Laboratory Evaluations for the Chronic Pain Patient

'Recommendations for a Standardized, Laboratory Based Workup for Pain Management'

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Abstract

Recent reviews indicate that total societal costs of chronic pain in the United States range from $560 to $635 billion annually. The cost of chronic pain due to direct medical treatments and lost productivity represents a greater economic burden than many of the nation's priority health conditions such as heart disease, cancer and diabetes (1). Despite the soaring costs of treating chronic pain, complete relief is uncommon due to the limited efficacy of current treatments. Subjective ratings have played a key role in the diagnosis and treatment of pain but profound individual differences in sensitivity complicate diagnosis and treatment (2). Due to the subjective nature of current pain assessments, limited efficacy of treatment options and risks associated with opioid abuse and diversion, the need for objective data to assist with chronic pain management has never been greater. Although substantial advances in our understanding of chronic pain pathophysiology have been made in recent decades, most of the research has identified relevant biochemical pathways and/or biomarkers with the aim of developing novel pain therapies. While these research efforts will most certainly prove beneficial to the pain community in years to come, it is imperative that clinical laboratories move toward developing pain specific laboratory evaluations which allow physicians to objectively identify underlying causes of chronic pain. A pain specific 'workup' which evaluates various biochemical pathways that are known to directly impact the development, worsening, and perception of pain will

provide pain practitioners with novel objective data and new modes of personalized pain treatment.

Introduction - Biomarkers of Pain

By definition, a biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic response to a therapeutic intervention (3). Biomarkers are employed across most medical specialties for purposes including but not limited to; identifying patients at risk of developing disease; disease diagnosis; prognosis; evaluation of treatment response and early stage drug development. Certain types of biomarkers are even employed as surrogate endpoints for clinical trials. Given the biopsychosocial complexity of chronic pain and the frequency of comorbid diagnoses related to depression and anxiety, it is not surprising that biomarker discovery related to physical pain has lagged behind other specialties in recent decades. Some researchers have even contested the validity of the search and concluded that finding biomarkers for pain is a sheer impossibility as pain, by definition, is a subjective experience (4). Most, if not all clinical researchers would agree that the experience of pain is always subjective and will never be quantifiable. Rather, the search for pain biomarkers is focused on identifying objective, measurable correlates to the neurobiological processes underlying painful conditions with the aim of enabling chronic pain diagnoses and treatments to be based on underlying pathophysiological mechanisms rather than symptomology (5-7). Successful identification of mechanistic biomarkers of pain (markers that reveal which pathophysiological mechanism is responsible for the pain) would not only improve our understanding and ability to accurately diagnose pain disorders but could also pave the way for the development of disease-modifying pain drugs.

While the endeavor to discover clinically suitable biomarkers of pain is no doubt a challenging journey, several things are clear: The chronic pain experience will always be subjective and no biomarker will ever replace patient self-report. Rather, mechanistic biomarkers of pain would provide physicians with objective information pertaining to the biochemical pathway responsible for the discomfort. Only with this information will providers be able to move away from the trial-and-error symptom control model and begin recommending medications and interventional procedures that will modulate the course of disease (5).

Objective biomarkers are the core elements of personalized medicine and the identification and validation of markers to assist with the diagnosis and treatment of chronic pain would significantly reduce healthcare costs worldwide. While the successful identification of any pain specific biomarker signifies an advancement in our understanding of pain pathophysiology, the most important and impactful biomarkers are likely to be those that can be modulated to change the course of disease. Our objective is to describe a panel of well-defined biomarkers chosen to assist physicians with determining the biochemical origins of pain. The panel is shown in Table 1, and each biomarker is discussed below. This panel evaluates markers of essential micronutrients critical for nerve health, chronic inflammation, oxidative stress/damage and neurotransmitter turnover. Abnormalities provide information about possible origins of neuropathic pain, inflammatory pain and altered pain perception. Each biomarker is directly linked to a pain relevant pathway such that abnormal results may reveal the biochemical basis for a patient's pain and indicate treatment strategies. Abnormal blood or urinary levels of these functional pain biomarkers can be corrected with safe, cost effective therapies which will

increase the likelihood of successful and prolonged pain management. Follow up testing should be ordered to ensure that the therapy of choice corrected any abnormal findings.

Biomarker	**Indication**
Methylmalonate	Vitamin B_{12} deficiency
Xanthurenate	Vitamin B_6 deficiency
Homocysteine	Deficiencies of folate, B_{12} or B_6
Quinolinate	Brain kynurenine pathway and NMDA agonization
Kynurenate	Aids interpretation of Xanthurenate and Quinolinate
Pyroglutamate	Glutathione response capacity
Vanilmandelate	Epinephrine & norepinephrine turnover
5-Hydroxyindoleacetate	Serotonin turnover

Table 1. Pain Management Laboratory Biomarker Profile.

Methylmalonic Acid – Methylmalonic acid (also known as MMA or Methylmalonate) is a sensitive and highly specific functional marker for the assessment of Vitamin B12 (cobalamin) status. Vitamin B12 status is an especially important consideration for pain practitioners as deficiencies of this essential vitamin can cause demyelination of nerves leading to painful neuropathies, axonal death and subacute combined degeneration of the spinal cord (8). Low levels of Vitamin B12 have been linked to polyneuropathy, trigeminal neuralgia, migraine, depression, mania, optic nerve atrophy, neuropsychiatric disorders, and various functional disabilities (9-13). Deficiencies in vitamin B12 can also result in elevated levels of homocysteine in the blood and urine due to its crucial role as a cofactor in amino acid metabolism. Elevated levels of homocysteine have been recognized as an independent risk factor for cardiovascular disease and stroke. An early diagnosis of vitamin B12 deficiency is crucial as response to treatment is dependent on the extent of nerve damage and the timing of replacement therapy (14).

The importance of screening chronic pain patients for vitamin B12 deficiency cannot be overstated due to the prevalence of malabsorption leading to deficiency. Other risk factors for Vitamin B12 deficiency include the use of medications such as proton pump inhibitors (PPIs), H2-receptor antagonists, metformin, colchicine, cholestyramine, and frequent or long-term use of anticonvulsants and/or antibiotics (10). Gastric surgery, intestinal bacterial overgrowth, hypothyroidism, diabetes and aging represent other risk factors for vitamin B12 deficiency. Due to the large number of medications (both prescription and over-the-counter), medical conditions and lifestyle choices that can precipitate vitamin B12 deficiency, combined with the ease, cost and effectiveness of replacement therapy, widespread screening is recommended within the chronic pain population. In addition to replenishing Vitamin B12 stores, treatment with various forms of cobalamin has been shown to provide pain relief, alleviate pain behaviors, improve nerve conduction and exert neuronal protection by promoting regeneration of injured nerves and antagonizing glutamate-induced neurotoxicity (15-20).

Evaluating levels of Methylmalonic acid is the most sensitive and specific method for assessing vitamin B12 status. Normalized urinary Methylmalonic acid levels greater than or equal to 2.3 µg/mg of creatinine are indicative of a functional vitamin B12 deficiency. Individuals with levels in the ‘high normal range’ should be monitored and retested or instructed to begin supplementation in order to decrease the likelihood of them becoming deficient. Replacement therapy for vitamin B12 deficiency is safe, convenient and cost effective. Options include oral, sublingual and/or intramuscular formulations. If malabsorption (opposed to inadequate intake of animal products) is suspected to be the precipitating factor for the deficiency, intramuscular injections will likely be the most effective formulation for replenishment.

Treatment Options for Abnormal Methylmalonic Acid

Result	Treatment*	Dosage
HIGH	Vitamin B12 as Methylcobalamin	2000 mcg/day
HIGH NORMAL	Vitamin B12 as Methylcobalamin	500 mcg/day

*HIGH Methylmalonic acid levels can also be treated with intramuscular injections. Dosage and frequency information presented in this table are for oral supplementation.

Xanthurenate – Xanthurenate (also known as Xanthurenic acid) is a sensitive marker of Vitamin B6 (pyridoxine) status. It is a byproduct of the hepatic kynurenine pathway that is strongly dependent on adequate pyridoxal phosphate, the active form of vitamin B6. Vitamin B6 is an essential vitamin required for the synthesis of proteins (including neurotransmitters such as serotonin and norepinephrine), the formation and integrity of the nerve insulating myelin sheath, the production of anti-inflammatory mediators and immune system function. A deficiency of vitamin B6 can cause peripheral neuropathy, migraine, chronic pain, depression, seizures and other neuropsychiatric disorders (21-23). Due to its central role in nerve health and function, an optimal level of vitamin B6 is necessary for successful and prolonged pain management and screening for deficiencies should be part of a laboratory based pain workup. While the direct impact of vitamin B6 deficiency on nerve health and function has been recognized for decades, recent research indicates that inflammation and the inflammatory process may actually drive a tissue-specific depletion of vitamin B6. Investigators conclude that the low circulating levels of vitamin B6 commonly seen in patients with inflammatory disease may result from the removal of vitamin B6 from circulation to meet the higher demand within certain tissues during the inflammatory process (24). This critical link between the inflammatory process and vitamin B6

status may explain why circulating B6 levels appear to exhibit an inverse correlation with the degree of pain in patient populations.

Evaluating levels of Xanthurenate is a sensitive and selective method for assessing vitamin B6 status. The metabolic conversion of Xanthurenate along the kynurenine pathway is heavily dependent on vitamin B6 such that a deficiency or insufficiency will manifest as an accumulation of Xanthurenate. In addition to its role as a marker of vitamin B6 status, recent research indicates that elevated levels of Xanthurenate, and therefore vitamin B6 insufficiency, may play a central role in the development of insulin resistance and its progression to type 2 diabetes (25, 26).

Treatment Options for Abnormal Xanthurenate

Result	Treatment	Dosage
HIGH	Vitamin B6 as pyridoxal-5-phosphase	50 mg/day

Homocysteine – Homocysteine is a sulfur amino acid which serves as a functional biomarker of B-vitamin (Folate, B6 and B12) status and as an individual risk factor for the early development of cardiovascular disease. Homocysteine metabolism can progress along two metabolic pathways, remethylation to methionine, which requires folate and vitamin B12 or transsulfuration to cystathionine, which requires vitamin B6. Due to the key role of these three B-vitamins in the metabolism and recycling of homocysteine, elevated urinary levels of this amino acid can indicate functional deficiencies of folate, vitamin B6 and/or vitamin B12 (27). All three of these essential B-vitamins are crucial for proper nerve health and function which should be an important consideration for the pain practitioner as deficiencies can lead to the

development and/or worsening of peripheral neuropathies. A crucial product of homocysteine transmethylation is the active methyl donor S-adenosylmehtionine (SAMe). SAMe is required for synthesis of phosphatidylcholine, a major constituent of neuronal membranes, and the biosynthesis of choline is one of the principal destinations of total body SAME methyl transfer. Early identification of elevated homocysteine is crucial as treatment with the appropriate B-vitamin(s) can decrease levels and therefore reduce the risk of cardiovascular disease. Homocysteine levels should be interpreted alongside markers of B-vitamin status (Xanthurenate and Methylmalonic acid) to determine which essential vitamins are lacking and require replenishment. While the majority of clinical significance is placed on elevated homocysteine levels and the associated increased risk of cardiovascular disease, abnormally low levels are also relevant in chronic pain patients as this can signal glutathione depletion and an increased risk of oxidative stress/damage (see pyroglutamate section) (28,29).

Treatment Options for Abnormal Homocysteine

Result	Treatment
HIGH	Treat abnormal Methylmalonic and/or Xanthurenate findings. The addition of folate (200mcg/day) is also recommended when Homocysteine levels are elevated
LOW	If accompanied by abnormal Pyroglutamate level treat according to Pyroglutamate section

Quinolinate & Kynurenate – Quinolinate and Kynurenate are neuroactive metabolites of the kynurenine pathway which is significantly upregulated in response to inflammation. As a result elevated levels of Quinolinate and Kynurenate serve as sensitive markers of chronic, systemic inflammation and the upregulation of this critical pathway has been shown to play a central role in the comorbidity of pain and depression (30). Up regulation of the kynurenine pathway in

response to inflammation, stress or chronic activation of the innate immune system impacts the development and severity of pain via two main mechanisms: Firstly, up regulation of this pathway results in a decreased production of serotonin as both pathways utilize tryptophan as a substrate. Decreased levels of serotonin not only lead to depression but also diminish the activity of descending inhibitory pain pathways which under normal serotonin supply act to inhibit pain (31-36). Secondly, up regulation of the kynurenine pathway increases circulating levels of Quinolinic acid which contributes to heightened nociception and an increased susceptibility to neurotoxicity via its interaction with glutamate receptors (37). Quinolinic acid is therefore not only a sensitive marker of systemic inflammation but also a bioactive modulator of pain perception due to its action on NMDA receptors linked to nociceptor systems. Kynurenate is an NMDA receptor antagonist that is also elevated during episodes of inflammation or chronic stress but appears to play a neuroprotective role by antagonizing the neurotoxic effects of Quinolinate. Therefore, the relative concentrations of these two kynurenine metabolites provides an indication of the potential risk for neuronal degeneration. As the QUIN/KYN ratio increases so too does the risk of nerve cell death as a result of systemic inflammatory disease. In the absence of inflammation, Quinolinate will not be elevated and high kynurenate reinforces abnormal Xanthurenate due to a B6 deficient impact on the hepatic kynurenine pathway. Magnesium and glycine are frequently helpful for antagonizing glutamatergic NMDA receptors, offsetting the Quinolinate agonistic effects.

Treatment Options for Abnormal Quinolinate & Kynurenate

Result	Treatment
HIGH QUIN	1. Anti-inflammatory/oxidant therapy 2. Magnesium glycinate
HIGH KYN	1. Anti-inflammatory/oxidant therapy/Nrf2 activator
BOTH HIGH	1. Anti-inflammatory/oxidant therapy 2. Magnesium glycinate

Pyroglutamate – Elevated levels of Pyroglutamate indicate glutathione depletion which renders nerve cells susceptible to oxidative damage. Glutathione is the most important and abundant intracellular antioxidant in all aerobic cells (38). Its powerful antioxidant effects are due to its dual roles as a scavenger of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and as a substrate for the detoxifying enzymes glutathione peroxidase (GPx) and GSSG reductase (GR) (39). Glutathione represents the most integral component of our natural antioxidant defense system and optimal levels are critical for combatting oxidative stress and ensuring cell survival. Oxidative stress is the direct consequence of an increased generation of free radicals and/or reduced physiological activity of our antioxidant defense system. The central role of oxidative stress in the development, maintenance and worsening of peripheral neuropathy is widely recognized and more recent studies have even identified free radicals as key players in the production of pain and the lowering of local nociceptor thresholds leading to hyperalgesia (40-42). Studies have also illustrated a direct link between levels of oxidative stress and the resulting susceptibility of skeletal muscle to fatigue and pain (40). As the body of literature describing the role of oxidative stress in chronic pain conditions continues to grow the capacity to maintain glutathione responses becomes a more crucial factor for the successful and prolonged

management of pain. This is especially true for patients who take regular doses of acetaminophen as the toxic metabolite of acetaminophen, N-acetyl-p-benzoquinone imine (NAPQI) is detoxified by glutathione. In the absence of sufficient levels of glutathione, NAPQI will combine with structural proteins leading to toxicity and hepatic damage. Not only does acetaminophen directly deplete glutathione through conjugation reactions but its metabolism and excretion also depletes glycine and the sulfur-containing amino acids that are required for glutathione synthesis (43). The depletion of glutathione by NAPQI and the consumption of necessary sulfated amino acids during acetaminophen metabolism is widely under recognized as a source of glutathione depletion and its relevance to pain management cannot be overstated. Evaluating glutathione status and replenishing deficiencies in order to decrease the susceptibility of cells to further oxidative damage can be achieved safely and cost effectively. Of the several interventions for improving glutathione capacity, one of the most highly effective strategies is combined oral N-acetyl-L-cysteine (NAC) augmented by taurine to spare the activity of that synthetic pathway along with glycine (another easily-depleted glutathione precursor) (43, 44).

Treatment Options for Abnormal Pyroglutamate

Result	Treatment	Dosage
HIGH	1. N-Acetyl-L-Cysteine 2. Taurine 3. Glycine	1. 1g daily 2. 2g daily 3. 2g daily
LOW	1. N-Acetyl-L-Cysteine 2. Taurine 3. Glycine	1. 1g daily 2. 2g daily 3. 2g daily

Vanilmandelate – Vanilmandelate is the urinary metabolite of norepinephrine, which along with serotonin is the principal mediator of endogenous pain inhibiting systems know as descending inhibitory pathways. Optimal levels of norepinephrine are required for the activation of

descending inhibitory pathways which act to inhibit pain. Deficient levels of this neurotransmitter system are believed to contribute to hyperactive pain processing while elevated levels of norepinephrine signify chronic stress and can lead to the generalized hypervigilant state often observed in patients with fibromyalgia (32, 45). Norepinephrine is a unique biomarker of pain as it can have a marked impact on the development, maintenance and perception of pain when present at sub optimal or elevated levels. Sufficient levels are required to activate descending inhibitory pathways which act to suppress ascending pain signals at the spinal level but an overproduction of norepinephrine can heighten ones sensitivity to pain as a result of generalized hypervigilance and perceptual amplification. While urinary levels of Vanilmandelate do not reflect or directly correlate to levels in the CNS, they do reflect overall rates of biosynthesis and breakdown and provide physicians with a non-invasive means to identify patients who may benefit from therapies designed to modulate the production of catecholamines.

Treatment Options for Abnormal Vanilmandelate

Result	Treatment
HIGH	1. Adrenal support, L-Theanine, Magnesium, Adaptogens
LOW	1. Catecholamine precursors, Adaptogens, Vitamin C

5-Hydroxyindoleacetate (5-HIAA) - 5-Hydroxyindoleacetate (5-HIAA) is the primary metabolite of serotonin which, along with norepinephrine is the principle mediator of descending inhibitory pathways which act to suppress pain. It is produced by synaptic monoamine oxidase action on serotonin, and SSRI therapies increase those losses due their inhibition of reuptake that increases serotonin synaptic dwell times. Abnormally low levels of 5-HIAA can indicate inadequate production of serotonin which has been linked to depression and is believed to

contribute to hyperactive pain processing (32). Serotonin is a monoamine neurotransmitter which is biochemically derived from dietary tryptophan but during episodes of inflammation, tryptophan is preferentially metabolized along the kynurenine pathway leading to decreased serotonin production. This inflammation induced 'shunt' away from serotonin and melatonin production results in greater circulating levels of neuroactive, brain-specific kynurenine metabolites such as Quinolinate and decreased levels of serotonin and melatonin. It is the deregulation of this pathway that provides the mechanistic link between systemic inflammation and diseases associated with low serotonin status such as chronic pain and depression. Patients with low levels of 5-HIA may benefit from serotonin precursor therapies such as 5-hydroxytryptophan or L-tryptophan. Elevated levels of 5-HIA are commonly observed in patients who are taking serotonin reuptake inhibitor medications such as SSRI's or SSNRI's.

Treatment Options for Abnormal 5-HIAA

Result	Treatment	Dosage
LOW	1. 5-Hydroxytryptophan (5-HTP) 2. Pyridoxal-5-Phosphate	1. 50 mg/day 2. 10 mg/day

Discussion/Conclusion

A standardized, laboratory based workup designed specifically for pain management provides objective information about the underlying biochemical mechanisms of pain while indicating novel, safe, personalized and cost effective pain treatments. Evaluating markers of nerve health, chronic inflammation, oxidative stress and neurotransmitter status and treating related deficiencies or disorders maximizes the likelihood of successful and prolonged pain management. Laboratory testing for functional biomarkers of pain is by no means intended to

replace traditional pain management techniques, however, evaluating such markers and recommending appropriate therapies will likely increase the efficacy of current techniques. Many of the recommended markers enable the early identification of deficiencies or disorders which progressively become worse if left untreated and, in the case of B-vitamin deficiencies, may even cause irreversible neurological damage. Given the severity and negative outcomes associated with long-term vitamin deficiencies, chronic inflammation, oxidative stress and neurotransmitter imbalance combined with the ease and minimal cost of early treatment, early identification through functional biomarker testing is recommended.

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