

Review began 03/15/2024
Review ended 07/17/2024
Published 07/19/2024

© Copyright 2024
Christopoulos et al. This is an open access
article distributed under the terms of the
Creative Commons Attribution License CC-
BY 4.0., which permits unrestricted use,
distribution, and reproduction in any
medium, provided the original author and
source are credited.

DOI: 10.7759/cureus.64942

Association Between Soluble Cell Adhesion Molecules (sP-Selectin, sE-Selectin, and sICAM-1) and Antibodies Against the Antigens of *Proteus mirabilis* in Rheumatoid Arthritis Patients

Georgios Christopoulos¹, Vasiliki Christopoulou², Konstantinos Stamatou³, Andreas Babionitakis⁴, John G. Routsias⁵

1. Department of Internal Medicine, Tzaneio General Hospital of Piraeus, Piraeus, GRC 2. Department of Internal Medicine – Propaedeutic, Attikon University Hospital, Athens, GRC 3. Department of Urology, Tzaneio General Hospital of Piraeus, Piraeus, GRC 4. Department of Pathophysiology, Medical School National & Kapodistrian University of Athens, Athens, GRC 5. Department of Microbiology, Medical School National & Kapodistrian University of Athens, Athens, GRC

Corresponding author: Georgios Christopoulos, drchristog@gmail.com

Abstract

Objective

The purpose of this study was to examine the association between the serum concentration of soluble cell adhesion molecules (CAMs) and antibodies against antigens of *Proteus mirabilis* (*P. mirabilis*) in rheumatoid arthritis (RA) patients, taking into consideration the implication of *P. mirabilis* in the etiopathogenesis of RA.

Methods

The serum levels of soluble P-selectin (sP-selectin), soluble E-selectin (sE-selectin), and soluble intercellular adhesion molecule-1 (sICAM-1) were determined by sandwich enzyme-linked immunosorbent assay (ELISA) in 59 RA patients and 36 healthy controls. Using the same ELISA method, the serum levels of class-specific antibodies against hemolysin (HpmB), urease C (UreC), and urease F (UreF) enzymes of *P. mirabilis* were also measured.

Results

In this study, increased levels of sP-selectin and sICAM-1 were observed in RA patients, while the levels of sE-selectin were increased in comparison with healthy controls but did not present a statistically significant difference. Moreover, increased levels of antibodies against HpmB, UreC, and UreF of *P. mirabilis* were found. Additionally, it was observed that the sE-selectin levels presented a significant correlation with IgG antibodies against the UreF antigen (there is no corresponding antigen in human tissue) in all the RA patients. A statistically significant correlation was observed between levels of soluble CAMs and antibodies against *P. mirabilis* in the different subgroups.

Conclusion

The observed correlation between soluble CAMs and antibodies against antigens of *P. mirabilis*, specifically in the subgroup of biologic therapy, indicates that *P. mirabilis* exists and provokes refractory in the treatment of RA.

Categories: Rheumatology

Keywords: sp-selectin, se-selectin, rheumatoid arthritis, proteus mirabilis, sicam-1, adhesion molecules

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of unknown etiology. It affects approximately 1% of the general population [1,2]. Manifestations of systemic inflammation are common in RA; however, chronic inflammation of the synovial tissues is the most prominent symptom [3].

The pathogenic mechanisms of this disease are not fully understood; nevertheless, it is known that a combination of synovial tissue destruction processes, which involve lymphocytes, macrophages, and fibroblasts, with increased angiogenesis rates and proliferation of the synovium lining layer characterizes the initiation of RA development [4-7]. The complex cascade of inflammatory interactions characterizing RA is mediated by cell adhesion molecules (CAMs), such as P-selectin, E-selectin, and intercellular adhesion molecule-1 (ICAM-1) [8-10]. These glycoproteins mediate cell-cell and cell-extracellular matrix adhesion,

How to cite this article

Christopoulos G, Christopoulou V, Stamatou K, et al. (July 19, 2024) Association Between Soluble Cell Adhesion Molecules (sP-Selectin, sE-Selectin, and sICAM-1) and Antibodies Against the Antigens of *Proteus mirabilis* in Rheumatoid Arthritis Patients. Cureus 16(7): e64942. DOI 10.7759/cureus.64942

which appears to play an important role in RA initiation and the perpetuation of synovial tissue inflammation [8].

More precisely, P-selectin, which is normally stored in the Weibel-Palade bodies of endothelial cells and in the alpha granules of platelets [11, 12], is released to the cell surface in response to the presence of autoantibodies and to inflammatory conditions in general [13, 14]. Released P-selectin produces CD40 ligand (sCD40L), which acts as a trigger for the activation of the endothelium [15, 16]. While P-selectin-induced endothelium activation results in a regular increase in vascular permeability, a synchronous motivation of activated platelets occurs. This leads to the migration of inflammatory agents to the joints. Indeed, low-affinity immunoglobulin (Ig) G receptors have been found to mediate platelet activation in the presence of anti-citrullinated protein antibodies [17].

Moreover, increased levels of soluble P-selectin (sP-selectin) and sCD40L found in the serum of RA patients correlated with RA severity [18, 19]. Using a mouse model of RA, Wipke et al. showed that after a few minutes of intravenous injection of autoantibodies, these antibodies migrated specifically to the joints even in the absence of pre-existing joint inflammation [20]. The specific homing of autoantibodies to the joints may contribute to the local activation of platelets, which in turn release important mediators of vascular permeability [21,22].

E-selectin is a cell adhesion molecule that is expressed on endothelial cells after activation by inflammatory cytokines or endotoxin [23,24]. Cell surface E-selectin contributes to the rolling attachment of leucocytes to the endothelium, a necessary step for leukocyte emigration into inflamed tissues, and has a main role in localized inflammatory responses [25, 26]. Soluble E-selectin (sE-selectin) is released from endothelial cells by proteolytic cleavage of the surface-expressed molecule [25,27].

Two studies found higher levels of sE-selectin in RA patients compared with healthy controls and osteoarthritis patients, respectively [28, 29]. In addition, a strong correlation between serum and synovial fluid concentrations of sE-selectin exists in RA patients [30]. Furthermore, synovial fluid levels of sE-selectin are higher in RA than in non-inflammatory joint diseases [31].

Inflammatory cytokines induce up-regulation of ICAM-1 expression on vascular endothelial cells, contributing to the adhesion of leucocytes to the local endothelial and subsequently to the migration of leukocytes to inflamed tissues [32,33]. Several authors demonstrated high soluble ICAM-1 (sICAM-1) levels in the serum of RA patients [28, 29], while others found that the increase in the serum of sICAM-1 in RA patients was not statistically significant [34,35].

Moreover, RA patients from various ethnic populations were found to have significantly elevated levels of antibodies against various antigens of *Proteus mirabilis* (*P. mirabilis*) [36]. In particular, it was observed that RA patients present asymptomatic bacteriuria more frequently in comparison to healthy controls. In addition, in the same study, it was observed that RA patients show increased levels of antibodies against *P. mirabilis* in their serum in comparison to healthy controls [37]. Similarly, in another study, it was found that RA patients present significantly increased levels of antibodies against *P. mirabilis* but not against *Escherichia coli* (*E. coli*) in comparison to healthy controls [38]. We found elevated levels of antibodies against hemolysin (HpmB), urease C (UreC), and urease F (UreF) enzymes of *P. mirabilis* in Greek RA patients [39]. In this study, we examined the existence of a correlation between the soluble cell adhesion molecules sP-selectin, sE-selectin, and sICAM-1 and the antibodies against HpmB, UreC, and UreF enzymes of *P. mirabilis* in RA patients.

Materials And Methods

This study included 59 RA patients (13 male and 46 female) with a mean age of 63.6 years and 36 healthy controls (10 male and 26 female) with a mean age of 60.9 years. The general characteristics of the participants are shown in Table 1. All patients included in this study had positive serum rheumatoid factor (RF) and fulfilled the diagnostic criteria for RA [40]. These were RA patients who attended the outpatient clinic of the rheumatology department. Patients with symptoms of urinary tract infections or patients who recently received antibiotic treatment were excluded from the study. Informed consent was obtained from all subjects.

	RA patients	Healthy controls
N	59	36
Age (years) mean (SD)	63.6 (10.57)	60.9 (9.08)
Male/Female	13/46	10/26
Disease duration (years) mean (SD)	8.49 (6.23)	N/A
ESR (mm/hr) mean (SD)	55 (29.12)	Normal
CRP (mg/dl) mean (SD)	34.3 (49.31)	Normal
Without treatment	23	N/A
Combined	17	
Biologic therapy (bDMARDs)	19	

TABLE 1: General characteristics of rheumatoid arthritis (RA) patients and healthy controls. The RA patients were subdivided into groups based on their received treatment.
SD: standard deviation; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; N/A: not applicable; bDMARDs: biologic disease-modifying anti-rheumatic drugs

The erythrocyte sedimentation rate (ESR), serum C-reactive protein (CRP), RF, total blood count, and platelet (PLT) count were determined by routine methods. The participants were divided into three groups according to the treatment they received. The patients in the first group did not receive any treatment, while those in the second group received a combined treatment of conventional synthetic disease-modifying anti-rheumatic drugs (csDMARDs) with cortisone. The third group received biologic treatment.

sP-selectin, sE-selectin, and sICAM-1 were quantified in all patients and controls using Quantikine commercial sandwich immunoassays (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. Briefly, a monoclonal antibody specific to the analyte was pre-coated onto a microplate. Standards, samples, and controls were pipetted into the wells. The dilution of the samples was 1/20 for sICAM-1 or sP-selectin assays and 1/10 for sE-selectin. A polyclonal antibody specific to the analyte, which had been conjugated to horseradish peroxidase, was used for detection. Following a wash to remove any unbound conjugated antibody, a substrate was added and color was developed, which was proportional to the analyte concentration. The intensity of the color was measured at 450 nm. The concentration of each sample was calculated from the standard curve and multiplied by the dilution factor.

The presence of antibodies against P. mirabilis in RA patients and healthy controls was determined by using enzyme-linked immunosorbent assays (ELISA) and three synthetic amino acid peptides that contained a homologous amino acid sequence with the P. mirabilis enzymes. Specifically, we used: (1) the peptide H2N-NGSSESRRALQDSQR-OH, which presents a homologous sequence with the HpmB of P. mirabilis and cross-reactivity with the human tissue antigen and specifically with the amino acid sequence EQK/RRAA (69-74) of the HLA-DRB1 molecule; (2) the peptide H2N-FAESRIRRETIAAED-OH, which presents a homologous sequence with the UreC of P. mirabilis and cross-reactivity with the human tissue antigen and specifically with the amino acid sequence LRREI (421-425) of α2(XI) collagens; and (3) the peptide H2N-ASRETKELRQEERQP-amide, which presents a homologous sequence with the UreF of P. mirabilis but not cross-reactivity with human tissue antigens.

For statistical analysis, the Student’s t-test was used to compare the mean values of the soluble adhesion molecule levels between RA patients and healthy control subjects. The same statistical test was also used to compare the mean values of the antibodies against P. mirabilis antigens between the same groups.

Similarly, a t-test was conducted to verify the age matching between patients and healthy controls, yielding t: 1.260 and p: 0.211. Since p >0.05, there was no significant difference in age between the two groups. Consequently, a chi-square test was performed to confirm gender matching, resulting in χ²: 1.577, p: 0.209. Similarly, the p >0.05 indicated no significant difference in gender distribution between patients and healthy controls. Thus, patients and healthy controls were well-matched in terms of both age and gender.

The samples were checked using D’Agostino-Pearson, Shapiro-Wilk, and Kolmogorov-Smirnov normality tests with GraphPad Prism version 9 (GraphPad Software, La Jolla, CA). These tests confirmed that the samples followed a normal (Gaussian) distribution. Therefore, a parametric analysis was performed.

Pearson’s correlation coefficient (r) was used for the detection of the relationship between the soluble adhesion molecules and antibodies against antigens of P. mirabilis, as well as ESR, CRP, and RF in the group and in all the subgroups of RA patients.

The local ethics committee of Laiko University Hospital approved the research protocol (approval number: 4837/21). Written and verbal informed consent was obtained prior to the study. All the procedures adhered to the ethical guidelines of the Declaration of Helsinki and its amendments.

Results

The comparison of the serum concentration of soluble CAMs between the RA patients and controls revealed differences between the two groups. The results of the serum concentration of soluble CAMs measured in the RA patients and controls are shown in Table 2. Specifically, the levels of sP-selectin were higher in the RA patients compared to those of healthy controls. The difference in sP-selectin levels between the two groups was statistically significant (t-test 6.023, p <0.001) (Figure 1). Similarly, the levels of sE-selectin were elevated in the RA patients but remained normal in the healthy controls. However, this difference was not statistically significant (p = NS) (Figure 1). The levels of sICAM-1 were also higher in the RA patients than in the normal controls. The difference in sICAM-1 levels between the two groups was statistically significant (t-test 6.496, p <0.001) (Figure 2).

	RA patients	Healthy controls	t-Test
	N=59	N=36	p-value
sP-selectin (ng/ml) Mean (SD)	39.74 (15.08)	25.79 (7.36)	p<0.001
sE-selectin (ng/ml) Mean (SD)	34.33 (17.01)	29.57 (10.70)	p = NS
sICAM-1 (ng/ml) Mean (SD)	227.10 (76.96)	148.41 (40.83)	p<0.001

TABLE 2: Results of the serum concentration of soluble adhesion molecules in RA patients and healthy controls

Mean value (standard deviation) of sP-selectin, sE-selectin, and sICAM-1 in rheumatoid arthritis (RA) patients and healthy controls and the results of statistical analysis t-test

sP-selectin: soluble P-selectin; sE-selectin: soluble E-selectin; sICAM-1: soluble intercellular adhesion molecule-1

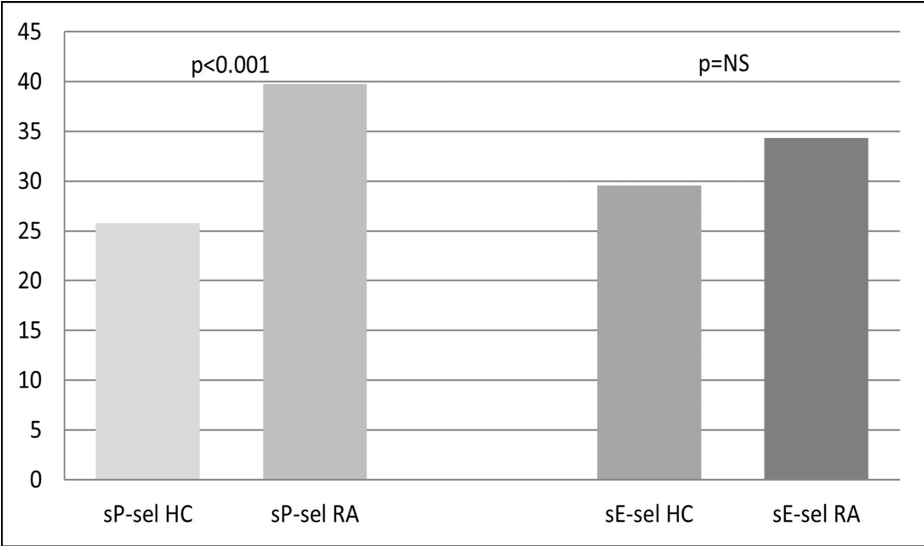


FIGURE 1: Serum concentrations of sP-selectin (sP-sel) and sE-selectin (sE-sel) in rheumatoid arthritis (RA) patients and healthy controls (HC)

sP-selectin: soluble P-selectin; sE-selectin: soluble E-selectin; NS: not significant

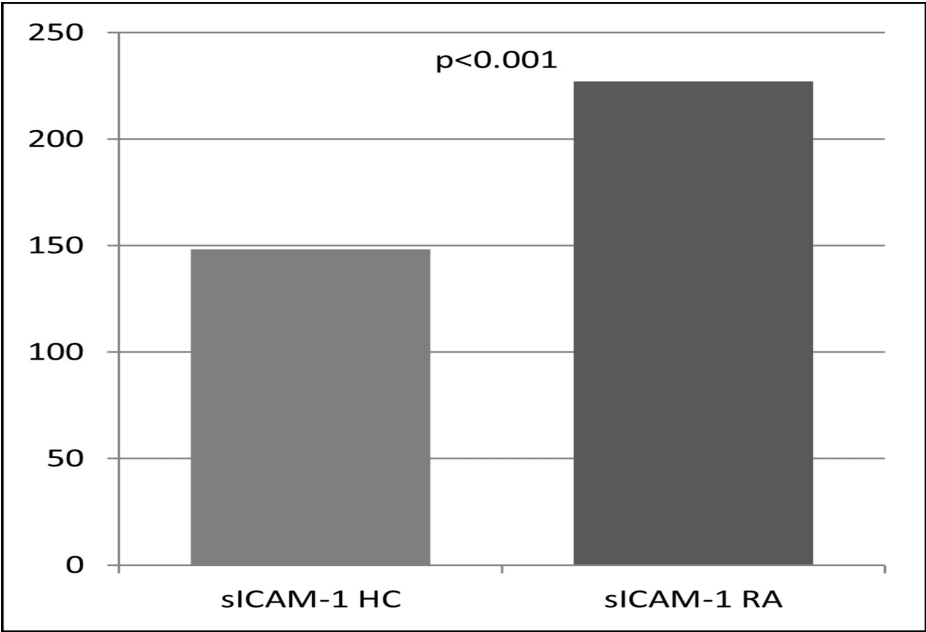


FIGURE 2: Serum concentrations of sICAM-1 in rheumatoid arthritis (RA) patients and healthy controls (HC)

sICAM-1: soluble intercellular adhesion molecule-1

Regarding antibodies against the antigens of P. mirabilis enzymes, their levels in the RA patients were significantly increased compared with the healthy control subjects (Table 3). In particular, the RA patients showed statistically significant raised levels of antibodies against HpmB compared with the healthy control subjects (Table 3). Likewise, the RA patients showed significantly higher levels of antibodies against UreC compared with the healthy control subjects (Table 3).

		RA patients	Healthy controls	t-test
		N=59	N=36	p-value
Anti-HpmB	IgM	0.91±0.61	0.66±0.31	p=0.008
	IgG	1.11±0.59	0.69±0.20	p<0.001
	IgA	0.37±0.21	0.22±0.07	p<0.001
Anti-UreC	IgM	0.62±0.39	0.45±0.23	p<0.01
	IgG	0.99±0.72	0.65±0.22	p=0.001
	IgA	0.26±0.11	0.19±0.05	p<0.001
Anti-UreF	IgM	0.95±0.74	0.65±0.37	p<0.01
	IgG	0.93±0.50	0.63±0.19	p<0.001
	IgA	0.30±0.12	0.21±0.06	p<0.001

TABLE 3: Results of the antibodies against antigens of Proteus mirabilis in rheumatoid arthritis (RA) patients and healthy controls

Mean values (MV±SD, OD 405) of the different classes of antibodies against antigens of P. mirabilis in RA patients and healthy controls, and the results of the statistical analysis t-test.

HpmB: hemolysin; UreC: urease C; UreF: urease F

Moreover, the RA patients showed significantly raised levels of antibodies against the UreF antigen; there is no corresponding antigen in the tissues of the human body. Similarly, the differences in antibody levels against UreF between the RA patients and the healthy control subjects were statistically significant. (Table 3).

In this study, the levels of sP-selectin, sE-selectin, and sICAM-1 were associated with the levels of antibodies against the three antigens of *P. mirabilis*: HpmB, UreC, and UreF. Our findings revealed that the sP-selectin levels in all the RA patients did not present a significant correlation. However, in the subgroup of RA patients without treatment, a significant correlation was observed between sP-selectin levels and IgM ($r = 0.661$, $p < 0.004$) and IgG antibodies ($r = 0.574$, $p < 0.01$) against HpmB. In the subgroup of RA patients treated with biologic therapy, an important correlation was also observed between sP-selectin levels and IgM antibodies against HpmB ($r = 0.469$, $p < 0.04$). The concentration of sE-selectin in all the RA patients showed a strong correlation with IgG antibodies against UreF ($r = 0.301$, $p < 0.02$). Equally strong was the correlation between the levels of sE-selectin and the IgA antibodies against HpmB in the RA patients treated with biologic therapy ($r = 0.515$, $p < 0.02$).

While the sICAM-1 levels in all the RA patients did not present a significant correlation with the levels of antibodies against antigens of *P. mirabilis*, in the subgroup of RA patients treated with biologic agents, a statistically significant correlation was shown between sICAM-1 concentration and IgM antibodies against HpmB ($r = 0.506$, $p < 0.02$). Moreover, a statistically significant correlation was found between sICAM-1 levels and CRP levels ($r = 0.317$, $p < 0.01$) in all the RA patients. Also, a statistically significant correlation was found between sICAM-1 and sP-selectin levels ($r = 0.232$, $p < 0.02$) in all the RA patients.

Discussion

Soluble forms of P-selectin, E-selectin, and ICAM-1 are known to exist in human serum and present elevated levels in numerous diseases. Previous studies have demonstrated that in RA, the levels of circulating sICAM-1 and sP-selectin were significantly elevated in RA patients relative to healthy controls [28]. Likewise, in this study, the levels of sP-selectin and sICAM-1 were significantly elevated in RA patients in comparison with healthy controls. While the levels of sE-selectin were increased in RA patients in comparison with healthy controls, this difference was not statistically significant. This result was in accordance with that of previous studies, which showed that the levels of sE-selectin were raised in RA patients compared with healthy controls, even though this difference was not statistically significant [28, 41].

Previous studies have shown that RA patients have elevated antibodies against various antigens of *P. mirabilis* [36, 42]. We demonstrated that the levels of sP-selectin, sE-selectin, and sICAM-1 in RA patients correlate with the levels of antibodies against HpmB, UreC, and UreF of *P. mirabilis*, further supporting the implication of *P. mirabilis* in the etiopathogenesis of RA [39]. In this study, we found increased levels of sP-selectin; this result is in accordance with previous studies [17,18,28]. Our finding of a statistically significant correlation of sP-selectin levels with IgM and IgG antibodies against HpmB in the subgroup of RA patients without treatment suggests that the antibodies against *P. mirabilis* through molecular mimicry and cross-reaction mechanisms with tissue antigens of the joints provoke an increased expression of sP-selectin, which contributes to the inflammation of the joints and the activation of RA.

Likewise, the significant correlation of sP-selectin with IgM antibodies against HpmB in the subgroup of biologic therapy shows disease activity despite treatment. This finding may indicate continuous local triggering by *P. mirabilis* and a rapid endothelial cell response consisting of the release of P-selectin [43, 44]. Indeed, it has been shown that the expression of P-selectin on the cell surface occurs within one minute after endothelial activation [14]. Moreover, the increased levels of sP-selectin found in the synovial liquid of RA patients are mainly released by synovial microvascular endothelial cells [28,45,46]. By contrast, decreased levels of sP-selectin in RA patients have been observed after infliximab infusions [47]. As shown in experimental models of inflammation, the blocking of P-selectin provokes a significant decrease in leukocyte rolling [48]. In our study, sP-selectin levels did not present a correlation with the antibodies against *P. mirabilis* in all the RA patients, obviously because most of them were receiving treatment, resulting in decreased expression of sP-selectin and also decreased production of antibodies against *P. mirabilis*.

Similarly, previous studies found that RA patients with active disease have higher levels of sE-selectin; however, after treatment with biologic agents or methotrexate, the levels of sE-selectin gradually decrease [49-51]. In our study, the significant correlation of sE-selectin levels with the IgA antibodies against HpmB in the subgroup of biologic therapy showed that sE-selectin collaborates with the antibodies in the development of inflammation in the joints. In addition, non-response to treatment with biological agents is probably due to the abovementioned triggering of joint inflammation by *Proteus*. This fact is strengthened by the significant correlation of sE-selectin with IgG antibodies against the UreF antigen of *P. mirabilis* in all the RA patients, whereas there is no corresponding antigen in the tissues of the human body. Consequently, the finding of these antibodies should be attributed to the presence of *P. mirabilis* in RA patients [29].

Furthermore, this study revealed a statistically significant difference in the levels of sICAM-1 between RA

patients and healthy controls. This finding is in accordance with those of previous studies [28,50,52]. However, other investigators have failed to demonstrate significant differences in sICAM-1 levels between RA patients and healthy controls [34,35,53].

Also in our study, sICAM-1 levels were significantly correlated with CRP levels. This result was similar to that of previous studies [52,54]. Notably, high circulating levels of sICAM-1 have been observed in RA patients with active disease, suggesting sICAM-1 levels as a marker for endothelial activation [55–57]. In confirmation of the above, a significant correlation was demonstrated between the serum levels of sICAM-1 and sP-selectin in this study. This correlation shows disease activity because sP-selectin is associated with disease activity in RA patients [17,18,28].

According to several researchers, RA patients under treatment have decreased levels of sICAM-1 [47,51,58]. This fact was also present in our study. In addition to decreased levels of sICAM-1, the patients under treatment also had decreased levels of antibodies against *P. mirabilis*. In the subgroup of RA patients who received biologic therapy, a significant correlation was observed between sICAM-1 level and IgM antibodies against HpmB, which suggests the triggering of RA by *P. mirabilis*. The consequent production of antibodies against *P. mirabilis* contributes to the expression of ICAM-1 in synovial tissues and the development of joint inflammation.

Antibodies against *P. mirabilis* were found in the serum of RA patients even in the initial stages of the disease. [59]. This bacterium can potentially be implicated in the induction of RA via molecular mimicry between bacterial and self-antigens that may lead to the production of antibodies targeting both bacteria and self-tissues [60]. Prior research has also shown that anti-*Proteus* antibiotics in combination with anti-rheumatic drugs in RA patients seem to have a better response to disease activity [61–63]. Finally, other anti-*Proteus* measures, in the form of vegetarian diets and phytotherapy, appear to have beneficial effects for RA patients [64–66].

The limitations of this study are a relatively small sample size. Further studies are required, with follow-up after antibiotic treatment in RA patients who present increased levels of antibodies against *P. mirabilis*.

Conclusions

Soluble CAMs are involved in the process of leucocyte adhesion and ingress into the rheumatoid synovium, causing the initiation and progression of the disease. Increased levels of antibodies against HpmB, UreC, and UreF of *P. mirabilis* and increased levels of sP-selectin, sE-selectin, and sICAM-1 were observed in this study. Moreover, the observed correlation between these antibodies and soluble CAMs suggests that *P. mirabilis* triggers the production of these antibodies in RA patients, which, through molecular mimicry and cross-reaction mechanisms with self-antigens of the joints, provokes activation of RA.

Additional Information

Disclosures

Human subjects: Consent was obtained or waived by all participants in this study. The local ethics committee of the Laiko University Hospital issued approval 4837/21. Written and verbal informed consent was obtained prior to the study. All the procedures adhered to the ethical guidelines of the Declaration of Helsinki and its amendments. . **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

References

1. Goronzy JJ, Weyand CM: Rheumatoid arthritis. *Immunol Rev.* 2005, 204:55–73. [10.1111/j.0105-2896.2005.00245.x](#)
2. Scott DL, Wolfe F, Huizinga TW: Rheumatoid arthritis. *Lancet.* 2010, 376:1094–108. [10.1016/S0140-6736\(10\)60826-4](#)
3. Lee DM, Weinblatt ME: Rheumatoid arthritis. *Lancet.* 2001, 358:903–11. [10.1016/S0140-6736\(01\)06075-5](#)
4. Gibofsky A: Overview of epidemiology, pathophysiology, and diagnosis of rheumatoid arthritis . *Am J Manag Care.* 2012, 18:S295–302.
5. Nourshargh S, Hordijk PL, Sixt M: Breaching multiple barriers: leukocyte motility through venular walls and the interstitium. *Nat Rev Mol Cell Biol.* 2010, 11:366–78. [10.1038/nrm2889](#)
6. Nourshargh S, Alon R: Leukocyte migration into inflamed tissues . *Immunity.* 2014, 41:694–707. [10.1016/j.immuni.2014.10.008](#)
7. Wehrens EJ, Prakken BJ, van Wijk F: T cells out of control-impaired immune regulation in the inflamed joint. *Nat Rev Rheumatol.* 2013, 9:34–42. [10.1038/nrrheum.2012.149](#)

8. Patel KD, Cuvelier SL, Wiehler S: Selectins: critical mediators of leukocyte recruitment . *Semin Immunol*. 2002, 14:73-81. [10.1006/smim.2001.0344](#)
9. Zarbock A, Ley K, McEver RP, Hidalgo A: Leukocyte ligands for endothelial selectins: specialized glycoconjugates that mediate rolling and signaling under flow. *Blood*. 2011, 118:6743-51. [10.1182/blood-2011-07-343566](#)
10. Ateş A, Kinikli G, Turgay M, Duman M: Serum-soluble selectin levels in patients with rheumatoid arthritis and systemic sclerosis. *Scand J Immunol*. 2004, 59:315-20. [10.1111/j.0300-9475.2004.01389.x](#)
11. Carlos TM, Harlan JM: Leukocyte-endothelial adhesion molecules. *Blood*. 1994, 84:2068-101.
12. Springer TA: Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell*. 1994, 76:301-14. [10.1016/0092-8674\(94\)90337-9](#)
13. Tedder TF, Steeber DA, Chen A, Engel P: The selectins: vascular adhesion molecules. *FASEB J*. 1995, 9:866-73.
14. McEver RP, Beckstead JH, Moore KL, Marshall-Carlson L, Bainton DF: GMP-140, a platelet alpha-granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel-Palade bodies. *J Clin Invest*. 1989, 84:92-9. [10.1172/JCI114175](#)
15. Henn V, Slupsky JR, Gräfe M, Anagnostopoulos I, Förster R, Müller-Berghaus G, Kroczeck RA: CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature*. 1998, 391:591-4. [10.1038/35393](#)
16. Semple JW, Italiano JE Jr, Freedman J: Platelets and the immune continuum . *Nat Rev Immunol*. 2011, 11:264-74. [10.1038/nri2956](#)
17. Habets KL, Trouw LA, Levarht EW, et al.: Anti-citrullinated protein antibodies contribute to platelet activation in rheumatoid arthritis. *Arthritis Res Ther*. 2015, 17:209. [10.1186/s13075-015-0665-7](#)
18. Cheng T, Li FF, Zhao S, Peng XC, Zhang XL: Soluble P selectin in synovial fluid level is correlated with the radiographic severity of knee osteoarthritis. *Clin Chim Acta*. 2010, 411:1529-31. [10.1016/j.cca.2010.05.009](#)
19. Ertenli I, Kiraz S, Arici M, Haznedaroglu IC, Calgüneri M, Celik I, Kirazli S: P-selectin as a circulating molecular marker in rheumatoid arthritis with thrombocytosis. *J Rheumatol*. 1998, 25:1054-8.
20. Wipke BT, Wang Z, Kim J, McCarthy TJ, Allen PM: Dynamic visualization of a joint-specific autoimmune response through positron emission tomography. *Nat Immunol*. 2002, 3:366-72. [10.1038/ni775](#)
21. Cloutier N, Paré A, Farndale RW, Schumacher HR, Nigrovic PA, Lacroix S, Boilard E: Platelets can enhance vascular permeability. *Blood*. 2012, 120:1334-43. [10.1182/blood-2012-02-413047](#)
22. Boilard E, Nigrovic PA, Larabee K, et al.: Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science*. 2010, 327:580-3. [10.1126/science.1181928](#)
23. Bevilacqua MP, Nelson RM: Selectins. *J Clin Invest*. 1993, 91:379-87. [10.1172/JCI116210](#)
24. Kansas GS: Selectins and their ligands: current concepts and controversies . *Blood*. 1996, 88:3259-87.
25. Wyble CW, Hynes KL, Kuchibhotla J, Marcus BC, Hallahan D, Gewertz BL: TNF-alpha and IL-1 upregulate membrane-bound and soluble E-selectin through a common pathway. *J Surg Res*. 1997, 73:107-12. [10.1006/jsre.1997.5207](#)
26. Lobb RR, Chi-Rosso G, Leone DR, et al.: Expression and functional characterization of a soluble form of endothelial-leukocyte adhesion molecule 1. *J Immunol*. 1991, 147:124-9.
27. Morandini R, Ghanem G, Portier-Lemarié A, Robaye B, Renaud A, Boeynaems JM: Action of cAMP on expression and release of adhesion molecules in human endothelial cells. *Am J Physiol*. 1996, 270:H807-16. [10.1152/ajpheart.1996.270.3.H807](#)
28. Littler AJ, Buckley CD, Wordsworth P, Collins I, Martinson J, Simmons DL: A distinct profile of six soluble adhesion molecules (ICAM-1, ICAM-3, VCAM-1, E-selectin, L-selectin and P-selectin) in rheumatoid arthritis. *Br J Rheumatol*. 1997, 36:164-9. [10.1093/rheumatology/36.2.164](#)
29. Klimiuk PA, Sierakowski S, Latosiiewicz R, Cylwik JP, Cylwik B, Skowronski J, Chwiecko J: Soluble adhesion molecules (ICAM-1, VCAM-1, and E-selectin) and vascular endothelial growth factor (VEGF) in patients with distinct variants of rheumatoid synovitis. *Ann Rheum Dis*. 2002, 61:804-9. [10.1136/ard.61.9.804](#)
30. Newman W, Beall LD, Carson CW, et al.: Soluble E-selectin is found in supernatants of activated endothelial cells and is elevated in the serum of patients with septic shock. *J Immunol*. 1993, 150:644-54.
31. Koch AE, Turkiewicz W, Harlow LA, Pope RM: Soluble E-selectin in arthritis. *Clin Immunol Immunopathol*. 1993, 69:29-35. [10.1006/clin.1993.1146](#)
32. Koch AE, Burrows JC, Haines GK, Carlos TM, Harlan JM, Leibovich SJ: Immunolocalization of endothelial and leukocyte adhesion molecules in human rheumatoid and osteoarthritic synovial tissues. *Lab Invest*. 1991, 64:313-20.
33. van Dinther-Janssen AC, Kraal G, van Soesbergen RM, Scheper RJ, Meijer CJ: Immunohistological and functional analysis of adhesion molecule expression in the rheumatoid synovial lining layer. Implications for synovial lining cell destruction. *J Rheumatol*. 1994, 21:1998-2004.
34. Aoki S, Imai K, Yachi A: Soluble intercellular adhesion molecule-1 (ICAM-1) antigen in patients with rheumatoid arthritis. *Scand J Immunol*. 1993, 38:485-90. [10.1111/j.1365-3083.1993.tb02592.x](#)
35. Voskuyl AE, Martin S, Melchers L, Zwinderman AH, Weichselbraun I, Breedveld FC: Levels of circulating intercellular adhesion molecule-1 and -3 but not circulating endothelial leucocyte adhesion molecule are increased in patients with rheumatoid vasculitis. *Br J Rheumatol*. 1995, 34:311-5. [10.1093/rheumatology/34.4.311](#)
36. Wilson C, Rashid T, Ebringer A: Worldwide links between proteus mirabilis and rheumatoid arthritis . *J Arthritis*. 2015, 4:1000142.
37. Senior BW, Anderson GA, Morley KD, Kerr MA: Evidence that patients with rheumatoid arthritis have asymptomatic 'non-significant' *Proteus mirabilis* bacteriuria more frequently than healthy controls. *J Infect*. 1999, 38:99-106. [10.1016/s0163-4453\(99\)90076-2](#)
38. Wilson C, Thakore A, Isenberg D, Ebringer A: Correlation between anti-*Proteus* antibodies and isolation rates of *P. mirabilis* in rheumatoid arthritis. *Rheumatol Int*. 1997, 16:187-9. [10.1007/BF01330294](#)
39. Christopoulos G, Christopoulou V, Routsias JG, Babionitakis A, Antoniadis C, Vaipopoulos G: Greek rheumatoid arthritis patients have elevated levels of antibodies against antigens from *Proteus mirabilis*. *Clin Rheumatol*. 2017, 36:527-35. [10.1007/s10067-016-3441-4](#)

40. Aletaha D, Neogi T, Silman AJ, et al.: 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis.* 2010, 69:1580-8. [10.1136/ard.2010.138461](https://doi.org/10.1136/ard.2010.138461)
41. Veale DJ, Maple C, Kirk G, McLaren M, Belch JJ: Soluble cell adhesion molecules--P-selectin and ICAM-1, and disease activity in patients receiving sulphasalazine for active rheumatoid arthritis. *Scand J Rheumatol.* 1998, 27:296-9. [10.1080/030097498442415](https://doi.org/10.1080/030097498442415)
42. Rashid T, Ebringer A, Wilson C: The link between *Proteus mirabilis*, environmental factors and autoantibodies in rheumatoid arthritis. *Clin Exp Rheumatol.* 2017, 35:865-71.
43. Pober JS, Cotran RS: The role of endothelial cells in inflammation . *Transplantation.* 1990, 50:537-44. [10.1097/00007890-199010000-00001](https://doi.org/10.1097/00007890-199010000-00001)
44. Hofer E, Stuhlmeier KM, Blakely ML, Van der Werf W, Hancock WW, Hunt BJ, Bach FH: Pathways of procoagulation in discordant xenografting. *Transplant Proc.* 1994, 26:1322.
45. Tak PP, Thirkow EW, Daha MR, Kluin PM, Smeets TJ, Meinders AE, Breedveld FC: Expression of adhesion molecules in early rheumatoid synovial tissue. *Clin Immunol Immunopathol.* 1995, 77:236-42. [10.1006/clin.1995.1149](https://doi.org/10.1006/clin.1995.1149)
46. To SS, Newman PM, Hyland VJ, Robinson BG, Schrieber L: Regulation of adhesion molecule expression by human synovial microvascular endothelial cells in vitro. *Arthritis Rheum.* 1996, 39:467-77. [10.1002/art.1780390315](https://doi.org/10.1002/art.1780390315)
47. Gonzalez-Gay MA, Garcia-Unzueta MT, De Matias JM, et al.: Influence of anti-TNF-alpha infliximab therapy on adhesion molecules associated with atherogenesis in patients with rheumatoid arthritis. *Clin Exp Rheumatol.* 2006, 24:373-9.
48. Johnson RC, Mayadas TN, Frenette PS, et al.: Blood cell dynamics in P-selectin-deficient mice. *Blood.* 1995, 86:1106-14.
49. Carson CW, Beall LD, Hunder GG, Johnson CM, Newman W: Soluble E-selectin is increased in inflammatory synovial fluid. *J Rheumatol.* 1994, 21:605-11.
50. Klimiuk PA, Fiedorczyk M, Sierakowski S, Chwiecko J: Soluble cell adhesion molecules (sICAM-1, sVCAM-1, and sE-selectin) in patients with early rheumatoid arthritis. *Scand J Rheumatol.* 2007, 36:345-50. [10.1080/03009740701406460](https://doi.org/10.1080/03009740701406460)
51. Klimiuk PA, Sierakowski S, Domyslawska I, Chwiecko J: Effect of etanercept on serum levels of soluble cell adhesion molecules (sICAM-1, sVCAM-1, and sE-selectin) and vascular endothelial growth factor in patients with rheumatoid arthritis. *Scand J Rheumatol.* 2009, 38:439-44. [10.3109/03009740903079321](https://doi.org/10.3109/03009740903079321)
52. Dessein PH, Joffe BI, Singh S: Biomarkers of endothelial dysfunction, cardiovascular risk factors and atherosclerosis in rheumatoid arthritis. *Arthritis Res Ther.* 2005, 7:R634-43. [10.1186/ar1717](https://doi.org/10.1186/ar1717)
53. Meyer MF, Schmidt O, Hellmich B, Schatz H, Klein HH, Braun J: Microvascular dysfunction in rheumatoid arthritis assessed by laser Doppler anemometry: relationship to soluble adhesion molecules and extraarticular manifestations. *Rheumatol Int.* 2007, 28:145-52. [10.1007/s00296-007-0397-3](https://doi.org/10.1007/s00296-007-0397-3)
54. Janssen BA, Luqmani RA, Gordon C, Hemingway IH, Bacon PA, Gearing AJ, Emery P: Correlation of blood levels of soluble vascular cell adhesion molecule-1 with disease activity in systemic lupus erythematosus and vasculitis. *Br J Rheumatol.* 1994, 33:1112-6. [10.1093/rheumatology/33.12.1112](https://doi.org/10.1093/rheumatology/33.12.1112)
55. Mason JC, Kapahi P, Haskard DO: Detection of increased levels of circulating intercellular adhesion molecule 1 in some patients with rheumatoid arthritis but not in patients with systemic lupus erythematosus. Lack of correlation with levels of circulating vascular cell adhesion molecule 1. *Arthritis Rheum.* 1993, 36:519-27. [10.1002/art.1780360412](https://doi.org/10.1002/art.1780360412)
56. Cush JJ, Rothlein R, Lindsley HB, Mainolfi EA, Lipsky PE: Increased levels of circulating intercellular adhesion molecule 1 in the sera of patients with rheumatoid arthritis. *Arthritis Rheum.* 1993, 36:1098-102. [10.1002/art.1780360810](https://doi.org/10.1002/art.1780360810)
57. Krenn V, Schedel J, Döring A, et al.: Endothelial cells are the major source of sICAM-1 in rheumatoid synovial tissue. *Rheumatol Int.* 1997, 17:17-27. [10.1007/pl00006846](https://doi.org/10.1007/pl00006846)
58. Klimiuk PA, Sierakowski S, Domyslawska I, Fiedorczyk M, Chwiecko J: Reduction of soluble adhesion molecules (sICAM-1, sVCAM-1, and sE-selectin) and vascular endothelial growth factor levels in serum of rheumatoid arthritis patients following multiple intravenous infusions of infliximab. *Arch Immunol Ther Exp (Warsz).* 2004, 52:36-42.
59. Newkirk MM, Goldbach-Mansky R, Senior BW, Klippel J, Schumacher HR Jr, El-Gabalawy HS: Elevated levels of IgM and IgA antibodies to *Proteus mirabilis* and IgM antibodies to *Escherichia coli* are associated with early rheumatoid factor (RF)-positive rheumatoid arthritis. *Rheumatology (Oxford).* 2005, 44:1433-41. [10.1093/rheumatology/kei036](https://doi.org/10.1093/rheumatology/kei036)
60. Wilson C, Rashid T, Tiwana H, et al.: Cytotoxicity responses to peptide antigens in rheumatoid arthritis and ankylosing spondylitis. *J Rheumatol.* 2003, 30:972-8.
61. O'Dell JR, Elliott JR, Mallek JA, et al.: Treatment of early seropositive rheumatoid arthritis: doxycycline plus methotrexate versus methotrexate alone. *Arthritis Rheum.* 2006, 54:621-7. [10.1002/art.21620](https://doi.org/10.1002/art.21620)
62. Ogrendik M: Antibiotics for the treatment of rheumatoid arthritis . *Int J Gen Med.* 2013, 7:43-7. [10.2147/IJGM.S56957](https://doi.org/10.2147/IJGM.S56957)
63. Saviola G, Abdi-Ali L, Campostrini L, et al.: Clarithromycin in rheumatoid arthritis: the addition to methotrexate and low-dose methylprednisolone induces a significant additive value-a 24-month single-blind pilot study. *Rheumatol Int.* 2013, 33:2833-8. [10.1007/s00296-013-2822-0](https://doi.org/10.1007/s00296-013-2822-0)
64. Kjeldsen-Kragh J, Rashid T, Dybwad A, Sioud M, Haugen M, Førre O, Ebringer A: Decrease in anti-*Proteus mirabilis* but not anti-*Escherichia coli* antibody levels in rheumatoid arthritis patients treated with fasting and a one year vegetarian diet. *Ann Rheum Dis.* 1995, 54:221-4. [10.1136/ard.54.3.221](https://doi.org/10.1136/ard.54.3.221)
65. Cock IE, van Vuuren SF: Anti-*Proteus* activity of some South African medicinal plants: their potential for the prevention of rheumatoid arthritis. *Inflammopharmacology.* 2014, 22:23-36. [10.1007/s10787-013-0179-3](https://doi.org/10.1007/s10787-013-0179-3)
66. Maki KC, Kaspar KL, Khoo C, Derrig LH, Schild AL, Gupta K: Consumption of a cranberry juice beverage lowered the number of clinical urinary tract infection episodes in women with a recent history of urinary tract infection. *Am J Clin Nutr.* 2016, 103:1434-42. [10.3945/ajcn.116.130542](https://doi.org/10.3945/ajcn.116.130542)