Observational Study

An Analysis of Biomarkers in Patients with Chronic Pain

Joshua Gunn, PhD[1], Melissa M. Hill, BS[1], Bradley M. Cotten, MS[1], and Timothy R. Deer, MD[2]

From: [1]Ethos Research & Development, Newport, KY; [2]Center for Pain Relief, Charleston, WV

Address Correspondence:
Joshua Gunn, PhD
Ethos Research & Development
29 East 6th St.
Newport, KY 41071
E-mail: DrGunn@ethosrd.com

Disclaimer: There was no external funding in the preparation of this manuscript. Conflict of interest: Each author certifies that he or she, or a member of his or her immediate family, has no commercial association (i.e., consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted manuscript.

Manuscript received: 04-17-2019
Revised manuscript received: 06-21-2019
Accepted for publication: 07-09-2019

Free full manuscript: www.painphysicianjournal.com

Background: Because of 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. Successful identification of mechanistic biomarkers would not only improve our understanding and ability to accurately diagnose pain disorders but would also facilitate the development of disease-modifying pain drugs.

Objectives: The objective of this study was to determine and evaluate the prevalence of abnormal biomarker findings in a population of patients with chronic pain.

Study Design: Retrospective, observational study.

Setting: Data analysis of biomarker test results was performed at a single industry site (Ethos Research & Development, Newport, KY) from clinical samples collected and analyzed from July to December 2018.

Methods: A novel, pain-specific biomarker test panel that evaluates biomarkers of systemic inflammation, oxidative stress, neurotransmitter turnover, and micronutrient status was employed to determine the prevalence of abnormal findings in 17,834 unique patient samples analyzed at a national reference laboratory (Ethos Laboratories, Newport, KY). Patient biomarker results were considered abnormal if they were outside of the 95% confidence interval reference ranges established using a healthy population of donors who had no history of chronic pain or opioid use.

Results: A total of 77% of patients with chronic pain exhibited at least one abnormal biomarker result (n = 13,765). The most common abnormal biomarker finding was elevated quinolinic acid, which was observed in 29% of patients (n = 5,107). Elevated pyroglutamate, indicative of glutathione depletion, was observed in 19% of patients (n = 3,314). Elevated xanthurenic acid, indicative of vitamin B6 insufficiency, was observed in 17% of patients (3,025). Elevated levels of the acrolein metabolite 3-hydroxypropyl mercapturic acid were observed in 21% of patients (n = 3,667). Elevated methylmalonic acid, indicative of a vitamin B12 deficiency, was observed in 10% of patients (n = 1,827), whereas abnormally low levels of neurotransmitter metabolites were observed in 8% of patients (n = 1,456).

Limitations: Medications and/or conditions other than those associated with chronic pain were not evaluated as potential causes of abnormal biomarker findings.

Conclusions: A novel biomarker assay that measures objective correlates to the neurobiological processes underlying chronic pain reveals a high prevalence of atypical biochemistry in a population of patients with pain. Abnormal biomarker findings presented here provide objective support for the role of cytokine-mediated inflammation, oxidative stress, abnormally low production of neurotransmitters, and micronutrient deficiencies in the development or worsening of chronic pain. This unique panel of functional pain biomarkers provides practitioners with novel, objective insight into the underlying causes of pain, which will pave the way for truly personalized pain medicine. Correcting abnormal biomarker findings with targeted, nonopioid therapies to improve patient function and alleviate pain potentially could lessen the opioid burden and drastically reduce health care costs.

Key words: Biomarker, pain, inflamation, oxidative stress, neurotransmitter, micronutrient deficiency, Kynurenine Pathway

Pain Physician 2020: 23:E41-E49

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 efficacy limitations of current treatments. Subjective ratings have played a key role in the diagnosis and treatment of pain, which is complicated by the profound individual differences in sensitivity and may complicate diagnosis and treatment (2). Because of 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. Although 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.

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 co-morbid 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. 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 health care costs worldwide. Although the successful identification of any pain-specific biomarker signifies an advancement in our understanding of pain pathophysiology, the most important and impactful biomarkers are those that can be modulated to change the course of disease.

The biomarker test panel used for this retrospective analysis evaluates markers of essential micronutrients critical for nerve health, chronic inflammation, oxidative stress/damage, and neurotransmitter turnover (Table 1). Abnormalities may 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.

Methods

Biomarker test results from 17,834 unique patients receiving opioids for the management of chronic pain were analyzed to determine the prevalence and clinical significance of abnormal findings. Patient test results were interpreted using reference ranges established at a large commercial laboratory (Ethos Laboratories, Newport, KY). Reference ranges for each biomarker included in this test panel were established using methods described in the Clinical and Laboratory Standards Institute's document Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition (C28-A3) (November, 2008). Specifically, refer-

Table 1. *Functional Biomarkers of Pain Test Panel offered by Ethos Labs (Newport, KY).*

Biomarker	Abnormal Result Indicates	Biomarker Class
Methylmalonic acid	Intracellular Vitamin B12 deficiency	Nerve Health
Xanthurenic acid	Intracellular Vitamin B6 deficiency	Nerve Health
Homocysteine	Deficiencies of Folate, Vitamin B6 or B12	Nerve Health
3-HPMA	Acrolein exposure	Nerve Health
Quinolinic acid	Brain Kynurenine Pathway and NMDA agonization	Inflammation
Kynurenic acid	Aids interpretation of Xanthurenic acid and Quinolinic acid	Inflammation
Pyroglutamate	Glutathione response capacity	Oxidative stress
Hydroxymethylglutarate	Coenzyme Q10 deficiency	Oxidative stress
Ethylmalonic acid	Carnitine and/or Vitamin B2 deficiency	Oxidative stress
5-Hydroxyindoleacetate	Serotonin turnover	Neurotransmitter status
Vanilmandelate	Epinephrine & Norepinephrine turnover	Neurotransmitter status

Table 2. *95% confidence interval reference ranges, mean ± SD and observed ranges of biomarker values in 17,834 unique patient samples. Reference intervals were established using healthy control subjects who had no history of chronic pain or opioid use.*

Biomarker	Reference Interval (µg/mg creatinine)	Pain Population	
		Mean ± SD	Range
Methylmalonic acid	0 – 2.3	1.3 ± 2.0	0 – 180
Xanthurenic acid	0 – 0.63	0.4 ± 0.3	0.01 – 7.95
Homocysteine	0 – 1.3	0.8 ± 1.3	0 – 83
3-HPMA	0 – 2.4	1.4 ± 2.1	0 – 53.7
Quinolinic acid	0 – 6.3	5.6 ± 3.0	0.6 – 71.5
Kynurenic acid	0 – 2.0	1.7 ± 0.9	0 – 14.4
Pyroglutamic acid	8 – 40	31.4 ± 16.9	0 – 698
Hydroxymethylglutarate	0 – 5.1	3.3 ± 1.8	0 – 68.5
Ethylmalonic acid	0 – 6.3	3.7 ± 3.2	0.1 – 105.4
5-Hydroxyindoleacetate	≥ <1.6	3.9 ± 7.61	0.1 – 827.7
Vanilmandelate	≥ <0.8	3.2 ± 1.5	0.1 – 19.6

ence intervals were adopted from accredited institutions who had performed large scale studies (minimum of 120 healthy donors) and were internally evaluated to confirm applicability to both our patient population and our analytic methodologies. Internal evaluation was performed by collecting samples from healthy, pain-free donors and establishing 95% double-sided, confidence intervals to compare directly to adopted reference ranges. Reference intervals determined internally closely resembled those ranges adopted from other institutions, and for several analytes the 2 intervals were almost identical (i.e., 95% confidence interval determined from internal donor samples was almost identical to reference interval employed by other laboratories). For analytes in which small differences existed at either end of the interval, internally determined 95% confidence intervals were employed as reference intervals to eliminate the possibility of variation due to different analytic methodologies. Reference intervals for each biomarker are presented in Table 2.

Results

Patient biomarker profiles were interpreted using established reference intervals presented in Table 2. Table 2 also details the mean, standard deviation, and range of observed values for each biomarker across the 17,834 unique patient samples. Analysis of the observed ranges for each biomarker provides important quantitative insight into the severity of some of the abnormal biomarker findings in this patient population. Distribution of abnormal findings across all biomarkers can be seen in Fig. 1.

Retrospective analysis of the 17,834 biomarker

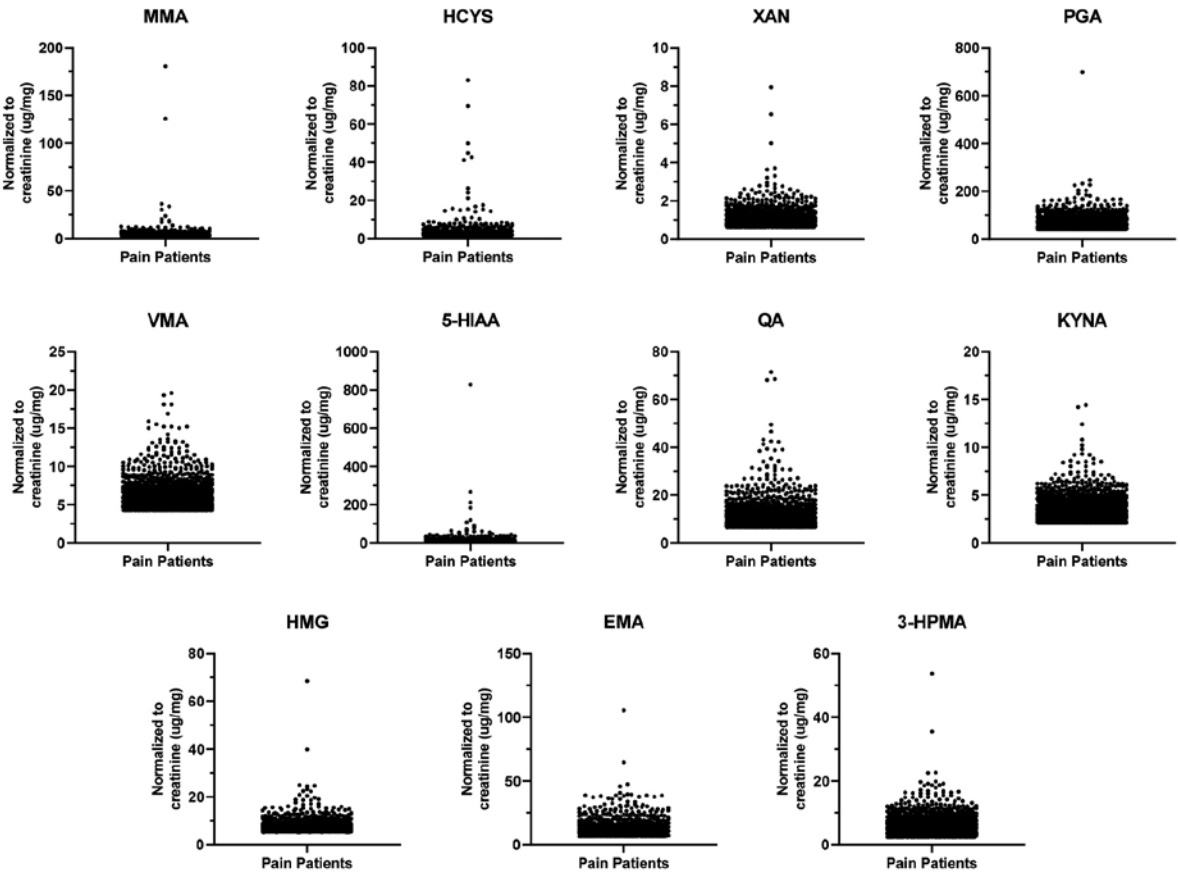

Fig. 1. *Scatterplots of abnormal biomarker results among patients with chronic pain. MMA, methylmalonic acid; HCYS, homocysteine; XAN, xanthurenic acid; PGA, pyroglutamic acid; VMA, vanilmandelate; 5-HIAA, 5-hydroxyindoleacetate; QA, quinolinic acid; KYNA, kynurenic acid; HMG, hydroxymethylglutarate; EMA, ethylmalonic acid; 3-HPMA, 3-hydroxypropyl mercapturic acid. All data points represent biomarker results that were outside the 95% confidence interval reference ranges.*

profiles revealed that 77% (n = 13,765) of patients exhibited at least one abnormal pain biomarker. Elevated quinolinic acid was the most common abnormal finding among the patient population with 29% (n = 5,107) exhibiting levels above the upper end of the established reference range. Another metabolite of the kynurenine pathway (KP), kynurenic acid, was elevated in 27% (n = 4,823) of patient samples, which further supports the role of an activated KP in the etiology of chronic pain. Elevated levels of the acrolein metabolite 3-hydroxypropyl mercapturic acid were detected in 21% (3,667) of patients. Elevated levels of pyroglutamate, indicative of glutathione depletion, were detected in 19% (n = 3,314) of patients. Elevated levels of xanthurenic acid, indicative of a vitamin B6 insufficiency, was detected in 17% (n = 3,025) of patients, whereas 10% (n = 1,827) of patients exhibited elevated levels of methylmalonic acid indicating a vitamin B12 deficiency. Metabolites of the pain-modulating neurotransmitters serotonin (5-hydroxyindoleacetate) and norepinephrine (vanilmandelate) were determined to be abnormally low in 8.2% (n = 1,456) of patients indicating a decreased rate of synthesis/turnover. The clinical relevance of decreased serotonin synthesis/turnover in the presence of elevated quinolinic acid will be discussed in detail later. Prevalence of abnormal findings for each of the 11 biomarkers included in Ethos Laboratories proprietary panel are presented in Table 3.

Table 3. *Prevalence of abnormal biomarker findings in 17,834 unique patient samples.*

Biomarker	Prevalence of Abnormal Results	Interpretation of Abnormal Result
Methylmalonic acid	10%	Vitamin B12 deficiency
Homocysteine	11%	Vitamin B6/B9 or B12 deficiency
Xanthurenic acid	17%	Vitamin B6 deficiency
3-HPMA	21%	Acrolein exposure
Quinolinic acid	29%	Chronic inflammation mediated by pro-inflammatory cytokines
Kynurenic acid	27%	Chronic inflammation mediated by pro-inflammatory cytokines
Pyroglutamate	19%	Glutathione depletion
Hydroxymethylglutarate	11%	Coenzyme Q10 deficiency
Ethylmalonic acid	10%	Carnitine/Vitamin B2 deficiency
5-Hydroxyindoleacetate	7.4%	Decreased synthesis/turnover of serotonin
Vanilmandelate	0.8%	Decreased synthesis/turnover of norepinephrine

Discussion

Our retrospective review of 17,834 pain biomarker profiles indicates a high prevalence of atypical biochemistry in patients with chronic pain. The importance of these findings cannot be overstated as each abnormal biomarker finding represents a possible underlying cause or worsening of chronic pain symptoms. Objective data directly implicating specific biochemical pathways, perturbed metabolic function, or micronutrient deficiencies as causes of pain would provide physicians with novel options for personized, nonopioid pain treatments.

Our analysis strongly implicates the KP (Fig. 2) and its various neuroactive metabolites in the etiology of chronic pain. The KP is significantly upregulated in response to inflammation, and as a result, elevated levels of KP metabolites serve as sensitive markers of chronic, systemic inflammation. This critical pathway, which has previously been shown to play a central role in the comorbidity of pain and depression (8), impacts the development and severity of pain via 2 main mechanisms. First, upregulation of this pathway results in a decreased production of serotonin as both pathways use tryptophan as a substrate. Decreased levels of serotonin not only lead to depression but also diminish the activity of descending inhibitory pain pathways that, under normal serotonin supply, act to inhibit pain (9-14). Second, upregulation of the KP increases circulating levels of quinolinic acid, which contributes to heightened nociception and an increased susceptibility to neurotoxicity via its interaction with glutamate receptors (15). Quinolinic acid is therefore not only a sensitive marker of systemic inflammation, but also a bioactive modulator of pain perception owing to its action on N-methyl-D-aspartate receptors linked to nociceptor systems. In our retrospective analysis, 29% of patients being treated for chronic pain had elevated levels of quinolinic acid.

Further supporting the role of an upregulated KP in the etiology of chronic pain was the finding that 27% of patients had elevated levels of kynurenic acid, and 17% had elevated levels of xanthurenic acid. Xanthurenic acid is a sensitive marker of vitamin B6 (pyridoxine) status. It is a byproduct of the hepatic KP 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 antiinflammatory mediators, and immune system function. A deficiency of vitamin B6 can cause peripheral neuropathy, migraine, chronic pain, depression, seizures, and other neuropsychiatric disorders (16-18).

Another key finding in this retrospective analysis was the prevalence of elevated pyroglutamate in patients with chronic pain. Elevated levels of pyroglutamate indicate glutathione depletion, which renders nerve cells susceptible to oxidative damage. 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. The central role of oxidative stress in the development, maintenance, and worsening of peripheral neuropathy is widely recognized, and more recent

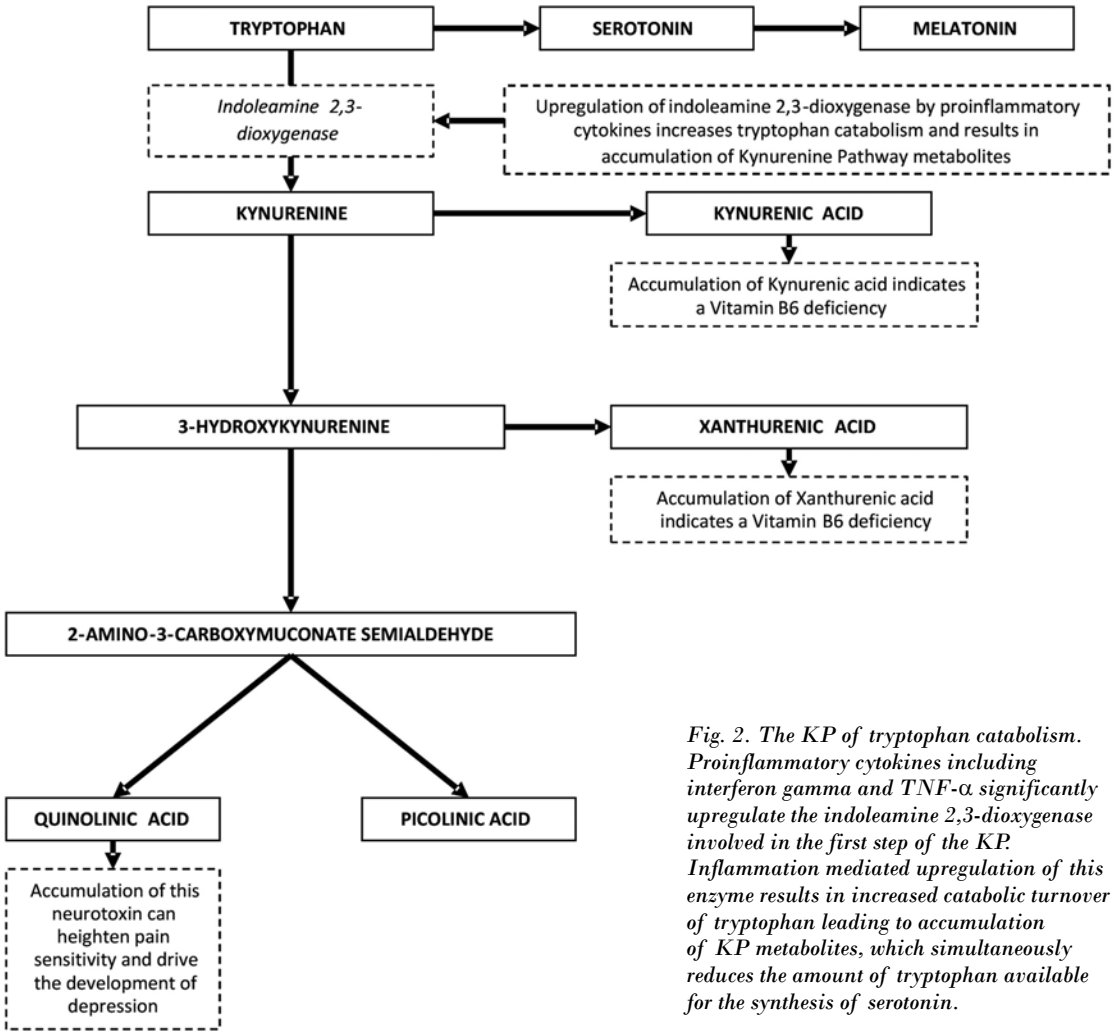

Fig. 2. The KP of tryptophan catabolism. Proinflammatory cytokines including interferon gamma and TNF-α significantly upregulate the indoleamine 2,3-dioxygenase involved in the first step of the KP. Inflammation mediated upregulation of this enzyme results in increased catabolic turnover of tryptophan leading to accumulation of KP metabolites, which simultaneously reduces the amount of tryptophan available for the synthesis of serotonin.

studies have even identified free radicals as key players in the production of pain and the lowering of local nociceptor thresholds, leading to hyperalgesia (19-21). Studies have also illustrated a direct link between levels of oxidative stress and the resulting susceptibility of skeletal muscle to fatigue and pain (19). 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. The depletion of glutathione by NAPQI is widely underrecognized as a source of glutathione depletion in patients with pain. Evaluating glutathione status and replenishing deficiencies to decrease the susceptibility of cells to further oxidative damage can be achieved safely and cost-effectively.

Retrospective analysis also revealed intracellular vitamin B12 deficiencies in 10% of patients. 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 (22). Low levels of vitamin B12 have been linked to polyneuropathy, trigeminal neuralgia, migraine, depression, mania, optic nerve atrophy, neuropsychiatric disorders, and various functional disabilities (23-27).

Risk factors for vitamin B12 deficiency include the use of medications such as proton pump inhibitors, H2-receptor antagonists, metformin, colchicine, cholestyramine, and frequent or long-term use of anticonvulsants and/or antibiotics (24). Gastric surgery, intestinal bacterial overgrowth, hypothyroidism, diabetes, and aging represent other risk factors for vitamin B12 deficiency. Because of 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 (28-33).

The metabolic and nutritional abnormalities observed in this study provide novel insights into the underlying biochemistry of chronic pain disorders and may help to explain the refractory nature of many chronic pain conditions as these biochemical abnormalities are not the target of current pain therapies. The high prevalence of perturbed biochemical patterns in this patient population raises several important questions: (1) Could these metabolic and nutritional abnormalities represent objective drivers of pain? (2) Could correcting abnormal findings result in significant improvement in pain or quality of life measures? (3) Could medication(s) be playing a role in the development of these metabolic and nutritional abnormalities given the high prevalence in this patient population? Although the first 2 questions will be the subject of subsequent investigations, the third question warrants consideration in the current discussion. The consideration of whether pain medications may cause or precipitate some of the biochemical abnormalities observed here does not subtract from the importance of these findings as the perturbed biochemical state can still act to worsen existing pain regardless of the cause (i.e., medication precipitated vs. diet/lifestyle), and should therefore still be considered a target for therapy/modulation. It is, however, important to consider such possibilities given the high prevalence of abnormal findings in a population of patients who are all prescribed opioid-based medications. Discovering "off-site" effects of opioids could shed light on the complex mechanisms of neurobiological phenomena, such as hyperalgesia if it were to be determined for example that opioids upregulate the KP leading to the accumulation of the neurotoxic metabolites such as quinolinic acid. To our knowledge, there have been no studies assessing the direct impact of opioids on circulating levels of KP metabolites, however, opioids have been shown to participate in neuroimmune signaling through toll-like receptors that, when activated, can stimulate the KP (34-38).

Also worthy of discussion is the relationship between elevated pyroglutamate (observed in 19% of patients) and the use of medications containing acetaminophen. Not only does acetaminophen directly deplete glutathione through conjugation reactions, but its metabolism and excretion also deplete glycine and the sulfur-containing amino acids that are required for glutathione synthesis (39). The depletion of glutathione during acetaminophen metabolism and excretion is widely underrecognized as a source of oxidative stress, and its relevance to pain management cannot be overstated because of the widespread use of acetaminophen containing medications.

Objective and robust correlates to the underlying neurobiology of chronic pain disorders would provide health care practitioners with novel information to assist with the diagnosis, treatment, and monitoring of chronic pain. The biomarkers introduced in this study provide objective information on metabolic and nutritional abnormalities that can cause or worsen pain. Perhaps the most exciting component of the biomarker patterns observed in this patient population is the fact that all of the aforementioned abnormalities can be corrected with targeted nutraceutical administration. Although abnormal biomarker findings along pathways related to chronic inflammation and neurotransmitter synthesis may be somewhat expected in this patient population, the prevalence and severity of micronutrient deficiencies and oxidative stress markers provide unique avenues of future adjuvant treatment strategies for the management of pain. Furthermore, future, prospective studies will aim to refine biomarker signatures in patient cohorts based on specific chronic pain diagnoses (i.e., type/location of pain) and preexisting or comorbid primary disease to further elucidate the role of these important biomarkers in the diagnosis, treatment, and monitoring of chronic pain.

Conclusions

A laboratory-based workup designed specifically for pain management reveals a high prevalence of atypical, pro-pain biochemistry in a population of patients with chronic pain. These findings support a role for measuring objective, quantifiable correlates to pain that can guide personalized, nonopioid pain treatments. This novel test panel provides objective information about the underlying biochemical mechanisms of pain while indicating novel, safe, and cost-effective pain treatments. Evaluating markers of chronic inflammation, oxidative stress, neurotransmitter turnover, and micronutrient status and correcting any abnormal findings with targeted therapies will increase the likelihood of successful and prolonged pain management. This novel concept of evaluating pain biochemistry will by no means replace traditional pain management techniques; however, evaluating such markers and recommending appropriate, targeted therapies will likely increase the efficacy of current techniques. Many of the biomarkers discussed in this analysis 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 neurologic damage. Given the severity and negative outcomes associated with long-term vitamin deficiencies, chronic inflammation, oxidative stress, and impaired neurotransmitter synthesis combined with the ease and minimal cost of early treatment, early identification through biomarker testing is recommended. If these findings prove to be reproducible in a prospective multicenter fashion, we believe it could have a major impact on long-term pain treatment outcomes and algorithms.

References

1. Gaskin D, Richard P. The economic costs of pain in the United States. *J Pain* 2012; 13:715-724.
2. Coghill R. Individual differences in the subjective experience of pain: New insights into mechanisms and models. *Headache* 2010; 50:1531-1535.
3. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther* 2001; 69:89-95.
4. Kalso E. Biomarkers for pain. *Pain* 2004; 107:199-201.
5. Backryd E. Pain in the blood? Envisioning mechanism-based diagnoses and biomarkers in clinical pain medicine. *Diagnostics* 2015; 5:84-95.
6. Backryd E. Pain and consciousness mocks philosophers and scientists. *Lakartidningen* 2012; 109:1039-1040.
7. Backryd E, Ghafoui B, Larsson B, Gerdle B. Do low levels of beta-endorphin in the cerebrospinal fluid indicate defective top-down inhibition in patient with chronic neuropathic pain? A cross-sectional, comparative study. *Pain Med* 2014; 15:111-119.
8. Kim H, Chen L, Lim G, et al. Brain Indoleamine 2,3-dioxygenase contributes to the comorbidity of pain and depression. *J Clin Invest* 2012; 122:2940-2954.
9. Iyengar S, Webster A, Hemrick-Luecke S, Xu J, Simmons R. Efficacy of duloxetine, a potent and balanced serotonin-norepinephrine reuptake inhibitor in persistent pain models in rats. *J Pharmacol Exp Ther* 2004; 311:576-584.
10. Marks D, Shah M, Patkar A, Masand P, Park G, Pae C. Serotonin-norepinephrine reuptake inhibitors for pain control: Premise and promise. *Curr Neuropharmacol* 2009; 7:331-336.
11. Miura H, Ozaki N, Sawada M, Isobe K, Ohta T, Nagatsu T. A link between stress and depression: Shifts in the balance between kynurenine and serotonin pathways of tryptophan metabolism and the etiology and pathophysiology of depression. *Stress* 2008; 11:198-209.
12. Myint A, Kim Y. Cytokine-serotonin interaction through IDO: A neurodegenerative hypothesis of depression. *Med Hypotheses* 2003; 61:519-525.
13. Mico J, Gibert-Rahola J, Casas J, Rojas O, Serrano M, Serrano J. Implication of beta 1 and beta 2-adrenergic receptors in the antinociceptive effect of tricyclic antidepressants. *Eur Neuropsychopharmacol* 1997; 7:139-145.
14. Sawynok J, Esser M, Reid A. Antidepressants as analgesics: an overview of central and peripheral mechanisms of action. *J Psychiatry Neurosci* 2001; 26:21-29.
15. Stone T, Perkins M. Quinolinic acid: A potent endogenous excitant at amino acid receptors in CNS. *Eur J Pharmacol* 1981; 72:411-412.
16. Levenson J. *Textbook of Psychosomatic Medicine, 2nd ed.* Arlington, American Psychiatric Publishing, 2011.
17. Hammond N, Wang Y, Dimachkie M, Barohn R. Nutritional neuropathies. *Neurol Clin* 2013; 31:477-489.
18. Beer M, Porter R, Jones T, Kaplan J, Berkwits M (eds.) *The Merck Manual. 18th ed.* Rahway, Merck Research Laboratories, 2006: pp. 26-61, 1036-1039, 1261-1263.
19. Vecchiet J, Cipollone F, Falasca K, et al. Relationship between musculoskeletal symptoms and blood markers of oxidative stress in patients with chronic fatigue syndrome. *Neurosci Lett* 2003; 335:151-154.
20. Khalil Z, Liu T, Helme R. Free radicals contribute to the reduction in peripheral vascular responses and the main-

tenance of thermal hyperalgesia in rats with chronic constriction injury. *Pain* 1999; 79:31-37.

21. Luo Z, Cizkova D. The role of nitric oxide in nociception. *Curr Rev Pain* 2000; 4:459-466.
22. Babior B, Bunn H. Megaloblastic anemias. In: Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL (eds). *Harrison's Principles of Internal Medicine, 15th ed.* New York, McGraw-Hill, 2001: pp: 674-680.
23. Sun A, Ni Y, Li X, Zhuang X, Liu Y, Liu X, Chen S. Urinary methylmalonic acid as an indicator of early vitamin B12 deficiency and its role in polyneuropathy in type 2 diabetes. *J Diabetes Res* 2014; 2014:e921616.
24. Briani C, Torre C, Citton V, et al. Cobalamin deficiency: Clinical picture and radiological findings. *Nutrients* 2013; 5:4521-4539.
25. Aggarwal A, Wood I. Low vitamin B12 syndrome in trigeminal neuralgia. *J Pain Relief* 2012; 1:5.
26. Ipcioglu O, Ozcan O, Gultepe M, Tekeli H, Senol M. Functional vitamin B12 deficiency represented by elevated urine methylmalonic acid levels in patients with migraine. *Turk J Med Sci* 2008; 38:409-414.
27. Oberlin B, Tangney C, Gustashaw K, Rasmussen H. Vitamin B12 deficiency in relation to functional disabilities. *Nutrients* 2013; 5:4462-4475.
28. Kikuchi M, Kashii S, Honda Y, Tamura Y, Kaneda K, Akaike A. Protective effects of methylcobalamin, a vitamin B12 analog, against glutamate-induced neurotoxicity in retinal cell culture. *Invest Ophthalmol Visual Sci* 1997; 38:848-854.
29. Kong X, Sun X, Zhang J. The protective role of mecobalamin following optic nerve crush in adult rats. *Yan Ke Xue Bao (Eye Science)* 1997; 20:171-177.
30. Akaike A, Tamura Y, Sato Y, Yokota T. Protective effects of a vitamin B12 analog, methylcobalamin, against glutamate cytotoxicity in cultured cortical neurons. *Eur J Pharmacol* 1993; 241:1-6.
31. Devathasan G, Teo W, Mylvaganam A, Thai A, Chin J. Methylcobalamin in chronic diabetic neuropathy: A double-blind clinical and electrophysiological study. *Clin Trials J* 1986; 23:130-140.
32. Kuwabara S, Nakazawa R, Azuma N, et al. Intravenous methylcobalamin treatment for uremic and diabetic neuropathy in chronic hemodialysis patients. *Intern Med* 1999; 38:472-475.
33. Ishihara H, Yoneda M, Yamamoto W. Efficacy of intravenous administration of methylcobalamin for diabetic peripheral neuropathy. *Med Consult N Remedies* 1992; 29:1720-1725.
34. Hutchinson M, Zhang Y, Shridhar M, et al. Evidence that opioids may have toll-like receptor 4 and MD-2 effects. *Brain Behav Immun* 2010; 24:83-95.
35. Shah M, Choi S. Toll-like receptor-dependent negative effects of opioids: A battle between analgesia and hyperalgesia. *Frontiers Immunol* 2017; 8:642.
36. Asp L, Holtze M, Powell S, Karlsson H, Erhardt S. Neonatal infection with neurotropic influenza A virus induces the kynurenine pathway in early life and disrupts sensorimotor gating in adult Tap1–/– mice. *Int J Neuropsychopharmacol* 2010; 13:475-485.
37. Clarke G, McKernan D, Gaszner G, Quigley E, Cryan J, Dinan T. A distinct profile of tryptophan metabolism along the kynurenine pathway downstream of toll-like receptor activation in irritable bowel syndrome. *Frontiers Pharmacol* 2012; 3:90.
38. Opitz C, Litzenburger U, Lutz C, et al. Toll-like receptor engagement enhances the immunosuppressive properties of human bone marrow-derived mesenchymal stem cells by inducing indoleamine-2,3-dioxygenase-1 via interferon-beta and protein kinase R. *Stem Cells* 2009; 27:909-919.
39. Emmett M. Acetaminophen toxicity and 5-oxoproline (pyroglutamic acid): A tale of two cycles, one an ATP-depleting futile cycle and the other a useful cycle. *Clin J Am Soc Nephrol* 2014; 9:191-200.