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Narrative Review

A Review of Etiological Biomarkers for Fibromyalgia and Their Therapeutic Implications

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Background: Fibromyalgia is a complex condition that has long puzzled the medical community. Hypotheses to explain the chronic widespread pain associated with the disease have evolved significantly over the years. However, research efforts to identify disease-specific biomarkers and develop effective treatments have been largely unsuccessful.

Objectives: The goals of this study were to review potential etiological biomarkers for fibromyalgia, focusing on micro-inflammation and metabolic syndrome, and to discuss the clinical implications of the review findings.

Study Design: A narrative review.

Methods: Relevant literature was obtained via Medline/PubMed, using the following search terms: fibromyalgia[ti] ("metabolic syndrome" OR "metabolic disease" OR biomarker*[ti] OR micro-inflammation OR sub-inflammation OR "low-level inflammation" OR "low-grade inflammation"). Results were filtered for the English language and screened for inclusion in the review.

Results: Articles included in the review covered the topics of pain, immune response/inflammation, micro-inflammation, metabolic syndrome, gut dysbiosis, oxidative stress, and stress response. Various molecules have been proposed as pain biomarkers for fibromyalgia, including neurotransmitters, neuropeptides, growth factors, and cytokines with possible etiological relevance. Recent genome-wide expression profiling suggests connections among low-level inflammation, termed "micro-inflammation," and the upregulation of genes involved in antibacterial and innate immune system response as well as those involved in clinical features, including high body mass index (BMI) and comorbid depression, in a subgroup of fibromyalgia patients. A set of 5 differentially expressed inflammatory genes have been identified as potential biomarkers of a micro-inflammation fibromyalgia subtype. Proposed triggers of micro-inflammation include bacterial disease and gut dysbiosis. Metabolic syndrome may be causative or consequential, while comorbid depression may be associated with dysbiosis and/or micro-inflammation through the gut-immune-brain axis. A potential new treatment approach based on this information has been proposed.

Limitations: External validation of potential etiological biomarkers is needed. Further investigations to ascertain the involvement of metabolic syndrome and gut dysbiosis and support the proposed treatment paradigm are warranted.

Conclusion: Fibromyalgia is likely the result of multiple causative factors, genetic and environmental. To date, no clear, reliable etiological biomarker for fibromyalgia has been identified. The considerable variability among patients suggests the presence of multiple disease subtypes with different pathophysiological mechanisms. Effective treatment therefore requires a multimodal, multidisciplinary approach that targets each individual patient's pathophysiological features. The proposed treatment paradigm attempts to address multiple factors that have been implicated more recently in the development and maintenance of fibromyalgia, such as micro-inflammation, metabolic syndrome, and gut dysbiosis.

Key words: Fibromyalgia, biomarkers, inflammation, innate immune response, metabolic syndrome, dysbiosis, etiology

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Fibromyalgia is a complex condition characterized by widespread musculoskeletal-type pain that is often associated with fatigue, cognitive impairment, and mood disturbances. The condition has been a conundrum to both physicians and patients since 1913, when the first conference on fibrositis was held by the Royal Society of Medicine (1). Over the years, hypotheses to explain the chronic widespread pain caused by fibromyalgia have progressed sequentially. The idea that fibrotic nodules were the root of the pain was supplanted by the notion of fibromyalgia as a psychiatric condition. Later came the explanation that fibromyalgia was a form of sympathetically maintained myofascial pain, and eventually there emerged the hypothesis that it was a condition of central sensitization (2).

Fibromyalgia remains poorly understood. A myriad of physiological changes in fibromyalgia patients have been documented, but very few have been shown to be causative or explanatory. Given our limited understanding of the etiology involved, accurate diagnosis remains a challenge, and effective treatment options have been absent. Antidepressants can improve comorbid depression but have variable effects on pain and other symptoms (3,5).

Significant effort has been dedicated to identifying biomarkers for fibromyalgia. Because fibromyalgia syndrome is clinically defined and encompasses a wide spectrum of presentations, distinguishing between individuals who meet the clinical criteria and those who exhibit similar symptoms but do not fit the formal diagnosis is difficult, and misdiagnosis is common. There is a need for validated biomarkers that would ideally not only have diagnostic or prognostic value but would also be linked to a fundamental etiological driver of the condition that may potentially be amenable to therapeutic intervention. Recent breakthroughs in this field have come from analyses of gene expression, metabolites, and gut microbiomes. Growing support suggests the widespread involvement of metabolic pathways, micro-inflammation, gut dysbiosis, immune activation pathways, and activation of the nociceptive system by inflammatory mediators in the development and maintenance of the condition (6-9). Several distinct molecular and pathophysiological factors that may represent potential etiological biomarkers have been recently identified (7).

Objective

The purpose of this article was to explore potential etiological biomarkers for fibromyalgia, particularly those associated with micro-inflammation and metabolic syndrome. The clinical significance and implications of the review findings are discussed.

Methods

Relevant literature was obtained via Medline/PubMed using the search term: fibromyalgia[ti] ("metabolic syndrome" OR "metabolic disease" OR biomarker*[ti] OR micro-inflammation OR sub-inflammation OR "low-level inflammation" OR "low-grade inflammation"). A search filter for the English language was applied. The search was performed on February 28th, 2024.

The search results were exported and annotated for screening purposes. To meet the inclusion criteria, the results had to be clinical studies, systematic reviews, or meta-analyses of molecular biomarkers for fibromyalgia. Results that met the exclusion criteria were nonclinical studies, interventional studies, other article types (e.g., correspondence), other biomarker studies (e.g., imaging, physiologic), and articles covering topics beyond the scope of this review. General biomarker reviews published more than 10 years prior to the search date were also excluded.

Additional relevant articles were obtained from the "similar articles" or "cited by" lists for PubMed search results and from the references of the reviewed articles. More information on potential biomarkers of interest was obtained from NCBI Gene and UniProtKB.

Results

The literature search returned 59 results. The final number of articles included after screening was 18. The main topics explored in these articles included pain, immune response/inflammation, micro-inflammation, metabolic syndrome, gut dysbiosis, oxidative stress, and stress response.

Pain Biomarkers

For a comprehensive review of potential biomarkers for fibromyalgic pain that extends beyond the metabolic and inflammatory markers covered here, we refer the reader to the paper by Favretti et al (6). Numerous pain biomarker candidates have been evaluated, including glutamate, substance P, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), mu-opioid receptor (MOR), neuropeptide Y (NPY), and pentraxin-3 (PTX-3). There is consistent evidence for altered levels of glutamate (CSF and several brain

regions), BDNF (blood), SP (CSF and blood), and NPY (blood) in fibromyalgia. However, more research is needed to assess the reliability of these metabolites to support their use as diagnostic biomarkers (6).

BDNF, a neurotrophic factor critical for brain plasticity that has been implicated in neuropathic and inflammatory pain mechanisms, has received significant attention as a modulator of pain in fibromyalgia and a possible etiological factor for it as well (10). A recently published systematic review and meta-analysis of 15 studies concluded that there was robust evidence that levels of circulating BDNF were significantly higher in fibromyalgia patients than in healthy controls (12). Furthermore, higher serum concentrations of BDNF and S100 calcium-binding protein B (S100B), another marker of brain plasticity, correlate with lower pressure pain threshold in women with fibromyalgia (11).

Another recently proposed pain biomarker is serum vascular endothelial growth factor (VEGF), a critical angiogenic molecule that is upregulated by inflammatory cytokines and can further enhance inflammatory processes (13). An observational study of female patients with fibromyalgia showed that their serum VEGF levels were significantly positively associated with the pressure pain thresholds of several tender points and might have therefore played a role in pain processing (14).

A longitudinal study of fibromyalgia patients found that changes in plasma concentrations of the chemokines interleukin-8 (IL-8) and monocyte chemotactic protein-1 (MCP-1) were correlated with changes in brief pain inventory severity scores and might have also affected the patients' pain processing (15).

A unique and promising avenue of research is the analysis of MOR expression on immune cells. The percentage of MOR-positive lymphocytes (termed the Mu-Lympho-Marker [MLM]) represents a potential biomarker for chronic pain in fibromyalgia (16,17). Malafoglia et al (16) and Raffaeli et al (17) reported lower proportions of MOR-positive B cells and natural killer (NK) cells in fibromyalgia patients than in pain-free healthy controls. The authors suggested a possible link between lower MOR expression and reduced pain thresholds (i.e., hyperalgesia) due to impaired endogenous opioid analgesia.

Immune Response and Inflammation

Although fibromyalgia is not considered a primary inflammatory disorder, there is ample evidence that the immune system is involved in the condition, and clinical studies indicate the existence of an inflammatory subtype of fibromyalgia (18). A concise review of potential biomarkers for immune and inflammation involvement is presented by Nagakura et al (19). Bäckryd et al (20) simultaneously assessed over 90 inflammation-related proteins in plasma and CSF samples from patients and control patients and identified several biomarker candidates for systemic inflammation and neuroinflammation. The study distinguished 4 proteins that were important for discriminating between fibromyalgia patients and controls in both plasma and CSF: C-X-C motif chemokine ligand 5 (CXCL5), CXCL6, MCP-2, and latency-associated peptide of transforming growth factor-beta 1 (LAP TGF-β1). CXCL5 and CXCL6 are involved in neutrophil activation, with CXCL6 having potent antibacterial properties (21). MCP-2 is involved in recruiting and activating a range of immune cells at sites of inflammation (22).

Cytokine levels in fibromyalgia patients have been studied extensively, albeit with mixed results. Systematic reviews and meta-analyses have reported that the fibromyalgia patients observed in them possessed significantly altered circulating levels of pro-inflammatory cytokines, including tumor necrosis factor (TNF) α, IL-1β, IL-6, IL-8, interferon gamma (IFN-γ), and C reactive protein (CRP), as well as anti-inflammatory cytokines, such as IL-1 receptor antagonist and IL-10, compared to healthy controls (20,23-25). The alteration of both pro- and anti-inflammatory cytokines, which are associated with comorbidities such as stress, depression, and primary inflammatory disorders, limits their potential clinical utility as disease biomarkers (24,25). Efforts have been made to identify a distinct disease-specific cytokine signature; however, more work is needed in this area (24), in which the potential of artificial intelligence could be utilized to unravel its complexities.

In their study of the inflammasome complex in the blood mononuclear cells of fibromyalgia patients, Cordero et al (26) observed that mitochondrial dysfunction and CoQ10 deficiency were involved in the activation of the NOD-like receptor protein 3 (NRLP3) inflammasome, a sensor of pathogen and cellular damage signals and regulator of the innate immune response. In addition, the inflammasomes showed increased caspase-1 activation and significantly increased serum levels of IL-1β and IL-18. IL levels were negatively correlated with CoQ10 levels and positively correlated with oxidative stress in mitochondria. The authors proposed that those inflammatory parameters, especially inflammasome activation, possibly represented potential new biomarkers for fibromyalgia diagnoses with etiological relevance (26).

More recently, researchers have turned their focus from identifying specific markers of inflammation toward exploring the potential role of chronic, low-grade inflammation (micro-inflammation) in the development and persistence of fibromyalgia.

Micro-Inflammation

Recently conducted genome-wide expression profiling analysis by Yao et al (7) provides valuable insights into several potential underlying molecular mechanisms and factors that may be crucial to the development and maintenance of fibromyalgia, particularly micro-inflammation. Yao et al (7) performed a secondary analysis of peripheral blood gene expression data generated by Jones et al (27), which consisted of 61 fibromyalgia patients and 68 healthy controls, and identified 54 differentially expressed inflammatory genes. Cluster analysis of the fibromyalgia patients based on the expression of those 54 genes revealed 2 distinct molecular subtypes: a "micro-inflammation subtype" and a "non-inflammation subtype." Patients in the micro-inflammation group had significantly greater mean BMI and prevalence of major depression as well as a higher number of activated dendritic cells and NK cells (according to the gene-set enrichment analysis). Yao et al (7) then compared gene expression profiles between the 2 patient groups and identified 106 differentially expressed genes. Subsequent gene ontology analysis revealed upregulation of genes responsible for innate immune and antibacterial responses in the micro-inflammation group. Using 3 machine learning algorithms, Yao et al (7) constructed a classification system for fibromyalgia subtypes based on BMI, major depression, and 5 differentially expressed inflammatory genes that possibly represented key biomarkers for micro-inflammation-type fibromyalgia: MMP8, ENPP3, MAP2K3, HGF, and YES1.

Matrix metalloproteinase-8 (MMP8) was ranked by one of the algorithms as the most important feature gene. This gene was significantly more upregulated in the fibromyalgia group than in the control group and twice as upregulated in the micro-inflammation subgroup as in the non-inflammation subgroup. Also known as neutrophil collagenase, MMP8 is a signifier of infection, with key roles in the remodeling of the extracellular matrix and the initiation and resolution of inflammation (28). Upregulation of MMP8 is reported in obesity (29) and various inflammatory diseases, including osteoarthritis, rheumatoid arthritis, and periodontitis (28), and has been proposed as a biomarker for the latter 2 conditions (30-32).

Mitogen-activated protein kinase kinase 3 (MAP2K3 or MKK3) was significantly upregulated in the fibromyalgia group and predominantly elevated in cases within the micro-inflammation subgroup. MAP2K3 is one of the main activators of the p38 MAPK pathway, which plays a major role in stress-induced inflammation (33) and in many chronic pain states, such as painful diabetic neuropathy (34). Research has shown that single-nucleotide polymorphisms in the MAP2K3 locus are strongly associated with BMI (35). High expressions of MAP2K3 were reported in the adipose cells and hypothalamus neurons of mice, indicating possible roles in adipogenesis, appetite regulation, and neuroinflammation of the hypothalamus (35). Positron emission tomography imaging of translocator protein (TSPO) and monoamine oxidase B (MAO-B) ligands has previously confirmed microglial neuroinflammation in several of the brain regions of fibromyalgia patients (36), further supporting the relevance of the activation of the p38 MAPK pathway.

MAP2K3 also affects mitochondrial function, which contributes to the inflammatory response to bacterial stimuli (37). Mitochondrial dysfunction has been reported in fibromyalgia patients' blood mononuclear cells and involves the activation of the NLRP3 inflammasome (26), possibly via MAP2K3.

Hepatocyte growth factor (HGF) was predominantly downregulated in the micro-inflammation group. HGF is critically involved in protecting tissue during inflammatory and immune diseases due to its direct effects on immune cells, especially dendritic cells and macrophages (40-42).

Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3) was significantly downregulated in the fibromyalgia group and predominantly decreased in cases within the micro-inflammation subgroup. ENPP3 plays a critical role in the suppression of chronic allergic inflammation by suppressing activated basophils and mast cells (44). This enzyme is also highly expressed on epithelial cells of the small intestine, where it controls the number of plasmacytoid dendritic cells, therefore regulating the immune response and protecting against chronic inflammatory disease (45). ENPP3 expression has also been reported in the small and medium DRG sensory neurons of rodents, where it plays a role in ATP metabolism and clearance, which may be important for normal nociceptive and anti-nociceptive signaling (46).

What relevance the proto-oncogene YES1 has to fibromyalgia is unclear. However, another gene that

received a high importance ranking in the Yao study was folate receptor beta (FOLR2), which was significantly more downregulated in the fibromyalgia group than in the control group. FOLR2 is anti-inflammatory and overexpressed in activated macrophages within healthy and inflamed tissue (47). FOLR2-positive macrophages are abundant in inflamed synovial tissue in chronic pain conditions, such as rheumatoid arthritis and osteoarthritis (48-50).

Few other studies have investigated the association between fibromyalgia and low-level systemic inflammation.

Oxidative Stress

As discussed previously, oxidative stress and mitochondrial dysfunction have been implicated in the pathophysiology of fibromyalgia, and several studies have examined oxidative stress markers (19,51). A study by Cordero et al (26) showed that in addition to increased mitochondrial reactive oxygen species, fibromyalgia patients' blood mononuclear cells also exhibited higher expression of the oxidative stress markers 8-oxoguanine glycosylase (OGG1) and 7,8-dihydro-8-oxoguanine (8-oxoG). Dos Santos et al (52) explored oxidative stress biomarkers in patients' blood samples, including lipid peroxidation products (LPO) and antioxidants, as determinants of fibromyalgia symptom severity. The results showed that reduced superoxide dismutase (SOD) and increased lipid peroxidation, along with worse muscle performance and quality of life, may independently contribute to greater widespread muscle pain. Shukla et al (53) found that fibromyalgia patients had significantly lower plasma levels of antioxidants, including SOD, glutathione reductase, and glutathione peroxidase, than did controls. The fibromyalgia patients also showed significantly higher levels of nitric oxide and LPO, which were positively correlated with the Revised Fibromyalgia Impact Questionnaire (FIQR) scores.

Obesity and Metabolic Syndrome

Studies have shown that women with fibromyalgia have a higher risk of metabolic syndrome (54,55) and that higher BMI is associated with worse scores and symptom severity on the FIQR (56-58). Importantly, obesity has been characterized as a state of chronic low-grade systemic inflammation (59). However, few studies have examined this link in fibromyalgia patients.

Ghafouri et al (60) investigated the connection between obesity and low-grade inflammation in women diagnosed with fibromyalgia by comparing the plasma levels of inflammatory biomarkers, muscle blood flow, metabolism, and pain assessment measures in patients with obesity to those in patients without obesity. The study identified 14 inflammatory proteins associated with obesity status. IL-6, IL-1RA, MCP-4, and macrophage inflammatory protein 1-beta (MIP-1β) levels were significantly higher in obese patients and positively correlated with BMI. In obese patients, plasma levels of several inflammatory proteins also correlated with sedentary behavior and Fibromyalgia Impact Questionnaire (FIQ) scores. This research provides support for an important pathophysiological relationship between obesity in fibromyalgia and micro-inflammation.

The aforementioned study by Yao et al (7) also suggests that fibromyalgia, metabolic syndrome, and micro-inflammation may be intrinsically intertwined and that a causal link may exist. Fibromyalgia patients in the micro-inflammation group had higher mean BMI and increased expression of MMP8 and MAP2K3. Positive associations between MMP8 and MAP2K3 expression and obesity have been reported previously (29,35). HGF expression was lower in the micro-inflammation group. HGF has been identified as an adipose cytokine and is strongly associated with obesity and metabolic syndrome (38,39). Research has shown that serum levels are associated with BMI, waist circumference, and elevated liver function tests and are inversely correlated with high-density lipoprotein (HDL) levels (38,39).

Xiao et al (61) assessed several inflammatory markers in the serum of fibromyalgia patients and reported that they exhibited significantly higher levels of CRP and higher BMI than did healthy controls. In fibromyalgia patients, serum CRP was significantly correlated with BMI, erythrocyte sedimentation rate (ESR), and serum IL-6 and IL-8. Okifuji et al (62) examined the association between obesity and neuroendocrine markers of fibromyalgia. Of the 38 fibromyalgia patients, 50% were obese, and a further 21% were overweight. BMI was strongly positively associated with IL-6 and epinephrine and weakly associated with CRP and cortisol. Together, these studies support an important link among obesity, inflammation, and stress in fibromyalgia.

Gut Dysbiosis

There are numerous reports of altered gut microbiome compositions in patients with fibromyalgia (8,63-65). Clos-Garcia et al (64) showed a significant reduction in the overall diversity of microbiota in fibromyalgia fecal samples. The researchers also analyzed the serum metabolome and found changes in the me-

tabolism of glutamate and other neurotransmitters. In their examination of the composition of fibromyalgia patients' gut microbiomes, Minerbi et al (65) observed changes in the relative abundance of numerous bacterial species, including a reduction in Faecalibacterium prausnitzii, a species with documented antinociceptive and anti-inflammatory effects (66). A systematic review of various biomarkers of altered gut microbiomes in fibromyalgia has provided support for associations between fibromyalgia and gut microbiota. The alterations in microbiome composition and microbial metabolism detailed in the review especially suggest a link, though more robust investigations are needed (8).

More recently, Martín et al (67) evaluated biomarkers of intestinal barrier function and inflammation in fibromyalgia patients. When compared to healthy controls, fibromyalgia patients had significantly higher plasma levels of anti-β-lactoglobulin antibodies (IgG anti-β-LGB) and zonulin-1 (ZO-1). These findings suggest increased intestinal permeability as well as lipopolysaccharides (LPS) and soluble CD14 (sCD14), indicators of increased microbial translocation. High plasma ZO-1 has also been reported in obese patients and shown to be positively correlated with BMI, fat mass, and fat percentage (68).

Stress Response

Because of the popular notion that fibromyalgia is a stress-related disorder, extensive research has been dedicated to identifying potential biomarkers associated with the stress response. Specifically, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis has been implicated in fibromyalgia (71,72). A recent systematic review and meta-analysis examined numerous indices of HPA axis dysregulation as potential stress biomarkers of fibromyalgia, including corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), cortisol, epinephrine, and norepinephrine (72). There was low-quality evidence for decreased levels of salivary and urinary cortisol and increased levels of norepinephrine in fibromyalgia. However, due to the high heterogeneity between studies and the partially contradictory results, the evidence for HPA axis dysregulation involvement in fibromyalgia is inconclusive. Relevantly, chronic stress and HPA axis dysregulation have been linked to the development of obesity and metabolic syndrome (73).

Discussion

Currently, no well-defined, reliable biomarker for fibromyalgia has been identified. More work is needed in this area, and future studies should consider combined the use of large longitudinal epidemiological datasets, such as the UK Biobank, with machine learning approaches to identify prognostic and diagnostic biomarkers (74). Importantly, the biomarker literature reviewed here reveals several interacting clinical and molecular factors involved in the pathophysiology of fibromyalgia. These interactions also suggest the existence of patient subsets that share distinct combinations of these factors. This possibility presents an opportunity to reconsider current hypotheses regarding the etiology of fibromyalgia.

Contemporary Hypotheses

The above findings imply that the establishment of "micro-inflammatory" fibromyalgia may involve bacterial infection and subsequent innate immune system response involving the activation and/or altered function of immune cells, such as neutrophils and dendritic, NK, and mast cells. We propose cumulative exposure to infections (bacterial or viral) could lead to the sustained activation of the innate immune system and chronic inflammation. Of relevance is the study by Ma et al (75), which showed that young patients with periodontitis had a 50% increased hazard ratio of developing fibromyalgia. This result suggests that bacterial infection of the gingiva may activate the innate immune system and cause the aforementioned chronic inflammation (possibly involving the elevation of MMP8 [30,32]).

Another possible cause of bacterial infection and immune system disturbance is gut dysbiosis (76). Studies have shown altered gut microbiome compositions in patients with fibromyalgia (8,63-65). These alterations might also explain fibromyalgia patients' high prevalence of comorbid depression, with accumulating evidence for involvement of gut dysbiosis in the development of mood disorders via the gut-immune-brain axis (77,78). Gut dysbiosis likely increases systemic inflammation, driving alterations in mood and pain through both humoral and vagal mechanisms (79-82). Potential involvement of the vagus nerve in fibromyalgia has been discussed (83). Recently, Martin et al (67) confirmed an association between biomarkers of gut barrier dysfunction (increased permeability) and bacterial infiltration with autonomic symptoms in fibromyalgia patients.

Furthermore, there appears to be a link between micro-inflammation and metabolic syndrome (possibly via elevation of MMP8, MAP2K3, and HGF) as well as

comorbid depression (7). Stress is also commonly implicated in the development of fibromyalgia (72) and is likely intricately linked with obesity (62). Notably, irritable bowel syndrome (IBS), which has been linked to gut dysbiosis, is associated with high prevalence of fibromyalgia (30%) as well as metabolic syndrome, obesity, anxiety disorder, and depression (69). The link between metabolic syndrome, gut dysbiosis, and inflammation has been summarized by Islam et al (84). Briefly, gut dysbiosis caused by high-fat-diet-induced obesity leads to the weakening of the intestinal barrier, thus allowing lipopolysaccharide (LPS; bacterial endotoxin) to leak into the circulation and translocate to white adipose tissue, triggering low-grade inflammation. Increased LPS concentration and tissue uptake correlate with systemic inflammation, metabolic syndrome, and obesity (85-87), possibly involving the inflammatory reflex (88).

We therefore hypothesize that, for a subset of patients with inflammatory-type fibromyalgia, disease onset involves the presence of metabolic syndrome, chronic bacterial infection load, and physical and/or mental stress, which may in turn lead to the onset of clinical depression. Innate immune system activation appears to be a core feature of this subgroup. A similar hypothesis was declared by Livshits and Kalinkovich in 2022 (89), implicating gut dysbiosis, neuroinflammation, and sarcopenia, with subsequent formation of the inflammatome (a large, representative set of genes associated with inflammation and immune responses, which overlap significantly with numerous diseases).

Clinical Implications

An ideal biomarker should have a high sensitivity and specificity and thus significant positive and negative predictive value for the condition. The biomarker should also be rigidly able to be determined rigorously by standard laboratory analysis. The means of measuring the biomarker should be relatively noninvasive, such as a saliva or blood sample, and should not involve costly equipment, such as advanced brain imaging techniques not widely accessible to global populations.

In light of recent biomarker research, attention should be paid to resolving stress and depression, reversing metabolic syndrome, and eliminating any ongoing bacterial infection load in the fibromyalgia patient. To this end, a new potential treatment paradigm protocol presents itself:

1) Treatment of depression and stress with the clinically appropriate combination of cognitive behavioral therapy and pharmacological therapy (e.g., duloxetine) (90,91).

2) Treatment of metabolic syndrome in the overweight or obese patient. Waist-circumference-to-height ratio should be calculated and ideal body weight determined. Since metabolic syndrome is most closely associated with the waist-circumference-to-height ratio, reduction of this ratio to the healthy range should be the primary goal of normalization. Aggressive weight loss may be achieved with bariatric surgery, the new class of weight-loss drugs (glucagon-like peptide 1 receptor agonists), caloric restriction, and the prescription of metformin (57,92,93).

3) Treatment with metformin, which offers potential dual benefits. Firstly, its use in the treatment of type 2 diabetes is associated with reductions in weight, hyperglycemia, hepatic glucose and lipids, and cardiovascular risk (93,94). Secondly, there is emerging evidence supporting the antinociceptive effects of metformin as well as its possible beneficial effects on comorbid depression, anxiety, and cognitive impairment (94). In addition, metformin has been shown to restore mitochondrial function and the expression of antioxidant enzymes in the fibroblasts of patients with fibromyalgia (95).

4) Treatment of any infection load could begin with a periodontist's review of the gingiva and treatment of any underlying periodontal disease. Emerging evidence indicates that gut dysbiosis may be treatable through a combination of intermittent fasting with the intake of insoluble fiber and consumption of a nutraceutical pairing of curcumin and zinc (84,96). Replenishment of significantly reduced bacterial genera (e.g., Bifidobacterium, Lactobacillus [64]) with probiotics may help rebalance the gut-brain axis. The potential benefits of prebiotic and probiotic supplementation in fibromyalgia patients have been demonstrated in a placebo-controlled randomized clinical trial (97).

5) For dual treatment of inflammation and depression, infliximab, a TNF-neutralizing antibody, should be considered. Infliximab has been shown to reduce major depressive disorder in patients with CRP above 5 mg/ml (i.e., high systemic inflammation) (98).

6) Confirmed vitamin deficiencies should be treated with supplements. There is strong evidence that vitamin D supplementation reduces fibromyalgia patients' pain (99). Oral vitamin B12 treatment can lower FIQR scores by approximately 20%, supporting a role for vitamin B12 supplementation in the presence of a vitamin deficiency (100). In a model of painful dia-

betic neuropathy, metformin demonstrated analgesic synergy with vitamin B12 (101). It may be worth testing for any synergistic analgesic responses in fibromyalgia models and then reviewing the potential for synergistic treatment effects in humans.

According to this paradigm, a multidisciplinary team consisting of a psychologist, personal trainer, dietician, periodontist, and primary care physician would be best suited to deliver treatment for the fibromyalgia patient, with possible referrals to bariatric surgeons for patients with morbid obesity. The multidisciplinary team should be routinely involved in treating patients and work toward the goals of achieving ideal body weight, reversal of metabolic syndrome, the remission of depression with effective management of lifestyle stress, and the elimination of sources of bacterial infection and immune activation.

In addition to the above, diagnostic workup to rule out secondary causes of chronic widespread pain should include the following:

1) Pathology testing of patients' CRP and ESR as well as hemoglobin A1C, fasting insulin, and celiac serology (transglutaminase and deamidated gliadin peptide antibodies), followed by small intestine biopsy and gastroscopy if the serology test is positive. Furthermore, circulating 25-hydroxy vitamin D should be measured, since abnormal levels thereof have been shown to potentially cause or exacerbate chronic widespread pain (102,103). Patients' vitamin B12 levels should also be recorded because research has shown an association between fatigue and neurological symptoms in fibromyalgia, with 42% of study patients having B12 deficiencies (104). The levels of vitamin C, zinc, and folate in serum and red blood cells may also be useful to record; deficiencies in these nutrients are prevalent in overweight or obese individuals and can contribute to gut dysbiosis (105).

2) Some patients with upper cervical spinal canal stenosis can present with 4-limb diffuse pain that mimics fibromyalgia, so it is important that patients aged 60 years and older who develop fibromyalgia be screened for symptomatic cervical spinal canal stenosis with appropriate CT and/or MRI imaging and undergo surgical treatment if appropriate (106-108).

Limitations

A large portion of this review is dedicated to discussion of the study by Yao et al (7), since we see it as a keystone piece of research that may not only point at new biomarkers but also at novel etiological drivers of different subtypes of fibromyalgia. We believe that this research opens new avenues of therapeutic research to pursue. While the model developed by Yao et al (7) had high precision, accuracy, specificity, and reliability, external validation of the potential biomarkers with an additional dataset is needed to support the proposed treatment plan. Further investigations to support contemporary hypotheses and the involvement of metabolic syndrome and gut dysbiosis are warranted.

Conclusion

At this stage, it is unlikely that the full story of fibromyalgia has been written. There is more to be discovered regarding factors contributing to the initiation and maintenance of the condition. The variability in clinical presentation and biological indices (e.g., immune/inflammation status) has always suggested the likelihood of multiple causative factors, and the paradigm proposed here does not alter that view. It is relevant for population health indices that attention to weight loss and the reversal of metabolic syndrome may also yield improvements in the prevalence of cardiovascular disease, cerebrovascular disease, and dementia as well as overall mortality. Thus, an argument can be made that the proposed treatment protocol may yield overall health benefits for patients regardless of their supposed disease subtype.

Of absolute importance is the need for validation of the work of Yao et al (7) in an external dataset, ideally one larger than the original dataset used to develop the model. If such validation is achieved, it may be appropriate to consider an open label trial to evaluate a multimodal approach that treats these newly implicated clinical variables. If efficacy is seen, comparative trials with existing fibromyalgia treatments could be performed to determine the rank order of efficacy of the treatment approaches.

We believe that, for the first time in a very long while, there is hope for fibromyalgia patients. With further, more robust research and biomarker validation, we may be able to develop an effective treatment strategy over the next few years that will revolutionize treatment for this condition.

Author Contribution

Marc Russo: conceptualization, writing—original draft preparation. Danielle Santarelli: writing—review and editing. Peter Georgius: writing—review and editing. Paul Austin: writing—review and editing.

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