-1Ra concentration in serum and synovial fluid with the degree of pain, joint dysfunction and the degree of radiographic changes.

Determine the sensitivity, specificity and other indicators which show the diagnostic value of measuring cytokines concentrations in serum and synovial fluid in patients with OA.

Material and Methods

The research was approved by the local bioethics committee.

The study group (OA) included 31 patients (mean age 71.0 ± 6.8 years; 26 women (83.87%) and 5 men (6.13%)) diagnosed with osteoarthritis of the knee, scheduled for total knee replacement. The reference group (INJ-Injury) consisted of 30 patients (mean age 51.1 ± 9.7 years, 13 women (43.33%) and 17 men (56.67%)) that were scheduled for knee arthroscopy because of different injuries of the knee joint.

Inclusion criteria for the study group:

Diagnosis of osteoarthritis of the knee based on clinical, radiological and laboratory parameters specified by the American Rheumatism Association. [12].

A positive qualification for total knee replacement.

Informed consent to perform the tests according to the protocol.

Discontinuation of non-steroidal anti-inflammatory drugs at least one week before obtaining the biological samples for research.

Inclusion criteria for the reference group:

A history of knee injury six months to three weeks prior to the examination.

No signs of OA based upon criteria mentioned earlier.

A positive qualification for elective knee arthroscopy.

Informed consent to perform the tests according to the protocol.

Discontinuation of non-steroidal anti-inflammatory drugs at least one week before obtaining the biological samples for research.

Exclusion criteria for study and reference groups:

The presence of hematoma in the knee.

Acute or chronic inflammatory disease of the knee joint including rheumatoid arthritis (according to diagnostic criteria of American Rheumatism Association [13]).

Systemic inflammatory and autoimmune disorders.

The use of immunosuppressive or steroid anti-inflammatory drugs by the patient.

Lack of informed consent for the test.

Clinical and Radiological Assessment

All patients from both groups underwent clinical examination which included the subjective assessment of knee pain using the visual analog scale (VAS) [14]. In addition, all patients in the study group (OA) were evaluated for clinical progression of knee osteoarthritis using the WOMAC index (Western Ontario and McMaster Universities Osteoarthritis Index). The WOMAC index ranges from "0" to "96", were "0" means no symptoms [15]. The radiological evaluation of the severity of osteoarthritis was performed using the Altman scale. It is a four-stage scale that describes radiographic changes in osteoarthritis. The scale is based upon the joint space width and the presence of osteophytes [16]. Altman score ranges from "0" to "3", where "0" means no radiographic signs of osteoarthritis and "3" represents the most advanced changes.

Immunosorbent Testing

The concentrations of IL1-β and IL-1Ra cytokines were measured in all patients from both groups. The unit used to express the concentration was pg/ml.

Prior to surgery 10 ml of venous blood was collected from the ulnar vein using a closed system–Vacutiner ®. The blood was collected not more than 24 hrs before the surgery, at the time when standard tests required for surgery were performed.

Synovial fluid was obtained by a puncture of the knee. It was done in the operating room just before the surgery, after preparation of the operative field. When it was possible the entire joint fluid was aspirated.

Blood and synovial fluid were collected to test tubes with an anticoagulant (heparin), then the tubes were centrifuged (2500 rev/min. for 10 min.). The supernatant was separated from the sediment, divided into parts with a volume of 300 μl, then immediately frozen in −72°C and stored in Eppendorf test tubes until further testing was performed.

After collecting the material from all subjects blood plasma and synovial fluid supernatant were thawed and the level of IL-1β and IL-1Ra was measured. The concentrations were determined using enzyme-linked immunosorbent assay (ELISA). It was done using

commercially available kits, in accordance with the manufacturer's specific protocols.

Description of the ELISA Method

The test samples and standards were pipetted to microplate wells coated with monoclonal antibodies specific for tested proteins, and incubated for 2 hrs. After thorough washing and filtration that removed unbound proteins, polyclonal detection antibodies bounded with horseradish peroxidase were added. After another two hours of incubation, washing and filtration color reaction was induced by adding substrates for the horseradish peroxidase (tetramethylbenzidine mixed in a 1:1 ratio with hydrogen peroxide). The reaction was carried out for 20 minutes in the dark and was stopped by adding 2N sulfuric acid. All stages of this procedure were performed at room temperature. The reading of extinction was made at a wavelength of 450 nm using a universal microplate reader El x 800, manufactured Bio-Tec, Inc., USA. The concentration of proteins was calculated from standard curves using the microplate reader software.

Statistical Analysis

In description of quantitative variables standard statistical parameters were given: arithmetic mean, standard deviation and median.

Distributions of the variables tested were significantly different from the normal distribution (tested with Shapiro-Wilk test). That is why the non-parametric U Mann-Whitney test was used to compare groups.

The correlation of quantitative variables was based on Spearman's test, the correlation coefficient and significance of the correlation result was obtained.

To assess the accuracy of diagnostic test the ROC curve (receiver operating characteristic) was used. The area under the ROC curve is a measure of how well a parameter can distinguish between two diagnostic groups (diseased/normal). An area of 1 represents a perfect test; an area of 0.5 represents a worthless test.

The result of the analysis was considered statistically significant if the level of significance p was less than or equal to 0.05.

Results

The study group (osteoarthritis-OA) consisted of patients with the mean age of 71 ± 6.8 years. Women prevailed in this group (84%). Age in the reference group of patients (injury-INJ) was significantly lower (51.1 ± 9.7 years) (p <0.000001) and the percentage of women was also lower (43.3%, p=0.001).

Evaluation of knee pain was performed in both groups. The VAS score in the OA group was 5.5 ± 2.6, while in the INJ group it was 3.8 ± 2.0 (p=0.01).

The WOMAC score was determined only for patients in the OA group, the mean was 58.4 ± 21.1. The values in the WOMAC index range from 0 to 96, where 96 means clinically severe osteoarthritis.

According to the four-level radiological Altman scale (0°,1°,2°,3°) nearly half of patients with OA group showed changes classified as grade 3° (45.2%). The others were classified as stage 2° (25.8%) or stage 1° (29.0%). There were no cases without radiological signs of osteoarthritis in the OA group (0° in the Altman scale).

Concentration of cytokines in serum and synovial fluid in the studied groups

Concentrations of IL-1β, and IL-1Ra in both groups of patients are presented in Table 1.

Cytokine	Study group (OA)	Reference group (INJ)	Statistical difference
IL-1β serum	5.24 ± 4.99 (3.83)	3.63 ± 2.31 (2.89)	NS (p=0.24)
IL-1β synovial fluid	2.97 ± 3.45 (1.58)	2.27 ± 1.15 (2.17)	NS (p=0.44)
IL-1Ra serum	624.38 ± 610.82 (492.55)	269.52 ± 144.25 (231.43)	p=0.0001
IL-1Ra synovial fluid	1127.15 ± 2598.44 (384.22)	414.63 ± 501.5 (284.82)	NS (p=0.63)
Cytokine concentration values are expressed in pg/ml.			
The values given are: arithmetic mean, standard deviation and median.			

Table 1: The concentration of cytokines in serum and synovial fluid in the two groups.

Statistical analysis showed that the concentration of IL-1Ra in serum was significantly higher in the OA group compared to the INJ group (p=0.0001). The concentration of this cytokine in the synovial fluid of patients with OA was also higher than those in INJ group, although in this case, the difference was not statistically significant.

Correlation of serum levels of cytokines with their concentration in the synovial fluid

The OA group had a positive correlation of IL-1β concenration in the serum and in the synovial fluid. The correlation coefficient (R) for IL-1β was 0.57 and the level of significance of this correlation was high, P=0.0009 (Figure 1). Similarly, the correlation was confirmed for its receptor antagonist (IL-1Ra), the coefficient R was 0.41 at the significance level of p=0.02 (Figure 2).
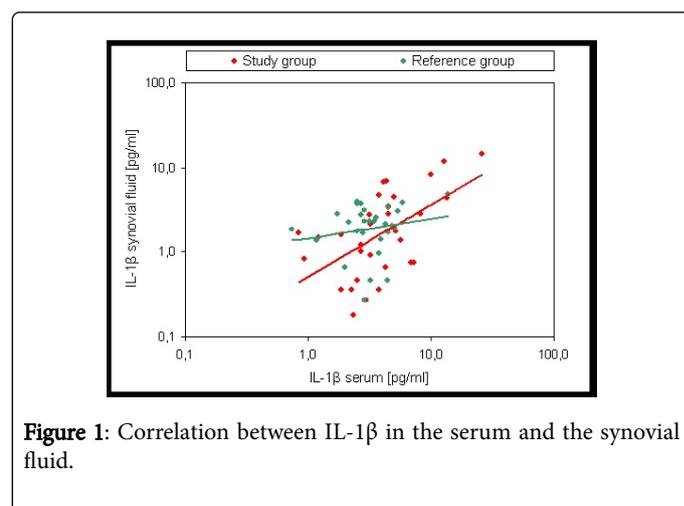

Figure 1: Correlation between IL-1β in the serum and the synovial fluid.

The reference group showed a positive correlation in serum and synovial fluid of IL-1Ra concentration, for which the correlation coefficient was 0.52 with significance p=0.03 (Figure 2), whereas correlation between the concentrations of IL-1β in this group was not observed (R=0.14, p=0.47).
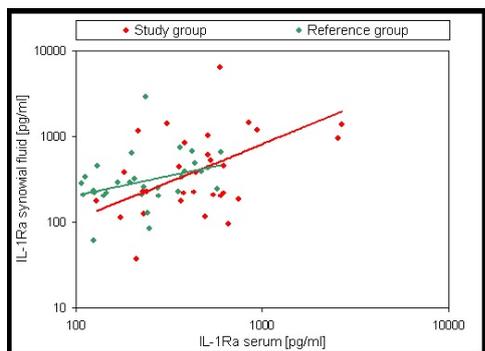
Figure 2: Correlation between IL-1Ra in the serum and the synovial fluid.

Correlation of cytokine concentration in serum and synovial fluid with age

There was no significant correlation between the levels of cytokines IL-1β and IL-1Ra with age in both studied groups (Table 2).

Parameter	Study group (OA)		Reference group (INJ)	
	Correlation coefficient R	Significance test	Correlation coefficient R	Significance test
IL-1β serum	-0.27	NS (p=0.14)	-0.09	NS (p=0.64)
IL-1β synovial fluid	-0.24	NS (p=0.20)	-0.14	NS (p=0.46)
IL-1Ra serum	0.11	NS (p=0.56)	0.14	NS (p=0.47)
IL-1Ra synovial fluid	0.07	NS (p=0.71)	0.05	NS (p=0.80)

Table 2: Correlations of cytokine concentrations with age in the studied groups.

Correlation between the used clinical and radiological scales with age

In both groups there was no significant correlation between clinical (VAS and WOMAC) and radiological scales (Altman) with age (Table 3).

Scale	Study group (OA)		Reference group (INJ)	
	Correlation coefficient R	Significance test	Correlation coefficient R	Significance test
VAS	-0.15	NS (p=0.41	-0.07	NS (p=0.72)
WOMAC	0.25	NS (p=0.18)	---	---
Altman	0.22	NS (p=0.23)	---	---

Table 3: The correlations of the analyzed scales with age.

Correlation of cytokine concentrations in serum and synovial fluid with the degree of pain expressed in VAS

In the OA group there was a very high negative correlation between the intensity of pain and the concentration of IL-1Ra in the synovial fluid (Table 4 and Figure 3). Furthermore similarly high negative correlation was observed between the VAS scale and level of this cytokine in serum (Table 4 and Figure 4). The observations in the reference group also showed a negative correlation of pain and IL-1Ra levels both in serum and in synovial fluid.

Cytokine	Study group (OA)		Reference group (INJ)	
	Significance test	Correlation coefficient R	Significance test	Correlation coefficient R
IL-1β serum	-0.14	NS (p=0.45)	-0.12	NS (p=0.51)
IL-1β synovial fluid	-0.26	NS (p=0.16)	-0.18	NS (p=0.35)
IL-1Ra serum	-0.75	p=0.000001	-0.70	p=0.00002
IL-1Ra synovial fluid	-0.86	p<0.000001	-0.88	p<0.000001

Table 4: Correlations of cytokine levels in the VAS groups.
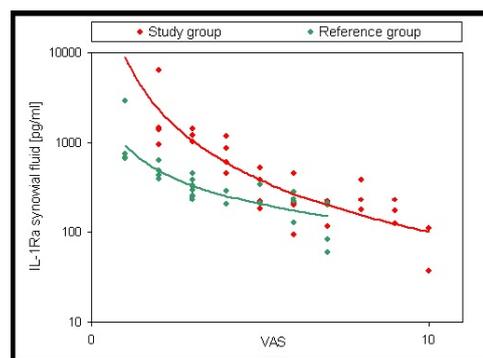

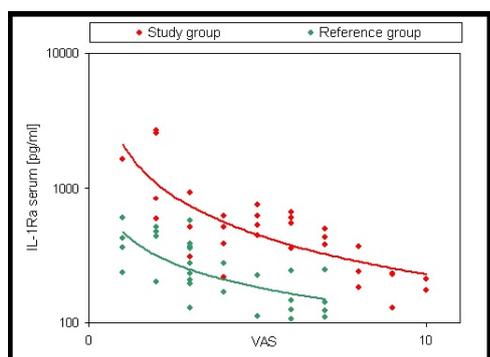**Figure 3:** Correlation of IL-1Ra level in the synovial fluid with VAS.

Figure 4: Correlation of IL-1Ra serum level with VAS.

Correlation of cytokine concentration in serum and synovial fluid with the WOMAC functional index and the Altman radiological scale.

The results showed a positive correlation between the serum concentrations of studied cytokines in the OA group with the WOMAC and Altman scale. These variables were correlated with each other at an average level (Table 5). WOMAC index correlated with IL-1Ra in the synovial fluid, while the correlation with the Altman scale concerned the synovial fluid concentrations of both IL-1β and IL-1Ra.

Cytokine	Study group (OA)		Reference group (INJ)	
	Significance test	Correlation coefficient R	Significance test	Correlation coefficient R
IL-1β serum	0.17	NS (p=0.35)	0.23	NS (p=0.22)
IL-1β synovial fluid	0.32	NS (p=0.08)	0.39	p=0.03
IL-1Ra serum	0.16	NS (p=0.38)	0.18	NS (p=0.34)
IL-1Ra synovial fluid	0.52	p=0.003	0.40	p=0.03

Table 5: Correlations of cytokine levels with the WOMAC functional scale and the Altman radiological scale in the OA group.

The diagnostic usefulness of IL-1Ra serum level in patients with OA

ROC curves analysis measured the sensitivity, specificity and other factors that determine the usefulness of measurement of IL-1Ra serum level for the diagnosis of osteoarthritis (Table 6). The sensitivity of the test was 71.0%, specificity 70.0%, positive prediction 71.0%, negative prediction of a result was 70.0%, odds ratio R=5.7. The area under the ROC curve for this ratio was 0.792 (Figure 5).
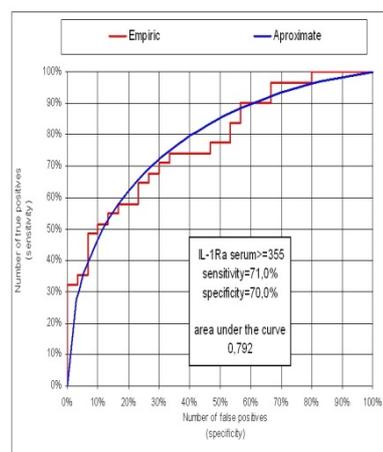

Figure 5: The ROC curve for the serum level of IL-1Ra.

Measures of the diagnostics test	The concentration of IL-1Ra in serum
Critical value	355
Abnormal values	≥ 320
Number of true positives	22
Number of true negatives	21
Number of false positives	9
Number of false negatives	9
McNemar's test	NS (p=0,81)
Sensitivity (95% CI)	71.0% (51.9%; 85.8%)
Specificity (95% CI)	70.0% (50.6%; 85.3%)
Prediction of a positive result (95% CI)	71.0% (51.9%; 85.8%)
Prediction of a negative result (95% CI)	70.0% (50.6%; 85.3%)
Odds ratio (95% CI)	5.7 (1.9; 17.5)
Area under the ROC curve (95% CI)	0.792 (0.685; 0.899)

Table 6: The concentration of IL-1Ra cytokine in serum as a diagnostic test.

Discussion

The studied group of patients with OA included people who, due to the severity of their disease, were qualified for total knee replacement. This group was compared with the reference group, which included patients after different knee injuries. This group cannot be treated as a control group, but as a distinct pathological condition in which the cytokines are also expressed. In a recently published article [17] the authors have described higher levels of proinflammatory cytokines (IL-6, CCL2, CCl 4, IFN-γ) in the synovial fluid of patients with symptomatic meniscal damage when compared with those who had clinically silent damage (in which cytokines were almost absent in the joint). At the same time a different group of authors [18] showed

that a significantly higher level of proinflammatory cytokines is present only a few days after the initial injury. Taking into account this fact, the reference group consisted only of patients who had the knee injury at least three weeks before the test.

The mean age in the OA group was higher than in the reference group. However, it did not matter in the interpretation of the results of cytokine concentrations in serum and synovial fluid because the correlation of the concentration with age of the patients was excluded (Table 2).

Functional limitation of knee joints differed among OA group of patients, however most patients showed significant impairment of the joint function with accompanying pain of the knee. Radiological features of knee joints were adequate to the late period of the disease. Scales describing the clinical (WOMAC and VAS) and radiological status (Altman scale) of patients were used to investigate their correlation with the concentration of cytokines in serum and synovial fluid. Based on the Spearman test, the correlation of these scales with age was excluded (Table 3).

From the group of known cytokines involved in inflammatory processes the cytokine with a proven catabolic potential on the cartilage was chosen - IL-1β.

In the context of the late period of osteoarthritis of the knee within the OA group, a representative anticatabolitic cytokine (IL-1Ra) was chosen. It came to mine interest because of its use in the biological treatment of OA.

From the literature it is known that IL-1β in the synovial fluid has been found in both OA and rheumatoid arthritis (RA), and its expression was confirmed in the cartilage, the synovial membrane and the subchondral bone layer [19-22]. Osteoarthritis in comparison with rheumatoid arthritis is characterized by a small degree of joint inflammation. That is why in the studied patients (OA group) concentration of IL-1β in serum and synovial fluid was relatively low, even lower than in the INJ group (Table 1). The importance of IL-1β (and TNF-α) in the pathogenesis of RA is more explicit and this cytokine is present in synovial fluid continuously at high concentrations [23].

Despite the low expression of IL-1β significant correlation of the concentration of this cytokine in the synovial fluid with the radiological Altman scale was found (Table 5). This confirms the contribution of joint inflammation, stimulated by these mediators, in the development of symptomatic OA [8].

Knee injury triggers a rapid release of IL-1β from chondrocytes, however in a chronic joint damage IL-1β is eliminated from the environment. The role of this cytokine is to initiate inflammation, which then in OA is sustained and modulated by other mediators including IL-17, IL-6 and chemokines [19,24-26]. In the study group we are dealing with a chronic process, which has a mechanism for limiting the inflammation. This is supported by the increased concentration of anti-catabolic cytokine (IL-1Ra) both in the synovial fluid and in blood serum of the patients.   Statistical analysis showed that the concentration of IL-1Ra in serum was significantly higher in the OA group compared to the INJ group (Table 1). The concentration of this cytokine in synovial fluid of patients with OA was also higher than those in INJ group, although in this case, the difference was not statistically significant. These results indicate the development of strong anti-catabolic response in patients from the OA group. Such direction of changes may prove to be characteristic of OA, in contrast

to RA [27]. The mechanism of action of IL-1β and anti-catabolic response to this cytokine is associated with the activity of membrane IL-1β receptor, which expression is increased in chondrocytes and synovial cells of people with OA. Of the two types of receptor, type I (IL-1RI) is important in the pathogenesis of the disease. It can be blocked by a specific biological inhibitor, the IL-1Ra. It is produced by many cells including chondrocytes and type B synoviocytes in a response to stimulation by inflammatory cytokines (IL-1β and IL-6), whereas there is no production after stimulation by anti-inflammatory cytokines (IL-4, IL-10) [7]. IL-1Ra acts as a competitive antagonist of interleukin 1, because it binds with the membrane receptors type I and type II but does not induce any cellular response. Balance of these two cytokines is important for the development of arthritic changes within the joint in patients with OA [28].

Recombinant IL-1Ra has been used in medical experiments in animals and in clinical studies (Anakinra and Orthokine ®) showing a protective effect against cartilage damage. Animals with experimentally induced OA showed a significant clinical and histological improvement after intraarticular administration of IL-1Ra [29,30]. Improvement in almost all determinants of the disease and reduction joint pain have been described in clinical studies conducted on patients treated with IL-1Ra [9]. These observations explain the observed high negative correlation between IL-1Ra concentration with the severity of VAS score in the OA study group (Table 4). A significant correlation was found for both concentrations of this cytokine in synovial fluid and serum (Figures 3 and 4).

In addition, the OA group had a positive correlation of IL-1Ra in the synovial fluid (but not in serum) with scales that describe the severity of OA. The explanation of this fact may be the increased production, as a protective response, of this anticatabolic cytokine in the advanced stages of the disease. As described in the literature, high levels of IL-1Ra can also be related with the radiological changes in some certain genotype varieties of this cytokine [9].

It has been proven that in advanced OA, the ratio between the concentration of IL-1Ra and IL-1β in the synovial fluid increases [31]. The results found in the current study-high concentrations of IL-1Ra compared to IL-1β in the synovial fluid - is a good confirmation of previous studies and provides evidence that this is an important indicator distinguishing OA from other inflammatory joint diseases. Moreover, these observations imply the use of IL-1Ra in the treatment of OA, as it may not be sufficient to block the postulated intracellular IL-1β functions. It is believed that intraarticular injections of IL-1Ra may be self-limiting in patients with knee OA and naturally high rate of IL-1Ra/IL-1β in the synovial fluid [31].

Significant difference between OA and the reference group obtained during the statistical analysis of IL-1Ra serum concentration led me to the evaluation of the diagnostic value of this measurement in patients with OA. The accuracy of a test to discriminate diseased cases from normal cases was evaluated using ROC curve analysis. IL-1β parameter was not analyzed using ROC because in most subjects the values were low, and in some cases remained on the border of the sensitivity of the test. Contrary, the mean concentration of IL-1Ra exceeded the determined threshold in all cases. Using ROC curves the sensitivity, specificity, odds ratio, the ability to predict a negative result, and the ability to predict a positive result of this test were measured (Table 6). Accuracy of a test is measured by the area under the ROC curve. According to the description of the ROC method an area of 1 represents a perfect test. Therefore the closer the ROC curve is to the upper left corner, the higher the overall accuracy of the test. For

measurements of IL-1Ra in serum the area under the ROC curve was 0.792 (Figure 5). These results suggest that measurement of IL-1Ra in serum is a reliable immunoassay test that determines the status of OA and can be an additional criterion for confirming late stage osteoarthritis.

Conclusion

Late stage osteoarthritis is characterized by a systemic inflammatory reaction with high concentration of interleukin-1β receptor antagonist (IL-1Ra) in serum, which is positively correlated with its concentration in the synovial fluid.

Pain in patients with late stage of OA may be reduced due to the high concentration of anti-inflammatory IL-1β receptor antagonist (IL-1Ra) both in the synovial fluid and in blood serum. This was confirmed by the presence of a negative correlation with VAS.

Determination of serum concentration of IL-1Ra can be an additional criterion for confirming late stage osteoarthritis, where due to the high degree of degeneration joint replacement is required.

Competing Interest

The author declares that he has no competing interest.

Authors' Contributions

AWP conceived of the study; assisted with patient recruitment; assisted with conduct, analysis, and interpretation of the assays; analyzed the data; performed the statistical analysis; made interpretation of the results and drafted the manuscript.

Author's Information

The author successfully defended his doctoral thesis on a topic: Expression of proinflammatory and anti-inflammatory cytokines in synovial fluid and blood serum, among patients with osteoarthritis of the knee, qualified for total knee replacement.

Acknowledgement

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