




How Well Do Current Laboratory Biomarkers Inform Clinical Decision-Making in Chronic Pain Management?

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Objective: Decision-making in chronic pain patients involves a combination of subjective and objective criteria, including patient history, physical examination, imaging, and patient response to prior treatments, clinical experience, probabilities, and recognition of patterns. However, there is a distinct lack of objective laboratory biomarkers in use in routine clinical care. The objective was to review the literature to identify and describe specific biomarkers in chronic pain management.

Methods: This is a narrative review of the literature regarding the use of laboratory biomarkers in chronic pain. A librarian-assisted literature search of the PubMed, Science Direct, and Google Scholar databases was performed and resulted in 304 possible manuscripts. We included manuscripts assessing laboratory collected biomarkers from urine, serum, cerebrospinal fluid, and saliva. After screening and review of the initial literature search results, a total of 75 manuscripts were included in the narrative review.

Conclusion: The studies reviewed suggested that specific biomarkers may help identify those patients at risk of disease development and function as a prognostic indicator for disease progression and treatment response. However, additional research is necessary before specific recommendations can be made, and current clinical decision-making is modified.

Keywords: chronic pain, biomarkers, outcomes

Introduction

Clinical decision-making in chronic pain patients involves a combination of subjective and objective criteria, including patient history, physical examination, medical imaging, and patient response to prior treatments, coupled with clinical experience, probabilities, and recognition of patterns (Table 1). In other areas of medicine, specific objective data (ie, blood pressure, lipid panel, blood glucose, etc) can be collected and analyzed, both for diagnostic purposes and assessing treatment response. For pain physicians, to date, this diagnostic option has not been available. Instead, pain management teams have relied on pain rating scales (eg, visual analog scale [VAS], numeric rating scale [NRS], patient-reported pain relief, among others), which are easily manipulated and not interchangeable.

Fortunately, researchers have been searching for pain-specific objective criteria and laboratory evaluations, and this includes biomarkers. Biomarkers are used as an indicator of normal and pathologic processes, therapeutic response to an intervention, identify individuals at risk of disease development, and to certify the diagnosis. Ideally for chronic pain, the biomarkers would be direct correlates to the

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Table 1 Traditional Clinical Decision-Making Pathway

Diagnostic Variable	Examples
Patient history	Pain focused; includes onset, timing, severity, location, quality, alleviating and provoking factors, and sensory or motor changes
Physical examination	Modified depending on site of pain; may include palpation, range of motion, sensory and motor testing, and specific maneuvers (eg, facet loading, straight leg raise, FABER test, etc)
Medical imaging	X-ray, computerized tomography (CT) scan, magnetic resonance imaging (MRI)
Specialty tests	Electromyography (EMG), quantitative sudomotor axon reflex test (QSART)
Patient response to prior treatments	Documented pain relief with length of time of past treatments

underlying biologic processes involved in chronic pain creation versus the subjective chronic pain measurements, including oxidative stress, systemic inflammation, nutritional status, and/or neurotransmitter creation and destruction. In doing so, treatments could become more targeted to the causative chronic pain mechanism.

Initial chronic pain biomarkers have been sought in laboratory studies, in addition to quantitative sensory testing, skin biopsy, and brain imaging.¹ This review will focus on laboratory collected biomarkers, including urinary, serum, cerebrospinal fluid (CSF), and salivary studies.

Methods

This is a narrative review of the literature regarding the use of laboratory biomarkers in chronic pain. The objective was to review the literature to identify and describe specific biomarkers in chronic pain management. A librarian-assisted literature search of the PubMed, Science Direct, and Google Scholar databases was performed to identify appropriate manuscripts. The terms “chronic pain”, “intractable pain”, “biomarkers”, “blood”, “serum”, “hematologic tests”, “urine”, “saliva”, “salivary”, “cerebrospinal fluid”, “spinal fluid”, and “biologic” were searched and potential manuscripts were collated. All results were in the English language and no date range was used. We also reviewed former narrative reviews to supplement the literature search. These parameters resulted in collection of 304 potential manuscripts (Figure 1). Manuscripts were included based on the level of evidence, quality of results obtained, impact of the conclusions, and if the study allowed increased breadth of the topic. Manuscripts were excluded if they did not relate to the subject matter. After removal of duplicates and screening the abstracts of the initial literature search

results, a total of 85 manuscripts underwent full-text review. Following full-text review, 75 manuscripts were included in the manuscript.

Literature Review of Chronic Pain Biomarkers

Urinary Biomarkers

Urinary biomarkers of acute and chronic pain states are of tremendous interest to researchers due to the non-invasive nature of urine collection and the relative simplicity of urine as a biological matrix for analysis. Table 2 lists the most studied urinary biomarkers.^{2–38} Urine contains cellular elements, biochemicals, metabolites, and proteins derived from glomerular filtration of plasma, and renal tube excretion that provide valuable insight into the metabolic and pathophysiological state of an individual.³⁸ Biomarker analysis in urine also affords certain analytical advantages over serum or plasma due to the concentrating ability of the kidneys, which often leads to higher levels of target analytes. Clinical utility of validated pain biomarkers is anticipated to range from diagnostic enrichment to predicting drug and/or interventional therapy efficacy in individual patients. Of particular interest to researchers and clinicians is the validation of actionable pain biomarkers, which could be monitored longitudinally to provide objective documentation of improvement, worsening, or response to a therapy. For such applications, urine represents the most highly desirable biospecimen due to the non-invasive collection techniques that can be conducted recurrently without specialized training, patient instruction, and/or expense.

High-throughput ‘omics’ technologies, facilitating the rapid exploration of genomic, proteomic, transcriptomic, and metabolomic data, has greatly facilitated the search of

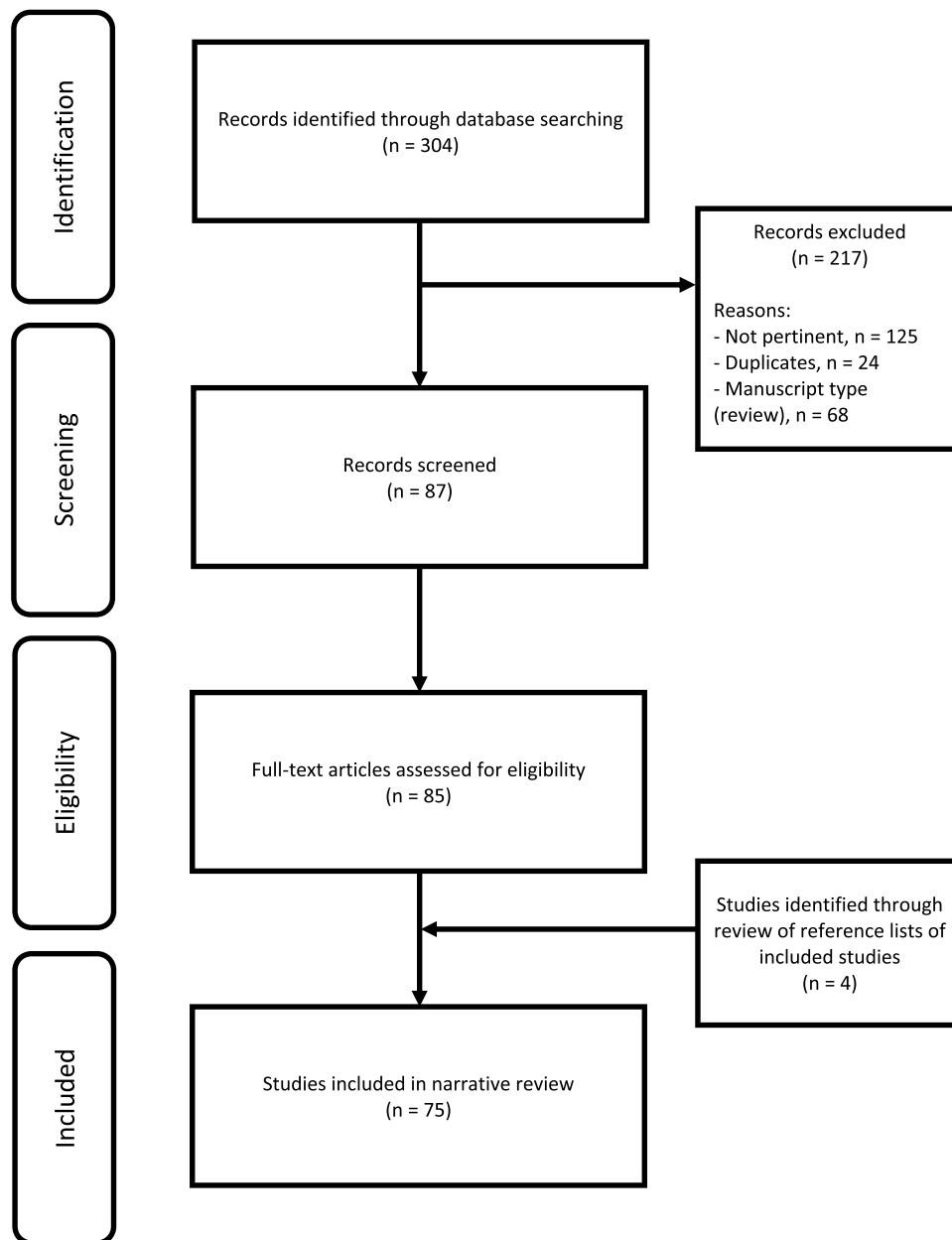


Figure 1 Flow Diagram of Screening and Selection Process.

clinically meaningful biomarkers in recent years. While much of the pain biomarker research utilizing CSF, serum, and plasma has focused on genomic, proteomic, and/or transcriptomic targets,^{39–43} much of the pioneering work involving urine has focused on metabolomic targets.^{44–46} Deer et al detailed the findings of a retrospective, observational study, which evaluated the prevalence of abnormal metabolomic biomarker findings in a cohort of 17,834 chronic pain patients. This study utilized a predefined, targeted urine panel comprising 11 endogenous biomarkers of systemic inflammation, oxidative stress, neurotransmitter

metabolism, and micronutrient deficiencies. Composite biomarkers of this unique panel all represent biochemical pathways known to impact the development or worsening of pain. Interestingly, each of the pain-pathways described in the study may also be “druggable” or modulated with existing treatment options such that abnormal biomarker findings could be corrected with existing compounds. The authors describe the panel as a cost-effective way to identify biochemical drivers of pain, which would afford safe, targeted, non-opioid therapy options. Findings of the retrospective analysis revealed that 77% of chronic pain

Table 2 Urinary Biomarkers and Relevance in Chronic Pain Pathology

Biomarker	Relevance	References
Methylmalonic acid	Elevated with vitamin B12 deficiency. Vitamin B12 deficiency leads to nerve damage and degeneration of the spinal cord. Peripheral neuropathy is the most common pain presentation.	[2–5]
Xanthurenic acid	Elevated levels indicate a vitamin B6 deficiency. Neuropathy due to vitamin B6 deficiency starts with numbness, paresthesias, or burning pain in the feet, which then ascends to affect the legs and hands.	[5–9]
Homocysteine	Elevated levels commonly indicate a B-vitamin (B6/B9/B12) deficiency. Elevated homocysteine results from B-vitamin deficiencies. Elevated homocysteine levels cause inflammation by increasing arachidonic acid and the proinflammatory prostaglandin E2 production.	[10–12]
3-HPMA	Elevated levels indicate increased exposure to acrolein. Acrolein contributes to inflammatory pain sensitivities through its binding and activation of the transient receptor potential ankyrin 1 receptor (TRPA1) in nerve fibers.	[13–15]
Quinolinic acid	Elevated levels indicate cytokine-mediated chronic inflammation. Quinolinic acid is a neuroactive kynurenine pathway (KP) metabolite which serves as a sensitive marker of chronic, systemic inflammation. Upregulation of this pathway has been shown to play a central role in the comorbidity of pain and depression. Quinolinic acid induces its depressive effects through its action on NMDA receptors.	[16–20]
Kynurenic acid	Elevated levels indicate cytokine-mediated chronic inflammation. Kynurenate is a neuroactive kynurenine pathway (KP) metabolite which serves as a sensitive marker of chronic, systemic inflammation. Upregulation of this pathway has been shown to play a central role in the comorbidity of pain and depression.	[16–20]
Pyroglutamate	Elevated levels indicate glutathione depletion. Glutathione depletion renders nerve cells susceptible to oxidative damage, which can lead to neuropathic pain.	[21–23]
Hydroxymethylglutarate	Elevated levels indicate a Coenzyme Q10 deficiency. Coenzyme Q10 deficiencies can cause muscle weakness and pain.	[24,25]
Ethylmalonate	Elevated levels indicate a carnitine deficiency. Carnitine deficiencies cause muscle aches and fatigue.	[26,27]
5-Hydroxyindoleacetate (5-HIAA)	Abnormally low levels indicate decreased synthesis/turnover of serotonin. Abnormally low synthesis/turnover of serotonin can heighten pain sensitivity.	[28–30]
Vanilmandelate	Abnormally low levels indicate decreased synthesis/turnover of norepinephrine. Abnormally low synthesis/turnover of norepinephrine can heighten pain sensitivity.	[31,32]
Prostatic exosomal proteins (PSEP)	Secreted by prostate corpuscles. Elevated PSEP was found to correlate with chronic prostatitis and chronic pelvic pain syndrome symptoms.	[33,34]
Matrix metalloproteinase (MMP), including MMP-9, MMP-9/NGAL, MMP-2	Involved with tissue remodeling and extracellular matrix degradation. Moderate key cellular processes, including angiogenesis, cytokine release, and inflammation. Studied in urological chronic pelvic pain syndrome and have been shown to positively correlate with severity of pain and symptoms in women with this pathology.	[35–38]
Neutrophil gelatinase-associated lipocalin (NGAL; also known as LCN 2)	Released by neutrophils in response to tissue inflammation and infection. Studied in urological chronic pelvic pain syndrome.	[35]

(Continued)

Table 2 (Continued).

Biomarker	Relevance	References
Vascular endothelial growth factor (VEGF, VEGF-R1)	Plays important role in modulating pain. Also involved with vascular permeability and angiogenesis. Studied in urological chronic pelvic pain syndrome and positively correlate with pain severity.	[34,36,37]

subjects exhibited at least one abnormal biomarker finding.⁴⁴

In the same year, authors of the aforementioned study completed a prospective, multi-site, cross-sectional study aimed at establishing the clinical validity of this 11-biomarker urine panel known as the Foundation Pain Index (Foundation PI).⁴⁵ Clinical validation of Foundation PI was established in a prospective, cross-sectional study designed to evaluate the criterion, discriminant, and face validity of this novel test offering in a population of chronic pain subjects. Studies confirmed that this multi-analyte assay with algorithmic analysis reveals novel objective data to guide targeted, non-opioid treatment strategies, including a number of different vitamins to treat underlying pathologies. Additionally, Foundation PI scores strongly segregate biomarker profiles of chronic pain subjects from pain-free controls and correlate with worsening clinical assessments of chronic pain. This information has not been previously available and provides objective, mechanistic insight into the pathology of chronic pain in a diverse population of chronic pain subjects.

Criterion validation analysis evaluated the significance of correlations between Foundation PI scores and validated clinical assessments for chronic pain. The 36-Item Short-Form Survey (SF-36) was selected as the most appropriate validated clinical assessment to perform criterion validation analysis as it evaluates both the physical and emotional impact of pain on subjects and is widely used in clinical practice. In a cohort of 153 chronic pain subjects and 334 age- and sex-matched pain-free controls, Foundation PI scores were significantly associated with overall SF-36 scores ($P = 0.041$) as well as with individual components of the SF-36, including general health ($P = 0.0457$), emotional well-being ($P = 0.0044$), and limitations due to emotional problems ($P = 0.0011$).⁴⁵ Correlations of Foundation PI scores with both physical and emotional components of the SF-36 are not surprising considering the diverse set of biochemical and metabolic

pathways represented by this biomarker set. Strong validation performance of Foundation PI indicates a central role for deranged biochemistry in the etiology of chronic pain, which is incredibly encouraging due to the reversible nature of biomarker abnormalities detected by this novel assay.

McCloy and colleagues conducted a comprehensive systematic review of the association between urinary biomarkers and pain, which was published as part of the International Federation for Medical and Biological Engineering (IFMBE) book series.⁴⁷ The authors reviewed findings from 277 studies comprising 22 randomized controlled trials (RCTs), 44 controlled observational studies, and 211 case studies or series. The RCTs involved 2177 patients with various pain diagnoses in which urinary biomarkers were measured as part of the study. The authors conclude that urinary biomarkers in painful conditions of bone turnover have consistently found statistically significant correlations between disease progression and pain but caution that chosen biomarkers may reflect the disease mechanism rather than pain levels.

Liang et al recently described the development of a urine-based biomarker for the detection of symptomatic progression of chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) in a retrospective multi-center study utilizing prostatic exosomal proteins (PSEPs) levels.³³ Spearman correlation coefficient showed a significant level of correlation between the NIH-Chronic Prostatitis Symptom Index (NIH-CPSI) and the urinary PSEP level ($r_s = 0.194$, $P = 0.0035$). The authors conclude that findings highlight the potential role of PSEP as a practical indicator of the symptomatic progression of CP/CPPS and clinical application of this assay may guide drug discovery and lead to better treatment options and improved quality of life.

In 2017, Dagher et al reported the findings of a large study aimed at comparing candidate Urological Chronic Pelvic Pain Syndrome (UCPPS) biomarkers among UCPPS subjects and control cohorts to gain new insights

into underlying pathophysiology.³⁷ Authors also examined associations of biomarkers with symptom severity within the UCPPS cohort and described the potential role of validated biomarkers in the development of new treatment strategies, as objective measures for patient classification schemes and evaluation of clinical outcomes, as well as further characterization of underlying disease mechanism. Results demonstrated that males with UCPPS had significantly higher normalized protein concentration of vascular endothelial growth factor (VEGF), VEGF receptor-1 (VEGF-R1), and matrix metalloproteinase 9 (MMP-9) than healthy male controls. Furthermore, males with UCPS had significantly higher concentrations of VEGF than female healthy controls. Additionally, pain and urinary symptoms were found to be positively associated with the MMP-9/NGAL complex in women.

Serum Biomarkers

The current literature regarding serum biomarkers is shown in Table 3.^{40,48–64,68} De Queiroz et al found that muscle damage and injury were associated with metabolic and inflammatory responses and the biochemical milieu of select inflammatory mediators, neuropeptides, and cytokines were found to be higher in subjects with painful muscle injury compared with those with latent injury or absent any muscle metabolic products (eg, creatine kinase).⁵³ Thus, the combination of inflammatory cytokines measured with muscle metabolism biomarkers may be used to differentiate the specific contributions of acute muscle injury to undefined back pain.

Pedersen et al studied the serum levels of Interleukin (IL)-6 (IL-6) and IL-8 in patients with lumbar radicular pain due to disc herniation.^{55,56} Samples were collected at subject recruitment and at 6-weeks or the 12-month follow-up time points, regardless of the treatment they received. They compared serum levels of IL-6 and IL-8 with patient-reported pain intensity on a 10 cm VAS. In this study, it was found that chronic lumbar radicular pain may be associated with a persistent increase of the proinflammatory cytokines IL-6 and IL-8 in serum after disc herniation, suggesting that these cytokines may be associated with the mechanisms underlying development of chronic pain due to disc herniation.

Current research surrounding low back pain (LBP) and associated inflammation and biomarkers focuses on chronic presentation of symptoms and the level of symptom severity. Multiple studies have observed significant differences in proinflammatory cytokines (eg, IL-6, tumor necrosis factor-

α [TNF- α], IL-8, and IL-1 β) in relation to pain intensity. Uçeyler et al compared cytokine profiles of patients with painful versus painless neuropathies and identified higher levels of proinflammatory cytokines in the serum of patients experiencing pain.⁵⁸ Patients were grouped on the basis of severity of patient-reported outcomes of LBP and were found to have significantly higher serum protein levels and serum mRNA levels of IL-6, IL-8, and TNF- α . Licciardone et al showed that IL-6 and IL-1 β were significantly correlated with both pain severity and somatic dysfunction.⁶⁴ In a study by Wang et al, patients were divided into groups of mild and severe sciatica, and both IL-6 and IL-8 were found to be greater in severe sciatica patients compared not only with controls but also with mild sciatica patients, suggesting that proinflammatory cytokine levels may contribute to sciatica intensity.⁶⁵

A study by Uçeyler et al showed that anti-inflammatory markers IL-4 and IL-10 were significantly lower in subjects experiencing lesser or no LBP and higher serum mRNA levels of IL-10 and IL-4 were found in painless neuropathy patients.⁵⁸ IL-4 and IL-10 are specific cytokines that are produced by activated macrophages and monocytes and considered to be anti-inflammatory because they can inhibit proinflammatory cytokine synthesis. In further work by Wang et al, elevated levels of IL-4 and IL-10 were found in mild sciatica patients as compared with severe cases.⁶⁵ This may suggest that there are analgesic effects of anti-inflammatory cytokines, which may also indicate a more favorable outcome to treatment.

Through the use of the McGill Pain Questionnaire, De Queiroz et al reported a positive correlation between TNF- α and current pain intensity, as well as “severity of pain in the last week.”⁵³ Serum protein levels and mRNA expression of TNF- α have been shown to be significantly higher in subjects experiencing greater intensity of chronic LBP. A study by Uçeyler et al showed a twofold higher TNF- α protein levels in patients with painful neuropathies compared to patients with painless neuropathies and healthy controls.⁵⁸ Wang et al showed, in a study that categorized patients by severity of sciatica pain, that those with severe sciatica had twofold higher TNF- α serum levels than those without sciatica and performed a prospective comparative longitudinal study, which found that there was a significantly higher proportion of TNF- α participants in the patient group than in the control group, which was sustained through multiple time points over a 6-month follow-up.⁶⁵ Additionally, they investigated the cross-sectional associations of depressive symptoms as a comorbidity that

Table 3 Serum Biomarkers and Relevance in Chronic Pain Pathology

Biomarker	Relevance	References
High-sensitivity C-reactive protein (hsCRP)	Acute phase protein. Positively associated with pain levels in acute LBP profiles.	[48,49]
Tumor necrosis factor α (TNF- α)	Cytokine that stimulates inflammatory responses, induces nerve swelling and neuropathic pain, and promotes cellular apoptosis via its cytotoxic effect. Serum protein levels and mRNA expression of TNF- α have been shown to be significantly higher in subjects experiencing greater intensity of LBP.	[50–52]
Soluble TNF receptor I (sTNFR I)	Positively correlated with measures of pain intensity or severity (acute pain).	[53]
Interleukin-6 (IL-6)	Helps mediate the acute-phase response to injury by promoting monocyte differentiation into macrophages and activating maturation of lymphocytes (acute pain). Exercise-induced injury models have found elevated serum levels of IL-6 released by injured skeletal muscle.	[54–57,64]
Interleukin-4 (IL-4)	Lower in subjects experiencing lesser or no LBP, may be elevated in those experiencing higher pain.	[58]
Interleukin-10 (IL-10)	Lower in subjects experiencing lesser or no LBP, may be elevated in those experiencing higher pain.	[58]
T helper 17 (TH17) cells and Interleukin-17 (IL-17)	Increased levels of TH17 cells and IL-17, leads to inflammation, further deterioration, and increased pain.	[59,60]
Interleukin-21 (IL-21), Interleukin-17 (IL-17), and Cyclooxygenase-2 (COX-2)	VAS pain scores were positively correlated with the serum levels. Higher expression was found in protein mRNA levels in disc tissue for disc herniation patients.	[61]
Regulated on activation, normal T cell expressed and secreted (RANTES)	Expressed in response to inflammatory stimuli and leads to catabolic activity. Higher levels of RANTES are associated with greater impairment and activity limitation, suggesting a link between inflammation and activity-related disability.	[62]
Apolipoprotein-M (APO-M), tetranectin (TN), and immunoglobulin light chain (IGL)	Found to be significantly lower in patients with disc herniation.	[63]
Apolipoprotein-LI (APO-LI)	Found to be significantly higher in patients with disc herniation.	[63]
Microfibril-associated protein 3 (MFAP3)	Decreased in blood in high pain states.	[40]
G protein subunit gamma 7 (GNG7)	Decreased in blood in high pain states. Decrease in blood is a strong predictor of future emergency room visits.	[40]
Contactin 1 (CNTN1)	Decreased in blood in high pain states.	[40]
Lymphocyte antigen 9 (LY9)	Increased in blood in high pain states.	[40]
Coiled-coil domain containing 144B (CCDC144B)	Decreased in blood in high pain states.	[40]
Guanylate binding protein 1 (GBPI)	Increased in blood in high pain states.	[40]
Interleukin-8 (IL-8) and Monocyte chemoattractant protein-1 (MCP-1)	Increased in blood in fibromyalgia patients and positively correlated with pain severity.	[68]

can contribute to levels of chronic LBP and potentially to inflammation, and found that although elevated TNF- α is associated LBP, these levels were not modulated by depression in patients.^{66,67}

Through investigating the involvement IL-21, IL-17, and cyclooxygenase-2 (COX-2) in disc herniation, Xue et al demonstrated that disc herniation patients exhibited significantly higher levels of serum IL-21 and IL-17.

Higher expression of IL-21, IL-17, and COX-2 was found in protein and mRNA levels in disc tissues from disc herniation patients than in normal disc tissues.⁶¹ They concluded that VAS pain scores, IL-17, and COX-2 were positively correlated with the IL-21 levels, implicating its role in the pathogenesis of lumbar disc herniation.

Regulated on activation, normal T cell expressed and secreted (RANTES) is expressed in response to inflammatory stimuli and leads to catabolic activity. Sowa et al showed significant associations between levels of RANTES after activity, and pain levels and pain-related functioning.⁶² The study found that higher RANTES levels were correlated with higher affective scores, a measure of pain interpretation. This indicates an important role of this systemic biomarker in the experience of chronic LBP. Furthermore, it was concluded that higher levels of RANTES are associated with greater impairment and activity limitation, suggesting a link between inflammation and activity-related disability.

Xie et al used proteomic analysis of blood samples to establish whether there are serum proteins associated with disc herniation.⁶³ Through the use of two-dimensional electrophoresis of blood samples from patients and control subjects, distinct protein spots were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Results showed that apolipoprotein-L1 (APO-L1), apolipoprotein-M (APO-M), tetranectin (TN), and immunoglobulin light chain (IGL) differed in patients with lumbar disc herniation. These investigators found that mean serum concentrations of APO-M, TN, and IGL were significantly lower in patients with disc herniation, whereas levels of APO-L1 were significantly higher. Very little is known about these biomarkers and similar to all the others mentioned, more long-term investigation is needed.

Niculescu et al sought to identify objective serum biomarkers for pain in psychiatric patients.⁴⁰ A total of 403 subjects were identified from the Indianapolis VA Medical Center and were followed longitudinally resulting in 493 blood samples. The authors reported being successful in discovering gene expression biomarkers that predicted pain and future emergency department visits. The study found that microfibril associated protein 3 (MFAP3) was the strongest predictor for pain, especially in females and males with post-traumatic stress disorder. Additionally, G protein subunit gamma 7 (GNG7), contactin 1 (CNTN1), lymphocyte antigen 9 (LY9), coiled-coil domain containing 144B (CCDC144B), and

guanylate-binding protein 1 (GBP1) were also viable markers for those individuals with chronic pain. GNG7 was identified as a strong marker for future emergency room visits for pain.

Interleukin-8 and monocyte chemotactic protein-1 (MCP-1) were found to be elevated in fibromyalgia patients in a study by Ang et al.⁶⁸ A total of 16 patients previously diagnosed with fibromyalgia provided two blood samples (week 1 and week 12) and the changes in IL-8 and MCP-1 concentrations were compared with pain severity at the two visits. The researchers found that the change in pain severity was positively correlated with changes in IL-8 and MCP-1 (ie, as pain severity increased, biomarker concentrations increased). The authors concluded that IL-8 and MCP-1 may assist in determining prognosis and monitoring treatment response.

Cerebrospinal Fluid Biomarkers

The list of reported CSF biomarkers is found in Table 4.^{69–76} The CSF contains various proteins, signaling molecules and peptides, metabolites, and other products that are in direct contact with the spinal cord and brain.⁷³ Fluctuations in these markers may reflect the biochemical changes in the central nervous system. In fact, the proteomics studies utilizing comprehensive mass spectrometry have reported 2500 to 3000 proteins within the CSF.^{73,77,78} While evidence remains limited, multiple studies have highlighted changes in biomarker levels that may reflect mechanisms related to acute and chronic pain manifestations.

Wahlbeck et al compared levels of angiotensin converting enzyme (ACE) and arginine vasopressin (AVP) between 15 patients with chronic pain disorder and 19 healthy control patients.⁷⁰ Serum AVP levels were significantly higher in chronic pain patients versus healthy controls. Concordant with this observation, serum osmolality which stimulates release of AVP was also elevated in chronic pain patients versus healthy controls. However, there was no significant in CSF ACE level between cohorts.

Brisby et al reported on 39 patients with lumbar disc herniation and sciatica in which CSF and serum concentrations of IL-1 β , IL-6, IL-8, interferon (IFN)- γ and TNF- α were investigated using the enzyme-linked immunosorbent assay (ELISA) technique.⁶⁹ CSF IL-8 concentrations were increased in 30.8% of the patients (12/39) and were correlated with a short duration of pain and disc extrusion or sequestration. The authors hypothesized that the increase in IL-8 is related to mechanical nerve root compression.

Table 4 Cerebrospinal Fluid Biomarkers and Relevance in Chronic Pain Pathology

Biomarker	Relevance	References
Interleukin-8 (IL-8)	Higher concentration is reported in patients with disc herniation.	[69]
Arginine vasopressin (AVP)	Higher in chronic pain patients.	[70]
Glial cell line-derived neurotrophic factor (GDNF)	Reduced expression in patients with fibromyalgia, chronic migraine, and probable analgesic abuse headache.	[71]
Somatostatin (SST)	Reduced expression in patients with fibromyalgia, chronic migraine, and probable analgesic abuse headache. Lower expression after burst spinal cord stimulation in patients with neuropathic pain.	[71,72]
Neural cell adhesion molecule LI	Higher levels associated with pain in fibromyalgia and rheumatoid arthritis.	[73]
Complement C4-A	Higher levels associated with pain in fibromyalgia and rheumatoid arthritis.	[73]
Lysozyme C	Higher levels associated with pain in fibromyalgia and rheumatoid arthritis.	[73]
Receptor-type tyrosine-protein phosphatase zeta	Higher levels associated with pain in fibromyalgia and rheumatoid arthritis.	[73]
Apolipoprotein-D	Higher levels associated with pain in fibromyalgia and rheumatoid arthritis.	[73]
Alpha-1-antichymotrypsin	Higher levels associated with pain in fibromyalgia and rheumatoid arthritis.	[73]
Granulins	Higher levels associated with pain in fibromyalgia and rheumatoid arthritis.	[73]
Calcium/calmodulin-dependent protein kinase type II subunit alpha	Higher levels associated with pain in fibromyalgia and rheumatoid arthritis.	[73]
Mast/stem cell growth factor receptor Kit	Higher levels associated with pain in fibromyalgia and rheumatoid arthritis.	[73]
Prolow-density lipoprotein receptor-related protein I	Higher levels associated with pain in fibromyalgia and rheumatoid arthritis.	[73]
Apolipoprotein-CI	Increased levels in neuropathic pain.	[74]
Autotaxin	Increased levels in patients with fibromyalgia.	[74]
Cystatin C	Elevated levels in obstetric patients in labor who were in severe pain.	[75]
Tumor necrosis factor beta (TNF-β)	Elevated levels in patients with trigeminal neuralgia and decreased after microvascular decompression surgery towards levels observed in healthy controls.	[76]
TNF-related apoptosis inducing ligand (TRAIL)	Elevated levels in patients with trigeminal neuralgia and decreased after microvascular decompression surgery towards levels observed in healthy controls.	[76]
Growth hormone AI (PRL)	Lower expression after burst spinal cord stimulation in patients with neuropathic pain.	[72]
Nucleobindin-2 (NUCB2)	Lower expression after burst spinal cord stimulation in patients with neuropathic pain.	[72]
Calbindin (CALB1)	Lower expression after burst spinal cord stimulation in patients with neuropathic pain.	[72]
Acyl-CoA binding protein (CBI)	Lower expression after burst spinal cord stimulation in patients with neuropathic pain.	[72]
ProSAAS (PCSK1N)	Lower expression after burst spinal cord stimulation in patients with neuropathic pain.	[72]
Endothelin-3 (END3)	Lower expression after burst spinal cord stimulation in patients with neuropathic pain.	[72]
Cholecystokinin (CCK)	Lower expression after burst spinal cord stimulation in patients with neuropathic pain.	[72]

Fibromyalgia is a heterogeneous condition that is characterized by widespread musculoskeletal pain, pain elicited on digital palpation of tender points, fatigue, sleep issues, and mood disorder. Despite decades of research on fibromyalgia, there continues to be limited data on its underlying mechanism of action, lack of diagnostic and prognostic markers, and limited treatment options. Several studies have highlighted measurable changes in CSF biomarkers that may have potential diagnostic and prognostic implications.^{71,73,74} Sarchielli et al studied 16 chronic migraine patients, 15 patients with probable chronic migraine and probably analgesic-abuse headache, 20 fibromyalgia patients, and 20 control patients.⁷¹ This study reported that compared to healthy control patients, there was lower levels of glial cell line-derived neurotrophic factor (GDNF) and somatostatin (SST). Khoonsari et al measured CSF biomarkers in 13 fibromyalgia patients and 11 rheumatoid arthritis patients, and identified 10 proteins that may be associated with pain including neural cell adhesion molecule L1, complement C4-A, lysozyme C, receptor-type tyrosine-protein phosphatase zeta, apolipoprotein D, alpha-1-antichymotrypsin, granulins, calcium/calmodulin-dependent protein kinase type II subunit alpha, mast/stem cell growth factor receptor kit, prolow-density lipoprotein receptor-related protein 1.⁷³ While the authors postulate that these biomarkers are known to be involved in pain mechanisms, these associations are novel in the context of fibromyalgia. Lind et al studied 40 fibromyalgia patients and 134 control patients, and reported increased levels of autotaxin in fibromyalgia patients.⁷⁴

A unique pre- and post-treatment study design was reported by Ericson et al, in which the levels of 92 CSF protein biomarkers related to inflammation were measured before and after microvascular decompression surgery in trigeminal neuralgia patients.⁷⁶ This study reported that TNF- β and TNF-related apoptosis inducing ligand (TRAIL) were elevated at baseline in patients with trigeminal neuralgia and decreased towards levels observed in healthy controls after patients underwent microvascular decompression surgery.

Similarly, a pre- and post-treatment study design was conducted by Royds et al.⁷² This study performed proteomic and neuropeptide CSF analysis in four patients with neuropathic pain who underwent burst spinal cord stimulation. Compared to baseline levels prior to spinal cord stimulator implant, there was decreased expression of growth hormone A1 (PRL), somatostatin (SST), nucleobindin-2 (NUCB2), calbindin (CALB1), acyl-CoA binding

protein (DBI), proSAAS (PCSK1N), endothelin-3 (END3) and cholecystokinin (CCK) after 8 weeks of burst spinal cord stimulation therapy.

Finally, Mannes et al studied obstetric patients experiencing severe labor pain and measured CSF levels of cystatin C, which is cysteine protease inhibitor belonging to the cystatin superfamily.⁷⁵ This study included five pregnant females with severe labor pain and five control pregnant patients who were presenting for elective Cesarean section and were not in severe pain. Compared to control patients, pregnant females with severe pain had elevated levels of cystatin C in the CSF. The study concluded that this observation is consistent with prior animal studies that report persistent pain inducing synthesis of cystatin C from the dorsal spinal cord and with subsequent overflow of surplus amounts into the CSF.

Salivary Biomarkers

The study of salivary diagnostic markers is an emerging field using nanotechnology and molecular studies to diagnose oral disease and systemic disorders (Table 5).⁷⁷⁻⁸⁷ Salivary biomarkers, along with urinary biomarkers, have the distinct advantage of non-invasive sampling. This makes clinical use of these laboratory studies more comfortable and accessible for patients.⁸⁸ Most biomarkers that are present in blood and urine are also detectable in saliva samples.⁸⁸

Several studies have examined the utility of salivary biomarkers as diagnostic and prognostic measures of pain in fibromyalgia patients. Catley et al compared salivary levels of cortisol, diurnal cycles of cortisol, and the impact of psychological stress on cortisol reactivity in 21 fibromyalgia patients versus 22 healthy control patients.⁷⁷ They reported that higher average levels of salivary cortisol were detected in fibromyalgia patients versus healthy control patients, although there was no difference in the diurnal cycle of cortisol or psychological stress reactivity. However, in chronic manifestations of fibromyalgia and widespread musculoskeletal pain, cortisol levels may actually be decreased with a blunted diurnal slope. This was depicted by Generaal et al which included 471 patients with chronic multisite musculoskeletal pain and 654 control patients, and compared their salivary cortisol levels at awakening, one-hour post-awakening, evening, as well as diurnal slopes.⁷⁸ They reported that patients with chronic musculoskeletal pain had lower cortisol levels on awakening and evening

Table 5 Salivary Biomarkers and Relevance in Chronic Pain Pathology

Biomarker	Relevance	References
Cortisol	Increased in early fibromyalgia and positively associated with pain, stress, and depression. Decreased in chronic fibromyalgia. Increased in dental pain (pulpal or periapical inflammation). Fluctuates depending on timing of sampling, but may be increased in fibromyalgia.	[77–80]
Alpha-amylase	Increased in burning mouth syndrome. Increased in temporomandibular disorder.	[81,82]
Transaldolase	Overexpression in female patients with fibromyalgia.	[83]
Phosphoglycerate mutase I	Increased in fibromyalgia. Decreased levels after treatment with mud-bath and balneotherapy in fibromyalgia patients.	[83,84]
Calgranulin	Increased calgranulina A and C in fibromyalgia.	[83]
Glutamate	Increased in chronic migraine.	[85]
Immunoglobulin A (IgA)	Increased in burning mouth syndrome.	[81]
Macrophage inflammatory protein-4 (MIP4)	Increased in burning mouth syndrome.	[81]
Uric acid	Decreased in burning mouth syndrome.	[81]
Ferric reducing activity of plasma (FRAP)	Decreased in burning mouth syndrome.	[81]
Nerve growth factor (NGF)	Decreased in temporomandibular disorder.	[82]
Brain-derived neurotrophic factor (BDNF)	Decreased in temporomandibular disorder. Decreased levels after treatment with mud-bath and balneotherapy in fibromyalgia patients.	[82,84]
Interleukin-1 beta (IL-1 β)	Increased in dental pain (pulpal or periapical inflammation).	[79]
Interleukin-6 (IL-6)	Increased in dental pain (pulpal or periapical inflammation).	[79]
C-reactive protein (CRP)	Decreased in chronic periodontitis.	[86]
Alpha-2-macroglobulin (alpha-2M)	Decreased in chronic periodontitis.	[86]
Toll-like receptor 2 (TLR-2)	Increased in burning mouth syndrome.	[87]

times, as well as a blunted diurnal slope. Some studies have even postulated that despite the chronicity of symptoms in fibromyalgia, there are fluctuating levels of biomarkers even within each day. To investigate these daily fluctuating biomarker levels, Fischer et al conducted a 14-day study on 32 female patients with fibromyalgia who recorded diary entries on momentary stress and pain levels, and collected samples during those respective for measurement of cortisol and alpha-amylase.⁸⁰ The study reported that momentary cortisol was associated with momentary pain, supporting that cortisol may impact diurnal fluctuation of pain levels in fibromyalgia patients.

In addition to cortisol, other notable salivary biomarkers were investigated in fibromyalgia patients. Bazzichi et al conducted a proteomic analysis of saliva in 22 fibromyalgia patients and 26 age- and sex-matched healthy control patients.⁸³ This study reported significant overexpression of salivary transaldolase, calgranulin A and C, and phosphoglycerate mutase I. To further assess changes in salivary biomarkers with treatment in fibromyalgia patients, another pre- and post-treatment study by Bazzichi et al measured phosphoglycerate mutase I levels in fibromyalgia patients who received mud-bath therapy (n=21) and balneotherapy (n=20).⁸⁴ This study reported

significant reductions in phosphoglycerate mutase I levels after both mud-bath and balneotherapy.

Similar to fibromyalgia, another manifestation of spontaneous pain with hyperalgesia, referred pain, and association with psychological comorbidities like depression and anxiety is temporomandibular disorder (TMD) myalgia.⁸² This leads to chronic masticatory pain and is three times more frequent in females compared to males.⁸² Jasim et al compared salivary samples from 39 patients with TMD and 39 pain-free healthy control patients.⁸² This study reported that TMD patients had both higher salivary and plasma levels of glutamate compared to healthy control patients. In addition, salivary nerve growth factor (NGF) and salivary brain-derived neurotrophic factor were decreased in TMD patients compared to healthy control patients.

Burning mouth syndrome is also a spontaneous pain manifestation characterized by intraoral burning and dysesthesias without any evidence of clinical lesions.⁸⁹ The symptoms are predominantly localized to the tongue and lips, and affected patients commonly have comorbid personality changes and mood disorders.⁸¹ Lopez-Jornet et al analyzed inflammatory biomarkers in saliva samples from 51 patients with burning mouth syndrome and compared them to saliva samples from 31 healthy control patients.⁸¹ In addition to increased salivary flow, salivary analysis in patients with burning mouth syndrome revealed increased levels of alpha-amylase, immunoglobulin A (IgA), macrophage inflammatory protein-4 (MIP4) compared to controls. Furthermore, there were decreased levels of uric acid and ferric reducing activity of plasma (FRAP) in patients with burning mouth syndrome versus controls. The authors conclude that burning mouth syndrome may affect inflammatory markers within the saliva and the resulting oxidative stress may correlate with the severity of pain.

Saliva also contains a variety of neuropeptides because of salivary gland innervation from nerves originating from the trigeminovascular system and thus can provide insight into nervous system pathology and various headache disorders.⁸⁵ Nam et al collected salivary samples from 46 females with chronic migraine, 50 females with episodic migraine, and 19 healthy control patients and compared salivary glutamate levels.⁸⁵ Glutamate is known to be one of the most abundant excitatory neurotransmitters present in the central nerve system and may be implicated in the pathogenesis of cortical spreading depression, activation of the trigeminal neuron, and central sensitization.⁹⁰ Mean salivary glutamate levels were significantly higher in the

chronic migraine cohort compared to the episodic migraine patients and control patients. The salivary glutamate level in the episodic migraine cohort was only marginally elevated compared to control patients.

Pulpal or periapical inflammation may lead to acute dental pain and may also be associated with increased expression of inflammatory biomarkers in the saliva. Haug et al compared 42 patients with pulpal or periapical inflammation seeking emergency dental treatment and 39 control patients.⁷⁹ After biomarker analysis of unstimulated saliva collection, results demonstrated increased levels of cortisol, IL-1 β , IL-6, as well as greater salivary flow in patients with pulpal or periapical inflammation versus control patients. Another study by Aurer et al measured inflammatory markers in the saliva from 9 edentulous patients, 10 chronic periodontitis patients, 18 aggressive periodontitis patients, and 14 health control patients.⁸⁶ Decreased levels of C-reactive protein (CRP) and alpha-2-macroglobulin (alpha-2M) were detected in edentulous patients and patients with chronic periodontitis. Thus, this study concludes that a reduction in host responsiveness in secreting inflammatory markers may play a role in the pathology of chronic manifestations of periodontitis.

Summary

The studies reviewed suggest that specific urinary, serum, cerebrospinal fluid, and salivary biomarkers may help identify those patients at risk of disease development, and function as a prognostic indicator for disease progression and treatment response. However, current individual and panel-based biomarkers have limited usability in regards to informing clinical decision-making at this time.

Future Directions

There is no doubt that the number of candidate biomarkers for complex disease, including chronic pain, will continue to increase as high throughput 'multi-omics' platforms become commonplace in government, academic, and industry research and development settings. Emerging high-efficiency techniques now allow for single-cell analysis and spatial metabolomics/transcriptomics, which will almost certainly further our understanding of the role of specific biomarkers and immune cells in chronic pain disorders.

Successful validation of candidate biomarkers is a rigorous, expensive, and continuous process that requires multiple, carefully designed clinical studies to evaluate performance criteria across the wide spectrum of chronic pain disorders. Throughout the evaluation process, individual candidate biomarkers and biomarker composite

panels must demonstrate clinical validity and utility if the outcome is to become part of routine clinical care. While the path from biomarker discovery to clinical adoption is often complex, slow, and expensive, few would question the potential impact that robust pain biomarkers would have on the field of pain management.

Additionally, as Backryd described in 2015, sub-classification of pain (ie, neuropathic, nociceptive, and nociplastic) and pertinent biomarkers should be an area of focus.⁹¹ Given that many chronic pain treatments are tailored to specific pain sub-classifications, the ability to collect and monitor unique biomarkers is imperative.

Conclusion

While the use of objective laboratory measures has become commonplace in other areas of medicine, it has been relatively lacking in the field of chronic pain medicine. Ultimately, a biomarker pain panel that highlights metabolic abnormalities, oxidative stressors, and nutritional deficiencies may help to identify the underlying treatable causes of pain and potentially provide evidence of treatment response. Certainly, additional research is necessary before specific recommendations can be made, but the potential benefits for the treatment of chronic pain make this an important and necessary area of future study.

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