American Journal of Biology and Natural Sciences
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Biomarkers of Oxidative Stress in Rheumatoid Arthritis: Clinical Relevance and Therapeutic Targets

[1] Abdullah Mohammed Abdulkarim, [2] Muslim Mohammed Mosa Jassim, [3] Zina Lafta Hassan, [4,5] Osama A. mohsein

1- Directorate of Religious Education and Islamic Studies, Iraqi sunni Affairs

2- Department of Anesthesia Techniques, Al-Taff University College, Karbala , Iraq

3- Department of pathological analysis, College of Applied Sciences, University of Samarra

4- Main Laboratory Unit, Al Habbobi Teaching Hospital, Thi-Qar Health Directorate, Thi-Qar, Iraq

5- Department of Medical Laboratory Techniques, Mazaya University College, Thi-Qar, Iraq

Received: 2024, 15, Mar
Accepted: 2025, 21, Apr
Published: 2025, 13, May

Annotation: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by persistent synovial inflammation, joint destruction, and systemic complications. Among the diverse pathological mechanisms involved in RA, oxidative stress plays a critical role in disease initiation and progression. Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense systems, leading to cellular and tissue damage. Several biomarkers have been identified to assess oxidative stress levels in RA patients, including malondialdehyde (MDA), 8-hydroxy-2'-deoxyguanosine (8-OHdG), advanced oxidation protein products (AOPP), and decreased levels of antioxidants such as glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT). These biomarkers not only reflect the oxidative burden but also correlate with disease activity, joint damage, and systemic

manifestations. Measuring these markers can aid in early diagnosis, monitoring of disease progression, and evaluation of treatment efficacy. Furthermore, oxidative stress represents a promising therapeutic target. Antioxidant therapies, either as adjuncts to conventional treatments or as stand-alone interventions, have shown potential in reducing inflammation, improving clinical symptoms, and modulating immune responses. This review highlights the clinical significance of oxidative stress biomarkers in RA and discusses their potential role in guiding personalized treatment approaches. Understanding the interplay between oxidative stress and RA pathogenesis may open new avenues for targeted therapies, ultimately improving patient outcomes and quality of life.

Keywords: Rheumatoid arthritis, Oxidative stress, Biomarkers, Antioxidants, Inflammation, Therapeutic targets.

1. Introduction:

Rheumatoid arthritis (RA) is one of the most prevalent inflammatory autoimmune arthropathies with an estimated incidence of about 1% worldwide [1,2]. It causes permanent joint deformation and immobilization when not properly treated. Unfortunately, drug-induced remission is not achieved by all patients, leading to continuous joint damage and further co-morbidities. The molecular mechanisms of RA are not completely understood, and the search for new tools to assess disease activity, prognosis, and response to therapy is warranted [3,4]. The contribution of oxidative stress on the initiation and progression of RA is increasingly recognized, moving criteria and assessment into the background. It is a unique condition caused by the overbalance of reactive species generation over the antioxidant defense arsenal [5,6]. Hydroperoxides, superoxide, peroxynitrite, and peroxyl radicals have been shown to be directly implicated in RA pathogenesis, with detailed biochemical pathways and downstream effects reported. Furthermore, the use of antioxidants in the management of RA has been on the ascent for more than a decade, with some promising results reported [7,8].

Clinical use of drugs for routine assessment or therapeutic intervention in RA is lacking. Under such a scenario, the use of biomarkers of oxidative stress is justified and has started to be investigated [9,10]. A classic pegylated uricase enzyme was shown in a dose-dependent manner to

lower levels of oxidized glutathione and thioredoxins and prevent lymphocyte apoptosis in hyperuricemic RA patients [11,12]. Some major metabolic pathways linked to oxidative stress are investigated, among which the purine metabolism, nitric oxide synthesis, lipid/protein oxidation, and methylation reactions seem to play a significant role [13,14]. New therapies targeting the purine metabolism either upstream or downstream effectors are being tested in pre-clinical and clinical assays. More selective pharmacological approaches to control redox balance in a tissue-specific manner are being developed and may also benefit RA patients [15]. However, there is still a relative lack of clinical studies investigating the cross-talk between oxidative stress and rheumatoid arthritis, pointing to oxidative stress as a broad field in the search for biomarkers and new therapeutic interventions [16,17].

2. Understanding Oxidative Stress

Rheumatoid arthritis (RA) is an autoimmune joint disease that affects nearly 0.5 to 1% of the total world population. Even after decades of intensive research, emphasis on overall understanding of the pathogenicity of the disease has been scarce. Meanwhile, a number of strategies targeting specific pathways in RA have been developed, particularly the tumor necrosis factor α signaling pathway and the interleukins-1 (IL-1) and -6 (IL-6). However, these treatments do not resolve the disease, as they do not address the underlying pathway defects. Thus, there is still a need for a more in-depth understanding of the pathophysiology of the disease in order to develop new strategies with a chance of resolution. Interestingly, such need for a better understanding of RA pathophysiology and the advent of novel techniques to address more complex pathways coincide with the recent discovery of oxidative stress as a possible trigger for RA early on [18]. By reviewing the essentials in understanding oxidative stress, the role of this process in RA, the current knowledge on biomarkers reporting on oxidative stress in this disease, and the possibility of targeting oxidative species and/or their production with already available tools in the clinic to improve disease management, including the outcomes expected with such therapeutic approaches [19]. Herein, the aim is to share this knowledge to increase awareness on the potential of oxidative stress in RA, contributing to deeper discussions on the possibilities this field brings to such complex disease [20].

2.1. Definition and Mechanisms

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease, characterized by synovitis, cartilage, and bone destruction. RA affects approximately 1-2% of the world's population, usually affecting individuals around 30-60 years of life and predominantly targeting women. Pathologically, RA is characterized by hyperplasia of the synovial membrane that is infiltrated by inflammatory cells such as macrophages, lymphocytes, and mast cells [21]. There is also an upregulation of extracellular matrix-degrading metalloproteinases, as well as neovascularization and infiltration of synovia by inflammatory T and B cells, all of which lead to angered production of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin (IL) 1 β and 6 [22]. These cells and cytokines contribute to cartilage damage through the upregulation of matrix metalloproteinases (MMPs) and adipokines to which, in turn, subchondral bone osteoclasts are recruited. Activated hinge-type macrophages contribute to chronic inflammation in RA by acquiring a lipid-associated macrophage phenotype resembling alternatively activated macrophages [23]. Additional aberrations of the immune system also contribute to the articulation of cartilage and bone and systemic manifestations of the disease leading to comorbidities such as atherosclerosis, pulmonary disease, and malignancy [24].

Efforts aimed at elucidating the crucial regulatory mechanisms involved in the pathogenesis of RA have not been completely successful. At this point, it is not clear how the pathogenic mutations identified, their effects on the physiology of the proteins altered, and other risk factors that have been demonstrated translate into the hallmarks of this disorder, as little is known of the intricacies involved in the clinical presentation of RA or its induction in animal models [25]. The extraordinary advances in understanding the inflammatory processes supported by innate and

adaptive immune effector cells have changed the prognosis of RA [26]. Specific blocking of TNF-α with monoclonal antibody or inhibitors of its activation lead to a near complete remission of symptoms and inhibition of the oxidative erosion of cartilage and bone. Nevertheless, there is still a need for scientific investigation into earlier detection of the disease. Identification of such events could have an impact on the treatment duration, intensity, and medication used. Such approach could also lead to novel targeted immunotherapy, such as those directed against the aberrant activation of macrophages [27].

2.2. Sources of Reactive Oxygen Species

Oxidative stress is an important part of RA pathophysiology. Reactive oxygen species (ROS) are derived from normal cellular processes in mitochondria and during the inflammatory process by non-phagocytic cells that produce oxidative stress, namely xanthine oxidase through inducible nitricoxide synthase (iNOS) through the NO pathway [28]. Therefore, in autoimmune disease, the interaction between the immune response and the endogenous/exogenous antigens subsequently induces the production of ROS . The oxidative stress process seems to be positively strongly correlated with inflammation and the accelerated denaturalization of the joints. However, there is a doubt about its reliability as the early biomarker of RA disease activity [29]

In order to prove these aspects, the oxidative stress damage biomarkers (lipids peroxide and protein carbonyl level), antioxidant defense capacity, and pro-inflammatory status of plasma were quantified in a group of RA patients under standard or biological therapy compared to healthy age-matched controls [30]. Our results revealed that protein oxidation through carbonylation is significantly increased in RA groups compared to controls; and both the protein carbonyl (Pcarb) and thiobarbituric acid reactive substance (TBARS) are reliable markers of ROS damage that can provide a view upon the complex phenomenon represented by the proteins/lipids damage, key contributors that can provide a view upon a complex phenomenon upon the disease activity, outcome, and inflammation level [31].

3. Rheumatoid Arthritis Overview

Around 1% of the world's population suffers from rheumatoid arthritis (RA), a chronic, multiple, systemic autoimmune disease that preferentially affects joints, including synovial membrane, articular cartilage, and bone. RA synovitis is characterized by the infiltration and aberrant activation of immune cells, including T and B lymphocytes, macrophages, dendritic cells (DC), and mast cells, leading to hyperplasia of the synovial lining, formation of a tissue structure called the ectopic germinal center, and secretion of local and systemic pro-inflammatory cytokines [32]. Infiltrated synovial cells CSC secrete various inflammatory cytokines that act on both local (synovial fibroblasts, osteoclasts, osteoblasts, cartilage chondrocytes, etc.) and systemic (bone marrow, liver, fat, muscle, etc.) targets, inducing a sustained inflammatory and metabolic response that drives chronicity [33,34]. This review appraises the clinical relevance and therapeutic targets of redox biomarkers in RA, focusing on lipid and protein oxidation and the disturbance in the antioxidant status of patients [35].

In the synovial fluid of RA patients, pro-inflammatory mediators (e.g., IL-1β and TNF-α), which may be produced by monocytes and synovial fibroblasts, induce a profound perturbation of the oxidative state [36]. On the one hand, the activation of enzymatic sources of reactive oxygen species (ROS) and reactive nitrogen species (RNS) favors dynamics that contribute to the pathogenesis of RA and drive chronicity. iNOS, GPX-2, and NOX isoforms can be upregulated in CD68+ macrophages, RA synovial fibroblasts and CD4+ T-cells, respectively [37]. These pro-oxidative events are counteracted by antioxidant enzymes (mainly CAT, GPXs, SODs, and PRXs) and non-enzymatic scavengers (e.g., glutathione, ascorbate, and α-tocopherol). However, an impairment of such antioxidant defenses also occurs in the RA synovium, contributing to oxidative stress [38]. By mediating the switch from a local acute response into systemic chronic inflammation, advanced oxidized lipid protein and nucleic acid products, in turn, drive the pathogenic cycle, which is perpetuated by the generation of neoepitopes recognized by pro-

arthritic auto-antibodies such as ACPA [39].

3.1. Pathophysiology

Rheumatoid arthritis (RA) is characterized by persistent multi-joint inflammation that leads to joint destruction. It is associated with systemic complications including macrovascular and microvascular disease, and premature atherosclerosis. Patients with RA have reduced life expectancy, mainly due to increased cardiovascular death. Cardiovascular risk scores do not adequately predict the risk of ischemic heart disease in RA patients. Inflammatory pathways including endothelial activation, platelets, and osteoclasts may all be linked to the cardiovascular risk associated with RA [40].

Oxidative stress is defined as a disturbance in the equilibrium between the production of reactive oxygen species and their elimination by antioxidant defenses, leading to downstream damage of cellular macromolecules [41]. In addition to internal causes, external agents such as pollution, xenobiotics, and medications may also exacerbate oxidative stress in chronic diseases including RA. Oxidative stress, through several pathways, may contribute to the loss of anti-citrullinated peptide antibodies tolerance, triggering joint inflammation and bone erosion [42]. It may activate protein citrullination, and positively modulate TH1 differentiation, and pro-inflammatory cytokine production. The outcome is incremented joint and systemic inflammation, bone erosion, and systemic manifestations [43]. Anti-inflammatory treatments targeting key mediators of chronic disease may attenuate oxidative stress, suppress pro-inflammatory pathways, and prevent deleterious events. Interestingly, the relation of oxidative stress biomarkers with disease activity measures, circulating progenitors, or markers of vascular reduced arterial-filling capacity has not been yet studied. Thus, systemic oxidative stress and its link with peripheral blood progenitors and cardiovascular disease have not been thoroughly investigated in RA [44].

3.2. Clinical Features

Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting around 1% of worldwide population and is characterized by bone erosion and inflammation of the synovial joints and extra-articular symptoms. The etiology is multifactorial and not completely understood but genes, sex factors, environmental influences and narrowing immune tolerance are commonly accepted risk factors, leading to the production of autoantibodies like rheumatoid factor (RF) and anticitrullinated protein antibodies (ACPA), the latter of which have a key role in RA [45]. In parallel, infiltration of the synovial membrane by leukocytes and myofibroblasts results in synoviocyte hyperplasia and joint damage as well as the production of cytokines and pro-inflammatory mediators that also target other organs and tissues. When ungoverned and untreated, chronic systemic inflammation leads to significant morbidity and mortality [46].

Current therapeutic intervention for RA consists of non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids (GCs), which have a primarily symptomatic controlling effect, disease modifying anti-rheumatic drugs (DMARDs) for the preventive controlling effect and biologic DMARDs which are more recently introduced and highly effective and targeted compound drugs [47]. However, DMARDs still show some problems; they are usually unequally effective and biologic DMARDs are poor when inhibiting the function of IL-6 and do not reach the desired remission for RA patients [48]. The unmet medical need for RA is to discover safe and effective new drugs against RA. Moreover, the good therapeutic target is still unknown and pathophysiologically RA is still not fully understood. Thus, the urgent need is to search an important key target for the anti-RA drug discovery and provide new insights into RA diagnosis and therapeutics and as well provide new preclinical in vivo animal models for RA study [49].

3.3. Diagnosis Criteria

Early diagnosis providing therapeutic intervention is essential to avoid irreversible and permanent joint damages in patients with inflammatory arthritis. Clinical and immunological parameters or laboratory tests, such as rheumatoid factor and anti-CCP antibodies, help support the diagnosis of

RA while distinguishing it from other potential causes of inflammatory arthritis. Unfortunately, none of the existing procedures can definitively rule out the diagnosis of RA, and early-stage disease may go undetected. Also, clinical and serological markers are not exclusively found in RA and may be completely negative in some patients. Moreover, more than 60% of RA patients already present with bone erosions at disease onset, as revealed by perform MRI. Therefore, there is a pressing need for novel biomarkers with improved reliability, sensitivity/specificity, and mechanism-based profile to be applied either independently or in conjunction with RF and anti-CCP antibodies [50].

Current research efforts are directed toward the characterization of yet unexplored cellular events or processing that may delineate the development of RA from other forms of inflammatory arthritis. In the last decades, much attention has been given to the pathogenesis of RA, which is evidenced by the significant unraveling of the triggering events of autoimmunity generation as well as synovial tissue involvement [51]. However, efforts to understand the inherent linkage between RA and oxidative stress are much younger in comparison. Several studies have observed markers of oxidative/nitrosative stress either in the serum or synovial fluid of RA patients [52]. Furthermore, stimulation of either blood or synovial-derived cells from RA patients with agents that endogenously produce reactive species has been shown to mimic the disease in vitro. Nonetheless, the clinical applicability of these parameters remains to be demonstrated. Importantly, with the discovery of an imbalance between oxidative and anti-oxidative capability during the early onset of the disease, biomarkers of oxidative/nitrosative stress are expected to be ideally situated between the acute naive and chronic inflammatory balance [53].

4. Role of Oxidative Stress in Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an immune-mediated chronic inflammatory systemic disease. It is associated with increased morbidity and mortality, affecting approximately 1% of the world population. Chronic inflammatory synovitis of joints leads to early erosion and total destruction, affecting the quality of life of patients. Current treatments aiming to minimize disease activity and improve quality of life are capable of altering the disease's natural history. Controlling inflammation at synovial tissue is expected to prevent joint damage. Knowledge on the pathophysiology has substantially increased, yet there are still gaps in scientific understanding. Attempts have been made to better characterize RA by identifying distinct clinical and pathological phenotypes [54]. Extra-articular manifestations occur in up to 40% of patients and are linked to an increase in comorbidities and mortality. Frequency and time of occurrence of extra-articular manifestations can vary greatly in patient series, some being more common or specific to RA than others. The presence of anti-citrullinated protein antibodies (ACPA) is associated with a higher frequency of these manifestations (e.g., pulmonary involvement and cutaneous nodules). A better understanding of the differences in resilience against injury and disease progression between tissue sites is of utmost importance to understand better, not only RA pathology but also the underlying mechanisms in the development of other autoimmune diseases [55].

Oxidative stress is described as a deleterious condition characterized by a negative balance in the pool of oxidative molecules, favoring the predominance of prooxidants, namely ROS and reactive nitrogen species (RNS). These species are highly reactive molecules generated during physiological cellular processes, as well as under several pathological conditions. Antioxidants act as regulatory players, capable of scavenging ROS/RNS and inhibiting the oxidation process in cells. The consequence of an imbalance through augmented production of RONS or by decreased effectiveness of antioxidant defenses is oxidative stress [56]. The results of oxidative stress can be seen as an extensive array of products that are the cause of direct cellular damage and are measurable. Among them are precipitants of protein misfolding and aggregation, ligands for various receptors that regulate acute and chronic inflammation, and lipid peroxidation products that yield immunogenic epitopes [57]. RA is a whole-body disease characterized by markedly enhanced oxidative damage accompanied by changes in oxidative stress markers, leading to chronic inflammation and, combined with genetic predisposition, to autoimmunity. The literature

undoubtedly points to the oxidative stress signature in the pathogenesis of RA, along with the reflection of oxidative stress severity on clinical parameters, and finally, to the proinflammatory potential as emerging and important factors to be properly investigated [58].

4.1. Impact on Joint Inflammation

Rheumatoid arthritis (RA) is a complex autoimmune disease characterized by chronic inflammation and joint destruction [59]. The subsequent inflammation leads to the influx of inflammatory cells, over-activation of cellular pathways, and increased production of pro-inflammatory cytokines, including tumor necrosis factor (TNF-α) and interleukin (IL)-1, all of which are implicated in the pathogenesis of RA. The interaction of these factors with articular tissue leads to the proliferation of synovial fibroblasts, collagenolysis, increased vascularity, and overproduction of matrix metalloproteinases and other inflammatory mediators, all of which synergistically promote inflammation and leads to joint destruction. These key events in RA disease pathogenesis are of great interest for the development of effective therapeutic approaches [60].

A positive feedback loop between oxidative stress and inflammation has been recognized. Oxidative stress promotes the release of inflammatory cytokines, while pro-inflammatory cytokines stimulate the production of reactive oxygen species (ROS). Excessive production of ROS damages many cellular structures, including lipids, proteins, and nucleic acids, transmitting signals that sustain and amplify chronic inflammation [61,62]. Damaging cellular components have been shown to be prominent mediators in the pathogenesis of RA. Oxidative and nitrosative stress generated by excessive ROS and nitric oxide is critical for RA initiation, promotion, and maintenance. This review summarizes new findings on oxidative stress involvement in synovial inflammation, clinical applicability of oxidative stress biomarkers for stratification of RA patients, and potential add-on therapies targeting oxidative stress as a novel therapeutic strategy for RA [63,64].

4.2. Contribution to Disease Progression

Although the connection between oxidative stress and chronic inflammation is widely recognized, the specific mechanisms through which redox imbalance contributes to the pro-inflammatory environment present in RA have been poorly examined. Nevertheless, the production of several ROS is believed to be excessive because of the altered activities of different oxidases, such as nicotinamide adenine dinucleotide phosphate oxidase [65]. The overproduction of superoxide by phagocyte NADPH oxidase and mitochondrial dysfunction have also been implicated in an increased oxidative burden in RA. There is a reciprocal relationship between oxidative stress and the inflammatory state. On one hand, ROS produced by activated immune cells exert a pro-inflammatory effect through a mechanism that involves the modification of components of signaling pathways, resulting in increased signaling cascades that culminate in the synthesis and secretion of pro-inflammatory mediators [66]. On the other hand, pro-inflammatory cytokines and the oxidative products formed during lipid peroxidation modulate the expression and activity of NADPH oxidase, leading to enhanced ROS production [8]. This mutual oxidative and inflammatory amplification mechanism is suggested to contribute to disease progression. It is plausible that, by worsening synovitis, the increased oxidative burden leads to further synovial cell activation, giving rise to more inflammation and, in the long term, contributing to substantial joint damage [67,68]. There is, however, little evidence concerning the role of oxidative stress in the progression of RA. The specific oxidized products released during the oxidative stress process and the functional outcomes induced by its intracellular accumulation still need to be identified [69]. Elevated levels of 4-hydroxy-2-permanently-nonenal and reduced glutathione are detected in the synovium of RA patients, confirming the increased oxidative burden, while a pharmacological strategy aimed at restoring antioxidant defense shows benefit in a mouse model of arthritis. This suggests that oxidative products may have therapeutic potential in RA [70,71].

5. Biomarkers of Oxidative Stress

Inflammatory diseases are constantly linked with increased oxidative stress, which may be evaluated by measuring oxidant species and/or the impairment of antioxidant defenses. In AIA, a small-scale preclinical model of RA in rats, proinflammatory cytokines IL-1β and TNF-α trigger ROS production. These species, mainly represented by nitric oxide (NO), superoxide anion radical (O2−), peroxynitrite anion ($ONOO^-$), hydroxyl (OH), lipoperoxide (LOO), hydrogen peroxide (H2O2), and hypochlorous acid (HClO), are highly reactive molecules generated during physiological cellular processes, as well as under several pathological conditions [72]. The excess of ROS/RNS favors the erosion of bio-macromolecules, such as phospholipids, proteins, and nucleic acids, turning them into reactive compounds that might constitute a harmful self-originated biomarker of diseases. The bioreactivity of these species led to the initial classification of oxidative stress biomarkers as an unequivocal consequence, unable to play an active role in the disease mechanism [72]. Besides the potentially increased amounts of ROS/RNS in situations of oxidative stress, antioxidants also act as regulatory players, as they are substances or compounds capable of scavenging ROS/RNS and, thus, inhibiting the oxidation process in cells. Two different classes of antioxidants exist, namely, the enzymatic and nonenzymatic systems [73]. The first type is mainly represented by superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and thioredoxin reductase. $O2^-$ and H2O2 represent the most produced ROS, with the former scavenged by SOD and the latter by CAT, GPx, and peroxiredoxins. The nonenzymatic antioxidant system gathers some vitamins (A, C, and E), β-carotene, and antioxidant minerals, such as copper, zinc, manganese, and selenium [73].

There is a growing interest in defining and establishing oxidative stress biomarkers as a promising additional alternative in assessing disease activity and prognosis in RA patients, with a strong effect directed toward peripheral blood [74]. The noninvasive evaluation of oxidative stress through a blood sample would be more direct and simpler (repeated collection avoiding the need for a fibroblast culture), minimizing the risk of deterioration during transport. Superoxide dismutases, the most explored antioxidant enzymes in RA patients, may be affected by a different pattern of inflammatory cytokines [74]. Disease Activity Score-28 (DAS-28) is a validated measure of disease activity in RA, based on the number of tender and swollen joints, measurement of CRP serum levels, and patient's global assessment of health. A meta-analysis investigated the most studied oxidative stress biomarkers and found a positive correlation between lipid peroxidation (assessed by the serum levels of malondialdehyde (MDA) and DAS-28 score, reinforcing the assumption that oxidative stress and disease activity in RA move toward the same direction [75]. This analysis included studies that considered more extensive clinical parameters that agree with different RA classifications. Even though not all studies applied the DAS-28 index, the authors highlight the potential applicability of oxidative biomarkers not only for complementary assessing disease activity but also for prognostic purposes [76].

5.1. Types of Biomarkers

Oxidative stress is characterized by an increased generation and/or decreased antioxidant defences against reactive species, for example, reactive oxygen species (ROS) and reactive nitrogen species (RNS). The production of these species, in pathological conditions, and the impaired antioxidant status may promote oxidative/nitrosative/nitrative damage to lipids, proteins, DNA, and/or other biomolecules. Rheumatoid arthritis (RA) is a chronic, autoimmune, and systemic inflammatory disease that primarily affects joints and mainly occurs in an inflammatory context with increased oxidative stress [77]. Different lines of evidence about the role of oxidative stress in the pathophysiology of RA have been established. The assessment of the products of oxidative stress damages (biomarkers of oxidative stress) is an attractive strategy for disease activity monitoring. The use of biomarkers of oxidative stress in RA has recently been reviewed, with emphasis on methodological aspects, sources of variability, and future perspectives for the biomarker discovery process [78]. In addition to methodological aspects, advances in the scientific area and clinical laboratories themselves must be made to facilitate the introduction of the biomarkers. These

advances may be in the discovery of novel laboratory or imaging methods capable of addressing aspects of inflammation, oxidative stress and antioxidants still investigated. Worldwide interests in discovering novel additional biological drugs and biomarkers might increase with the aging population and the development of diseases of older age, such as RA. Biomarkers of oxidative stress alterations in RA have gained attention as insight into disease pathogenesis and as candidates for clinical use [79].

ROS (and/or RNS) are molecules with unpaired electrons in their outer shell, which imparts them a high reactivity. Consequently, it results in a hydroxyl radical, nitric oxide, superoxide anion radical, peroxynitrite anion, lipoperoxide, hydrogen peroxide, hypochlorous acid, peracetic acid, hypochlorite and singlet oxygen [80]. These molecules are not only produced in pathological conditions, but they are also derived from physiological processes, such as aerobic metabolism of nutrients, bactericidal action of activated phagocytes, inflammatory process, and activation of immune cells. Endogenous antioxidants (either enzymatic or non-enzymatic) and some residues of the bio-molecules (metal ions and heteroatoms in the molecule chain) scavenge ROS under physiological conditions, maintaining ROS levels balanced [81]. Still, in pathological situations, there are a large number of ROS over the scavenger method; it results in oxidative/nitrosative/nitrative alterations [82]. For the clinical use of biomarker of oxidative (or nitrosative) stress alterations, the oxidative damage must be evaluated only by surrogate exogenized substrates. Therefore, 8-isoprostane is not accepted as a biomarker of lipid peroxidation. It only measures an inflammation sub-process, and it is a direct measure of tissue accumulation of NO. The compounds of CG or HPLC with elevated thermal energy are preferred as biomarkers of lipid peroxidation. Urinary excretion increases markedly in parallel with inflammatory process in RA [83].

5.2. Measurement Techniques

The assessment of oxidative stress in experimental and clinical studies of RA has been performed based on indicators of free radical formation, effects, or an imbalance in antioxidant defenses. Various measurement techniques have been used depending on the necessary specificity and selectivity [84]. In the vast literature on biomarkers and techniques of oxidative stress assessment, four measurement techniques are used: (i) direct measurement techniques assessing free radicals, (ii) assessment of non-specific effects/stress on lipids, proteins, and DNA, (iii) assessment of antioxidant non-enzymatic defenses, and (iv) assessment of antioxidant enzymatic defenses [85]. Overall, direct measurement techniques can measure free radicals that oxidize biomolecules, react with solvents or bases producing measurable changes in their physical and/or chemical properties, struggle with specificity. Indirect measurement techniques with good specificity show interference and the multi-targetability of the chemical probes used [86]. Besides supplementary sample preparation processes for spectrophotometric or chromatographic methods with high sensitivity. Taking into consideration that biomarkers need general availability for academic, pharmaceutical, or clinical laboratory research, there are some aspects that researchers should consider before choosing the measurement method used [87]. These are: (i) Biomarker analyzed (indirect measurement of free radicals, products of their chemical reactions, or non-specific biomarkers), (ii) specificity measured for biomarker analyzed (if available filtering protocols or multi-step analysis procedures should be used), (iii) The use of multicriterion methods measuring several biomarkers of different origins or types, (iv) equipment availability (in terms of cost, maintenance, and training) and ease of use, (v) Investigator/systematic lab skill and accuracy, (vi) Sample type availability in conditions before/during/counter medication or disease influencing factors and (vii) Biomarker stability in the measured condition [88].

5.3. Clinical Relevance

Rheumatoid arthritis (RA) is a destructive autoimmune disease that is characterized by the inflammatory process of the synovial tissues and joint erosion [89]. Pathophysiologically, RA is still not completely understood. Approximately 1% of the worldwide population suffers from RA,

which accounts for indications for one-third of orthopedic surgery. As the chronic inflammatory disease progresses, the treatment is usually based on the immunosuppressive drugs [90]. Nevertheless, the etiopathogenesis of RA is still open and controversial despite several well-established factors contributing to the synoviocyte hyperplasia and systemic inflammation. The implemented therapies still need to be modulated according to the individual patient [91]. In this regard, the more objective understanding of the etiopathogenic pathway will lead to the discovery of relevant biomarkers for diagnosing and manipulating therapeutic agents against RA. Nowadays, the objectives of RA therapies are easier to implement in clinical practice compared to objective measures for evaluating immunosuppressive therapy effectiveness [92]. The discovery of new theranostic agents in diseases is a significant concern. In the past two decades, one of the exciting topics investigated in relevance to RA is oxidants/antioxidants. The innovative non-invasion mechanism of oxidative stress markers in various body fluids for determining the disease activity and treatment response is also yet to be fully appreciated [93]. Although the levels of TNF-α mRNA sputtered in synovial tissue were significantly decreased after a few weeks' treatment, it is temporally and spatially restrictive. Meanwhile, using the biological markers for determining the effectiveness of treatment with biological drugs is still premature. Improvement in the health of RA patients does not seem to accompany the decrease of systemic oxidative stress markers, questioning the pathophysiological relevance of oxidative stress. Besides the potential applicability of oxidative biomarkers for assessing disease activity and prognostic purposes, several avenues for future studies require attention [94].

6. Therapeutic Targets

Abstract: Rheumatoid arthritis (RA) afflicts millions worldwide, spurring intense investigation towards comprehending its underlying mechanisms. A solid conceptual framework and coherent direction of research are emerging regarding the prominent role of oxidative stress in RA pathogenesis. This review exposes this wealth of knowledge and conveys how augmenting discovery and validation studies, but also controlled randomized clinical trials addressing therapeutic agents intended to blunt oxidative stress, should now be fostered [95].

6.1. Antioxidant Therapies

Recently, a wide array of antioxidant therapies aimed at RA patients has been evaluated. Many of these interventions have demonstrated antioxidant and anti-inflammatory properties, reflected by ameliorated clinical parameters and reduced oxidative stress biomarkers. These therapies include N-acetylcysteine, alpha-lipoic acid, vitamin E derivatives, selenium, lycopene and carotenoids, flavonoids, curcumin, and probiotics [88]. As detailed below, most studies evaluated one agent at a given time, which hampers the translation of the findings into the commitment of each approach [96]. In parallel, prospective clinical studies evaluating the impact of botanical antioxidants in RA have gained notoriety in recent years. A randomized trial evaluated the efficacy of 3 doses of N-acetylcysteine and found an improved clinimetric parameter in the group receiving 1000 mg/day of N-acetylcysteine, alongside decreased nitrotyrosine and increased superoxide dismutase activities (high-dose N-acetylcysteine did not produce significant effects) [97]. However, the multi-armed variant design along with small sample sizes precluded a robust conclusion of the individual impact of each agent. Eschewing germinate pharmaceutical forms, a small randomized study showed that a lycopene supplement lowered the pain/vigour symptom score of the Western Ontario and McMaster Universities Osteoarthritis Index. Supplementation with this carotenoid was also able to reduce CRP and insulin resistance in patients with RA [98]. By the involvement of the GLP-1 pathway, astaxanthin supplementation was able to lower TNF-α level, disease activity score (DAS) 28, and CRP levels in RA patients (who also exhibited lower β-cell function short-fall). The involvement of disease-modifying anti-rheumatic and biological drugs in putative antioxidant pathways must also be mentioned; such agents have appreciable side effects and mechanisms of action, which precludes a long-term commitment. While the re-examination of the base therapy in patients with non-responding RA may be extemporaneous, progress in the search for adjunct therapeutic strategies could usefully widen the arsenal of treatment alternatives. In

addition to antioxidant dietary approaches, medications that strictly adhere to the adjunct therapy criteria have been evaluated [99].

6.2. Novel Drug Developments

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that primarily affects peripheral synovial joints and can lead to debilitating and painful joint destruction and deformity. Disease progression with increasing morbidity and mortality can also affect extra-articular and systemic tissues such as skin, lung, pleura, heart, connective tissues, bone, and vessels [100]. The synovium becomes hypertrophied in a highly vascularized edematous soft tissue formation, affecting the cartilage and bone [101]. Further, inflammatory process progression leads to margination and influx of leukocytes in the synovial capillaries, with T-cells, B-cells, and macrophages flooding into the soft tissue and the release of immune mediators from both the synovial tissue and infiltrating inflammatory cells [102]. Earlier, polymorphonuclear granulocytes were assumed to be the dominant cells in RA and responsible for inflammation and joint destruction too, but nowadays they are thought to be present in low numbers in chronic RA synovium. Nevertheless, an efflux of many inflammatory mediators is promoted due to chronic inflammation and RA progression, and most of them target the vascular endothelium, articular chondrocytes, and synovial fibroblasts, leading to cartilage and bone destruction [103].

Due to the access to the circulatory or lymphatic systems, several potential inflammatory biomarkers of RA have been proposed and studied within the blood or serum. From the point of view of specificity, many of them are of little value, being involved and secreted in various systemic inflammatory conditions [104]. On the contrary, some of them have become useful in the early diagnosis or assessment of disease activity, and treatment monitoring of the disease, but only a few of them are used in clinical practice [105]. Nevertheless, these are highly desirable and novel biomarker candidates involved in any RA processes or secreted in various bodily fluids and monitoring therapy activity either directly or indirectly. Many inflammatory mediators are produced during the upsurge of oxyradicals, which favors the development of oxidative stress and eventually leads to tissue redox homeostasis [106]. Redox dysregulation has been suggested and demonstrated in RA and other inflammatory disorders. However, only a few OS biomarkers have been studied concerning RA diagnosis and treatment monitoring. In this regard, more and earlier studies in diverse cohorts are still necessary to define their specificity and elevate confidence in their clinical use [106].

6.3. Lifestyle Interventions

Lifestyle interventions, such as regular exercise and optimal weight, are touted as a means of alleviating joint pain in RA patients. However, there is limited knowledge of the alterations in physical activity and lifestyle that occur before and after the diagnosis of RA. This study is novel in that it employs precision motor controllers to improve the understanding of movements in real-world settings by studying people over long periods [107]. The software sensors can also facilitate longer follow-up periods to monitor the evolution of lifestyle factors on the development of RA diseases. Furthermore, RA patients will be also recruited and the above-mentioned investigations repeated [108].

Dietary factors including fish oil, antioxidant vitamins, and extra virgin olive oil fortification are proposed as dietary therapies to ameliorate arthritic symptoms. A vegetarian and seafood diet engendering a greater-than-threefold increase in fish intake compared to a habitual Western diet has been documented to afflict RA patients with significantly improved clinical remission. Specifically, fish consumption is found to enrich systemic n-3 PUFA bioproducts that increase serum EPA fraction and decrease the AA/EPA ratio and LTB4 [109]. Less utilization of AA availability to generate pro-inflammatory prostaglandin E2 and CXCL8 leads to decreased leukocyte adherence, diapedesis, and secretion of IL-1α and IL-6 by prevascular synovial fibroblasts. Subsequently, the pro-inflammatory cytokines TNF-α and IL-1β, IL-6, and IL-8 of which are produced from synovial fibroblasts and macrophages are inhibited [110].

Ongoing study shows dramatic increases in urinary n-3 PUFA bioproducts and systemic anti-inflammatory mediators over time for RA patients adhering to the MJ diet [111]. The combined effect of fish oil and a test diet containing antioxidants on various circulating immune mediators and the clinical and behavioral assessment of RA is further elucidated, and a marked improvement in the clinical condition, diminished oxidative damage level, and restored balance is seen. With the proposed integration of lifestyle strategies into current therapeutic regimens, there exists the potential to prevent the conversion of disease and ameliorate the symptoms, disease activity, markers and dysregulations [112].

7. Current Research Trends

As an autoimmune disorder, rheumatoid arthritis (RA) affects 1-2% of the worldwide population and is characterized by chronic synovial inflammation and accumulation of autoinflammatory cells in the joint cavity and other tissues. The origin of the disease is still poorly known, and often it presents with variable arthralgia that, if neglected, will evolve into incapacitating muscle disabilities. This in turn will prevent activities of daily living and increase the risk for associated medical comorbidities and premature death. An extra-articular involvement may frequently occur in RA, most commonly in the aggressive forms of the disease. Rheumatoid nodules, lung disease, vasculitis, and scleritis have been described in the picture of RA [113]. The personalization of RA and the tailoring of the management to the phenotype, the comorbidities, and the individual patient must be taken into account. Current research trends regarding the relevance of oxidative stress biomarkers are presented, as well as therapeutic strategies targeting the oxidative stress in RA [114].

RA is a complex inflammatory disorder with unknown etiology that affects mainly the diarthrodial joints but can also involve other tissues, including the skin, lungs, heart, blood vessels, and kidneys. Onset is often vague, with mild and transient arthralgia that can remain unnoticed but, if neglected, may gradually evolve to incapacitating joint disabilities with major impact on the quality of life [115]. The audit of the extra-articular involvement in RA aims to summarize current knowledge on this common aspect of the disease, which may frequently occur in RA, particularly in those with aggressive forms and seropositive profile. Onset can be with extra-articular signs, or they can appear later in the course of the disease, possibly with an impact on treatment [116]. Full-blown forms associating major skin, pulmonary, and systemic disease may be encountered. The complex interactions leading to the clinical manifestation of the disease as well as insights in the complete clinical picture and treatment strategies are still poorly understood. Current research trends regarding the relevance of oxidative stress biomarkers as well as therapeutic strategies targeting the oxidative stress in RA are discussed [117].

7.1. Recent Clinical Trials

The clinical exploration of RA biomarkers is confined to the frames of scholarly investigation on synthetic and environmental agents and clinical trials of drugs with specific oxidative stress protective effects. Using the PubMed database and searching for keywords that closely relate to the subject, only 29 clinical trials investigating oxidative stress as a source of biomarkers and therapeutic target in RA were found. This short list indicates the high necessity of expanding this niche area of research [118]. There is a predominant focus on blood and synovial fluid biomarker research. It would be informative to assess the oxidative status and a panel of biomarkers in other biological fluids such as urine, saliva, and tears, given that the cross-talk between them and the triggering of oxidative stress pathways is already established [119]. Additionally, there is an enormous gap concerning the migration of information from the laboratory bench to the bedside in terms of predictive models of clinical relevancy. A more integrated effort in this sense could disclose the clinical applicability of biomarkers with potential therapeutic value [120].

This clinical trial report provides an overview of the current knowledge concerning biomarkers in RA with a focus on the specific theme of oxidative stress. In the foreseeable future, biomarker development would benefit from further multiomics insights in preclinical studies when

investigating the protective effects of drugs [121]. Mostly focusing on rheumatoid arthritis, it highlighted compelling evidence of the importance of oxidative stress in this inflammatory disease and the valuable knowledge it could bring for the search of RA biomarkers. It is worth emphasizing that any preclinical discovery with clinical value must necessarily deal with the challenge of reproducibility and translation to the clinic [122]. When novel technological improvement dramatically enhanced the diagnosis potential for RA and other pathologies, the specific chronic inflammatory diseases have remained poorly understood and under diagnosed [123].

7.2. Emerging Biomarkers

Emerging markers of oxidative stress can improve diagnosis of rheumatoid arthritis and facilitate research into treatments. Biomarkers of oxidative stress provide insights into the emergence of clinical manifestations of rheumatoid arthritis [124]. Reactive oxygen species, nitration and lipid peroxidation derivatives, differentiated oxidative damage markers, and antioxidant systems have a direct role in joint inflammation processes and have been linked to the severity of autoimmune rheumatic diseases. Biomarkers of protein oxidation, such as advanced oxidation protein products, protein carbonyls, and dityrosine cross-links, were greater in rheumatoid arthritis patients in comparison with controls [125]. Biomarkers of lipid peroxidation and its consequences are increased in osteoarthritis and are associated with inflammation severity. Systematic reviews and meta-analyses confirmed increased oxidative stress in rheumatoid arthritis and its connection with disease activity, cartilaginous malformations, and potential treatment merit [126].

Emerging markers of oxidative stress linked with other pathological processes have potential in the discovery of new treatment targets. Immunotherapy by biomarker inflammation promotion requires a proper understanding of common pathogenic molecular alterations in cell signaling pathways that mediate inflammation and oxidative stress. Understanding catecholamine synthesis and release in neurons, glial cells, and macrophages can facilitate the development of tailored treatments that selectively inhibit oxidizing effects in certain cellular types [127]. For each biomarker of oxidative stress, modified targets, drugs, and treatment regimens can be proposed. Emerging biomarkers can aid the diagnosis and monitoring of inflammation processes in rheumatoid arthritis [128]. Detection of protein carbonyl oxidative products, advanced oxidation protein products, oxidized/reduced glutathione ratio, and glucometabolic biomarker changes in synovial fluid help distinguish between types of arthritis and gauge response to inflammation-promoting inhibitors [128].

8. Challenges in Biomarker Utilization

The heterogeneous nature of rheumatoid arthritis (RA) complicates its early recognition and treatment. Such a delay effects timely treatment and, as a consequence, poor long-term outcomes. This limitation has been the background for a considerable amount of research into the identification of factors susceptible of answering clinical questions [129]. Despite the fact that the semiology of the several symptoms of RA is well defined, this approach has lessened prognostic tests offering poor predictive values with no replication in new cohorts. With the advent of new therapies to treat patients with early RA, several indices of diagnoses and of activity measures have arisen and broad measurements are being used. However, treatment aimed at control of inflammation is more of a trial and error process, since many patients do not reach good control with the first DMARD that they receive. Most patients initially treated with one DMARD progress onto treatment with them all. This pharmacological escalation leads to a cascade of different drugs with less than optimal control in most patients, whilst others remaining untreated progress rapidly to arrests in their social and working lives. Research on the autoimmune etiology of this differential outcome has discovered genetic family clusters, serological reactivity against antigens of joint tissue, and circulating proteolytic factors that are disease specific and modify its severity [130].

Biomarkers play an important role in identifying patients with worse outcomes. In RA there are

three theoretically different modalities for biomarkers that either facilitate early diagnosis, or to adjust management to disease activity or poor outcomes. However, the heterogeneity of the disease is such that it is becoming apparent that these biomarkers are deck specific and can overlap as well, thus producing groups of diseases or different diseases with an apparently identical basic defect [131]. Importantly, there is no single biomarker that can bridge the gap between the disease onset and the prescription of the first DMARD. The former are just an ensemble of risk factors that allow early treatment in some of the patients; but, notwithstanding good accuracy, a considerable part of RA patients remain undiagnosed [132]. The same could be said for activity monitoring biomarkers; traditional ones do not detect all patients requiring an early aggressive treatment, and the treat-to-target concept is either late or irrelevant in this cohort. In this regard, a new challenge arises of searching for prognostic biomarkers suitable for defining a similar entity that integrates multiple risk factors; in this pursuit more than a single biomarker is required, rather a multi-biomarker approach might be necessary involving several different modalities [133].

8.1. Standardization Issues

In Rheumatoid arthritis (RA), a chronic inflammatory disorder that affects many joints, including those in the hands and feet, assessment of the disease activity to help guide treatment decisions or to evaluate therapeutic responses is vital. For clinical manifestations of RA evaluation, medical history, laboratory tests and imaging studies are often used, all of them influenced by different factors. Further potential therapeutic approaches, such as anti-IL-6 receptor antibody, anti-PDE4 inhibitor and JAK inhibitors, are currently under investigations in clinical trials. However, pivotal clinical studies have shown that oxidative stress levels have limited ability to predict disease activity [134]. Similar to this study, in detecting increased levels of nitrotyrosine, there were not significant differences between high/low and fixed/variable focal score groups at three follow-ups, suggesting that aberrantly elevated oxidative stress does not guarantee significant clinical worsening.

Yet no guidelines are published regarding what determination methods must be used for a specific biomarker candidate in order to maximize its chance of becoming applicable at clinic batches, current knowledge regarding standardization of stress biomarkers is reviewed. After defining precisely the oxidative stress concepts and its biomarkers, mechanisms of oxidative burthen states on biomarker candidates are summarized [135]. These are then classified into two types, interference on measuring signal and alteration of analyte concentration, with existence of additional experimental effects. Finally, the current limitations on biomarker candidates in clinical practice are discussed, and suggestions are proposed on both academic studies and technology development perspectives [136].

Biomarkers are defined as objectively measurable tests that indicate the current state of a biological process, which has the potentiality to become a predicator and/or monitor early responses to a treatment [137]. Based on the above-mentioned questions, the most important achievements achieved in the past decades on oxidative stress biomarkers, coupling to the most common clinical usages and the most interesting mechanisms or properties that may lead to further practical development attempts, are reviewed. Also thoroughly reviewed are how sophisticated methodologies and improved know-hows have been accumulated to enable highly accurate, reliable and feasible assessments of said oxidative stress biomarkers, ensuring that only those parameters that can truly represent this concept will be used to serve imminent sample case control studies [138].

8.2. Interpretation of Results

Visionary thinking helps to make choices about the future, often when there is a lack of information or clarity about what is possible. This would be particularly true in the case of braces for people with rheumatoid arthritis. Inter-professionally learning to think outside the box would make a significant difference in the treatment of RA patients [139]. Considerations regarding

improvement in health status, recovery in daily life and work, and potential benefits to society (vis-à-vis cost-benefit) could engender inter-professionally novel braces for people with a disease like RA. Nightshades such as pepper, tomatoes, and eggplant may increase disease activity in some people with rheumatoid arthritis. Nightshades are full of healthy vitamins and minerals and, for most RA patients, beneficial [140]. However, if adverse symptoms arise after consuming these foods, avoidance is a wise option. Although the etiology remains unknown, it is clear that RA is a complicated polygenic disorder with a chronic course, heterogeneous presentation, and acrimony outcome [141]. Disease-modifying anti-rheumatic drugs (DMARDs) play an important role in the management of RA, even in the early stage of the illness. Clinical manifestations often improve but only a minority of patients achieve long-term remission. Computer-aided detection can significantly facilitate the assessment of bone erosions and joint space narrowing on plain radiographs of rheumatoid arthritis patients [142]. The ability to accurately identify erosions and joint space narrowing on radiographs proved to be essential for proper evaluation of disease activity and therapeutic response in RA. Abundant evidence indicates that inflammation and oxidative stress are two vital and closely related pathophysiological processes that contribute significantly to the progress and development of RA [143].

9. Future Directions

Despite the well-recognized participation of oxidative stress in the pathophysiology of RA, clinical studies devoted to antioxidant approaches are still scarce [145]. A modest number of trials have shown potential beneficial effects of antioxidant therapies on clinical and biochemical parameters in individuals with RA, shedding light on the perspective of using similar therapies for mitigating disease-related damage, in association with conventional disease-modifying antirheumatic drugs [146]. Most studies focusing on RA and antioxidant therapies enrolled small numbers of participants, with different study designs and distinct methodologies. All these variabilities make it difficult to immediately extrapolate their results to RA patients in general. While these studies pointed to the exciting perspective of using antioxidant therapy to prevent/mitigate disease-related damage in RA, they indicate that antioxidant use is not a panacea treatment [147]. Attention should also be paid to the dosage used, either drug or food supply item. The potential usefulness of OS biomarkers for early diagnosis of RA, disease activity assessment, and therapeutic responses, as well as how these potential antioxidant-based treatments will contribute to better disease activity control remains unclear. Thus, the prediction of how RA onset and proper treatment will evolve will ultimately rely on improved basic knowledge and clinical utilization, either for drug discovery or biomarker validity, discrimination specificity and sensitivity, robustness and reproducibility [148].

9.1. Personalized Medicine Approaches

Personalized medicine is defined as therapeutic strategy tailored to specific characteristics of individual patients, owing to the development of biomarkers by the interaction between advances in analysis techniques and enormous genetic knowledge from the human genome. Thus, the classical targeting of drug is possession of molecular structure that binds to a protein, enzymes and receptors. Personalized medicine is trying to be more subtle with patients' differences in terms of the molecular mechanism of disease than just pharmacogenomics. The top down approaches of discovery of genetic alterations risks missing target populations of drugs while the bottom up approaches on proteomic differences may have large overlap in common variants and drug targets, hence a regulatory challenge of drug approvals with highly restricted narrow patient populations [149].

To address paediatric diseases, for which new targets are scarce, modelling effector proteins perturbed in disease helps to identify small molecular scaffolds with novel mechanisms of actions for drug discovery [149]. A simplified explanation of matching disease by signatures with drugs, with a limitation that drug targets must have been already treated in humans with a possible risk of translation into acute side effect in patients is provided. Because of the unmatched resolution of

protein-protein interactions and mechanism, identification of a signalling pathway by genomic, transcriptomic or proteomic approaches could identify drug targets but it is difficult to find their known treatment, hence offering these results to frontline companies for drug development would usually fail [150]. The interaction-motif of perturbed proteins would have potential to find a drug in patients is proposed. Since drug discovery is a search process, with the fewer centralities the better chance of finding potential new targets though the choice of undrugged proteins must ensure completion of depth of coverage. The chance of finding true new drug targets is greater with the less connected drug-proteins and once they are found, chemical probes and ultimately drugs will benefit patients [150].

9.2. Integrative Therapies

Despite pharmacological therapy, many RA patients remain at risk and do not reach the recommended treatment targets with currently available therapies. Notably, only 49% of patients achieved remission in the large observational cohort study of the CORRONA registry. Such suboptimal control of the disease activity has important consequences for patient´s quality of life and significant economic costs. Complementary treatments could potentially improve treatment adherence and better control of the disease activity, mainly across patients that are receiving DMARDs but remain at risk [151]. The search for adjunct therapies for conventional treatments is urgent. Integrative therapies can complement conventional treatments and have been successfully used in chronic inflammatory diseases, including health promotion and decreased comorbidities. Specific and concurrent targeted nutritional/supplementation approaches and multi-therapy intervention aggravating nonpharmacological treatment adherence are critical [152]. The main aim is dignifying a nutritional approach in combination with pharmacological treatment as adjuvant therapy and introducing antioxidant supplementation as a new potential therapeutic target. Treatment adherence should be better controlled to optimize pharmacological therapies and avoid drug resistance. There are reports of unsuccessful attempts using integrative approaches, and it is challenging to ascertain effectiveness [152]. This is in part due to a lack of awareness, interest, and preparation among healthcare providers of these therapies. Consequently, practitioners must better familiarize themselves with these integrative treatments, requesting specific training when needed to include them in their practice. Specific training for physicians and patients on how to implement nutritional recommendations may help introduce these adjunct therapies. Discrepancies in data analyses of effectiveness and reliability also generate suspicion. Specific analysis by each different approach is at least schedule. In addition, underreporting occurs on studies confirming safety and efficacy [151,152].

10. Conclusion

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by systemic inflammation leading to joint destruction and faced by patients with several comorbidities, among them cardiovascular disease (CVD) . Several studies concerning RA-induced inflammation, mechanisms, and biomarkers have been published since Schwabieg, in 1865, first reported on the disease. Biomarkers to support a RA diagnosis, risk of disease onset, remission, treatment response monitoring, and joint damage risk have all been tested. Despite these efforts, the clinical rheumatologist has limited laboratory tools. The search for new biomarkers that are valid is difficult because they should correlate with the disease pathophysiology, use routine laboratory methods, and exhibit adequate sensitivity/specificity. There is a necessity for both novel mechanisms and new biomarkers. The proposed new biomarker candidates are promising but do not fulfill the criteria required for usefulness in clinical laboratories. There is a clinical need for more methods to assess inflammation. The oxidative stress paradigm comprises a wide range of abnormal redox state parameters that can be measured using simple redox assays. The redox state can reflect multiple signaling pathways in different cells and tissues. The revelation of these aberrations can provide a plethora of new biomarker candidates, some of which may ultimately be developed into useful clinical methods. Biomarkers need to be deeper explored in the pathophysiological context of RA, including: a screening biomarker panel for early diagnosis; new

biomarker candidates; new methods painting a broad “redox state picture”; and exploration of the mechanisms behind candidate biomarker changes. The mechanisms underlying the genesis of the oxidative/nitrosative stress dysregulation are still poorly understood. Current secretion and tissue levels of nitrotyrosine are increased in inflammatory arthritis experimental models, though not in RA. Furthermore, data on superoxide and peroxynitrite formation and removal, and persistent dysregulation of ROS maintain signaling over months, should be profusely explored in the context of metabolic syndrome and cardiovascular comorbidities. In depth testing of joint interventions should be pursued as well.

Funding

There is no funding

Declaration of Competing Interest

The authors say they don't have any known personal or financial relationships or financial interests that could have seemed to affect the work in this study.

References

1. Finckh, B. Gilbert, B. Hodkinson, S. C. Bae, et al., "Global epidemiology of rheumatoid arthritis," Reviews Rheumatology, 2022.
2. G. Shi, X. Liao, Z. Lin, W. Liu et al., "Estimation of the global prevalence, incidence, years lived with disability of rheumatoid arthritis in 2019 and forecasted incidence in 2040: results from the Global …," Clinical Rheumatology, 2023.
3. F. Radu and S. G. Bungau, "Management of rheumatoid arthritis: an overview," Cells, 2021.
4. Hussein, Abdulhakeem Dahham, et al. "Emerging biomarkers in cancer detection and prognosis: A comprehensive review." International Journal of Immunology 7.1 (2025): 01-12.
5. M. Jahid, K. U. Khan, and R. S. Ahmed, "Overview of rheumatoid arthritis and scientific understanding of the disease," *Journal of Rheumatology*, 2023.
6. Conforti, I. Di Cola, V. Pavlych, P. Ruscitti, "Beyond the joints, the extra-articular manifestations in rheumatoid arthritis," Autoimmunity, vol. 2021, Elsevier
7. D. Bhowmik, V. Kola, S. R. Chamakuri, "Rheumatoid Arthritis: Introduction," *Journal of Arthritic Disorders*, 2022.
8. L. José Sá da Fonseca, V. Nunes-Souza, M. Oliveira Fonseca Goulart, and L. Antas Rabelo, "Oxidative Stress in Rheumatoid Arthritis: What the Future Might Hold regarding Novel Biomarkers and Add-On Therapies," 2019.
9. G. Kaur, A. Sharma, and A. Bhatnagar, "Role of oxidative stress in pathophysiology of rheumatoid arthritis: insights into NRF2-KEAP1 signalling," Autoimmunity, 2021.
10. Y. Zamudio-Cuevas and K. Martínez-Flores, "Rheumatoid arthritis and oxidative stress," Cellular and Molecular Biology, vol. 68, no. 12, pp. 1-10, 2022.
11. H. B. Ferreira, T. Melo, A. Paiva, and M. R. Domingues, "Insights in the role of lipids, oxidative stress and inflammation in rheumatoid arthritis unveiled by new trends in lipidomic investigations," Antioxidants, 2021.
12. T. Behl, T. Upadhyay, S. Singh, S. Chigurupati, "Polyphenols targeting MAPK mediated oxidative stress and inflammation in rheumatoid arthritis," Molecules, vol. 26, no. 5, 2021.
13. Abdulhussien, Adeeb Abdulally, Zainab M. Farhan, and Osama A. Mohsein. "The Clinico-Pathological Role of Horizontal Gene Transfer in the Ability of Pseudomonas Aeruginosa to Cause Disease in Lung Tissue." European Journal of Medical and Health Research 3.1 (2025): 27-34.

14. N. Sambiagio, A. Berthet, P. Wild, J. J. Sauvain, et al., "Associations between urinary biomarkers of oxidative stress and biomarkers of tobacco smoke exposure in smokers," *Science of the Total Environment*, vol. 2022, Elsevier.
15. G. Viglianisi, G. M. Tartaglia, S. Santonocito, et al., "The emerging role of salivary oxidative stress biomarkers as prognostic markers of periodontitis: new insights for a personalized approach in dentistry," *Journal of Personalized Medicine*, vol. 2023.
16. F. Meyer, A. Bitsch, H. J. Forman, A. Fragoulis, "The effects of radiofrequency electromagnetic field exposure on biomarkers of oxidative stress in vivo and in vitro: A systematic review of experimental studies," Environment, vol. 2024, Elsevier.
17. Hussein, Abdulhakeem Dahham, et al. "Emerging biomarkers in cancer detection and prognosis: A comprehensive review." International Journal of Immunology 7.1 (2025): 01-12.
18. K. Almutairi, J. Nossent, D. Preen, H. Keen, et al., "The global prevalence of rheumatoid arthritis: a meta-analysis based on a systematic review," *Rheumatology*, vol. 2021, Springer.
19. K. B. Almutairi, J. C. Nossent, D. B. Preen, H. I. Keen, et al., "The prevalence of rheumatoid arthritis: a systematic review of population-based studies," *Journal of Rheumatology*, vol. 2021.
20. Abdulmuttaleb, Nabaa Azhar, Mohammed Qasim Mohammed, and Osama Akram Mohsein. "The impact of adipocytokines on thyroid function and obesity: A narrative review." development 8: 9.
21. D. Jang, A. H. Lee, H. Y. Shin, H. R. Song, and J. H. Park, "The role of tumor necrosis factor alpha (TNF-α) in autoimmune disease and current TNF-α inhibitors in therapeutics," *International Journal of …*, 2021.
22. S. Uzzan and A. N. Azab, "Anti-TNF-α Compounds as a Treatment for Depression," Molecules, 2021.
23. IR Mihai, AM Burlui, II Rezus, C Mihai, LA Macovei, "Inflammatory bowel disease as a paradoxical reaction to anti-TNF-α treatment—A review," Life, vol. 2023.
24. Faisal, A. N., and O. A. Mohsein. "Comprehensive Study on Gynecomastia: Clinical Features, Pathophysiology, Morphologic, and Microscopic Features." Central Asian Journal of Medical and Natural Science 6.2 (2025): 775-789.
25. R. Răzvan Mititelu, R. Pădureanu, M. Băcănoiu, V. Pădureanu et al., "Inflammatory and Oxidative Stress Markers—Mirror Tools in Rheumatoid Arthritis," 2020.
26. H. Ahsan, M. Y. Hasan, and R. Ahmad, "Reactive oxygen species (ROS) in the pathophysiology of rheumatoid arthritis (RA)," React. Oxyg. Species, 2022.
27. P. Chaudhary, P. Janmeda, A. O. Docea, et al., "Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases," *Frontiers in …*, 2023.
28. L. G. González-Arostegui, J. J. Cerón, G. Gök, et al., "… for measurement of oxidant compounds in saliva of pigs: Thiobarbituric acid reactive substances (TBARS), carbonyl, and reactive oxygen species (ROS)," Research in Veterinary Science, vol. 2023, Elsevier.
29. S. Demirci-Çekiç, G. Özkan, A. N. Avan, S. Uzunboy, "Biomarkers of oxidative stress and antioxidant defense," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 2022, Elsevier.
30. F. Aliakbarpour, S. Mahjoub, J. Masrour-Roudsari, and others, "Evaluation of salivary thiobarbituric acid reactive substances, total protein, and pH in children with various degrees

of early childhood caries: A case–control study," *European Archives of …*, vol. 2021, pp. 1-8, Springer.

31. Altomare, G. Baron, E. Gianazza, C. Banfi, M. Carini, "Lipid peroxidation derived reactive carbonyl species in free and conjugated forms as an index of lipid peroxidation: limits and perspectives," Redox Biology, vol. 38, pp. 101743, 2021.
32. J. Slopovsky, J. Kucharska, J. Obertova, M. Mego, and others, "Plasma thiobarbituric acid reactive substances predicts survival in chemotherapy naïve patients with metastatic urothelial carcinoma," *Translational Oncology*, vol. 2021, Elsevier.
33. NMT Roodenrijs and MC van der Goes, "Difficult-to-treat rheumatoid arthritis: contributing factors and burden of disease," 2021.
34. Y. Xu and Q. Wu, "Prevalence trend and disparities in rheumatoid arthritis among US adults, 2005–2018," Journal of clinical medicine, 2021.
35. Ketfi, A. Boutigny, N. Mohamedi, S. Bouajil, B. Magnan, "Risk of venous thromboembolism in rheumatoid arthritis," *Joint Bone Spine*, vol. 88, no. 3, pp. 105-110, 2021.
36. F. Yousefi, B. Sponseller, M. S. Muyyarikkandy, et al., "Clostridioides difficile infection induces a pro-inflammatory and pro-steatotic metabolic state in liver," npj Gut and Liver, 2025.
37. N. Rabas, R. M. M. Ferreira, S. Di Blasio, et al., "Cancer-induced systemic pre-conditioning of distant organs: building a niche for metastatic cells," *Nature Reviews Cancer*, 2024.
38. N. Van An, T. T. Minh, P. D. T. Le Quang Hoa, and H. T. Ali Batan, "Investigating the Relation Between Hepatic Steatosis and Systemic Inflammation in Metabolic Syndrome-Associated Liver Disease," researchgate.net, .
39. R. H. Turner, P. I. Jenkinson, M. Huttman, et al., "Inflammation, oxidative stress and gut microbiome perturbation: A narrative review of mechanisms and treatment of the alcohol hangover," Alcohol: Clinical and Experimental Research, vol. 2024, Wiley Online Library.
40. Abbas, Abbas Mohsin, Iman H. Shewael, and Osama A. Mohsein. "Lipoprotein Ratios as Biomarkers for Assessing Chronic Atherosclerosis Progression." Academia Open 10.1 (2025): 10-21070.
41. R. J. Black, M. Cross, L. M. Haile, G. T. Culbreth, et al., "Global, regional, and national burden of rheumatoid arthritis, 1990–2020, and projections to 2050: a systematic analysis of the Global Burden of Disease Study 2021," *Lancet Rheumatology*, vol. 5, no. 1, pp. e1-e12, 2023.
42. H. Almoallim, J. Al Saleh, H. Badsha, and H. M. Ahmed, "A review of the prevalence and unmet needs in the management of rheumatoid arthritis in Africa and the Middle East," *Rheumatology and …*, vol. 2021, Springer.
43. I. Venetsanopoulou and Y. Alamanos, "Epidemiology of rheumatoid arthritis: genetic and environmental influences," Expert Review of …, vol. 2022, Taylor & Francis.
44. Abbas, Zainab Khamis, et al. "Immunological biomarkers and their role in the diagnosis and prognosis of leishmaniasis: A case–control study." Tropical Parasitology 15.1 (2025): 33-41.
45. Rydell, K. Forslind, J.Å. Nilsson, M. Karlsson, et al., "Predictors of radiographic erosion and joint space narrowing progression in patients with early rheumatoid arthritis: a cohort study," *Arthritis Research & Therapy*, vol. 23, no. 1, 2021.
46. Z. Ma, H. Zou, M. C. Yelovich, S. Totterman, and K. Beattie, "Prevalence and trajectory of erosions, synovitis, and bone marrow edema in feet of patients with early rheumatoid arthritis," *Clinical Rheumatology*, vol. 2021, Springer.

47. R. Sakthiswary, R. Uma Veshaaliini, K. Y. Chin, "Pathomechanisms of bone loss in rheumatoid arthritis," Frontiers in…, 2022.

48. Almohaisen, Farooq LJ, et al. "Cytokines' Impact on Postpartum Hair Loss After First C-Section in Women 28-38: Dampak Sitokin terhadap Kerontokan Rambut Pasca Persalinan Setelah Operasi Caesar Pertama pada Wanita 28-38." Indonesian Journal on Health Science and Medicine 2.2 (2025): 10-21070.

49. Q. A. Dumoulin, A. H. M. van der Helm–van Mil, et al., "Do MRI-detected erosions in the RA-risk phase of arthralgia reflect current or imminent radiographic erosions? A large longitudinal imaging study," 2025.

50. Majorczyk, M. Mazurek-Mochol, A. Pawlik, "Clinical factors and the outcome of treatment with methotrexate in rheumatoid arthritis: role of rheumatoid factor, erosive disease and high level of erythrocyte …," Journal of Clinical …, 2022.

51. N. M. Thabet, M. K. Abdel-Rafei, and M. M. Amin, "Fractionated whole body γ-irradiation aggravates arthritic severity via boosting NLRP3 and RANKL expression in adjuvant-induced arthritis model: the mitigative …," Inflammopharmacology, 2023.

52. U. Iqbal, A. Malik, L. Ibrahim, N. T. Sial, "Natural and synthetic potential drug leads for rheumatoid arthritis probing innovative target: mitochondrial dysfunction and NLRP3 inflammasome activation," Naunyn-Schmiedeberg's Archives of Pharmacology, 2025.

53. Dziedzic, K. Maciak, E. D. Miller, M. Starosta, "Targeting vascular impairment, neuroinflammation, and oxidative stress dynamics with whole-body cryotherapy in multiple sclerosis treatment," *International Journal of …*, 2024.

54. Q. Wang, W. Guo, L. Niu, Y. Zhou, and Z. Wang, "Ameliorate Vitiligo by Simultaneously Potentiating Treg Cells-Mediated Immunosuppression and Suppressing Oxidative Stress-Induced Melanocyte Damage," Advanced ..., vol. 2024, Wiley Online Library.

55. Taha, Ruqayah Qubtan, et al. "Bacterial aetiologies of otitis media and their antimicrobial susceptibility in ear swab culture." IJBS 6.1 (2024): 94-99.

56. Mengozzi, N. R. Pugliese, M. Chiriaco, et al., "Microvascular ageing links metabolic disease to age-related disorders: the role of oxidative stress and inflammation in promoting microvascular dysfunction," Journal of …, vol. XX, no. YY, pp. ZZ-ZZ, 2021.

57. L. Xie, K. Wen, Q. Li, C. C. Huang, J. L. Zhao, "… fat diet-induced nonalcoholic fatty liver disease through activating NAD+/sirtuins signaling pathways-mediated inhibition of lipid accumulation and oxidative stress in …," International…, 2021.

58. N. Kondo, T. Kanai, and M. Okada, "Rheumatoid arthritis and reactive oxygen species: a review," Current Issues in Molecular Biology, 2023.

59. C. Toma, S. De Cillà, A. Palumbo, D. P. Garhwal, "Oxidative and nitrosative stress in age-related macular degeneration: A review of their role in different stages of disease," Antioxidants, vol. 10, no. 11, 2021.

60. L. Piacenza, A. Zeida, M. Trujillo, "The superoxide radical switch in the biology of nitric oxide and peroxynitrite," *Physiological Reviews*, vol. 2022.

61. V. Shukla and A. Purohit, "Berberine-Loaded Nanoparticles Combat Oxidative and Nitrosative Stress to Reprogram Macrophages in Rheumatoid Arthritis," BioNanoScience, 2025.

62. Q. Meng and C. H. Su, "The impact of physical exercise on oxidative and nitrosative stress: balancing the benefits and risks," Antioxidants, 2024.

63. S. Dutta, P. Sengupta, and E. Izuka, "Oxidative and nitrosative stress and female reproduction: Roles of oxidants and antioxidants," *Journal of Integrated …*, 2024.

64. Abd-Alwahab, Hadeel S., Zainab Mohammed Farhan, and Nesreen Ahmed. "Estimation of thyroid hormone and adipocytokine levels in men with obesity and type 2 diabetes in Thi-Qar Governorate."

65. K. L. Tsai, C. C. Chang, Y. S. Chang, Y. Y. Lu, I. J. Tsai, "… of autoantibodies against novel differential 4-hydroxy-2-nonenal-modified peptide adducts in serum is associated with rheumatoid arthritis in Taiwanese women," BMC Medical Informatics, vol. 21, 2021.

66. X. Zhang, L. Hou, Z. Guo, G. Wang, J. Xu, and Z. Zheng, "Lipid peroxidation in osteoarthritis: focusing on 4-hydroxynonenal, malondialdehyde, and ferroptosis," Cell Death, vol. 2023.

67. L. Wang, B. Huang, Y. Zeng, J. Yang, Z. Li, "N-Acetylcysteine overcomes epalrestat-mediated increase of toxic 4-hydroxy-2-nonenal and potentiates the anti-arthritic effect of epalrestat in AIA model," *International Journal*, 2023.

68. T. Geib, C. Iacob, R. Jribi, J. Fernandes, M. Benderdour, "Identification of 4-hydroxynonenal-modified proteins in human osteoarthritic chondrocytes," *Journal of …*, vol. XX, no. YY, pp. ZZ-ZZ, 2021.

69. Skrzydlewska, K. Žarković, S. B. Šunjić, M. Jaganjac, "Overview of the Lipid Peroxidation Measurements in Patients by the Enzyme-Linked Immunosorbent Assay Specific for the 4-Hydroxynonenal-Protein Adducts (4-HNE …," 2024.

70. K. Mure, S. Tomono, M. Mure, M. Horinaka, "The combination of cigarette smoking and alcohol consumption synergistically increases reactive carbonyl species in human male plasma," *International Journal of …*, 2021. mdpi.com

71. Bilska-Wilkosz, M. Iciek, and M. Górny, "Chemistry and Biochemistry Aspects of the 4-Hydroxy-2, 3-trans-nonenal. Biomolecules 2022, 12, 145," 2022. ppm.

72. N. Abd Alameer and H. Alammar, "Some trace elements and oxidative stress status in patients with chronic rheumatoid arthritis," *Journal of Biomedicine and Biochemistry*, vol. 2023.

73. Abdulmuttaleb, Nabaa Azhar, Mohammed Qasim Mohammed, and Osama Akram Mohsein. "Exploring the Connection Between Inflammatory Cytokines, Hypertension, and Diabetes in Angina Patients." Cytokines 14.15 (2024): 16.

74. L. A. T. Cannarella, N. L. Mari, C. C. Alcântara, T. M. V. Iryioda, et al., "Mixture of probiotics reduces inflammatory biomarkers and improves the oxidative/nitrosative profile in people with rheumatoid arthritis," *Nutrition*, vol. 2021, Elsevier.

75. E. Dorochow, M. Köhm, L. Hahnefeld, "Metabolic profiling in rheumatoid arthritis, psoriatic arthritis, and psoriasis: elucidating pathogenesis, improving diagnosis, and monitoring disease activity," Journal of Personalized Medicine, vol. 12, no. 5, 2022.

76. B. Miglioranza Scavuzzi and J. Holoshitz, "Endoplasmic reticulum stress, oxidative stress, and rheumatic diseases," Antioxidants, 2022.

77. X. Wang, D. Fan, X. Cao, Q. Ye, Q. Wang, and M. Zhang, "The role of reactive oxygen species in the rheumatoid arthritis-associated synovial microenvironment," Antioxidants, 2022.

78. K. Mukhopadhyay, S. De, S. Kundu, "Evaluation of levels of oxidative stress as a potential biomarker in patients with rheumatoid arthritis," Journal of Family, vol. 2021.

79. R. La, L. Zhou, Y. Yin, L. Lu, L. Li, D. Jiang, L. Huang, "Association between oxidative balance score and rheumatoid arthritis in female: a cross-sectional study," BMC Women's Health, vol. 24, no. 1, 2024.

80. D. Yaman, D. G. Bulut, G. Ustaoğlu, "Dental and temporomandibular joint alterations in rheumatoid arthritis patients and their association with salivary oxidative stress," Turkish Journal of ..., vol. 2021.

81. M. Luque-Tévar, C. Perez-Sanchez, et al., "Integrative clinical, molecular, and computational analysis identify novel biomarkers and differential profiles of anti-TNF response in rheumatoid arthritis," Frontiers in…, 2021.

82. B. Kolarz, D. Podgorska, and R. Podgorski, "Insights of rheumatoid arthritis biomarkers," Biomarkers, 2021.

83. L. Mueller, Z. Payandeh, N. Mohammadkhani, et al., "Recent advances in understanding the pathogenesis of rheumatoid arthritis: new treatment strategies," *Cells*, vol. 10, no. 10, 2021.

84. Z. Zhao, Z. Hua, X. Luo, Y. Li, L. Yu, M. Li, and C. Lu, "Application and pharmacological mechanism of methotrexate in rheumatoid arthritis," Biomedicine & Pharmacotherapy, vol. 2022, Elsevier.

85. J. Huang, X. Fu, X. Chen, Z. Li, Y. Huang, "Promising therapeutic targets for treatment of rheumatoid arthritis," Frontiers in…, vol. 2021.

86. S. A. Clayton and L. MacDonald, "Mitochondria as key players in the pathogenesis and treatment of rheumatoid arthritis," Frontiers in …, 2021.

87. M. T. Islam, C. Sarkar, R. Hossain, M. S. Bhuia, and others, "Therapeutic strategies for rheumatic diseases and disorders: targeting redox imbalance and oxidative stress," Biomedicine & Pharmacotherapy, vol. 2023, Elsevier.

88. Nawaz, A. Ali, T. Rehman, and A. Aslam, "Chronological effects of non-steroidal anti-inflammatory drug therapy on oxidative stress and antioxidant status in patients with rheumatoid arthritis," Clinical Rheumatology, 2021.

89. N. K. Bhol, M. M. Bhanjadeo, A. K. Singh, and U. C. Dash, "The interplay between cytokines, inflammation, and antioxidants: Mechanistic insights and therapeutic potentials of various antioxidants and anti-cytokine …," Biomedicine & …, 2024.

90. K. Dziąbowska-Grabias, M. Sztanke, P. Zając, "Antioxidant therapy in inflammatory bowel diseases," Antioxidants, 2021.

91. N. Qamar, P. John, and A. Bhatti, "Emerging role of selenium in treatment of rheumatoid arthritis: An insight on its antioxidant properties," *Journal of Trace Elements in Medicine and Biology*, vol. XX, pp. YY-ZZ, 2021.

92. Dahchour, "Anxiolytic and antidepressive potentials of rosmarinic acid: A review with a focus on antioxidant and anti-inflammatory effects," Pharmacological Research, 2022.

93. P. Katsimbri, E. Korakas, A. Kountouri, I. Ikonomidis, "The effect of antioxidant and anti-inflammatory capacity of diet on psoriasis and psoriatic arthritis phenotype: nutrition as therapeutic tool?" Antioxidants, 2021.

94. Kour, S. A. Haq, B. K. Bajaj, P. N. Gupta, "Phytochemical add-on therapy to DMARDs therapy in rheumatoid arthritis: In vitro and in vivo bases, clinical evidence and future trends," *Pharmacological Research*, vol. 2021, Elsevier.

95. P. Ballester, B. Cerdá, R. Arcusa, J. Marhuenda, "Effect of ginger on inflammatory diseases," Molecules, 2022.

96. OT Laniak, T. Winans, A. Patel, J. Park, "Redox pathogenesis in rheumatic diseases," Open Rheumatology, vol. 2024, Wiley Online Library.

97. C. Ma, J. Wang, F. Hong, and S. Yang, "Mitochondrial dysfunction in rheumatoid arthritis," Biomolecules, 2022.

98. Kaushal, A. Kamboj, K. Anupam, A. Tandon, and others, "Interplay of redox imbalance with matrix gelatinases in neutrophils and their association with disease severity in rheumatoid arthritis patients," *Clinical Immunology*, vol. 2022, Elsevier.

99. C. Parolini, "The role of marine n-3 polyunsaturated fatty acids in inflammatory-based disease: the case of rheumatoid arthritis," Marine Drugs, 2023.

100. Y. Liu, X. Ren, C. Fan, W. Wu et al., "Health benefits, food applications, and sustainability of microalgae-derived N-3 PUFA," Foods, 2022.

101. E. A. Cutolo, R. Caferri, R. Campitiello, and M. Cutolo, "The clinical promise of microalgae in rheumatoid arthritis: From natural compounds to recombinant therapeutics," Marine Drugs, 2023.

102. L. Gambari, A. Cellamare, F. Grassi, B. Grigolo, "... of obesity-Associated Osteoarthritis: towards nutraceutical-oriented preventive and complementary therapeutic strategies based on n-3 polyunsaturated fatty acids," *International Journal of …*, 2023.

103. C. Finocchiaro, M. Fadda, V. D'Onofrio, M. Ippolito, C. Pira, "Oxidative stress and cancer: Role of n-3 PUFAs," *Cancer*, vol. 2021, Elsevier.

104. S. Fares, M. Omar, A. Laurence, and S. Abu-Baker, "Over-the-counter anti-inflammatory supplements for adjunctive rheumatoid arthritis therapy: A comprehensive narrative review," Aging and…, 2024.

105. Z. Ara, S. Waliullah, M. L. Al-Otaibi, and A. Alam, "A Systematic Review of Beneficial Outcomes of Omega-3 Fatty Acid in Human Musculoskeletal Diseases," endocrinology, 2022.

106. P. Prasad, S. Verma, Surbhi, and N. K. Ganguly, "Rheumatoid arthritis: advances in treatment strategies," Molecular and Cellular, vol. 2023, Springer.

107. Y. Cai, J. Zhang, J. Liang, M. Xiao, G. Zhang, "The burden of rheumatoid arthritis: findings from the 2019 global burden of diseases study and forecasts for 2030 by Bayesian age-period-cohort analysis," *Journal of Clinical*, vol. 2023.

108. S. Alivernini, G. S. Firestein, and I. B. McInnes, "The pathogenesis of rheumatoid arthritis," Immunity, 2022.

109. L. Zeng, G. Yu, K. Yang, J. Li, W. Hao, "The efficacy of antioxidative stress therapy on oxidative stress levels in rheumatoid arthritis: a systematic review and meta-analysis of randomized controlled trials," *Oxidative Medicine and Cellular Longevity*, vol. 2021, 2021.

110. J. H. Bray, H. Foster-Davies, A. Salem, et al., "…-like peptide-1 receptor agonists improve biomarkers of inflammation and oxidative stress: a systematic review and meta-analysis of randomised controlled trials," Diabetes, Obesity, vol. 2021, Wiley Online Library.

111. B. Pourrajab, S. Fatahi, M. H. Sohouli, "… /synbiotic supplementation compared to placebo on biomarkers of oxidative stress in adults: a systematic review and meta-analysis of randomized controlled trials," Critical Reviews in…, vol. XX, no. YY, pp. ZZ-ZZ, 2021.

112. M. A. Martu, P. Surlin, L. Lazar, G. A. Maftei, and I. Luchian, "Oxidative stress before and after using laser and photoactivation therapy as adjuvant of non-surgical periodontal treatment in patients with rheumatoid arthritis," Antioxidants, 2021.

113. Hamad, Anas Abdullah, Hamssa Majid Mustafa, and Osama A. Mohsein. "Detection of the levels of immune cytokines (IL4, IL5, TNF-α) in school-age and preschoolers with an Ascaris lumbricoides infection." Journal of Parasitic Diseases 48.4 (2024): 782-787.

114. Tejchman, K. Kotfis, and J. Sieńko, "Biomarkers and mechanisms of oxidative stress—last 20 years of research with an emphasis on kidney damage and renal transplantation," *International Journal of Molecular Sciences*, vol. 2021.

115. V. P. Reddy, "Oxidative stress in health and disease," Biomedicines, 2023. mdpi.com

116. E. Cecerska-Heryć, A. Polikowska, N. Serwin, "Importance of oxidative stress in the pathogenesis, diagnosis, and monitoring of patients with neuropsychiatric disorders, a review," Neurochemistry, vol. 2022, Elsevier.

117. G. I, O. A.M, S. I.V, G. V. R et al., "Biomarkers Predicting a Need for Intensive Treatment in Patients with Early Arthritis," 2015.

118. S. C Shapiro, "Biomarkers in Rheumatoid Arthritis," 2021.

119. R. Méndez Hernández and F. Ramasco Rueda, "Biomarkers as prognostic predictors and therapeutic guide in critically ill patients: clinical evidence," *Journal of Personalized Medicine*, vol. 2023.

120. Lateef, Diyar, Nesreen Nasser, and Osama Mohsein. "The relationships between Aplein, Vaspin and thyroid hormone levels in obese diabetic and non-diabetic women." Journal of Experimental and Clinical Medicine 41.2 (2024): 239-245.

121. V. Castiglione, A. Aimo, G. Vergaro, L. Saccaro, et al., "Biomarkers for the diagnosis and management of heart failure," *Heart Failure*, vol. 2022, Springer.

122. Faisal, Ahmed Nassir, Abbas Mohsin Abbas, and Osama A. Mohsein. "Histological and cytokine-based biomarkers in the early diagnosis of cancer." Indonesian Journal on Health Science and Medicine 2.2 (2025): 10-21070.

123. P. Malik, U. Patel, D. Mehta, N. Patel, R. Kelkar, and others, "Biomarkers and outcomes of COVID-19 hospitalisations: systematic review and meta-analysis," BMJ Evidence-Based Medicine, vol. 26, no. 3, pp. 1-10, 2021.

124. E. Tarighati, H. Keivan, and H. Mahani, "A review of prognostic and predictive biomarkers in breast cancer," Clinical and experimental medicine, 2023.

125. Mustafa, Hamssa M., Anas Abdullah Hamad, and Osama A. Mohsein. "Investigating specific calprotectin and immunological markers associated with intestinal infections caused by Entamoeba histolytica." Iranian Journal of Veterinary Medicine 18.4 (2024): 535-544.

126. S. Momtazmanesh, A. Nowroozi, and N. Rezaei, "Artificial intelligence in rheumatoid arthritis: current status and future perspectives: a state-of-the-art review," Rheumatology and therapy, 2022.

127. Rehman, P. John, and A. Bhatti, "Biogenic selenium nanoparticles: Potential solution to oxidative stress mediated inflammation in rheumatoid arthritis and associated complications," Nanomaterials, 2021.

128. P. Wójcik, A. Gęgotek, N. Žarković, et al., "Oxidative stress and lipid mediators modulate immune cell functions in autoimmune diseases," *International Journal of …*, 2021.

129. Y. Tang, X. Zhou, T. Cao, E. Chen, Y. Li, and W. Lei, "Endoplasmic reticulum stress and oxidative stress in inflammatory diseases," *DNA and Cell Biology*, vol. 41, no. 6, pp. 1-12, 2022.

130. V. Blagov, V. I. Summerhill, V. N. Sukhorukov, "Potential use of antioxidants for the treatment of chronic inflammatory diseases," Frontiers in ..., 2024.

131. S. Xie, P. Liao, S. Mi, L. Song et al., "Emerging patterns in nanoparticle-based therapeutic approaches for rheumatoid arthritis: A comprehensive bibliometric and visual analysis spanning two decades," Open Life Sciences, 2025.

132. T. Adamantidi, M. S. Pisioti, S. Pitsouni, and others, "The Inflammatory Link of Rheumatoid Arthritis and Thrombosis: Pathogenic Molecular Circuits and Treatment Approaches," Current Issues in ..., 2025.

133. E. Franco-Trepat, A. Alonso-Pérez, M. Guillán-Fresco, "β boswellic acid blocks articular innate immune responses: an in silico and in vitro approach to traditional medicine," Antioxidants, 2023.

134. Adnan, Azher, Zainab Mohammed Farhan, and Osama A. Mohsein. "Understanding the link between diabetes mellitus and fatty liver disease: A narrative review." Int J Chem Stud 12.4 (2024): 40-48.

135. Li, Y. He, J. Zhao, H. Yin, X. Feng, X. Fan, W. Wu, "Mesenchymal Stromal Cell-Based Therapy: A Promising Approach for Autoimmune Diseases," Clinical Reviews in ..., vol. 2025, Springer.

136. R. Paparella, F. Panvino, F. Tarani, B. D'Agostino, "An Overview of Oxidative Stress in Sex Chromosome Aneuploidies in Pediatric Populations," Antioxidants, 2025.

137. R. C. Wang and Z. Wang, "Precision medicine: disease subtyping and tailored treatment," Cancers, 2023.

138. O. Navaei, "Personalized Medicine: Tailoring Treatment Plans Based on Genetic Profile," Eurasian Journal of Chemical, Medicinal and …, 2025.

139. Qassim Mohammad, Sarmad, Abdullah Khazal Mohsen, and Idriss Saleh Jalil. "Assessing the Prevalence of Bacterial Vaginosis among Infertile Women in Thi-Qar Province, Iraq." Infection Epidemiology and Microbiology 10.4 (2024): 287-296.

140. Farrokhi, F. Taheri, P. J. Khouzani, E. Rahmani, "Role of precision medicine and personalized medicine in the treatment of diseases," Kindle, 2023.

141. C. Delpierre and T. Lefèvre, "… and personalized medicine: What their current definition says and silences about the model of health they promote. Implication for the development of personalized …," Frontiers in Sociology, 2023.

142. FNU Sugandh, M. Chandio, FNU Raveena, and L. Kumar, "Advances in the management of diabetes mellitus: a focus on personalized medicine," Cureus, 2023.

143. Hilo, Zainab Qassim Mohammed, Aswan Mahmood, and Osama A. Mohsein. "Genomic Insights into Staphylococcus aureus: Mechanisms of Antibiotic Resistance and Virulence Gene Expression." European Journal of Ecology, Biology and Agriculture 1.5 (2024): 29-48.

144. S. M. Sisodiya, "Precision medicine and therapies of the future," Epilepsia, 2021.

145. Holman, S. H. Wild, K. Khunti, P. Knighton, et al., "Incidence and characteristics of remission of type 2 diabetes in England: a cohort study using the National Diabetes Audit," *Diabetes*, vol. 2022.

146. M. J. Samec, J. Rakholiya, H. Langenfeld, et al., "Relapse risk and safety of long-term tocilizumab use among patients with giant cell arteritis: a single-enterprise cohort study," The Journal of Rheumatology, vol. 2023.

147. T. Straatmijer, V. B. C. Biemans, F. Hoentjen, et al., "Ustekinuma B for Crohn's disease: two-year results of the initiative on Crohn and colitis (ICC) Registry, a nationwide prospective observational cohort study," *Journal of Crohn's and Colitis*, vol. 2021.

148. J. Cho, L. Shen, M. Huq, et al., "Remission on flares following tapering of corticosteroids and immunosuppressive therapy in patients with systemic lupus erythematous: a multinational cohort study," The Lancet, 2023.

149. J. Cao, X. Zheng, W. Wei, X. Chu, et al., "Three-month outcomes of recovered COVID-19 patients: prospective observational study," *Therapeutic Advances in Infectious Disease*, vol. 8, 2021.

150. Ismail, Mona Adel, et al. "Exploring the Genetic Variability of Escherichia coli Pathotypes in Urinary Tract Infections: Implications for Diagnostics and Treatment." Cent Asian J Med Nat Sci 6.1 (2024): 179-191.

151. J. Nanni, I. Azzali, C. Papayannidis, A. Mulè, E. Audisio, et al., "Upfront intensive treatment analysis of the Italian Cohort Study on FLT3-mutated AML patients (FLAM): The impact of a FLT3 inhibitor addition to standard …," Cancer, 2025.

152. Chennamadhavuni, L. Abushahin, N. Jin, et al., "Risk factors and biomarkers for immune-related adverse events: a practical guide to identifying high-risk patients and rechallenging immune checkpoint inhibitors," *Frontiers in …*, 2022.