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Review Article

Diagnostic Biomarkers for Upper Extremity Chronic Pain Conditions

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Hand surgery patients often experience chronic pain conditions. However, there are few reliable ways to measure pain, making diagnosis and subsequent management of these conditions notably challenging for the hand surgeon. Various diagnostic biomarkers have been actively studied in the chronic pain management field with promising results. This review discusses the development of diagnostic biomarkers for chronic pain conditions of the upper extremity, including complex regional pain syndrome, osteoarthritis, and neuropathic pain. Techniques involving the measurements of heart rate variability, molecular biomarkers including inflammatory and noninflammatory molecules, metabolites, and exosomes, magnetic resonance imagining and electroencephalography, as well as skin biopsy, are discussed. Future potential applications are proposed.

The International Association for the Study of Pain defines pain as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage."[1] It is important to distinguish pain, a subjective phenomenon affected by psychological, sociological, and cultural factors, from nociception that represents the encoding and processing of noxious stimuli by the central nervous system.[2] Pain may thus be viewed as the cognitive and emotional response to nociception and may exist without nociception, and vice versa. The psychological input on the pain experience cannot be understated. In patients with complex regional pain syndrome (CRPS), for example, depression levels are predictive of greater pain severity, and patients with CRPS display higher levels of emotional distress than healthy persons.[3]

Acute pain, such as pain following surgery, has a rapid onset and quick resolution. Acute pain is distinct from chronic pain, which persists beyond the normal expected time of tissue healing, which is typically within 2 months after the initial insult.[4] Acute tissue injury, such as trauma, surgery, or thermal injuries, results in the activation of peripheral pain receptors, along with a local and systemic inflammatory response, and leads to complex biochemical changes that are usually reversible once tissue healing has occurred. In some patients, acute pain does not resolve and instead progresses to chronic pain; this is thought to be because of alterations in pain processing, both at the peripheral and central levels.[4]

Patients with chronic pain conditions present a quandary for hand surgeons. Currently, the diagnosis of chronic pain conditions such as CRPS depends on the presence of clinical factors with no agreed upon standardized criteria, leaving hand surgeons with no reliable tools to adequately diagnose and manage these challenging patients. The identification and implementation of diagnostic biomarkers—defined as measurable elements in an organism indicative of the presence of disease—for chronic pain conditions has been an active area of research, with great potential for improved identification, understanding, and management of patients with chronic pain. Such biomarkers may also help eliminate the stigma associated with chronic pain and help foster improved care. In this narrative review, we present advancements in biomarkers for chronic pain conditions with an emphasis on clinically relevant conditions for hand surgeons,

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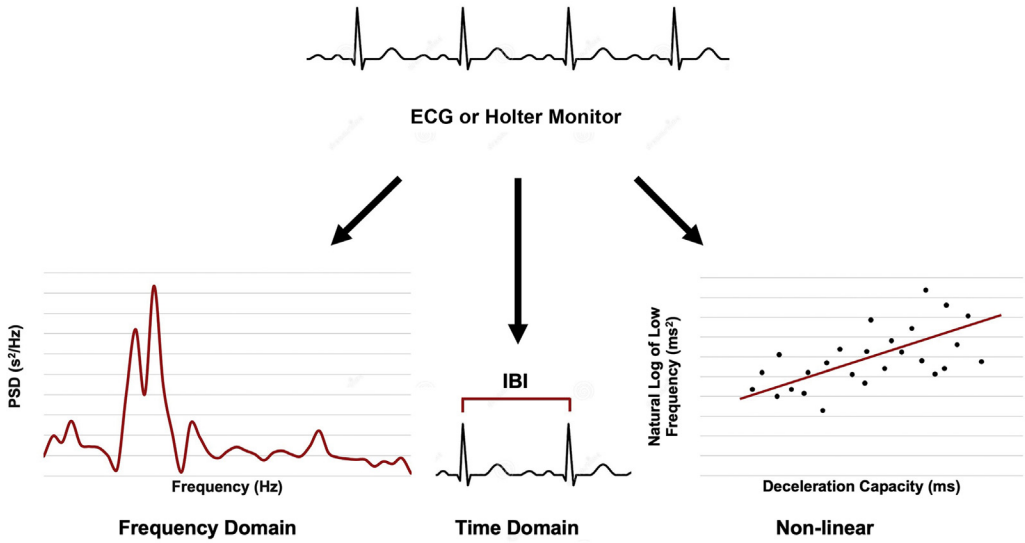

Figure. Modalities for analyzing the HRV. Frequency domain measurements estimate the distribution of power into 4 frequency bands: the ultralow frequency band (<0.003 Hz), very LF band (0.003–0.04 Hz), LF band (0.04–0.15 Hz), and HF band (0.15–0.4 Hz). The LF band corresponds to sympathetic input, whereas the HF band corresponds to parasympathetic input. Power spectral density (seconds^2/Hz) is the signal energy found in a particular band and can be calculated by summing the area under the curve for a specific frequency range (band). Time domain indices of HRV quantify the amount of variability in measurements of the IBI, which is the time between successive heartbeats. This may be measured as the SD or the root mean square of the IBI (time between successive R waves or R-R interval) on an ECG. Nonlinear analysis involves logarithmic modeling and focuses on 3 clinically relevant measurements: heart rate turbulence that measures the heart rate response to premature beats, deceleration capacity (milliseconds) that measures the ability of the heart to decelerate in certain time scales, and fractal scaling that analyzes fractal organization of the heart.

with the caveat that the research is still in its infancy with little direct clinical applications presently available.

Vital Signs and Heart Rate Variability

Pain signal processing and the autonomic nervous system are interconnected neural elements. In response to noxious stimuli, the sympathetic nervous system (SNS) output increases, whereas the parasympathetic nervous system (PNS) output decreases, leading to increased blood pressure, heart rate, pupillary dilation, diaphoresis, and heightened attention, among other responses.[2] In patients with chronic pain, studies have identified a positive correlation between the presence and intensity of pain and comorbid hypertension.[5] However, given that hypertension is prevalent in the general population and associated with other factors, blood pressure is not a specific marker for chronic pain. The same lack of specificity for chronic pain conditions may be applied to the association with resting heart rate, which is influenced by both SNS and PNS input, among a multitude of other factors.[6] Subsequently, studies have focused on a different cardiac biomarker for chronic pain—heart rate variability (HRV).

Heart rate variability is a measure of the variation in the time interval (ie, interbeat interval [IBI]) between consecutive heartbeats. Heart rate variability attempts to tease out the individual contributions of SNS and PNS autonomic reactivity to nociception and has been proposed as a more specific biomarker of acute and chronic pain rather than blood pressure and heart rate alone.[2] Heart rate variability is most commonly measured by obtaining an electrocardiogram (ECG) or with a Holter monitor, and software is used to analyze the IBI sequences.[2] Three main methods of quantifying HRV exist: frequency domain, time domain, and nonlinear. Frequency domain involves generating a curve based on the ECG input that displays how often certain frequencies of IBIs are detected in an ECG sequence (Fig.). Spectral densities are then quantified within predefined bands by calculating the area under the curve for the band of interest. The clinically relevant bands include both high-frequency (HF) and low-frequency (LF) bands that correspond to the PNS and SNS inputs, respectively. Time domain measures include measuring the standard deviation (SD) or the root mean square of the SD (RMSSD) of an IBI sequence within a time interval. Nonlinear analysis involves logarithmic modeling and focuses on the following 3 clinically relevant measurements: (1) heart rate turbulence that quantifies heart rate response to premature beats, (2) deceleration capacity that estimates the ability of the heart to decelerate on specific time scales, and (3) fractal scaling that analyzes the fractal organization of heart rate regulation.[7]

A recent meta-analysis of patients with chronic pain conditions, including musculoskeletal pain and CRPS, found that patients with chronic pain in general displayed no difference in LF-HRV, a decrease in HF-HRV, and an increased LF to HF-HRV ratio, compared with healthy controls, suggesting autonomic dysfunction and reduced parasympathetic output as the primary homeostatic alterations.[8] The study also noted that patients with CRPS and diabetic neuropathy specifically had reduced IBI and SD and reduced RMSSD, respectively, compared with healthy controls (Table).[8] A study evaluating HRV in patients with CRPS reported decreased SD and RMSSD in patients with CRPS compared with controls.[6] The same study also noted a nonsignificant increase in LF-HRV, decrease in HF-HRV, and increase in the LF to HF-HRV ratio in this CRPS cohort; these values did not attain statistical significance, perhaps secondary to a lack of power. An additional study did find a significantly higher LF to HF-HRV ratio in patients with CRPS than in healthy controls, further associating autonomic disturbance in the pathogenesis of CRPS.[9]

Heart rate variability is a promising diagnostic biomarker for chronic pain conditions that is supported by a growing body of literature. The primary barrier to clinical application, besides additional confirmatory studies for specific conditions treated by hand surgeons, is the ease and rapidity of HRV measurement and analysis. It is a difficult measure to use as a spot check and perhaps may be better for tracking pain over time. Additionally, although

Table
Diagnostic Biomarkers for Hand-Related Chronic Pain Conditions

Condition	Molecular Biomarkers (Inflammatory Markers, Metabolites, Exosome Contents)	Nonmolecular Biomarkers (HRV, structural MRI, functional MRI, EEG, skin biopsy)
CRPS	↑Osteoprotegerin ↓ Bilirubin + IgG antibodies[*] ↑ Glutamate, aspartate ↓ Tryptophan, arginine ↑ miR-21-3p, miR-146a, and miR-146b	↓ IBI, SD, RMSSD ↑ LF:HF-HRV ↓ Dorsal insula, left orbitofrontal complex, cingulate cortex thickness ↓ Functional connections in the forebrain areas related to motor, affective, cognitive, and pain inhibitory/modulatory processes ↑ TNF-α, IL-6, mast cell accumulation[‖]
Osteoarthritis	↑IL-7, IL-8, IL-10, IL-12, IL-13, interferon-γ, C-reactive protein, C-reactive protein metabolite, C-X-C motif chemokine ligand 10, MMP-3 MMP-13, tissue inhibitor of metalloproteinase-1, C-terminal cross-linked telopeptide of type II, cartilage oligomeric matrix protein, brain-derived neurotrophic factor ↓ IL-1b ↑ Fructose, citrate ↓O-acetylcarnitine, N-phenylacetylglycine, methionine, ethanol, creatine, malate, ethanolamine, 3-hydroxybutyrate, hexanoylcarnitine[†] ↓ arginine ↓ Production TNF-α, IL-6, prostaglandin-E2, nitric oxide ↑ Production IL-10	↓ Prefrontal motor cortex gray matter volume ↓ Spontaneous brain activity in the prefrontal limbic area ↓ Functional connectivity between the dorsolateral prefrontal cortex and the pain matrix[¶]
Neuropathic pain	↑ Haptoglobin isoforms[‡] ↓ Transthyretin and α2 macroglobulin[‡] ↑ Fos, Tp53, Csk, and Map2k2[‡] ↑ Phosphocholine, alanine, and taurine[§] ↑ Ccl3 ↑ Complement 5a, intercellular adhesion molecule 1[‡]	↓ RMSSD ↑ Small, unmyelinated fibers

[*] Activate β2 adrenergic, M2 muscarinic receptors.
[†] Synovial fluid, rather than serum, for sample.
[‡] Mouse model.
[§] Urine, rather than serum, for sample.
[‖] Acute, not chronic, CRPS.
[¶] Pediatric population.

ECGs and Holter monitors are readily available, the software and personnel needed for HRV analysis are not readily available to most hand surgeons. There have been recent developments of inexpensive and free software tools available for broad use in HRV analysis; nevertheless, they have not yet been thoroughly validated for clinical diagnostic purposes.

Molecular Biomarkers

Molecular biomarkers may be found in the tissue, blood, urine, synovium, and/or other body fluids and include inflammatory and noninflammatory mediators. Metabolites have been studied as potential biomarkers of chronic pain conditions.[10] Additionally, exosomes—nanosized extracellular vesicles that carry proteins, lipids, and nucleic acids important in cellular communication—may serve as diagnostic markers and therapeutic targets for various chronic pain conditions.[11]

Patients with osteoarthritis (OA) have pain with joint movement that may occur at any stage of disease progression.[12] Initial OA pain is derived from a nociceptive response to joint damage, whereas later changes in the severity and location of pain result from peripheral sensitization that occurs when proinflammatory cytokines at the joint increase the reactivity of nociceptors.[12] Central sensitization may also occur over time.[12] Given that inflammation has been implicated in both the onset and progression of OA pain, it is not surprising that a positive correlation exists between serum levels of proinflammatory cytokines, interleukin (IL) 7, IL-8, IL-12, IL-13, interferon γ, C-reactive protein, and C-X-C motif chemokine ligand 10. Interestingly, both positive and negative correlations between pain severity and serum levels of the proinflammatory markers IL-6 and tumor necrosis factor alpha (TNF-α) and a negative correlation between pain severity and serum levels of IL-1b have been reported (Table).[12] IL-10, an anti-inflammatory marker, has been positively correlated with OA pain, suggesting some self-modulation of the inflammatory response.[12] Matrix metalloproteinases (MMPs) including MMP-3 and MMP-13, markers of cartilage/bone turnover including tissue inhibitor of metalloproteinase-1, C-terminal cross-linked telopeptide of type II collagen, and cartilage oligomeric matrix protein, as well as additional growth factors and adhesion molecules have been positively correlated to pain severity.[12] Levels of C-reactive protein metabolite increased significantly with the degree of central sensitization, and brain-derived neurotrophic factor has been reported as a reliable indicator of central sensitization.[12]

With regard to metabolites, the synovial fluid of patients with chronic symptomatic OA have elevated levels of fructose and citrate and decreased levels of O-acetylcarnitine, N-phenylacetylglycine, methionine, ethanol, creatine, malate, ethanolamine, 3-hydroxybutyrate, and hexanoylcarnitine.[10] Significantly depleted serum arginine levels were found in a separate cohort of patients with knee OA.[10] Exosomes derived from mesenchymal stem cells in patients with OA carry substances that decrease the production of inflammatory mediators, including TNF-α, IL-6, prostaglandin-E2, and nitric oxide, and increase the production of the anti-inflammatory marker IL-10, in the chondrocytes.[11]

Complex regional pain syndrome typically occurs after an inciting injury to the upper extremity and presents with persistent pain and hyperalgesia not explained by the initial injury. Persistent inflammation leads to peripheral sensitization; some mediators can sensitize neurons in the spinal cord, eventually leading to central sensitization.[13] Given the different types of CRPS, the variable symptomatology, and temporal disease progression, a single diagnostic biomarker is unlikely.[13] Rather, research has focused on biomarkers that predict the development of CRPS after trauma and characterize the early and late stages.

Studies have not found elevated serum levels of proinflammatory cytokines (eg, TNF-α) in patients with predominately

early CRPS compared with patients with upper extremity fractures or upper extremity pain of other origin (Table).13 One study did report elevated serum levels of osteoprotegerin, a cytokine receptor involved in regulating bone turnover, in patients with CRPS compared with healthy controls and to patients who had normal healing after a long bone fracture (sensitivity = 74%, specificity = 79% for the diagnosis of CRPS).13 Patients with CRPS also have lower levels of serum bilirubin compared with healthy controls.[14] Two groups have shown that approximately 70% of patients with CRPS have the presence of antiautonomic immunoglobulin G serum antibodies that activate β-2 adrenergic or M2 muscarinic receptors.[13] With regard to metabolites, changes in plasma levels of amino acids involved in glutamate receptor activation were reported in patients with CRPS; specifically, increased levels of glutamate and aspartate, decreased levels of tryptophan, an increase in the kynurenine to tryptophan ratio, and a decrease in arginine levels were reported.[10] Finally, serum derived exosomes were found to have significantly different levels of expression of 127 different micro RNAs in patients with CRPS compared with healthy controls; 3 particular micro RNAs (miR-21-3p, miR-146a, and miR-146b), known to be involved in overactivation of the innate immune response, were found to be overexpressed.[11]

Neuropathic pain, characterized by the presence of allodynia, spontaneous pain, sensory loss, and/or hyperalgesia, may occur after nerve injury.[15] As observed in patients with chronic OA pain and CRPS, neuropathic pain involves both peripheral and central sensitization.[16] One study reported significantly elevated serum levels of 4 different haptoglobin isoforms and significantly decreased serum levels of transthyretin and α-2 macroglobulin in nerve compression model mice compared with healthy controls (Table), with normalization of levels in compression model mice treated with gabapentin.[16] Another study noted changing gene expression levels of Fos, Tp53, Csk, Map2k2, Stat3, Ccl2, Pxn, Tgfb1, Notch1, and Prkabc, among others, in both nerve compression and nerve transection mice compared with healthy controls.[15] Metabolomic studies have noted increased levels of phosphocholine, alanine, and taurine in the urine of patients with neuropathic pain.10 Ccl3, a chemokine potentially mediating peripheral and central sensitization in neuropathic pain, was identified as being transferred in exosomes from Schwann cells to peripheral blood in patients with neuropathic pain.[11] Furthermore, there is significant upregulation of complement 5a and intercellular adhesion molecule 1 in exosomes of spared nerve injury mice compared with sham controls.[11]

Inflammatory and noninflammatory molecules, metabolites, and contents of exosomes have potentially promising applications as diagnostic biomarkers for chronic pain conditions. Primary barriers to clinical implementation include the identification of expression patterns specific to particular pain conditions (eg, OA, CRPS, and neuropathic pain). Additionally, although most hand surgeons will have access to common laboratory tests, the evaluation of most metabolites and exosome contents require highly specific assays and other molecular tools not available in a typical hand surgery practice.

Neuroimaging—Magnetic Resonance Imaging and Electroencephalography

Central sensitization—long-term brain reorganization that leads to altered responses to nociception—is a key concept underlying chronic pain research and why structural magnetic resonance imaging (MRI), functional MRI, and electroencephalography (EEG) have been studied.[17] Structural MRI provides information regarding the anatomical features of central nervous system gray and white matter.[18] Patients with various chronic pain conditions have structural abnormalities in the medial prefrontal cortex, hippocampus, postcentral gyrus, and the inferior frontal gyrus, compared with healthy controls (Table).[18] Patients with OA were found to have predominately lower volumes of prefrontal motor cortex gray matter (Table).[18] For patients with CRPS, both positive and negative correlations with pain intensity and cortical thickness in the dorsal insula, left orbitofrontal cortex, and cingulate cortex have been described.[13] The diagnostic accuracy of structural MRI ranges from 68% to 84% for patients with various chronic pain conditions, with sensitivity ranging from 65% to 83% and specificity ranging from 67% to 75%.[19]

Functional MRI detects central nervous system signal changes associated with perfusion, since neuronal activation and cerebral blood flow are highly coupled processes.[18] The primary form of functional MRI is blood oxygen level–dependent functional MRI, and it measures small changes in blood oxygenation. Because increased neuronal activity is associated with increased metabolic demand, increased blood oxygenation serves as a proxy for increased neural activity. For patients with chronic pain conditions, functional MRI differences can be seen in the insula, postcentral gyrus, thalamus, middle cingulate cortex, amygdala, hippocampus, and posterior cingulate cortex.[18] Patients with OA also have disrupted spontaneous brain activities in the prefrontal limbic regions, as well as disrupted functional connectivity between the dorsolateral prefrontal cortex and the pain matrix (Table).[18] In pediatric patients with CRPS, functional connections in the forebrain areas related to motor, affective, cognitive, and pain inhibitory/modulatory processes are reduced.[13] The diagnostic accuracy of resting state functional MRI for patients with various chronic pain conditions ranges from 58% to 96%, with sensitivity and specificity ranging from 68% to 83% and from 56% to 90%, respectively.[19]

Electroencephalography detects electrical activity in the brain. Waveforms are measured via a network of transcutaneous electrodes on the scalp. Quantitative EEG (brain mapping) measures electrical activity represented by waveforms.[20] Quantitative EEG evaluates 4 basic frequency waves associated with different levels of cortical arousal: beta (14–30 Hz, awake states with high levels of environmental stimulation), alpha (8–13 Hz, awake states with low levels of environmental stimulation), theta (4–7 Hz, light sleep states), and delta (1–3 Hz, deep sleep). A meta-analysis examining quantitative EEG findings for patients with neuropathic pain, fibromyalgia, migraines, musculoskeletal pain, and chronic abdominal pain, among others, evaluated power density and peak frequencies and noted increased theta, alpha, beta, and delta band power at rest in these patients compared with healthy controls.[20] Finally, most studies cited in a meta-analysis did reveal that patients with chronic pain had enhanced power spectra at the frontal and parieto-occipital electrode locations.[20] In patients with chronic pain conditions, the diagnostic accuracy of EEG ranges from 83% to 92%.[19]

Structural MRI, functional MRI, and quantitative EEG represent potentially useful tools for detecting chronic pain and/or tracking it longitudinally. Within these modalities, more specific markers for upper extremity chronic pain conditions, including neuropathic pain/CRPS and osteoarthritis, must be developed to be clinically useful for the hand surgeon. Furthermore, EEG may be challenging for hand surgeons who do not have a relationship with a neurology practice. Advanced MRI may also not be readily available.

Skin Biopsy

Skin biopsies have been used to evaluate for tissue markers that correlate with the presence and severity of chronic pain conditions. Skin biopsies are typically performed using a 3-mm full thickness punch that is sent for processing and immunohistochemical

staining.[21] In patients with acute CRPS, the upregulation of TNF-α and IL-6 and mast cell accumulation were observed in skin samples from the affected extremity compared with the contralateral unaffected extremity (Table).[13] These differences were not seen in patients with chronic CRPS compared with healthy controls; instead, a downregulation of Langerhans cells was reported.[13] Skin biopsy may also be useful for the early diagnosis of neuropathic pain. Small fiber neuropathy, or the loss of small, unmyelinated fibers, may precede the loss of large fibers in patients with impaired glucose tolerance or diabetes mellitus, and studies have noted that small fiber neuropathy in skin biopsy was detected in such patients despite normal large fiber testing.[21] Although skin biopsies may represent an attractive modality for evaluating diagnostic chronic pain biomarkers, their invasive nature and need for postbiopsy staining, processing, and interpretation may limit practical applicability.

Genetics

Genetic and epigenetic factors have been studied as contributors to the development of upper extremity chronic pain conditions. It is well established that OA has a large genetic component of its multifactorial etiology.[22] Numerous genes, including those coding for cartilage oligomeric matrix protein, chondroadherin-like protein, and type XI collagen have been implicated.[22] However, most risk variants for OA occur in the nonprotein coding regions of the genome, suggesting that OA is primarily driven by changes to regulation of gene expression rather than to protein structure itself.[22] Epigenetic changes, or changes to the genome without alterations to the DNA sequence, including DNA methylation and histone modification, have been implicated as well.[22] Some patients also have a genetic predisposition for CRPS.[3] Studies of familial aggregation of CRPS support that CRPS may be heritable.[3] Other evidence includes associations between the presence of particular human leukocyte antigen (HLA), notably HLA-DQ1, HLA-DQ8, HLA-DR6, and HLA-DR-13, and HLA-B62, and the development of CRPS.

In conclusion, pain management is a critical component of upper extremity surgery practice. This is especially true, considering the prevalence of OA and the notable challenges presented by patients with CRPS and neuropathic pain. The development of diagnostic biomarkers for chronic pain conditions frequently encountered by hand surgeons, although still nascent, present an exciting potential for improved diagnostic accuracy for these complex, variable, and poorly understood problems. Such diagnostic modalities could serve to improve care, not only in identifying the chronic pain condition but also in tracking progress over time as various treatments are attempted. Nevertheless, the techniques involving the measurement of HRV, molecular biomarkers, and functional MRI/EEG modalities need further refinement before they can be applied clinically. Condition-specific diagnostics must be developed and evaluated. Additional barriers to clinical implementation include access to modalities (eg, MRI) and temporal delays from the time of testing to analysis and ultimate results. How we can connect the identification of biomarkers and physiologic changes to subsequent treatments is not well understood. Nevertheless, considering the resources required to treat patients with chronic pain conditions and the difficult life experiences that these patients endure, any opportunity to improve the diagnosis and management for these patients could have substantial impact and importance.

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