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Research Article

Evaluation of Serum Sestrin 2 Levels and Its Correlation with Sclerostin in Women with Rheumatoid Arthritis

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ABSTRACT

Background: Rheumatoid arthritis (RA) is an autoimmune disorder that is characterized by chronic inflammation of the joints and other systemic issues. Interconnection between oxidative stress, inflammation and bone metabolism takes an important place in the development of RA. Sestrin 2, a protein involved in the response to stress, and sclerostin, the key protein that regulates bone formation, have recently appeared as the potential biomarkers of many inflammatory diseases. But their relationship in rheumatoid arthritis is not well studied yet.
Objectives: The present study was designed to determine the levels of serum Sestrin 2 in women with RA in comparison with healthy individuals, to estimate the ability of Sestrin 2 and sclerostin to serve as biomarkers of RA and to evaluate the correlation between the biomarkers and other parameters of disease activity.
Materials and Methods: Case-control study included 42 females with RA and 42 healthy age- and body mass index- matched control subjects. Levels of Sestrin 2, sclerostin, anti-cyclic citrullinated peptide (ACCP), high-sensitivity C-reactive protein (hs-CRP), erythrocyte sedimentation rate (ESR), and uric acid were determined.
Results: The concentrations of serum Sestrin 2 in patients with RA were significantly reduced compared to the control group (4.416 ± 1.350 ng/mL vs. 6.453 ± 1.666 ng/mL, $p < 0.0001$), while sclerostin was significantly increased (905.128 ± 138.450 pg/mL vs. 597.042 ± 171.459 pg/mL, $p < 0.0001$). In addition, ACCP and hs-CRP also demonstrated statistically significant differences ($p < 0.0001$). The ROC analysis confirmed that the best diagnostic parameters had hs-CRP (AUC = 1.000), ESR (AUC = 0.994), ACCP (AUC = 0.910), and sclerostin (AUC = 0.899). For Sestrin 2, AUC was 0.184 with the cut-off value of less than 5.458 ng/mL having 73.17% sensitivity and 69.05% specificity. Pearson correlation analysis revealed significant correlations of the examined biomarkers with clinical parameters.
Conclusion: It can be concluded that Sestrin 2 and sclerostin are promising biomarkers of the pathology in RA. In particular, sclerostin has shown good diagnostic capabilities, whereas the Sestrin 2 demonstrated specific characteristics of decreased concentration in RA patients.

KEYWORDS: Rheumatoid arthritis; Sestrin 2; Sclerostin; Biomarkers; Diagnostic performance; Disease activity

INTRODUCTION

Introduction Rheumatoid arthritis (RA) is an autoimmune disease that mainly affects synovial joints. The disease causes inflammation, cartilage destruction, and bone erosion [1]. The world prevalence of rheumatoid arthritis is 0.5-1% and it is mostly seen in women at perimenopausal stage [2]. The pathogenesis of RA is associated with a complex process of genetic predisposition, environment factors, and immune

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system regulation that results in development of autoantibodies like rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (ACCP) [3]. Pathogenesis There are several mechanisms involved in the pathogenesis of rheumatoid arthritis like inflammation, oxidative stress and bone metabolism. Inflammation is maintained by pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1β, which not only promote joint inflammation, but also contribute to the development of other systemic problems and bone loss [4]. The connection between inflammation and bone metabolism is rather complex and includes bone resorption and formation suppression. Sclerostin, encoded by SOST gene, is a glycoprotein that is an inhibitor of Wnt/β-catenin signaling pathway, which is needed for osteoblasts function and bone formation [5]. The protein binds LRP5/6 co-receptors and blocks Wnt ligands from signaling, thus decreasing osteoblast function and stimulating bone resorption [6]. Increased production of sclerostin under the influence of inflammatory cytokines such as TNF-α and IL-6 could contribute to localized and systemic bone loss in RA [7]. The increased serum concentration of sclerostin in RA patients has been found. Also, increased sclerostin concentration is associated with high level of disease activity, bone erosion and bone mineral density loss [8,9]. Moreover, sclerostin can serve as a diagnostic marker because of its superior potential compared to classical markers like RF and ACCP [10]. Sestrin 2 (SESN2) belongs to a group of stress-induced proteins along with Sestrin 1, 2 and 3 [11]. SESN2 is induced by different kinds of cellular stresses like oxidative stress, DNA damage, hypoxia through p53 and Nrf2 signaling pathways [12]. Sestrin 2 serves as a key regulator of cellular homeostasis by controlling the mTORC1 signaling pathway, activating AMPK and autophagy [13]. Sestrin 2 has antioxidative effect due to peroxiredoxin regeneration and ROS reduction [14]. The role of Sestrin 2 in inflammatory and autoimmune diseases is being revealed lately. Sestrin 2 protects cells from tissue damage and reduces inflammatory reactions in many inflammatory conditions [15]. Its expression in RA and relationship with the disease activity is not well understood yet. Some researchers indicate that Sestrin 2 is suppressed in inflammatory conditions, which could result in the increase of oxidative stress and inflammation [16]. Others report increased concentration of Sestrin 2 as the response to the cellular stress [17]. Sestrin 2 influence on bone metabolism is rather interesting. It influences osteoblast differentiation and bone formation through mTORC1 signaling regulation [18]. Besides that, Sestrin 2 could interact with Wnt/β-catenin signaling pathway which is targeted by sclerostin [19]. But their interaction in the RA is poorly investigated now. Currently, there are a variety of diagnostic techniques for RA including physical examination, imaging and lab markers such as RF, ACCP, ESR, CRP [20]. They have proven their utility but still, there is the need of additional markers to improve diagnostic accuracy and reveal new features of RA pathogenesis and progression. ACCP has become the most specific marker for RA, having sensitivity and specificity about 70-80% and 95-98%, respectively [21]. However, there are some disadvantages of this marker like the existence of seronegative forms of RA and inability to predict disease progression. ESR and CRP are known markers of systemic inflammation, used for the assessment of disease activity and treatment control [22]. They lack specificity and their relationship with bone metabolism in RA needs more research. A lot of Iraqi studies are devoted to inflammatory diseases [23], [24], [25]. But this data was used to study new inflammatory parameters in Iraqi women with RA.

With the constraints associated with existing biomarkers and the need for better diagnostic tests, the present study has been undertaken to determine the role of serum Sestrin 2 and sclerostin in female patients with RA and to identify their utility as diagnostic and prognostic markers. Objectives of this study included:

1. Comparison of the serum level of Sestrin 2, sclerostin, ACCP, hs-CRP, ESR, and uric acid in RA patients compared to healthy individuals.
2. Determining the diagnostic utility of these markers individually and collectively through ROC curve analysis.
3. Assessing the association of Sestrin 2, sclerostin with other clinical parameters in RA patients.
4. Determining the possible link between Sestrin 2 and sclerostin in the pathogenesis of RA.

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METHODOLOGY

STUDY DESIGN AND PARTICIPANTS

This study was carried out in Fallujah teaching hospital from March 2025 to June 2025 to assess serum levels of Sestrin 2 and sclerostin in patients with RA and healthy controls. It comprised 42 female patients with RA defined by the ACR/EULAR classification criteria from 2010 [3]. In addition, 42 healthy female controls matched in age and body mass index (BMI) were included in the study. This research was approved by the ethics committee of the institution, and informed consent was received from all participants before inclusion in the study.

The subjects with RA were recruited among the patients attending rheumatology outpatient clinic, and healthy controls were chosen among those undertaking routine health screening. Participants were all women with ages ranging from 18 to 70 years. RA patients eligible for inclusion in the study had a confirmed diagnosis of RA and did not receive biologic therapy or any immunosuppressive drugs which might alter the serum levels of biomarkers under investigation. Healthy control group was recruited among those who had never been diagnosed with autoimmune, inflammatory and chronic diseases that can affect inflammatory and bone metabolism markers.

The exclusion criteria for both patient and control groups include being pregnant or breast-feeding, having infection, malignant tumors, chronic kidney and liver diseases, endocrine disorders related to bone metabolism and medication known to affect bone metabolism (bisphosphonates, denosumab, teriparatide). Patients with other types of inflammatory arthritis, such as psoriatic arthritis or ankylosing spondylitis, were also not enrolled in the study.

CLINICAL ASSESSMENT

Data about demographics and clinical characteristics of all subjects were gathered, which included their age, BMI, disease duration, medications used and other clinical characteristics. Activity of RA was determined through clinical tests. DAS28, a score used to determine disease activity of RA, was measured according to the number of tender joints, number of swollen joints, patient's overall assessment of disease activity, and either ESR or CRP according to pre-established formula [26].

LABORATORY MEASUREMENTS

Blood samples were collected from all participants after an overnight fast. Serum was separated by centrifugation and stored at -80°C until analysis. The following laboratory parameters were measured:

Routine Laboratory Tests: ESR was measured using the Westergren method, and uric acid levels were determined using standard enzymatic methods. High-sensitivity C-reactive protein (hs-CRP) was measured using immunoturbidimetric assays.

Anti-Cyclic Citrullinated Peptide Antibodies (ACCP): Serum ACCP levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Euroimmun, Lübeck, Germany) according to the manufacturer's instructions. The normal reference range for ACCP was < 15 U/mL.

Sclerostin (SOST): Serum sclerostin levels were measured using a quantitative sandwich ELISA kit (R&D Systems, Minneapolis, MN, USA) with a detection range of 62.5-4000 pg/mL. The assay was performed according to the manufacturer's protocol, with samples diluted as necessary to fall within the standard curve range. The intra-assay and inter-assay coefficients of variation were < 8% and < 10%, respectively.

Sestrin 2 (SESN2): Serum Sestrin 2 levels were measured using a commercially available ELISA kit (Cloud-Clone Corp., Houston, TX, USA) specifically designed for human Sestrin 2 detection. The assay

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range was 0.156-10 ng/mL, with a sensitivity of 0.053 ng/mL. The intra-assay and inter-assay coefficients of variation were < 10% and < 12%, respectively. All samples were analyzed in duplicate to ensure accuracy.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 8.0 (GraphPad Software, La Jolla, CA, USA). Data were assessed for normality using the Shapiro-Wilk test. Normally distributed data were expressed as mean ± standard deviation (SD), while non-normally distributed data were reported as median (interquartile range). Comparisons between RA patients and healthy controls were performed using independent Student's t-test for normally distributed data and Mann-Whitney U test for non-normally distributed data.

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of each biomarker and determine optimal cut-off values for distinguishing RA patients from healthy controls. The area under the ROC curve (AUC) was calculated with 95% confidence intervals (CI). Sensitivity and specificity were calculated at the optimal cut-off point, defined as the value that maximized the Youden index (sensitivity + specificity - 1).

The Pearson correlation test was applied in order to study the correlation between biomarker levels and clinical indicators in patients suffering from rheumatoid arthritis. Coefficients of correlation (r) were obtained, and p-values were presented. Significance level was considered as $p < 0.05$.

RESULTS

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

In the study, 42 females with RA and 42 healthy females were selected as controls. From Table 1, it can be seen that the ages of the two groups of participants did not differ significantly (RA patients: 51.756 ± 7.513 years; controls: 50.286 ± 6.106 years; p=0.330). Likewise, BMI of RA patients and the controls was also found to be similar (23.664 ± 1.942 kg/m² vs. 23.003 ± 1.262 kg/m²; p=0.07), which clearly confirms that successful matching has been done.

COMPARISON OF BIOMARKER LEVELS BETWEEN RA PATIENTS AND CONTROLS

Inflammatory markers: As expected, patients with RA have exhibited significantly higher levels of inflammatory markers in comparison with controls. ESR was significantly higher in patients with RA (23.334 ± 3.665 mm/hr) in comparison with controls (13.535 ± 2.268 mm/hr; $p < 0.0001$). The same can be said about the levels of hs-CRP, which are significantly elevated in patients with RA (9.375 ± 2.024 mg/L) compared with controls (0.9678 ± 0.2463 mg/L; $p < 0.0001$). Such results allow one to speak about the presence of active systemic inflammation in RA patients and confirm the effectiveness of the usage of these markers for diagnostics purposes. At the same time, levels of serum uric acid did not differ significantly between the two groups (RA: 3.723 ± 0.778 mg/dL; controls: 3.981 ± 0.9149 mg/dL; $p = 0.171$). Thus, uric acid cannot be considered as a useful RA marker.

RA-specific autoantibody: Levels of ACCP were significantly higher in patients with RA (20.307 ± 7.198 U/mL) than in controls (8.741 ± 4.459 U/mL; $p < 0.0001$). Such result confirms the presence of specific autoimmunity to RA in the RA patient group.

Sclerostin: Serum levels of sclerostin were significantly elevated in RA patients (905.128 ± 138.450 pg/mL) in comparison with healthy controls (597.042 ± 171.459 pg/mL; $p < 0.0001$). Increased level of sclerostin in RA patients suggests that alteration of bone metabolism, including suppression of bone formation through inhibition of Wnt pathway, is an important aspect of the RA pathology.

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Sestrin 2: To our surprise, levels of serum Sestrin 2 were significantly reduced in RA patients (4.416 ± 1.350 ng/mL) compared with healthy controls (6.453 ± 1.666 ng/mL; $p < 0.0001$) (Table 1). This decrease in the level of Sestrin 2 in RA patients is rather remarkable, taking into account the function of this protein.

Table 1. The demographics and blood levels of the parameters examined in patients with rheumatoid arthritis (RA) and healthy controls.

Group Statistics					
	no	**N**	**Mean**	**Std. D.**	***p value***
Age years	controls	42	50.286	6.106	0.330
	RA Patients	42	51.756	7.513	
BMI (kg/m^2)	controls	42	23.003	1.262	0.07
	RA Patients	42	23.664	1.942	
ESR (mm/hr)	controls	42	13.535	2.268	<0.0001
	RA Patients	42	23.334	3.665	
Uric Acid (mg/dL)	controls	42	3.981	0.9149	0.171
	RA Patients	42	3.723	0.778	
ACCP (U/mL)	controls	42	8.741	4.459	<0.0001
	RA Patients	42	20.307	7.198	
hs-CRP (mg/L)	controls	42	0.9678	0.2463	<0.0001
	RA Patients	42	9.375	2.024	
Sclerostin (SOST) (pg/mL)	controls	42	597.042	171.459	<0.0001
	RA Patients	42	905.128	138.450	
Sestrin2 (SESN2) (ng/mL)	controls	42	6.453	1.666	<0.0001
	RA Patients	42	4.416	1.350	

DIAGNOSTIC PERFORMANCE OF BIOMARKERS

Diagnostic Ability of RA-Specific and Inflammatory Markers: ROC curve analysis confirmed very high diagnostic ability for both hs-CRP (AUC = 1.000, 95% CI: 1.000 to 1.000) and ESR (AUC = 0.994, 95% CI: 0.9847 to 1.000) in discriminating RA patients from the control group. Using the optimal cut-off of > 3.910 mg/L, the sensitivity and specificity of hs-CRP were 100% and 100%, respectively. Similarly, the ESR levels above the cut-off of > 17.01 mm/hr indicated 95.12% sensitivity and 95.24% specificity. It is evident that these established inflammatory markers have a strong diagnostic ability in RA.

ACCP was found to have good diagnostic ability (AUC = 0.910, 95% CI: 0.8432 to 0.9768). The cut-off of > 15.21 U/mL resulted in 87.80% sensitivity and 90.48% specificity. This finding confirms the known role of this very specific marker of RA despite a little lower sensitivity than that of hs-CRP and ESR.

Sclerostin: Good diagnostic ability was shown by sclerostin (AUC = 0.899, 95% CI: 0.8336 to 0.9643). Using the optimal cut-off of > 743.7 pg/mL, sclerostin exhibited 85.37% sensitivity and 78.57% specificity. Although the diagnostic ability of sclerostin was a little bit lower than that of hs-CRP, ESR, and ACCP, it was still quite good. Figure 1 shows ROC curves for ACCP, hs-CRP, sclerostin, and Sestrin 2.

Sestrin 2: On the contrary, Sestrin 2 showed AUC of 0.184 (95% CI: 0.7278 to 0.9040), which indicates that this biomarker has poor diagnostic ability as a single marker due to its inverse correlation with RA. Using the optimal cut-off of < 5.458 ng/mL, Sestrin 2 indicated 73.17% sensitivity and 69.05% specificity. Low AUC value approaching 0 indicates an inverse relationship between Sestrin 2 levels and RA; the lower the level of Sestrin 2, the higher the risk of RA. Although the finding looks quite odd mathematically, it means that Sestrin 2 cannot be used as a diagnostic marker due to its inverse correlation with RA. Nevertheless, this fact that Sestrin 2 levels are significantly lower in RA patients can be interesting biologically.

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Uric Acid: Uric acid showed poor diagnostic performance (AUC = 0.426, 95% CI: 0.4471 to 0.7004), with a cut-off of < 3.670 mg/dL demonstrating only 58.54% sensitivity and 59.52% specificity as shown in table 2 and figures 1 and 2. The near-chance AUC value confirms that uric acid has limited utility as a diagnostic marker for RA, consistent with the lack of significant difference between groups observed in the group comparison.

Table 2. Serum hs-CRP, ESR, ACCP, Sclerostin, Sestrin2 and Uric Acid diagnostic performance in differentiating individuals with rheumatoid arthritis from healthy controls

Variable	AUC	Positive if Cut of Value	Sensitivity%	Specificity%	95% Confidence Interval
hs-CRP (mg/L)	1.000	> 3.910	100.0	100.0	1.000 to 1.000
ESR (mm/hr)	0.994	> 17.01	95.12	95.24	0.9847 to 1.000
ACCP (U/mL)	0.910	> 15.21	87.80	90.48	0.8432 to 0.9768
Sclerostin (pg/mL)	0.899	> 743.7	85.37	78.57	0.8336 to 0.9643
Sestrin2 (ng/mL)	0.184	< 5.458	73.17	69.05	0.7278 to 0.9040
Uric Acid (mg/mL)	0.426	< 3.670	58.54	59.52	0.4471 to 0.7004

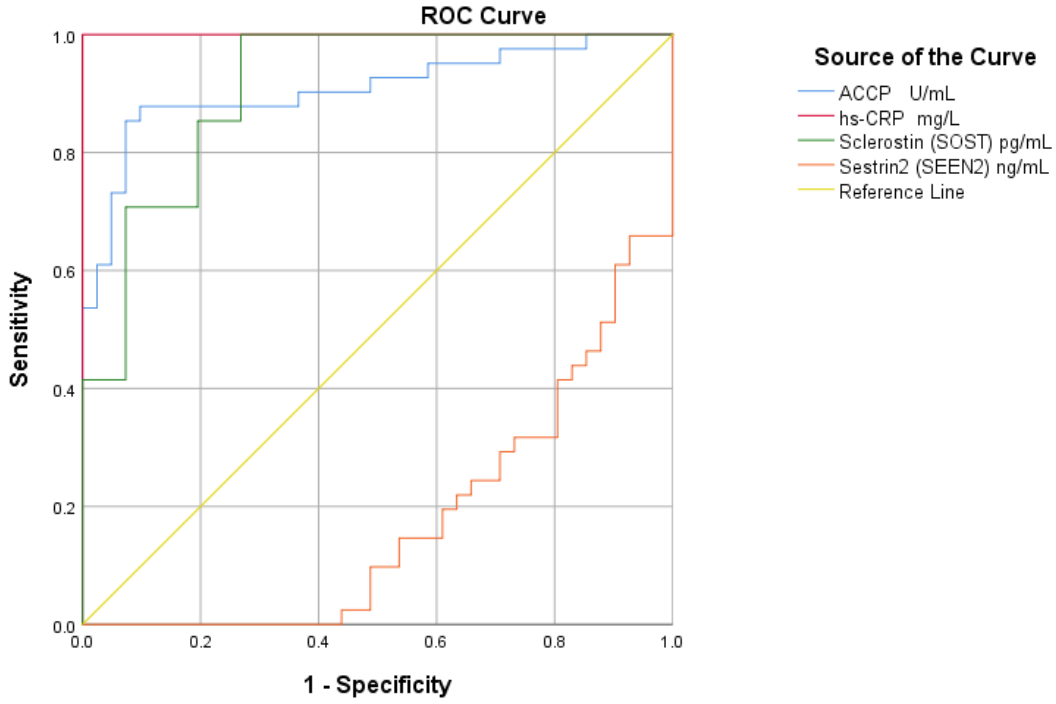

Figure 1. Receiver operating characteristic (ROC) curves of serum ACCP, hs-CRP, Sclerostin and Sestrin2 for the diagnosis of rheumatoid arthritis.

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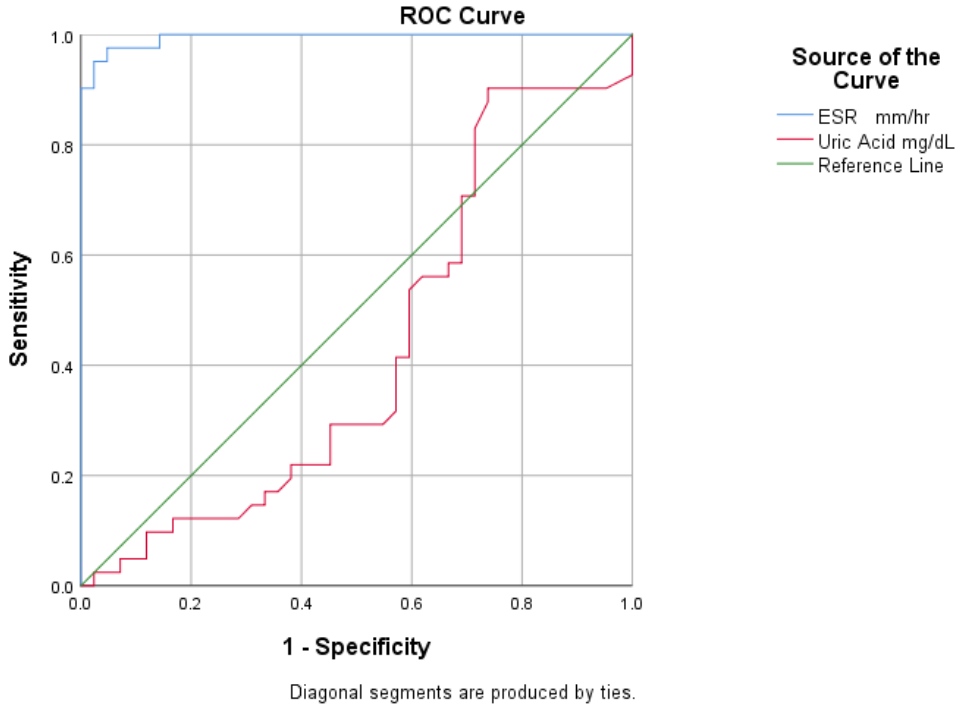

Figure 2. Receiver operating characteristic (ROC) curves of s ESR and Uric Acid for the diagnosis of rheumatoid arthritis.

CORRELATION ANALYSIS

As seen in Table 3, the results of the Pearson correlation analysis indicated significant relationships between the biomarkers studied in RA patients. There were complicated connections between inflammatory biomarkers, bone remodeling markers and disease activity parameters observed in the correlation matrix.

ESR demonstrated high positive correlations with the level of hs-CRP ($r = 0.81$, $p < 0.001$) and ACCP ($r = 0.58$, $p < 0.05$), which is explained by the association of systemic inflammation, disease activity and autoantibodies production. Similarly, hs-CRP was found to correlate positively with the levels of ACCP and sclerostin. Increased correlation of sclerostin with ACCP, ESR and hs-CRP suggests the role of inflammation and autoimmune reaction in elevation of sclerostin levels and inhibition of bone remodeling. On the other hand, negative correlations of Sestrin 2 with inflammatory biomarkers such as ESR and hs-CRP confirmed the hypothesis about the link between decreased Sestrin 2 levels and increased inflammation and oxidative stress. Finally, the negative correlation of Sestrin 2 with sclerostin needs further clarification.

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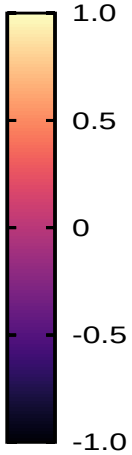
Table 3. Pearson correlation matrix of the evaluated serum biomarkers and clinical parameters in rheumatoid arthritis patients

	Age years	BMI kg/m2	ESR mm/hr	ACCP U/mL	Uric Acid mg/dL	hs-CRP mg/L	Sclerostin pg/mL	Sestrin2 ng/mL
Age years	1.00	-0.09	0.08	0.02	0.05	0.02	0.25	-0.10
BMI kg/m2	-0.09	1.00	0.23	0.05	0.06	0.19	0.24	-0.15
ESR mm/hr	0.08	0.23	1.00	0.58	-0.22	0.81	0.59	-0.53
ACCP U/mL	0.02	0.05	0.58	1.00	-0.19	0.70	0.34	-0.38
Uric Acid mg/dL	0.05	0.06	-0.22	-0.19	1.00	-0.09	-0.12	0.02
hs-CRP mg/L	0.02	0.19	0.81	0.70	-0.09	1.00	0.69	-0.51
Sclerostin pg/mL	0.25	0.24	0.59	0.34	-0.12	0.69	1.00	-0.40
Sestrin2 ng/mL	-0.10	-0.15	-0.53	-0.38	0.02	-0.51	-0.40	1.00



DISCUSSION

This work offers new insights regarding the potential involvement of Sestrin 2 and sclerostin as biomarkers in RA as well as the connection between them and RA activity. The current study has established the expression profile of the above-mentioned biomarkers in RA patients compared with healthy controls, and its importance for the pathogenesis of RA and improvement of diagnostic and monitoring of RA activity should be emphasized.

As for the elevated serum levels of sclerostin in RA patients compared to healthy controls (905.128 ± 138.450 pg/mL vs. 597.042 ± 171.459 pg/mL, $p < 0.0001$), these results correlate with the previously published reports where sclerostin expression was found to be elevated in RA patients [7,8]. It should be noted that sclerostin is a protein encoded by SOST gene and serves as a very strong inhibitor of the Wnt/β-catenin pathway, which plays an important role in osteoblasts differentiation and bone formation [5].

The mechanisms original improved sclerostin expression in RA are multifactorial. Pro-inflammatory cytokines, particularly TNF-α and IL-6, which are elevated in RA, have been shown to upregulate SOST expression in osteocytes [27]. This cytokine-mediated upregulation of sclerostin contributes to the uncoupling of bone remodeling, favoring bone resorption over formation, which is a hallmark of RA-associated bone loss [28]. Additionally, the chronic inflammation in RA may directly affect osteocyte function and survival, further contributing to dysregulated sclerostin production [29].

The diagnostic performance of sclerostin in our study (AUC = 0.899, 85.37% sensitivity, 78.57% specificity at a cut-off of > 743.7 pg/mL) suggests that it may serve as a useful adjunctive biomarker in RA diagnosis. While the diagnostic accuracy of sclerostin was slightly lower than that of hs-CRP (AUC = 1.000) and ESR (AUC = 0.994), it demonstrated comparable performance to ACCP (AUC = 0.910), which is currently considered one of the most specific biomarkers for RA [21]. But it is necessary to state that perfect sensitivity and specificity of hs-CRP in this cohort can be affected by the chosen population and may be not applicable to other studies.

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It is important to mention that significant correlations between sclerostin, ESR, hs-CRP and ACCP demonstrate that inflammation and autoimmune processes stimulate the production of sclerostin. These results correspond to the idea that cytokines involved in inflammatory responses and produced due to the presence of RA increase bone remodeling and, thus, sclerostin expression [7]. The correlation between sclerostin and disease activity markers indicates that it may become a new indicator of RA activity and progression and help in planning the strategy of management and monitoring [10].

The significantly decreased levels of serum Sestrin 2 in RA patients (4.416 ± 1.350 ng/mL vs. 6.453 ± 1.666 ng/mL, $p < 0.0001$) are a novel discovery in the field of RA. Sestrin 2 is a stress-induced protein playing an essential role in regulating cell homeostasis, antioxidants system and inflammation [11]. Lowering Sestrin 2 levels in RA indicate an impairment of the response to cellular stress and oxidative stress in patients suffering from RA.

The induction of Sestrin 2 in cells exposed to different kinds of stress, such as oxidative stress, DNA damage, and inflammation occurs mainly via p53 and Nrf2 pathways [12]. Sestrin 2 helps in adaptation of cells to stressful conditions by affecting mTORC1 signaling pathway and activation of AMPK [13]. Besides, this protein possesses antioxidant properties because of its ability to regenerate peroxiredoxins and reduce ROS accumulation [14]. Considering the high level of oxidative stress and inflammation in RA patients, the decreased levels of Sestrin 2 create a vicious circle consisting of oxidative stress, inflammation and damage of tissues.

The negative correlation between Sestrin 2 and inflammatory markers observed in our study supports the concept that decreased Sestrin 2 expression is associated with increased inflammation in RA. This is consistent with previous studies that have demonstrated anti-inflammatory effects of Sestrin 2 in various conditions [15]. The potential mechanisms by which Sestrin 2 exerts anti-inflammatory effects include inhibition of NLRP3 inflammasome activation, suppression of NF-κB signaling, and modulation of macrophage polarization [30].

Interestingly, Sestrin 2 has been shown to influence osteoblast differentiation and bone formation through regulation of mTORC1 signaling and interaction with the Wnt/β-catenin pathway [18,19]. The negative correlation between Sestrin 2 and sclerostin observed in our study suggests a potential functional relationship between these two markers in the context of RA. Given that sclerostin inhibits the Wnt pathway, and Sestrin 2 may enhance Wnt signaling, the decreased Sestrin 2 levels in RA patients could further contribute to the suppression of bone formation by means of impaired Wnt signaling, thus possibly contributing to increased effects of the elevated sclerostin. However, since the diagnostic value of Sestrin 2 in this study was quite low (AUC = 0.184), we conclude that this protein is not a suitable diagnostic marker for RA. The low AUC value, caused by the inverse relationship (the lower the concentration of this protein, the more likely the subject has RA), means that Sestrin 2 alone cannot distinguish between RA patients and healthy controls. This is consistent with the fact that stress-response proteins are generally considered poor diagnostic markers because of numerous factors affecting their levels. Still, the statistically significant difference in Sestrin 2 between RA patients and healthy controls requires additional research into the role of this protein in disease pathology and multi-marker panels.

The results of the correlation analysis demonstrate the interesting connections between the studied biomarkers and RA disease activity. The positive correlations between sclerostin and the inflammatory and autoimmune parameters (ESR, hs-CRP and ACCP) suggest that increased inflammation and autoimmunity are associated with higher concentrations of sclerostin, which is associated with greater bone loss. This finding is consistent with previously found connection between the inflammatory burden and bone erosion in RA [31].

The negative correlation between Sestrin 2 and the inflammatory markers demonstrates the plausibility of the idea that reduced levels of this protein are associated with increased inflammation. The inverse

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relationship between Sestrin 2 and sclerostin looks especially interesting, and deserves further research into the potential mechanisms behind it. It is possible that the same inflammatory signals which increase the production of sclerostin decrease the production of Sestrin 2, or that Sestrin 2 and sclerostin influence bone metabolism in opposite ways through similar signaling pathways.

The results obtained in this study have several practical implications. The diagnostic performance of sclerostin suggests that it can be used as an additional biomarker in RA, especially in cases where the conventional markers are inconclusive. The correlation between sclerostin and the disease activity markers suggests that this protein can be helpful in monitoring disease progression and treatment efficacy. Still, the lack of reference ranges and moderate sensitivity and specificity of sclerostin limit its immediate use in clinical practice.

The reduced levels of Sestrin 2 in RA patients emphasize the importance of oxidative stress and stress responses in the development of this disease. Although Sestrin 2 alone is not a suitable diagnostic marker, it can play an important role in multi-marker panels or be a good target for therapy. Treatment strategies aimed at the increased Sestrin 2 levels or increased activity of this protein can potentially restore the cellular stress adaptation and decrease inflammation in RA.

It is important to note some limitations of the current study. Firstly, its cross-sectional nature makes it impossible to draw the conclusions about the causal relationship between Sestrin 2 and sclerostin and RA, and about any temporal changes in the levels of these biomarkers. The longitudinal studies are necessary to determine how Sestrin 2 and sclerostin levels change with the disease progression or treatment. Secondly, the relatively small number of participants (42 patients, 42 controls) makes the statistical analysis somewhat underpowered and reduces the generalizability of the obtained results. The larger, multicenter studies are necessary to confirm these results and develop the reference ranges. Thirdly, the current study includes only female patients because of the gender difference in the frequency of RA. However, this fact makes the obtained results non-generalizable to male patients with RA. Fourthly, there are no data about DAS28 scores, radiological examination and medications in this study. Thus, the possibility to analyze the correlation between these clinical parameters and biomarkers is limited. Fifthly, the lack of adjustments for multiple comparison in the correlation analysis increases the risk of type I error.

Further research directions should include the multicenter prospective studies to validate the obtained results and evaluate the usefulness of Sestrin 2 and sclerostin as the markers of disease prognosis. The mechanistic studies will be needed to understand the molecular pathways through which Sestrin 2 and sclerostin work and cause RA pathogenesis. The potential of Sestrin 2 as the target for RA therapy should be studied in preclinical models. Besides, the usefulness of multi-marker panels including Sestrin 2, sclerostin, and other biomarkers in RA diagnosis and prognosis should be studied.

The diagnostic performance of sclerostin (AUC = 0.899) in this study was comparable to that of ACCP (AUC = 0.910), consistent with the findings from previous studies which suggested that sclerostin is a good diagnostic biomarker for RA [10]. The slight superiority of ACCP (higher sensitivity – 87.80% vs. 85.37%, and specificity – 90.48% vs. 78.57%) still makes this parameter the best one in the current cohort. However, sclerostin provides the advantage of giving the information about the bone metabolism which plays the crucial role in RA pathogenesis and can be used for the assessment of the disease activity and bone damage prediction.

The excellent diagnostic performance of hs-CRP (AUC = 1.000) and ESR (AUC = 0.994) in this study is associated with the presence of inflammation in the RA patient cohort. However, it should be noted that these markers are not specific for RA and can be increased in numerous other conditions [22]. Thus, these markers are most useful when interpreted in the context of other clinical and laboratory data.

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The poor diagnostic performance of uric acid (AUC = 0.426) in separating RA patients from healthy controls proves that this marker has no use in RA diagnosis. Despite the fact that uric acid levels can be influenced by inflammation, and the studies exist that suggest the relationship between uric acid and RA disease activity [32], these findings are not enough to consider this parameter as a diagnostic marker for RA.

CONCLUSION

This research proves that the concentration of Sestrin 2 is significantly lower in RA patients whereas sclerostin concentration is significantly higher in them than in healthy controls. Sclerostin has shown good diagnostic value with an AUC of 0.899, whereas Sestrin 2 by itself is not a proper marker for diagnosing the condition despite its significant variations between RA patients and controls. The association of the biomarkers with other well-known indicators of the disease proves their ability to help track the development and progress of RA. The inverse association of Sestrin 2 with sclerostin and inflammation indicators hints at a protective role of the protein against these factors in RA.

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