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Review Article

Galectin-3, Matrix Metalloproteinase-3 and TLR-2 Receptor as Novel Biomarkers in the Diagnosis of Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disorder that activates arthritogenic immune responses, along with many of the systemic inflammatory cascades that result in synovitis and the progressive irreversible destruction of affected joints. Studies have demonstrated the pathogenic role of some biomolecules and autoantibodies in RA disease. Some other markers, like erythrocyte sedimentation rate (ESR), acute phase reactant protein (CRP), and rheumatoid factor (RF), have also been used successfully to diagnose and treat RA. These are the anticyclic citrullinated peptide (ACPA) autoantibody, tumor necrosis factor-alpha (TNFα), and interleukin 1 and 6 (IL-1, IL-6). Many others are still under study. In this review, we focused on a few biomolecules that could either directly or indirectly contribute to the pathogenesis of RA, aiming to demonstrate their diagnostic characteristics and capacity to forecast the disease. These are Galectin-3 (Gal-3), matrix metalloproteinase-3 (MMP-3) and toll-like receptor 2 (TLR-2). After reviewing peer-reviewed studies from 24 years ago, we concluded that these markers could potentially serve as prognostic factors for RA disease activity in the future and have reasonable diagnostic power. We believe that combining these markers with traditional ones could enhance the accuracy and clarity of clinical diagnosis, as well as track the effectiveness of current therapies.

Keywords: Galectin-3, Matrix metalloproteinase-3, TLR2, Rheumatoid arthritis.

الكالكتين-3 , ماتريكس ميتالو بروتينيز- 3 و المستقبل TLR2 علامات حيوية جديدة في تشخيص التهاب المفاصل الرثوي

الخلاصة

التهاب المفاصل الروماتويدي (RA) هو اضطراب مزمن في المناعة الذاتية ينشط الاستجابات المناعية لالتهاب المفاصل مع افراز العديد من السايتوكينات الالتهابية الجهازية التي تؤدي إلى التهاب الغشاء المفصلي، وهو تدمير تدريجي لا رجعة فيه للمفاصل المصابة. لقد ثبت أن بعض الجزيئات الحيوية والأجسام المضادة الذاتية تلعب دورًا ممرضًا في هذا المرض. تم استخدام الأجسام المضادة الذاتية للببتيد السيتروليني المضاد الحلقي(ACPA) ، وعامل نخر الورم -ألفا (TNF-α) ، والإنترلوكينات 1 و6 حاليا بنجاح في التشخيص السريري وعلاج المرض، بالإضافة إلى العلامات التقليدية ESR ، CRP وRF . العديد من الجزيئات الاخرى لا تزال قيد الدراسة. لقد تم التركيز في هذه المراجعة الادبية على بعض الجزيئات الحيوية التي قد تكون متورطة بشكل مباشر أو غير مباشر في التسبب في مرض التهاب المفاصل الروماتويدي، وحاولنا إظهار ميزتها التشخيصية وقدرتها على التنبؤ بالمرض. هذه الجزيئات هي الكالكتين Gal-3 وانزيم الماتركس ميتالوبروتينيز MMP-3 ، والمستقبلTLR-2 . بعد اجراء مراجعة للدراسات السابقة التي تمت مراجعها من قبل النظراء خلال 24 عامًا، كشفت وخلصت هذه المراجعة إلى أن هذه العلامات قد يكون لها في المستقبل سمة تنبيه وانذار لنشاط مرض التهاب المفاصل الروماتويدي ولها قوة تشخيص جيدة نوعا ما. نحن نعتقد أنه عندما يتم دمج هذه العلامات مع المستخدمة حاليا في تشخيص المرض قد يعطي المزيد من الدقة والوضوح في التشخيص السريري للمرض ومراقبة نشاط العلاجات المستخدمة حاليًا .

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder with an unknown etiology [1,2]. It triggers arthritogenic immune responses and numerous systemic inflammatory cascades, leading to synovitis and the irreversible, progressive destruction of the affected joints [3–7]. Its primary manifestation is symmetric inflammatory arthritis, polyarticular pain and swelling, typically involving the small joints of the hands and feet [8–10]. The disease prevalence ranges approximately from 0.5% to 1.3% [11–15] and women are more affected than men [16].

METHODS

We conducted a comprehensive search of peer-reviewed scientific publications from 2000 to 2024

using the Scopus database, Google Scholar, and PubMed. Firstly, we structured this article to highlight the primary definition of RA disease and the factors that contribute to its pathogenesis. Secondly, it reviewed the disease diagnosis and the current traditional markers used. Lastly, the paper looked at the new biomarkers Gal-3, MMP-3, and TLR2 to show what they are, how they can be used in clinical settings, and how they might be useful as disease markers. This review aims to examine the diagnostic characteristics of these new biomarkers in disease diagnosis, as well as their potential for global approval as traditional RA disease markers. The search strategy employed key words such as "rheumatoid arthritis," "RA factors," "RA diagnosis and scoring," "galectins," "GAL-3," "MMPs," and "TLRs," either in combination or alone. The exclusion criteria included comorbidities of RA disease, such as sarcopenia, cardiovascular disease, musculoskeletal, neurological, malignancy, renal dysfunction, or other overlapping autoimmune diseases.

Factors of RA pathogenesis

The invasion of provoked cells into the synovium, which secretes inflammatory cytokines and articular cartilage-destroying enzymes [17], essentially describes RA and leads to the destruction of bone and articular cartilage [18]. Both genetic and ecological factors are involved in RA pathogenesis [19–23]. There are genes like the HLA DRB1 gene (also called MHC-I) [16], CTLA-4 (also known as major histocompatibility class 4), and PTPN22 (also known as protein tyrosine phosphatase non-receptor type 22) [19,24] that help immune cells do their job. These genetic structures, environmental risk factors [20, 24, 25], and the microbiome [25, 26] can all interact in different ways to cause RA. These interactions can alter self-antigens, which in turn cause abnormal production of cellular and humoral immune response products as well as the migration of T and B cells into the synovium [25]. When these things happen, they include autoreactive T and B cells [12], rheumatoid factor RF autoantibody, and other autoantibodies that fight post-translational modifications (PTM) processes such as citrullination, carbamylating, and acetylation [27]. Serological observation of these autoantibodies can occur for approximately 4.5 years prior to the onset of joint inflammation symptoms during the pre-RA phase [28].

RA Diagnosis

The clinical diagnosis of RA is based on several criteria, including physical symptoms, joint radiographs, and serological tests [29]. We need to find four of the seven main signs of the disease right now: stiff joints that last more than an hour in the morning, inflammation in at least three joint areas, arthritis in both feet and hands, rheumatoid nodules, autoantibodies such as RF and serum anticyclic citrullinated peptide ACPA, and a radiographic changes exam [30]. The American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) defined RA in 2010 as having synovitis in at least one joint, even if there wasn't a different diagnosis that explained the synovitis better, and getting a total score of 6 or more out of a possible 10 scores in four different areas [31,32]. There are four groups that the ACR used to rate these domains, and each one had a point value: joint symptoms, CRP and/or ESR, and serological tests of RF and/or ACPA as a biomarker that predicts aggressive disease. The American College of Rheumatology (ACR) suggested using six different ways to measure the activity of RA. These are the Disease Activity Score with a 28-joint count (DAS 28), the Simplified Disease Activity Index (SDAI), the Clinical Disease Activity Index (CDAI), the Routine Assessment of Patient Index Data with 3 Measures (RAPID3), the Patient Activity Scale (PAS), and the PAS-II [33]. The ACR categorized the disease activity into four stages: remission, low, moderate, and high activity, assigning a score to each stage based on the aforementioned six measurements. Conceptually, the clinical remission stage implies an absence of articular and extra-articular inflammation [34]. The CDAI measures four parameters: the number of painful and swollen joints (0–28) and the 10-point global disease activity assessments for both patients and doctors. This makes it a good, simple, and accurate way to measure the activity of the RA disease. This evaluation easily yields the CDAI value, which remains independent of the acute-phase reactant parameter. In certain clinics, patients may not always have immediate access to ESR and CRP measurements, limiting the use of DAS-28 [35,36]. Recently, researchers developed the multi-biomarker disease activity (MBDA) scoring, a commercial blood test, to assess RA disease activity [37]. Some of the 12 biomarkers that show how RA starts are acute-phase reactants (like serum A amyloid and CRP), hormones (like leptin and resistin), growth factors (like vascular endothelial VEGF and epidermal growth factor EGF), vascular cell adhesion molecules (VCAM1), skeletal-related proteins (like YKL-40), matrix metalloproteinases (like MMP-1 and MMP-3), and cytokine-related proteins (like IL-6 and tumor necrosis factor receptor 1 [37–39]. The collected algorithm for these 12 individual serum biomarkers implicated in the pathogenesis of RA was selected to mimic the individual DAS28-CRP [40, 41].

Traditional diagnosis

Serological inflammatory tests, specifically ESR and CRP, are the traditional diagnostic tools for RA disease. These tests are inexpensive and widely available, serving as both a preliminary routine diagnosis of inflammatory diseases and follow-up tools for monitoring the therapy response. They provide clinicians with valuable information and support the clinical symptoms and signals of inflammation [42–44]. In the positive acute phase, the two tests work together, and in the negative acute phase, they can either rise or decrease in response to

inflammation [45]. First recognized in 1930 with a pneumococcal pneumonia infection, the CRP releases cytokines, particularly IL-6, during the infection, stimulating tissue inflammation and hepatocyte synthesis. It rises within 4-6 hours of inflammation or injury onset, making it more useful for disease diagnosis. Meanwhile, the ESR rises for 24–48 hours before slowly decreasing. The two are more valuable for prognosticating a response to treatment and monitoring disease activity [43,46]. Elevated ESR and CRP levels, along with joint count assessments, can indicate the presence of an active inflammatory response. Many autoantibodies are involved in the disease pathogenesis due to an autoimmunity mechanism and assist in the diagnosis and prediction of RA. These are RF, ACPA and nuclear antigen (anti-RA33) [47]. About 75 years ago, researchers discovered an antibody specific to the antigen-antibody complex [48]. Researchers first used it to diagnose RA disease [48–50], targeting antigen epitopes that are part of the immunoglobulin IgG. However, individuals or patients with autoimmune and infectious diseases may observe its lack of specificity. Individual increases in diseases with age exceed 25% among elderly individuals over the age of 85 years [50]. Peptide arginine deiminases (PADs) initiate the citrullination reaction, which transforms arginine into citrulline via the deamination process [7,51–54]. Dysregulated citrullination leads to the production of autoantibodies, known as ACPAs, as part of an immune response [55]. Early stages [56] revealed the presence of ACPA, as the persistent protein in the joint or circulation facilitated the formation of the immune complex, leading to joint swelling and damage [57,58]. The 2010 ACR/EULAR RA classification criteria included it, demonstrating strong specificity for RA [7,59]. Its sensitivity ranges from 41–66% for early RA and 41–77% for established RA, while its specificity ranges from 88–98% [60]. Furthermore, over 80% of RA patients exhibit elevated levels of ACPA antibodies [52].

New Novel Future Markers

Galectin -3 (Gal-3)

Galectins are an old group of different proteins in the lectin family. They have the ability to identify carbohydrates and specifically bind to oligosaccharide structures on cell surfaces, the extracellular matrix (ECM), and secreted glycoproteins [61–63]. Recently, they became known as an important group of soluble proteins with amino acid sequences that have stayed the same over time and can recognize β-galactoside carbohydrate structures [62,64–66]. They are a masterful, powerful regulator that modulates immune response homeostasis and has a key role in the expansion and/or quenching of inflammation, including RA disease [64,67]. They are extensively present in different tissues and organs, with the highest expression types in the immune system. Galectins control signaling within and between cells, as well as in ECM spaces, by attaching to their receptors [68]. The endocytotic pathway quickly brings galectins back into cells [69]. There, they sort glycoconjugates that are free and those that are bound to them on the membrane. Galectin-3 (Gal-3) is primarily found in the cytoplasm, where it shuttles into the nucleus and secretes itself to the cell surface and elevated biological fluids such as serum and urine [70–74]. It can mediate various processes during inflammation, such as enhancing monocyte chemotaxis and macrophage activation, neutrophil activation, adhesion, chemotaxis, and opsonization. It also plays a role in degranulation and superoxide production, contributing to the development of innate immunity in both acute and chronic inflammatory pathways [3,75]. Among the galectin family, Gal-3 molecules have a unique configuration with two compositional domains: The N-terminal area, which contains the phosphorylation position for subunit interactions, facilitates oligomerization. This area is sensitive to proteolysis by matrix metalloproteinases and may participate in interactions with other intracellular proteins [70]. The C-terminal part, on the other hand, contains the carbohydrate recognition domain (CRD), responsible for carbohydrate binding [62,76-78]. Gal-3 stands out in its family because of its strong association with RA pathogenicity [79]. Researchers have discovered that RA and osteoarthritis release and express Gal-3 through the inflamed synovium [80]. This changes the activity and inflammatory features of osteoclasts, T-cells, and stromal cells [78]. Early detection of RA increases it, and this correlates with ACPA positivity and MRI bone erosion scores in RA patients [81].

Clinical importance of Gal-3

In 2003, Ohshima and colleagues reported an increase in Gal-3 serum levels in RA sera and synovial fluids, which they correlated with CRP levels. Not only does it activate inflammation, but it also plays a role in inducing synovial fibroblasts [82]. In 2015, Issa and colleagues analyzed serum Gal-3 in long-standing LRA and newly diagnosed ERA (duration < 6 months) and compared it with healthy controls. They found that Gal-3 levels were increased in LRA and there was no difference between ERA and control under a daily physical activity program applied to study whether serum Gal-3 shows daily variation and/or responds to exercise in the RA and control populations [83]. In 2017, Issa and colleagues measured the concentration of Gal-3 in undifferentiated arthritis UA patients, which they followed up on for at least 12 months and reclassified according to appropriate criteria. The study showed an increase in serum Gal-3 levels in patients with early UA of pre-RA origin. ROC analysis showed a potential for Gal-3 to discriminate between pre-RA and non-RA within 12–23 months [84]. In 2019, Mendez-Huergo and his colleagues checked the amount of Gal-3 in the blood of RA patients who were taking disease-modifying anti-rheumatic drugs (DMARDs) and/or corticoid medications. They observed no significant difference in Gal-3 levels between RA class I and II patients and

the control group, but they did observe a difference between RA class III and the control group. Researchers have also found that a different way of controlling Gal-3 may help the pain-relieving effects of DMARDs and corticoid treatment in people with RA [64]. In 2020, Gruszewska and her colleagues aimed to evaluate the diagnostic efficacy of Gal-3 by comparing it to the routine RA tests CRP and ESR. The study demonstrated that Gal-3 is a useful tool for RA diagnosis and treatment because of its higher diagnostic power, which can serve as a valuable alternative marker for rheumatic diseases, particularly RA [77]. In 2022, Abdel Baki and his colleagues found that serum Gal-3 levels were higher in people with RA compared to controls. These levels were also higher in people with high disease activity (HDA), suggesting they could be useful biomarkers for RA [85]. In 2023, Pedersen and his colleagues looked at the levels of Gal-3 in plasma and SF from people with chronic RA. They found that the plasma levels of Gal-3 were the same as those in a healthy group, but the SF levels were very high [78]. The study's aim was not to identify the diagnostic potency of Gal-3. However, the results showed that mononuclear cells in blood and SF demonstrated Gal-3. Thus, high Gal-3 levels in the complex synovial microenvironment may have acceptable diagnostic power. In 2023, Amer and his colleagues looked at how high Gal-3 levels were in RA samples compared to controls and came to the conclusion that it is a decent way to tell if someone has RA [86].

Matrixmetalloproteinase-3

Genetic susceptibility and some activating mechanical stress events can induce initial pro-inflammatory MMP genes in joints [87]. It can occur by either directly damaging chondrocytes or activating them to produce abnormal levels of MMPs, which have been seen as a factor in articular cartilage degeneration in RA [88,89]. As a result, the joint cavity receives microcrystals, osteochondral fragments, and ECM degradation products. They then call on swollen synovium cells (neutrophils, macrophages, and synoviocytes) and invite them into the joints, where they release a lot of inflammatory cytokines, chemokines, and lipid mediators, along with more ROS and MMPs [90–92]. Matrix metalloproteinases MMPs are a group of endopeptidase tissue-degrading enzymes that are involved in many diseases, including arthritis, cancer, and neurological disorders [91]. They break down internal peptide linkages in polypeptide chains through extracellular pathways. Their catalytic domains have a similar structure and depend on zinc [87,93–96]. In healthy and unhealthy bodies, they play a big part in controlling things like trophoblast implantation, embryogenesis, angiogenesis, bone growth, wound healing, tissue regeneration, injury and repair [94,97], cell migration, and the splicing of cytokines [93,94,98,99]. Furthermore, within the MMP family, some members protect against disease, suggesting an anti-inflammatory role [90]. The ECM is an active structure that includes lipids, enzymes, structural proteins and antimicrobial peptides. All its constituents are required to maintain the valid function of the barrier [100]. MMPs cause ECM breakdown, while inhibitors reassemble matrix components and alter the shape of ECM [94,100,101]. MMPs help the immune system control the inflammatory process and make it easier for white blood cells to get to the inflammation site [100]. They are also involved in a number of inflammatory diseases because they activate tissue enzymes during an inflammatory response [94]. Consequently, the pathogenesis of various diseases, including RA, implicates MMPs [93]. Six groups have classified the MMP family based on their structure and substrate specificity, resulting in a diverse range of 23 types [87,90]. These include collagenases, gelatinases, stromelysins, matrilysins, and membrane-type MT-MMPs. We divide them into two subgroups: the transmembrane-types and the glycosyl phosphatidylinositol GPI-anchored types. There are also unclassified MMPs, such as enamelsin and macrophage metalloelastase [87, 90, 93–95]. Among this family, MMP-1 and MMP-3 are key enzymes in RA related to disease activity and cartilage and bone destruction [87,91,102,103]. Fibroblasts of synovial and chondrocytes in joints can synthesize and secrete MMP-3, also known as stromelysin-1 [104] [99,105–108]. In RA, active MMP-3 can speed up joint damage by breaking down different extracellular substances, including aggregate nucleoprotein, cartilage-linked protein, fibronectin, laminin, and collagen IV, VII, IX, and XI [99,101,105–107]. It also activates other pro-MMPs, such as pro-MMP-7, pro-MMP-8, and pro-MMP-9 [101,107]. Aggrecan-based proteoglycans undergo degradation when MMP-3 and MMP-9 work in tandem. Proteoglycan degeneration at the surface and further destruction of collagen fibers by MMP-1 and MMP-13 in the deep position both contribute to articular cartilage damage [87]. Those with high progression had significantly higher MMP-3 base levels, making it a powerful predictive marker of RA disease activity and an early predictor variable for the gradual joint damage that occurs locally in the inflamed joint and enters the bloodstream [101,106]. Disease activity, histological synovitis, and synovial MMP-3 expression positively correlate with the elevated serum MMP-3 concentration [105]. Therefore, researchers have studied serum MMP-3 as an indicator of RA disease activity [99,105]. Increasing MMP3 levels in serum are not unique to RA; they are just seen as a useful way to keep an eye on synovial inflammation and other pathological processes that are key to joint destruction in RA [109]. Also, serum MMP-3 was strongly connected to a lot of inflammatory interleukins, like IL-8, IL-6, IFN-γ, and CRP, which destroyed cartilage [103].

Clinical significance of MMP-3

In 2000, Yoshihara and colleagues assessed the baseline level of seven classes of MMP in the SF of patients with RA or osteoarthritis OA. The study found that RA patients had significantly higher levels

of MMP-3, which can serve as a marker for diagnosing RA [88]. In 2014, Sun and colleagues developed a sensitive assay to measure the active form of MMP-3 in both ex vivo and human sera to obtain information about RA progression and an effective response to therapy. Serum levels of active MMP-3 in RA patients, both pre- and post-treatment, were significantly lower in patients receiving anti-TNF therapy compared to baseline levels, indicating the treatment's anti-inflammatory effects. This reduction can serve as a marker for both disease progression and treatment follow-up [110]. Also in 2014, Da Ma and his colleagues looked at the MMP-3 level in RA patients' serum and SF using various tests. They discovered that serum MMP-3 levels were higher in RA patients compared to controls and higher in RA patients with high-grade synovitis than in those with low-grade synovitis [111]. In 2014, Mahfouz and his colleagues investigated the link between MMP-3 levels and the development of joint damage in RA patients. They discovered that the baseline MMP-3 concentration was significantly higher in the high progress group compared to the low progress group, and there is a positive relationship between MMP-3 levels and erosion score [112]. In 2015, Ma and colleagues followed up on the serum MMP-3 level for one year and evaluated its value to predict radiographic progression. The study found that having high levels of MMP-3 in the blood for 3 to 6 months can predict radiographic progress after one year. This means that keeping an eye on changing levels of MMP-3 in the blood along with other signs of disease activity may be more helpful for coordinating radiographic progress and treatment decisions in RA [105]. Ma and colleagues conducted a study in 2015 that showed a higher serum level of MMP-3 in RA patients compared to controls, and a marked elevation in active RA patients compared to relief period patients [113]. In 2016, Fadda and his colleagues discovered that serum MMP-3 levels were higher in RA patients compared to controls. These levels were especially higher in RA patients who had positive CRP, RF, and ACPA sera. It reports a significant relationship between MMP-3 and the DAS28 score, which represents disease activity. Furthermore, a significant difference also appeared in those with erosions when compared to those without. The elevated MMP-3 serum levels indicate the disease activity of RA patients and serve as a specific marker for joint damage [101]. Skacelova and colleagues in 2017 investigated serum MMP-3 levels in RA patients and found highly significant differences compared to healthy individuals [114]. In 2019, Nachvak and his colleagues looked at the levels of MMP-3 in the RA placebo group and the CoQ10 supplementation group to see what effect CoQ10 supplementation had on serum matrix metalloproteinases (MMPs). The MMP-3 levels significantly decreased in the CoQ10 group and increased in the placebo group. Researchers concluded that CoQ10 could serve as a new complementary treatment for RA patients [91]. The 2019 study by Tuncer and colleagues found that people with RA had much higher levels of MMP-3 than the control group. These levels were linked to ESR, CRP, DAS28, and HAQ scores. Researchers found that patients with moderate or high disease activity had significantly higher MMP-3 concentrations than those with low disease activity. In the early stages of the disease, elevated serum MMP-3 levels can be used as an indicator for erosion damage and to monitor disease activity [99]. Hattori *et al*. conducted a study in 2019 to investigate the potential of normal serum MMP-3 levels in predicting clinical remission and normal physical function in the daily lives of RA patients. It was found that MMP-3 levels were better than CRP levels at predicting both clinical remission (SDAI≤ 3.3) and normal function (HAQDI≤ 0.5), as well as both of these things. They came to the conclusion that normal serum MMP-3 levels, along with CRP levels or disease activity, can help doctors predict when a person with RA will be better and be able to do normal things with their bodies [115]. Takemoto *et al*. looked into the MMP-3 level in 2020 to see if it could tell them if RA patients who had switched to abatacept treatment would reach low disease activity (LDA) at 52 weeks. The area under the receiver-operating curve (AUC) value showed that MMP-3 improvement rates at 12 weeks in bio-switch patients had the highest value, with a cut-off value of 20.0% for predicting LDA attainment at 52 weeks. In the bio-switch RA group, a 20% drop in MMP-3 levels after 12 weeks was a strong predictor, as was DAS28-CRP at the start. In the bio-naïve group, which consisted of patients who had never received a biological drug before, DAS28 was the only predictor. Patients who showed a 20% decrease in MMP-3 levels at 12 weeks had significantly higher LDA performance rates at 52 weeks, compared to those who did not see a 20% improvement in the bio-switch group. The research suggested that a drop in MMP-3 levels could help doctors guess how well abatacept therapy will work and keep track of how well other treatments are working [106]. Hamdy *et al*. studied MMP-3 levels in two groups of RA patients in 2022: one with low disease activity (LDA) according to DAS28 <3.2; the other was in clinical remission. They wanted to see if MMP-3 levels could predict sonographic activity. The results indicated that there was a significant difference in MMP-3 concentration between patients and controls, but there was no difference between clinical remission patients and LDA in RA patients. Serum MMP-3 tended to be higher in patients with sonographic activity than in those with sonographic remission [108]. Table 1 presents evidence, as represented by receiver-operating curve (ROC) analysis, from some of the studies that validated the potential use of Gal-3 and MMP-3 as biomarkers for RA disease. The area under curve AUC is divided into five levels: 1.0 is perfect, excellent (0.9–0.99), good (0.8–0.89), fair (0.7–0.79), poor (0.51–0.69), and 0.5 is of no value [116].

Toll-like receptor-2

Toll-like receptors (TLRs) are effective functional biomolecules and pioneers in the first-line defense

immune system in a variety of hosts, from insects to mammals, as in humans [117]. In mammals, including humans, TLRs are transmembrane proteins with preserved structures and evolutionary modifications with a dual function within innate and adaptive immune systems [117,118]. Innate immune system cells use the TLR family to recognize specific molecules from microbes [119, 120]. This is how they tell the difference between self-antigens and non-self-antigens. They are recognition receptors in the host's early defense response. These receptors look for certain molecular models in microbes, which are grouped into three groups: microbe-associated, external pathogen-associated, and damaged molecules released from host cells, which are called internal damage-associated molecular patterns [121–123]. They are found on many innate immune cells [124], and when they are activated, they start signaling cascades that quickly release cytokines that cause inflammation, like TNFα, IL-1, IL-6, and IFN-1 [125,126]. Researchers have identified ten types of TLRs in humans and twelve types in mice [127–129]. These TLRs have two regulatory roles in cell physiopathology, and when they are out of whack, they can lead to a number of pathophysiological diseases [118]. TLR-2 has emerged as an important regulator of autoimmune-associated inflammation. Generally, signaling enhances immune cell responsiveness, leading to tissue inflammation, which is preferable for infection-fighting. But if you don't control the TLR-2 signaling pathway properly, it can lead to an overactive inflammatory response that could be harmful in cases of inflammation and autoimmune diseases [130].

Table 1: Potential use as a biomarker for Gal-3 and MMP-3

Markers	Authors / Ref.	Level use	ROC Statistical issues			Potential use as a biomarker.
			AUC	Sens.%	Spec.%	
Gal-3	Issa *et al*., [84]	Serum	Poor	NP	NP	- Acceptable Gal-3 predictor for pre-RA and non-RA.
	Mendez-Huergo *et al*., [64]	Serum	Fair	↑	↑	- Successful Gal-3 to differentiate HAD-Class III of RA from controls.
	Gruszewska *et al*., 2020 [77]	Serum	Excellent	↑	↑	- Great diagnostic power of Gal-3 in rheumatic diseases including RA.
	Abdel Baki *et al*., [85]	Serum	Excellent	↑	↑	- Promising Gal-3 biomarker for RA in high disease activity HDA.
	Amer *et al*., [86]	Serum	Excellent	↑	↑	- Excellent Gal-3 predictor for RA diagnosis
MMP-3	Ma *et al*., [105]	Serum	Fair	NP	NP	- MMP-3 with core disease activity indicator acceptable predictor for radiographic progression
	Tuncer *et al*., [99]	Serum	NP	↑	↑	- MMP-3 prognostic tool for joint erosion progression at early stage - predictor for disease activation, monitor to treatment response.
	Hattori *et al*., [115]	Serum	Male: Fair	↔	↑	- MMP-3 is a predictor to clinical remission and normal physical function in both genders of RA patients.
			Female: poor	↔	↔	
	Takemoto *et al*., [106]	Serum	Bio naïve: poor	NP	NP	- MMP-3 a predictive biomarker to achieve the low disease activity in Bio-switch RA patients.
			Bio-switch: close to fair	NP	NP	
	Hamdy *et al*., [108]	Serum	Fair	↓	↔	- MMP-3 prognostic marker to differentiate RA patients from others.
	Ma *et al*., [111]	Synovial	Close to good	↑	↔	- MMP-3 is an alternative biomarker of histological synovitis and helpful for diagnosis of RA.
		Serum	Fair	↑	↓	
	Mahfouz *et al*., [112]	Serum	Good	↑	↔	- powerful prognostic MMP-3 marker for disease activity - Early predictor of progressive joint devastation.

ROC; receiver-operating curve, Sens; sensitivity; Spec; specificity; ↑; higher (>70 to 100%), ↔: moderate level (50 - 70) %, ↓; low (≤to 50%), NA; not provided in article.

Human plasma, breast milk, amniotic fluid, and monocyte culture supernatant all contain extracellular soluble forms of TLR2 [122,131]. These are recognized as key modulators, aiding in TLR signaling pathways through a first-line negative regulatory pathway by inhibiting the proinflammatory activity of cell surface TLRs [117,132,133]. In other words, sTLR acts as bait and can attract foreign antigens. It acts as a fake receptor, stopping the activation of TLR-ligand signaling pathways and controlling defense mechanisms. This stops the release of too many cytokines and the activation of TLRs [117,134–136]. Natural sTLR2 plays a crucial role in identifying various microbe types and has demonstrated the ability to alter cell responses to bacterial lipopeptides [137]. The sTLR2 and sTLR4 biomolecules reduce inflammation by inactivating proinflammatory responses linked to TLR molecules. Accordingly, one can suggest the protective proteins sTLR-2 and sTLR-4 as diagnostic biomarkers for the diseases [117].

Clinical significance of TLR-2

The next literature review is mostly about the part that the TLR2 signaling pathway plays in the development of RA. However, it doesn't talk about how the soluble form of sTLR2 is evaluated in different body fluids. This is because the former has many previous studies but the latter does not. In 2003, Seibl and his colleagues found that IL-1β, TNF-α, and LPS raised the level of TLR2 expression in SFs from RA patients who were treated with them as activation ligands. An in situ hybridization study on the SF tissue of RA patients revealed this [138]. Nic Ultaigh and colleagues (2011) investigated how active TLR-2 was in SF cells of RA tissue culture by blocking it with an agonist. They found a reduction in the spontaneous secretion of TNF-a, IL-1b, IFN-g and IL-8 from RA synovial tissue. Therefore, they consider TLR-2 as a

potential therapeutic target for treating RA patients [139]. In 2016, Lacerte and colleagues found that RA patients had more TLR1/2 expression in their blood and SF monocytes than healthy volunteers [140]. Eser and colleagues also found that RA patients had 3.8 times more TLR1/2 expression than healthy controls [141]. A study by Thwaites and colleagues in 2020 showed that stimulating the TLR1/2 signaling pathway increased IL-6 and TNF-α levels significantly in RA blood monocytes compared to healthy monocytes. TLR1/2-activated IL-6 was linked to disease activity [142].

The link between the three biomarkers

In general, TLRs-2 are the first-line defense immune system modulators that activate in response to different molecular recognition patterns, as we mentioned previously. TLRs can recognize Gal-3 [123]. It is a strong signal that causes inflammation that some cells make in large amounts in response to different inflammatory stimuli. It can affect inflammatory cells in either an autocrine or paracrine way [62]. In humans, the protein and mRNA of Gal-3 are highly expressed in the synovial membrane close to joint damage and are highly associated with CRP, IL-6, TNF-α, MMP-3, and many cytokines and chemokines [143]. Joint fibroblasts from people with RA can use Gal-3 to help lipopolysaccharide (LPS) release IL-6 by using TLRs as sensors. In macrophages, TLR2 and Gal-3 work together to increase cytokine production in response to pathogen recognition patterns [144]. Extracellular Gal-3 activates many types of immune cells, including macrophages, mast cells, neutrophils, and lymphocytes, and mediates cell-cell and cell-matrix communication [72,143,145–147]. When macrophages are activated, they can release Gal-3 into the extracellular space. This starts a vicious cycle that sets off pro-inflammatory signaling cascades [148]. There is a protein called COMP made by chondrocytes that helps form cartilage. Adhering synovial fibroblasts release more Gal-3, which makes the inflammation in the synovium worse [4]. Further, in the extracellular space of chondrocytes, Gal-3 stimulates MMP-3 and ADAMTS5 synthesis. The two fundamental enzymes are responsible for proteoglycan devastation in cartilage [78,149,150]. In addition, the cytokines IL-6 and MMP-3 are two effectors produced by immune cells in response to TLR2 activation [20].

Conclusion

Gal-3 has good diagnostic power for the disease diagnosis. Its sensitivity ranged approximately (71%–96%) and specificity (71%–100%), with the AUC ranging from 0.64-0.98. The MMP-3 biomarker is also very good at diagnosing RA; it has an AUC range of 0.586 to 0.831 and a sensitivity range of 48% to 93%. Its specificity range is 48% to 82%. Soluble TLR-2 has not undergone clinical evaluation since its discovery, necessitating additional evidence. We believe that Gal-3 and MMP-3 possess reasonable diagnostic power for RA disease, and their combination with traditional markers can enhance the accuracy and clarity of the clinical diagnosis. Although sTLR2 lacks statistical evidence, we believe it has a predictive role for RA disease.

Conflict of interests

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