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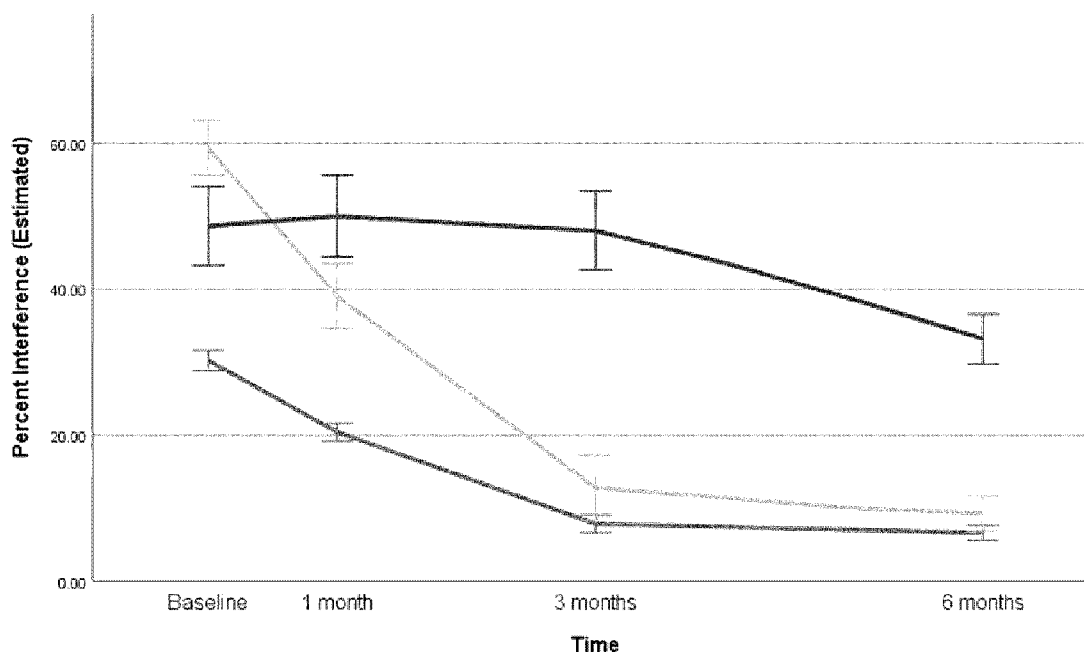


Figure 1

(57) Abstract: A panel of biomarkers is provided for predicting functional recovery after musculoskeletal trauma. The panel comprises BDNF and TGF- β 1, wherein the levels of these markers, when present together, are inversely correlated with recovery. Also provided are assays, kits, and related methods and uses, such as methods for predicting risk of chronic pain.

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**BIOMARKERS ASSOCIATED WITH FUNCTIONAL RECOVERY AFTER
MUSCULOSKELETAL TRAUMA AND RELATED METHODS**

Field

The present invention relates to musculoskeletal trauma. In particular, the present
5 invention relates to biomarkers associated with pain and recovery after musculoskeletal
trauma, and related products and methods.

Background

The burden of chronic pain represents problems on both the national and global
stages, leading to competing public health crises of undermanaged pain and suffering and
10 over-prescription of opioid drugs to treat it. Obvious structural lesions that are clearly
associated with symptoms are rare in many chronic pain conditions, and with few exceptions
most intervention strategies have failed to produce consistent evidence of strong
effectiveness. Faced with a growing epidemic of suffering from chronic pain coupled with few
effective interventions to manage it, healthcare providers and decision makers have made
15 the identification of mechanisms to explain chronic pain a priority.

While the etiology of chronic pain can be difficult to clearly identify, many cases are
at least anecdotally linked to an inciting traumatic event, such as a road traffic collision, a
workplace injury, sporting injury, or injuries incurred during activities of daily living.
Consistent evidence indicates that for those who experience such injuries, the majority of
20 recovery occurs within the first 6-12 weeks and then plateaus and this appears to be
independent of the type of injury or the body region affected. In conditions such as traumatic
neck (e.g., 'whiplash') or low back pain, recent trajectory analyses reveal that rapid recovery
occurs in less than 50% of sufferers, and that 15 to 30% are expected to report ongoing
severe symptoms 6 to 12 months following the trauma which would then be commonly
25 labelled 'chronic pain'.

Armed with this information, researchers have been working for the past two decades
to identify the 'at risk' patient who is unlikely to recover smoothly, with the larger goal of
understanding the mechanisms to explain the transition from acute-to-chronic pain such that
interventions can be created to prevent chronicity. To date, the most consistent predictors of
30 non-recovery have been classed within the psychosocial domain, including high self-rated
pain intensity, disability, fear, or distress. While useful for classifying patients into predicted
recovery groups, this knowledge has yet to translate into improved outcomes. This may be
due to ineffective interventions for targeting maladaptive post-traumatic psychology, or it
may be because maladaptive psychology is a proxy for other confounding mechanisms yet
35 to be understood. Biomarkers for pain and distress have been explored for many years now,

with mixed levels of success. For example, Sterling and colleagues explored the potential explanatory value of 3 markers known to be associated with stress or inflammation in explaining recovery status following whiplash: tumour necrosis factor-alpha (TNF α), Interleukin 1-beta (IL-1 β) and C-Reactive protein (CRP). They found that both CRP and TNF α were elevated following trauma in a group of 44 participants when compared to a control group of n=18, but that when the trauma group was separated into recovered/non-recovered by scores on 3-6 month outcomes, none of the markers save for a small negative association with TNF α (high TNF α , less likely to be recovered) were able to discriminate between the groups. More recently, Klyne and colleagues conducted a similar analysis in a sample of n = 109 participants with acute low back pain and found divergent results from that of Sterling in that higher CRP and IL-6 were associated with the recovered group while high TNF α was again associated with non-recovery in their low back pain group.

U.S. Patent Application Publication Nos. 2003/0157099, 2009/0117589, 2012/0122717, 2016/0136310, 2016/033442, 2017/0016913, 2017/0035844, 2017/0161441, and 2018/0271943 and U.S. Patent Nos. 8,518,649 and 9,605,073 generally identify BDNF, IL-1 β , and other biomarkers as candidates to assess pain, whereas TGF- β 1 was identified as a molecule for treatment of pain and inflammation in a dose dependent manner.

A need exists for the development of an effective panel of biomarkers and related methods for predicting the recovery of a subject following musculoskeletal trauma as well as alternative biomarker panels, prognostic protocols, and related methods.

Summary of the Invention

In accordance with an aspect, there is provided a panel of biomarkers for predicting functional recovery after musculoskeletal trauma, the panel comprising BDNF and TGF- β 1, wherein the levels of BDNF and TGF- β 1 are inversely correlated with recovery.

In an aspect, the panel further comprises IL-1 β .

In an aspect, the panel further comprises one or more of TNF- α , IL-6, IL-10, cortisol, and CRP.

In an aspect, the panel comprises all of BDNF, TGF- β 1, IL-1 β , TNF- α , IL-6, IL-10, cortisol, and CRP.

In an aspect, the panel consists of BDNF, TGF- β 1, IL-1 β , TNF- α , IL-6, IL-10, cortisol, and CRP.

In an aspect, the biomarkers are blood biomarkers.

In an aspect, the biomarkers are detected as protein.

In an aspect, the biomarkers are detected as mRNA.

In an aspect, low levels of BDNF and TGF- β 1 as compared to control values assigns the subject to a some recovery group and high levels of BDNF and TGF- β 1 as compared to
5 control values assigns the subject to a no or minimal recovery group.

In an aspect, low or moderate levels of BDNF, TGF- β 1, and IL-1 β as compared to control values assigns the subject to a 'some recovery group' (rapid recovery group and delayed recovery group) and high levels of BDNF and TGF- β 1 as compared to control values assigns the subject to a no or minimal recovery group.

10 In an aspect, low levels of BDNF, TGF- β 1, and IL-1 β as compared to control values assigns the subject to a rapid recovery group or a delayed recovery group and high levels of BDNF and TGF- β 1 as compared to control values assigns the subject to the no or minimal recovery group.

In an aspect, the control values are sample-specific control values.

15 In accordance with an aspect, there is provided an assay comprising probes for detecting the panel of biomarkers described herein for predicting functional recovery after musculoskeletal trauma.

In accordance with an aspect, there is provided a kit for detecting the biomarkers described herein for predicting functional recovery after musculoskeletal trauma.

20 In accordance with an aspect, there is provided a method for predicting functional recovery after musculoskeletal trauma in a subject, the method comprising measuring the levels of a panel of biomarkers comprising BDNF and TGF- β 1 in the subject, wherein the levels of BDNF and TGF- β 1 are inversely correlated with recovery.

In an aspect, the panel of biomarkers further comprises IL-1 β .

25 In an aspect, the panel of biomarkers further comprises one or more of TNF- α , IL-6, IL-10, and cortisol.

In an aspect, the panel of biomarkers further comprises BDNF, TGF- β 1, IL-1 β , TNF- α , IL-6, IL-10, and cortisol.

In an aspect, the panel of biomarkers consists of BDNF, TGF- β 1, IL-1 β , TNF- α , IL-6,
30 IL-10, and cortisol.

In an aspect, the panel of biomarkers excludes CRP.

In an aspect, the biomarkers are blood biomarkers.

In an aspect, the biomarkers are detected as protein.

In an aspect, the biomarkers are detected as mRNA.

5 In an aspect, low levels of BDNF and TGF- β 1 as compared to control values assigns the subject to a some recovery group and high levels of BDNF and TGF- β 1 as compared to control values assigns the subject to a no or minimal recovery group.

10 In an aspect, low or moderate levels of BDNF, TGF- β 1, and IL-1 β as compared to control values assigns the subject to a 'some recovery group' (rapid recovery group and delayed recovery group) and high levels of BDNF and TGF- β 1 as compared to control values assigns the subject to a no or minimal recovery group.

15 In an aspect, low levels of BDNF, TGF- β 1, and IL-1 β as compared to control values assigns the subject to a rapid recovery group or a delayed recovery group and high levels of BDNF and TGF- β 1 as compared to control values assigns the subject to the no or minimal recovery group.

In an aspect, the method further comprises assessing factors associated with the subject.

20 In an aspect, the factors comprise one or more of sex, age, BMI, anatomical region of trauma, employment status, household income, educational attainment, post-traumatic distress, and pre-existing physical or psychological comorbidities.

In an aspect, the method further comprises treating the subject based on the predicted functional recovery.

25 In accordance with an aspect, there is provided a prognostic phenotyping protocol for predicting rate of recovery in MSK trauma in a subject, the protocol comprising measuring the levels of a panel of biomarkers comprising BDNF and TGF- β 1 in the subject, wherein low levels of BDNF and TGF- β 1 as compared to control values assigns the subject to a "some recovery group" (rapid recovery and delayed recovery) and high levels of BDNF and TGF- β 1 as compared to control values assigns the subject to a no or minimal recovery group.

30 In an aspect, the panel further comprises IL-1 β and wherein low or average levels of BDNF, TGF- β 1, and IL-1 β as compared to control values assigns the subject to a rapid

recovery group or a delayed recovery group and high levels of BDNF and TGF- β 1 as compared to control values assigns the subject to the no or minimal recovery group.

In accordance with an aspect, there is provided a method for predicting risk of chronic pain in a subject, the method comprising measuring the levels of a panel of
5 biomarkers comprising BDNF and TGF- β 1 in the subject, wherein the levels of BDNF and TGF- β 1 are positively correlated with the likelihood of the subject experiencing chronic pain.

In accordance with an aspect, there is provided a method for predicting and/or estimating the current severity of pain in a subject, the method comprising measuring the levels of a panel of biomarkers comprising IL-1 β and TGF- β 1 in the subject, wherein the
10 levels of IL-1 β and TGF- β 1 are negatively correlated with the predicted and/or estimated severity of pain that the subject is currently experiencing.

In accordance with an aspect, there is provided a method for predicting and/or estimating current pain interference in a subject, the method comprising measuring the levels of a panel of biomarkers comprising TNF- α and CRP in the subject, wherein the levels
15 of TNF- α and CRP are negatively correlated with the predicted and/or estimated pain interference that the subject is currently experiencing.

In an aspect, the subject has an injury affecting the axial spine.

The novel features of the present invention will become apparent to those of skill in the art upon examination of the following detailed description of the invention. It should be
20 understood, however, that the detailed description of the invention and the specific examples presented, while indicating certain aspects of the present invention, are provided for illustration purposes only because various changes and modifications within the spirit and scope of the invention will become apparent to those of skill in the art from the detailed description of the invention and claims that follow.

25 **Brief Description of the Drawings**

The present invention will be further understood from the following description with reference to the Figure, in which:

Figure 1 shows the recovery trajectories for the entire sample (axial and extremity injuries combined) showing the 3 class quadratic model: Dark Grey (Rapid recovery), Light
30 Grey (Delayed recovery), Black (High intercept & little or no recovery by 6 months).

Figure 2 shows a graphical representation of pain severity moderators. A. Prior employment; solid line = employed for pay; dashed line = not employed for pay. B. Pre-

existing psychopathology; solid line = absent; dashed line = present. C. Sex; solid line = male; dashed line = female.

Figure 3 shows a graphical representation of pain interference moderators. A. Pre-existing pain; solid line = absent, dashed line = present. B. Region of injury; solid line = axial spine; dashed line = extremity. C. Region of injury; solid line = axial spine; dashed line = extremity. D. Region of injury; solid line = axial spine; dashed line = extremity. E. Peri-traumatic stress; solid line = low pre-trauma life stress; dashed = high pre-trauma life stress.

Figure 4 shows a graphical representation of the 3-class latent profile solution along with the frequencies of each class, using Z-transformed values so all fit on the same scale.

All 8 target markers presented in a 3-class profile solution were labeled accordingly: Class 1 = Low concentration of all markers (32.8% of the sample), Class 2 = Average Concentration of all markers (49.0%), Class 3 = High Concentration of BDNF and TGF- β 1 (18.2%). Relative concentration represents z-transformed values. Biomarkers: Brain-Derived Neurotrophic Factor (BDNF), Transforming Growth Factor-beta 1 (TGF- β 1), C-reactive protein (CRP), Tumour Necrosis Factor-alpha (TNF- α), Interleukins 1-beta (IL-1 β), 6 (IL-6) and 10 (IL-10), and cortisol.

Figure 5 shows a graphical representation of the 3-class latent profile solution adequately described by 3 of the 8 markers. Classes were labelled accordingly: Class 1 = Low concentration of all markers (41.5% of the sample), Class 2 = Average Concentration of all markers (42.4%), and Class 3 = High concentration of BDNF and TGF- β 1 (16.1%). Relative concentration represents z-transformed values. Biomarkers: Interleukin-1 β (IL-1 β), Brain-Derived Neurotrophic Factor (BDNF), and Transforming Growth Factor β 1 (TGF- β 1).

Detailed Description

Definitions

Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes V*, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew et al. (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8). Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present invention, the

typical materials and methods are described herein. In describing and claiming the present invention, the following terminology will be used.

It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting. Many patent applications, patents, and publications may be referred to herein to assist in understanding the aspects described. Each of these references is incorporated herein by reference in its entirety.

In understanding the scope of the present application, the articles “a”, “an”, “the”, and “said” are intended to mean that there are one or more of the elements. Additionally, the term “comprising” and its derivatives, as used herein, are intended to be open ended terms that specify the presence of the stated features, elements, components, groups, integers, and/or steps, but do not exclude the presence of other unstated features, elements, components, groups, integers and/or steps. The foregoing also applies to words having similar meanings such as the terms, “including”, “having” and their derivatives.

It will be understood that any aspects described as “comprising” certain components may also “consist of” or “consist essentially of,” wherein “consisting of” has a closed-ended or restrictive meaning and “consisting essentially of” means including the components specified but excluding other components except for materials present as impurities, unavoidable materials present as a result of processes used to provide the components, and components added for a purpose other than achieving the technical effect of the invention. For example, a composition defined using the phrase “consisting essentially of” encompasses any known acceptable additive, excipient, diluent, carrier, and the like. Typically, a composition consisting essentially of a set of components will comprise less than 5% by weight, typically less than 3% by weight, more typically less than 1%, and even more typically less than 0.1% by weight of non-specified component(s).

It will be understood that any component defined herein as being included may be explicitly excluded from the claimed invention by way of proviso or negative limitation.

In addition, all ranges given herein include the end of the ranges and also any intermediate range points, whether explicitly stated or not.

Terms of degree such as “substantially”, “about” and “approximately” as used herein mean a reasonable amount of deviation of the modified term such that the end result is not significantly changed. These terms of degree should be construed as including a deviation of at least $\pm 5\%$ of the modified term if this deviation would not negate the meaning of the word it modifies.

Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below. The abbreviation, "e.g." is derived from the Latin *exempli gratia* and is used herein to indicate a non-limiting example. Thus, the abbreviation "e.g." is synonymous with the term "for example." The word "or" is intended to include "and" unless the context clearly indicates otherwise.

Brain Derived Neurotrophic Factor (BDNF) is a protein encoded by the *BDNF* gene in humans. BDNF is a member of the neurotrophin family of growth factors found in the brain and periphery. BDNF in the short-term promotes pain, however in the long-term acts as an inhibitor of pain-sensitivity.

Transforming Growth Factor beta-1 (TGF β 1 or TGF-beta1) belongs to the transforming growth factor beta family and has been associated with diverse-pain related processes. TGF β 1 can regulate factors involved in nociception, sensitization and Ca²⁺ influx. In addition, it also serves as a protective factor for nerves and can help to regenerate nerves after injury, thereby coupling it to pain.

Interleukins are a group of cytokines that were first seen to be expressed by leukocytes. Interleukin-1beta (IL-1 β or IL-1beta) belongs to the family of cytokines and is characterized as a potent inducer of pain and inflammation. IL-1 β has been implicated in pain, inflammation and autoimmune conditions. However, its role in pain is underappreciated. Dysregulation of the innate immune system with an increase in IL-1 β gives rise to a spectrum of symptoms marked by inflammation and pain.

IL-6 is a cytokine involved in a wide variety of biological functions. It plays an essential role in the final differentiation of B cells into immunoglobulin-secreting cells, as well as inducing myeloma/plasmacytoma growth, nerve cell differentiation, and, in hepatocytes, acute-phase reactants.

IL-10 is a protein that inhibits the synthesis of a number of cytokines, including IFN-gamma, IL-2, IL-3, TNF, and GM-CSF produced by activated macrophages and by helper T cells.

C-reactive protein (CRP) is an annular (ring-shaped), pentameric protein found in blood plasma, whose levels rise in response to inflammation. It is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion by macrophages and T cells. Its physiological role is to bind to lysophosphatidylcholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via C1q.

Cortisol is a steroid hormone, in the glucocorticoid class of hormones. It is produced in humans by the zona fasciculata of the adrenal cortex within the adrenal gland. It is released in response to stress and low blood-glucose concentration. It functions to increase blood sugar through gluconeogenesis, to suppress the immune system, and to aid in the metabolism of fat, protein, and carbohydrates. It also decreases bone formation.

TNF- α is a cell signaling protein (cytokine) involved in systemic inflammation and is one of the cytokines that make up the acute phase reaction. It is produced chiefly by activated macrophages, although it can be produced by many other cell types such as CD4+ lymphocytes, NK cells, neutrophils, mast cells, eosinophils, and neurons. The primary role of TNF α is in the regulation of immune cells. TNF α , being an endogenous pyrogen, is able to induce fever, apoptotic cell death, cachexia, inflammation and to inhibit tumorigenesis and viral replication and respond to sepsis via IL1 & IL6 producing cells. Dysregulation of TNF production has been implicated in a variety of human diseases including Alzheimer's disease, cancer, major depression, psoriasis and inflammatory bowel disease (IBD).

As used herein, the term "biomarker" is intended to encompass a substance that is used as an indicator of a biologic state and includes genes (and nucleotide sequences of such genes), mRNAs (and nucleotide sequences of such mRNAs) and proteins (and amino acid sequences of such proteins). A "biomarker panel" includes a plurality of biomarkers, the expression of each of which is measured in order to provide a quantitative or qualitative summary of the expression of one or more biomarkers in a subject, such as in comparison to a standard or a control. Probes, such as nucleic acid probes or proteins such as antibodies, for example, may be used for measuring the biomarkers or the biomarkers may be measured directly using mRNA or DNA, for example.

The terms "increased" or "increased expression" and "decreased" or "decreased expression", with respect to the expression pattern of a biomarker(s), are used herein as meaning that the level of expression is increased or decreased relative to a constant basal level of expression of a household, or housekeeping, protein, whose expression level does not significantly vary under different conditions. A nonlimiting example of such a household, or housekeeping, protein is GAPDH. Other suitable household, or housekeeping, proteins are well-established in the art. In other aspects, these terms refer to an increase or decrease in the level of expression as compared to that observed in a control population, such as a subject or pool of subjects who have not undergone recent musculoskeletal trauma. In more typical aspects, these terms refer to an increase or decrease in relative concentrations in relation to the mean values of the sample in question.

As used herein, the term "musculoskeletal tissue" refers to muscles, bones, ligaments, or tendons in an animal body. As such, the term "musculoskeletal trauma" refers to muscles, bones, ligaments, and/or tendons that have been injured or damaged. Non-limiting examples of possible types of damage include a broken or bruised bone; a torn, pulled, or bruised muscle; a tendon with a tendinopathy, and so on.

The term "subject" as used herein refers to any member of the animal kingdom, typically a mammal. The term "mammal" refers to any animal classified as a mammal, including humans, other higher primates, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, goats, rabbits, etc. Typically, the mammal is human. In specific aspects, the biomarkers and methods described herein can be used in non-human animals. It will be understood that the biomarkers may not be completely conserved between the human versions described herein and equivalent animal versions, however, given the descriptions and examples provided here in it is understood that a skilled person could modify the biomarkers to be suitable for a desired animal population.

Biomarkers and Panels

Described herein are biomarkers useful for predicting functional recovery after musculoskeletal trauma. More specifically, the biomarkers described herein may find use in predicting the likelihood of a subject developing chronic pain after a traumatic event and may assist in classifying patients into recovery classes, including low intercept with smooth recovery, high intercept with rapid recovery, or high intercept with no or little recovery over at least 6 months. The biomarkers described herein may assist in predicting distal outcomes. Distal outcomes can be described by subject reports of ongoing pain related interference 6 months after an inciting trauma. These can be most easily classed as fully recovered / no disability (scores <5% on a disability scale), moderate ongoing disability (5-20% of the scale), or persistent severe disability (>20% on the scale). The biomarkers described herein may in aspects be used to predict these single-point distal outcomes rather than trajectories, according to clinical context. Additionally, the biomarkers may be used either individually or in combination to predict the severity of a current pain experience without regard to the long-term outcomes in certain subgroups of the injured population, especially effective in those who also describe considerable other life stressors or pre-existing health conditions around or before the inciting trauma, or who report trauma and pain primarily affecting the axial spine (neck, upper or lower back regions). The biomarkers may be combined in a panel, which in aspects may comprise a single platform upon which all of the biomarkers are measured at once in a single test or, alternatively, one or all of the biomarkers may be measured individually and separately from the others.

Typically, the panel of biomarkers comprises at least TGF- β 1 and, optionally, BDNF. The levels of BDNF and TGF- β 1 are inversely correlated with recovery, meaning that high levels of these biomarkers are typically associated with worse recovery and low levels are associated with better recovery, both in terms of the extent of recovery (distal outcome) and the speed of recovery (trajectory). Likewise, high levels tend to be associated with an increased likelihood of the subject experiencing chronic pain, while low levels tend to be associated with a lower likelihood of chronic pain in the subject. TGF- β 1 in particular may also be useful in isolation or in combination with other markers like IL-1 β for predicting current pain severity in certain subgroups of the population, including the unemployed or those with a pre-existing mood disorder where, unlike for longer term prognosis, in the acute pain state higher TGF- β 1 is associated with lower pain intensity in those groups when both are measured at the same time.

In aspects, the overall levels of the biomarkers are useful for assigning a subject to a specific recovery group. For example, low levels of BDNF and TGF- β 1 as compared to certain predetermined control values assign the subject to a group designated "some recovery expected". These subjects are expected to make a full to moderate recovery over a rapid or intermediate time period. In contrast, high levels of BDNF and TGF- β 1 as compared to certain predetermined control values assign the subject to a group designated "no or minimal recovery". These subjects are more likely to report persistent problems 6 months later. Any recovery that is expected will also happen over a longer time period in these subjects. These subjects are more likely to experience chronic pain than those assigned to the "some recovery" group.

In aspects, the panel may further comprise IL-1 β . In such cases, IL-1 β is particularly useful for further defining the 3 different classes of biomarker groupings, in that IL-1 β can be used to describe two additional groups: a 'low concentration of all markers' group and an 'average concentration of all markers' group, in comparison to certain normative values. IL-1 β may be particularly useful for predicting current pain in certain subgroups of the population, including those who are overweight (BMI > about 25 kg/m²) or those with a pre-existing pain problem present before the most recent injury. In other aspects, together TGF- β 1 and IL-1 β function to predict current pain severity in populations with pre-existing mood disorders. In other aspects, lower levels of TGF- β 1 and IL-1 β are associated with higher pain severity in the acute post-trauma phase in those populations.

Other biomarkers may also be included in the panel, for example for purposes of predicting current or future pain severity and related interference. Specific examples include one or more of TNF- α , IL-6, IL-10, C-Reactive Protein (CRP), and cortisol. Typically, low levels of these biomarkers are correlated to good outcomes, including rapid and/or full

recovery, whereas high levels are correlated to worse outcomes, such as slow and/or little to no recovery, though there are instances where these relationships are reversed in certain subgroups of the population. The panel may comprise any or all of BDNF, TGF- β 1, IL-1 β , TNF- α , IL-6, IL-10, CRP, and cortisol or it may consist of any combination or all of BDNF, TGF- β 1, IL-1 β , TNF- α , IL-6, IL-10, CRP, and cortisol. Surprisingly, not all of the identified biomarkers function in the same way or affect the same pathways, and some are in very different pathways such that some may exist in high concentrations while others are low in the same person. It is only when combinations of these biomarkers are present (such as at least 2, 3, 4, or more) in the same person, is the higher risk identified. Without wishing to be bound by theory, it appears that there is a synergistic relationship amongst these biomarkers that correlates with worse outcomes.

The biomarkers are typically isolated from blood, but it is understood that they may be isolated from any body tissue or fluid, in line with known practices for any given biomarker. Examples include blood, blood plasma, blood serum, hemolysate, spinal fluid, urine, lymph, synovial fluid, saliva, sperm, amniotic fluid, lacrimal fluid, cyst fluid, sweat gland secretion, and bile. It is not necessary that all measured biomarkers be isolated from the same source, for example, one biomarker may be isolated from blood and another from a urine sample. However, it is generally most convenient for all tested biomarkers to be measured from a single blood draw.

Typically, it is the protein biomarker that is measured. It is also possible to measure mRNA using known methods. Typically, the protein biomarkers are measured using antibodies, for example, in an ELISA or Luminex-based method. Methods for detecting and measuring the biomarkers are known to a skilled person and certain typical methods are exemplified herein.

For example, in methods of generating a result useful for functional recovery classification, the expression pattern in blood, serum, etc. of the biomarkers provided herein is obtained. The quantitative data associated with the biomarkers of interest can be any data that allows generation of a result useful for functional recovery classification, including measurement of DNA or RNA levels associated with the markers but is typically protein expression patterns. Protein levels can be measured via any method known to those of skill in the art that generates a quantitative measurement either individually or via high-throughput methods as part of an expression profile. For example, a blood-derived patient sample, e.g., blood, plasma, serum, etc. may be applied to a specific binding agent or panel of specific binding agents to determine the presence and quantity of the protein markers of interest.

The quantitative data associated with the biomarkers of interest typically takes the form of an expression profile. Expression profiles constitute a set of relative or absolute expression values for a number of biomarker products corresponding to the plurality of markers evaluated. In various embodiments, expression profiles containing expression patterns of at least about 2, 3, 4, 5, 6, 7, 8 or more markers are produced. The expression pattern for each differentially expressed component member of the expression profile may provide a particular specificity and sensitivity with respect to predictive value, e.g., for diagnosis, prognosis, monitoring treatment, etc.

Numerous methods for obtaining expression data are known, and any one or more of these techniques, singly or in combination, are suitable for determining expression patterns and profiles in the context of the present disclosure.

For example, DNA and RNA (mRNA, pri-miRNA, pre-miRNA, miRNA, precursor hairpin RNA, microRNP, and the like) expression patterns can be evaluated by northern analysis, PCR, RT-PCR, Taq Man analysis, FRET detection, monitoring one or more molecular beacon, hybridization to an oligonucleotide array, hybridization to a cDNA array, hybridization to a polynucleotide array, hybridization to a liquid microarray, hybridization to a microelectric array, cDNA sequencing, clone hybridization, cDNA fragment fingerprinting, serial analysis of gene expression (SAGE), subtractive hybridization, differential display and/or differential screening. These and other techniques are well known to those of skill in the art.

The present disclosure includes nucleic acid molecules, typically in isolated form. As used herein, a nucleic acid molecule is to be "isolated" when the nucleic acid molecule is substantially separated from contaminant nucleic acid molecules encoding other polypeptides. The term "nucleic acid" is defined as coding and noncoding RNA or DNA. Nucleic acids that are complementary to, that is, hybridize to, and remain stably bound to the molecules under appropriate stringency conditions are included within the scope of this disclosure. Such sequences exhibit at least 50%, 60%, 70% or 75%, typically at least about 80-90%, more typically at least about 92-94%, and even more typically at least about 95%, 98%, 99% or more nucleotide sequence identity with the sequences for the biomarkers disclosed herein, and include insertions, deletions, wobble bases, substitutions, and the like. Further contemplated are sequences sharing at least about 50%, 60%, 70% or 75%, typically at least about 80-90%, more typically at least about 92-94%, and most typically at least about 95%, 98%, 99% or more identity with the biomarker sequences disclosed herein

Specifically contemplated within the scope of the disclosure are genomic DNA, cDNA, RNA (mRNA, pri-miRNA, pre-miRNA, miRNA, hairpin precursor RNA, RNP, etc.)

molecules, as well as nucleic acids based on alternative backbones or including alternative bases, whether derived from natural sources or synthesized.

The present disclosure further provides fragments of the disclosed nucleic acid molecules and/or proteins. As used herein, a fragment of a nucleic acid molecule refers to a small portion of the coding or non-coding sequence. The size of the fragment will be determined by the intended use. For example, if the fragment is chosen so as to encode an active portion of the protein, the fragment will need to be large enough to encode the functional region(s) of the protein. For instance, fragments which encode peptides corresponding to predicted antigenic regions may be prepared. If the fragment is to be used as a nucleic acid probe or PCR primer, then the fragment length is chosen so as to obtain a relatively small number of false positives during probing/priming.

Protein expression patterns can be evaluated by any method known to those of skill in the art which provides a quantitative measure and is suitable for evaluation of multiple markers extracted from samples such as one or more of the following methods: ELISA sandwich assays, flow cytometry, mass spectrometric detection, calorimetric assays, binding to a protein array (e.g., antibody array), or fluorescent activated cell sorting (FACS).

In one embodiment, an approach involves the use of labeled affinity reagents (e.g., antibodies, small molecules, etc.) that recognize epitopes of one or more protein products in an ELISA, antibody-labelled fluorescent bead array, antibody array, or FACS screen. Methods for producing and evaluating antibodies are well known in the art.

A number of suitable high throughput formats exist for evaluating expression patterns and profiles of the disclosed biomarkers. Typically, the term high throughput refers to a format that performs at least about 100 assays, or at least about 500 assays, or at least about 1000 assays, or at least about 5000 assays, or at least about 10,000 assays, or more per day. When enumerating assays, either the number of samples or the number of markers assayed can be considered.

Numerous technological platforms for performing high throughput expression analysis are known. Generally, such methods involve a logical or physical array of either the subject samples, or the protein markers, or both. Common array formats include both liquid and solid phase arrays. For example, assays employing liquid phase arrays, e.g., for hybridization of nucleic acids, binding of antibodies or other receptors to ligand, etc., can be performed in multiwell or microtiter plates. Microtiter plates with 96, 384 or 1536 wells are widely available, and even higher numbers of wells, e.g., 3456 and 9600 can be used. In general, the choice of microtiter plates is determined by the methods and equipment, e.g.,

robotic handling and loading systems, used for sample preparation and analysis. Exemplary systems include, e.g., xMAP® technology from Luminex (Austin, Tex.), the SECTOR® Imager with MULTI-ARRAY® and MULTI-SPOT® technologies from Meso Scale Discovery (Gaithersburg, Md.), the ORCA™ system from Beckman-Coulter, Inc. (Fullerton, Calif.) and
5 the ZYMATE™ systems from Zymark Corporation (Hopkinton, Mass.), miRCURY LNA™ microRNA Arrays (Exiqon, Woburn, Mass.).

Alternatively, a variety of solid phase arrays can favorably be employed to determine expression patterns in the context of the disclosed methods, assays and kits. Exemplary formats include membrane or filter arrays (e.g., nitrocellulose, nylon), pin arrays, and bead
10 arrays (e.g., in a liquid “slurry”). Typically, probes corresponding to nucleic acid or protein reagents that specifically interact with (e.g., hybridize to or bind to) an expression product corresponding to a member of the candidate library, are immobilized, for example by direct or indirect cross-linking, to the solid support. Essentially any solid support capable of
15 withstanding the reagents and conditions necessary for performing the particular expression assay can be utilized. For example, functionalized glass, silicon, silicon dioxide, modified silicon, any of a variety of polymers, such as (poly)tetrafluoroethylene, (poly)vinylidenedifluoride, polystyrene, polycarbonate, or combinations thereof can all serve as the substrate for a solid phase array.

In one embodiment, the array is a “chip” composed, e.g., of one of the above-
20 specified materials. Polynucleotide probes, e.g., RNA or DNA, such as cDNA, synthetic oligonucleotides, and the like, or binding proteins such as antibodies or antigen-binding fragments or derivatives thereof, that specifically interact with expression products of individual components of the candidate library are affixed to the chip in a logically ordered manner, i.e., in an array. In addition, any molecule with a specific affinity for either the sense
25 or anti-sense sequence of the marker nucleotide sequence (depending on the design of the sample labeling), can be fixed to the array surface without loss of specific affinity for the marker and can be obtained and produced for array production, for example, proteins that specifically recognize the specific nucleic acid sequence of the marker, ribozymes, peptide nucleic acids (PNA), or other chemicals or molecules with specific affinity.

30 Microarray expression may be detected by scanning the microarray with a variety of laser or CCD-based scanners, and extracting features with numerous software packages, for example, IMAGEGENE™ (Biodiscovery), Feature Extraction Software (Agilent), SCANLYZE™ (Stanford Univ., Stanford, Calif.), GENEPIX™ (Axon Instruments).

High-throughput protein systems include commercially available systems from
35 CIPHERGEN Biosystems, Inc. (Fremont, Calif.) such as PROTEIN CHIP™ arrays, and

FASTQUANT™ human chemokine protein microspot array (S&S Biosciences Inc., Keene, N.H., US).

Quantitative data regarding other dataset components, such as clinical indicia, metabolic measures, and genetic assays, can be determined via methods known to those of skill in the art.

Various analytic processes for obtaining a result useful for making a functional recovery classification are described herein, however, one of skill in the art will readily understand that any suitable type of analytic process is within the scope of this disclosure.

In assigning subjects to specific recovery groups, where low intercept, smooth recovery is Class 1, high intercept, rapid recovery is Class 2, and high intercept, little to no recovery is Class 3, it will be understood that these are typically more of a continuum than a discrete line delineating one class from another and, therefore, there is often overlap between the marker levels and associated classes. However, typically, BDNF, TGF- β 1, IL-1 β , TNF- α , IL-6, IL-10, cortisol, and CRP levels are positively correlated with increasing class, meaning that higher levels tend to indicate worse predicted outcome.

In aspects, BDNF levels of up to about 2500 pg/mL are associated with Class 1, of from about 2500 to about 5000 pg/mL are associated with Class 2, and of from about 5000 pg/mL or higher are associated with Class 3.

In aspects, TGF- β 1 levels of up to about 21,000 pg/mL are associated with Class 1, of from about 19,000 to about 30,000 pg/mL are associated with Class 2, and of from about 30,000 pg/mL or higher are associated with Class 3.

In aspects, IL-1 β levels of up to about 2.5 pg/mL are associated with Class 1, of from about 2.5 pg/mL or higher are associated with Class 2, and of from about 2.5 pg/mL or higher are associated with Class 3.

In aspects, TNF- α levels of up to about 5.2 pg/mL are associated with Class 1, of from about 5.2 pg/mL or higher are associated with Class 2, and of from about 5.2 pg/mL or higher are associated with Class 3.

In aspects, IL-6 levels of up to about 85 pg/mL are associated with Class 1, of from about 75 pg/mL or higher are associated with Class 2, and of from about 75 pg/mL or higher are associated with Class 3.

In aspects, IL-10 levels of up to about 16 pg/mL are associated with Class 1, of from about 16 pg/mL or higher are associated with Class 2, and of from about 16 pg/mL or higher are associated with Class 3.

5 In aspects, cortisol levels of up to about 120000 pg/mL are associated with Class 1, of from about 105000 pg/mL or higher are associated with Class 2, and of from about 85000 pg/mL or higher are associated with Class 3.

In aspects, CRP levels of up to about 4000 ng/mL are associated with Class 1, of from about 2200 ng/mL or higher are associated with Class 2, and of from about 1500 ng/mL or higher are associated with Class 3.

10 The panels of biomarkers described herein may be provided as an assay for predicting functional recovery after musculoskeletal trauma and/or for predicting likelihood of a subject experiencing chronic pain or functional interference after a traumatic event. Further, provided herein are kits for detecting the biomarkers of these panels. The kits may include a single platform including all of the biomarkers to be measured, along with suitable
15 reagents and/or instructions for use. They may alternatively include individual components for measuring each biomarker of interest separately and/or in any desired combination.

Methods

Also described herein are methods for predicting functional recovery after musculoskeletal trauma in a subject. These methods typically comprise measuring the levels
20 of a panel of biomarkers comprising BDNF and TGF- β 1 in the subject, wherein the levels of BDNF and TGF- β 1 are inversely correlated with recovery.

Typically, as has been described above, low levels of BDNF and TGF- β 1 as compared to control values assigns the subject to a "some recovery" group and high levels of BDNF and TGF- β 1 as compared to control values assigns the subject to a "no or minimal
25 recovery group."

The panel may further comprise IL-1 β for further discriminating between a 'low concentration of all biomarkers' group and an 'average concentration of all biomarkers' group. IL-1 β may work in synergy with TGF- β 1 to predict current pain severity especially in people with existing mood disorders.

30 In the methods described herein, further biomarkers may be measured. These typically include one or more of TNF- α , IL-6, IL-10, CRP and cortisol. Thus, typically, the panel of biomarkers comprises or consists of one or more of BDNF, TGF- β 1, IL-1 β , TNF- α ,

IL-6, IL-10, CRP, and cortisol and, typically, comprises or consists of BDNF, TGF- β 1, and IL-1 β .

In aspects, the methods described herein further comprise assessing factors associated with the subject. These factors often comprise one or more of employment
5 status, household income, educational attainment, post-traumatic distress, and pre-existing physical or psychological comorbidities, such as sex, BMI, and/or mood disorders, and are assessed using questionnaires, by asking the subject or family members questions, by reviewing medical charts, and other known methods.

In aspects, the methods described herein further comprise treating the subject based
10 on the predicted functional recovery. For example, if the subject is classified into the rapid recovery group, the treatment and follow up he may receive would be expected to be quite different from if he had been classified into the no or minimal recovery group. Such subjects would be expected to have dramatically different needs and outcomes and would require different treatments. Being able to predict at a very early stage which outcome is expected
15 can assist in providing targeted treatment to the subjects that would benefit most from that and avoid over-treating subjects that do not necessarily need certain interventions.

Treatment decisions are best made by the treating clinicians, but in those who are predicted to be in the no or minimal recovery group, these may include more targeted pain management strategies, cognitive-behavioural interventions, or graded activity and exercise
20 in accordance with known medical and rehabilitation principles.

Also described herein is a prognostic phenotyping protocol for predicting rate of recovery in MSK trauma in a subject, the protocol comprising measuring the levels of a panel of biomarkers comprising BDNF and TGF- β 1 in the subject, wherein low levels of BDNF and TGF- β 1 as compared to control values assign the subject to a some recovery
25 group and high levels of BDNF and TGF- β 1 as compared to control values assigns the subject to a no or minimal recovery group. As explained above, the panel may further comprise at least IL-1 β , TNF α , IL-6, IL-10, cortisol, and/or CRP. Generally, lower levels of these markers as compared to control values assign the subject to a rapid recovery group, and high levels of these markers as compared to control values assign the subject to the no
30 or minimal recovery group, though these associations may change or even be reversed in certain subgroups of the population.

The methods described herein may also find use in predicting risk of chronic pain in a subject. The method comprises measuring the levels of a panel of biomarkers comprising BDNF and TGF- β 1 in the subject, wherein the levels of BDNF and TGF- β 1 are positively
35 correlated with the likelihood of the subject experiencing chronic pain.

The methods described herein may also find use in predicting and/or estimating the current severity of pain in a subject. The method comprises measuring the levels of a panel of biomarkers comprising IL-1 β and TGF- β 1 in the subject, wherein the levels of IL-1 β and TGF- β 1 are negatively correlated with the predicted and/or estimated severity of pain that the subject is currently experiencing. In typical aspects, the levels of these biomarkers are considered alongside an assessment of factors associated with the subject, as outlined herein.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples. These examples are provided for purposes of illustration only and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

The following examples do not include detailed descriptions of conventional methods, such as those employed in the construction of vectors and plasmids, the insertion of genes encoding polypeptides into such vectors and plasmids, or the introduction of plasmids into host cells. Such methods are well known to those of ordinary skill in the art and are described in numerous publications including Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989), *Molecular Cloning: A Laboratory Manual*, 2nd edition, Cold Spring Harbor Laboratory Press, which is incorporated by reference herein.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out the typical aspects of the present invention and are not to be construed as limiting in any way the remainder of the disclosure.

Examples

Example 1: Describing Post-Traumatic Recovery through Trajectory and Distal 6-month Outcomes

The current analysis was undertaken to describe the outcomes of recovery following acute musculoskeletal trauma using two different approaches: 1. The identification of distal outcomes to describe the magnitude of pain or functional interference as reported by participants when measured at a single point 6 months after the inciting trauma, and 2. The identification of meaningful trajectories in the data to describe how participants transitioned from acute trauma through to either recovery or persistent pain and interference.

Data for this observational cohort study were drawn from the longitudinal SYMBIOME (Systematic Merging of Biology, Mental Health and Environment) databanking study (clinicaltrials.gov ID no. NCT02711085). The methods of data collection were, in brief, eligible participants were identified from emergency or acute-care clinicians within a local hospital urgent care centre. Participants were eligible for inclusion if they presented to the centre within 3 weeks of sustaining any non-catastrophic injury affecting the musculoskeletal system. Non-catastrophic was defined as any injury that was managed conservatively and did not require inpatient admission or surgery. These included slips and falls, road traffic injuries, occupational injuries, sports injuries, or other related trauma. Other inclusion criteria were age of 18-66 years, able to speak and understand conversational English, and free of major systemic disease or disorder that would logically affect recovery from MSK trauma, such as cancer, major organ disease, or neuromuscular disorder such as stroke or amyotrophic lateral sclerosis. Pregnancy was not a reason for exclusion, though no pregnant women were recruited during the duration of this study.

After being medically cleared and discharged, interested participants gave permission for a member of the research team to describe the study, answer questions, and enroll prior to leaving the hospital. Prior to leaving, consenting participants had two vials of antecubital blood drawn into 4mL K2 EDTA BD vacutainer tubes by a trained phlebotomist and immediately stored on ice for transfer and storage at a wet laboratory facility. Baseline characteristics including age, sex, and current symptom intensity were also captured. Participants were then sent home with a more fulsome package of clinical questionnaires and kits for capturing samples of saliva, hair and stool (the latter two were optional components).

The questionnaires included tools to measure pain intensity and functional interference (Brief Pain Inventory), depressive symptoms (Patient Health Questionnaire-9), acute stress reactions (Acute Stress Disorder Scale), trauma-specific distress (Traumatic Injuries Distress Scale), and several questions pertaining to patient metadata (age, sex, work status, educational attainment, medicolegal status, household income and family status), peri-traumatic lifestyle (stress, activity, diet) and health (medications, comorbidities) variables. All participants provided informed, written consent prior to participation.

The primary outcome used to define recovery was the Brief Pain Inventory (BPI). The BPI is one of the most widely used pain-interference scales globally and has adequate evidence of validity across many clinical populations including musculoskeletal pain. It provides two subscale scores: one measuring pain severity and another measuring pain-related interference.

Follow-up occurred at 1, 2, 3, 6 and 12 months from injury, with the biological samples collected at 3, 6 and 12 months only, and participants were reimbursed up to \$300 in expenses and compensation for time for participation in the entire 12-month study.

For purposes of defining the recovery trajectories only (no biomarker data), data from an additional cohort were included. These data came from a very similar observational cohort study run in Chicago, IL, with the primary differences being a focus on post-traumatic neck pain (i.e. whiplash-associated disorder) in which pain severity was measured with a 0-10 Numeric Rating Scale (analogous to the BPI Pain Severity score) and interference was measured using the Neck Disability Index (a region-specific tool analogous to the BPI Pain Interference score when both are converted as a percentage of max possible score). Another subtle difference was in timing of outcome, meaning that follow-up points were binned across the two databases into baseline (<3 weeks for injury), 2-4 weeks, 3 months, and 6-12 months. The 2-month score from our cohort was dropped from the trajectory analysis as it added unnecessary noise when less than half the sample had data for that point.

In both cohorts, any intervention was provided at the discretion of the treating healthcare provider. Broad categories of treatment received were captured during follow-up in the SYMBIOME cohort, but as they appeared to have no material effect on outcomes or trajectories, were not explored further here.

Analysis

Distal Outcomes: The primary outcome was functional interference using the relevant interference score from each cohort (BPI or NDI) converted to a percentage of max possible score. Disability thresholds were used here to create 3 categories of distal outcome: full recovery (<5% disability), moderate persistent problems (5-20% disability) and severe persistent problems (>20% disability). Each participant was assigned to one the 3 categories (coded 1, 2, or 3) based on their 6 month score.

Trajectories: Latent Growth Curve Analysis using the Growth Mixture Modeling function in MPlus v6.12 software (Muthen and Muthen) was used to identify the latent trajectories within the data. Raw, non-transformed data were used for the analysis, and any participant with at least one data point was included in the analysis. Missing data were not imputed with the exception of those participants who scored a 0% disability at the second-to-last, in which case the 0 was carried forward under the assumption that they had recovered. Preliminary analysis indicated that the models were best evaluated when those with axial problems (neck or low back injuries) were disaggregated from those with extremity (upper or lower extremity) injuries. Different class models were explored, including both linear and

quadratic curves, and when all parameters were free to vary or were constrained in a serial fashion until the best model fit was obtained. In this case, 'best' was defined as having the lowest AIC and BIC values, high entropy (>0.80), classification accuracy of 85% or higher across all classes, no class with fewer than 10 participants, a bootstrapped LMR Likelihood ratio test with a significant (<0.05) p value when the k class solution was compared to the k-1 class solution, and clinical interpretability of the class structure.

Participant demographic and metadata were explored descriptively, including means, range, and frequencies as appropriate.

Results

Across the two cohorts, 231 participants had at least one data point and were included in the analyses. These broke down by follow-up period as follows: baseline (n = 231), 1 month (n = 180), 3 months (n = 173) and 6-12 months (n = 147). The demographic data of these are described in Table 1 below. There were 145 participants with primarily axial injuries, and 96 with primarily extremity injuries.

15	Table 1: Participant demographics of the full longitudinal cohort.																						
	<table> <tr> <th data-bbox="363 1059 464 1093">N = 231</th><th data-bbox="1002 1059 1157 1093">Description</th></tr> <tr> <td data-bbox="363 1093 564 1126">Sex (% female)</td><td data-bbox="1002 1093 1082 1126">54.9%</td></tr> <tr> <td data-bbox="363 1126 608 1160">Age (mean, range)</td><td data-bbox="1002 1126 1182 1160">39.7 (18 to 66)</td></tr> <tr> <td data-bbox="363 1160 608 1193">BMI (mean, range)</td><td data-bbox="1002 1160 1228 1193">26.1 (14.4 to 51.5)</td></tr> <tr> <td data-bbox="363 1193 576 1227">Region of injury</td><td></td></tr> <tr> <td data-bbox="387 1227 703 1261"> Axial (neck or low back)</td><td data-bbox="1002 1227 1082 1261">60.2%</td></tr> <tr> <td data-bbox="387 1261 735 1294"> Extremity (upper or lower)</td><td data-bbox="1002 1261 1082 1294">39.8%</td></tr> <tr> <td data-bbox="363 1294 735 1328">Employed for pay (FT or PT)</td><td data-bbox="1002 1294 1082 1328">73.7%</td></tr> <tr> <td data-bbox="363 1328 719 1361">Post-Secondary Education</td><td data-bbox="1002 1328 1082 1361">75.0%</td></tr> <tr> <td data-bbox="363 1361 975 1395">Pre-trauma Psychopathology (Mood Disorder)</td><td data-bbox="1002 1361 1082 1395">26.1%</td></tr> <tr> <td data-bbox="363 1395 715 1429">Pre-trauma Pain condition</td><td data-bbox="1002 1395 1082 1429">17.1%</td></tr> </table>	N = 231	Description	Sex (% female)	54.9%	Age (mean, range)	39.7 (18 to 66)	BMI (mean, range)	26.1 (14.4 to 51.5)	Region of injury		Axial (neck or low back)	60.2%	Extremity (upper or lower)	39.8%	Employed for pay (FT or PT)	73.7%	Post-Secondary Education	75.0%	Pre-trauma Psychopathology (Mood Disorder)	26.1%	Pre-trauma Pain condition	17.1%
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Distal Outcomes: Owing to attrition over time, 65% of the sample at baseline provided data at the final follow-up period. In order to classify the remaining 35%, estimated distal values were extracted from the recovery curves defined by LGCA. The estimated models appeared accurate as the correlation between the estimated and available observed data was $r = 0.94$. Using our pre-defined thresholds against the observed or estimated 6-12 month outcomes, 44.9% of the overall sample was fully recovered (<5% disability), 35.1% were moderately disabled (5-20% disability) and 20.0% were highly disabled (>20% disability).

Trajectories: After comparing multiple iterations, a 3-class quadratic model was accepted for the combined axial and extremity injury group. In the combined group, variance in the quadratic term was constrained to zero for trustworthy model convergence while all

other parameters remained free. The 3 trajectories had similar characteristics (shown for the whole cohort in Figure 1). These were labeled: Low intercept, smooth recovery (rapid recovery) (34.9% of the sample), High intercept, rapid recovery (delayed recovery) (19.2% of the sample), and High intercept, little or no recovery by 6 months (no or minimal recovery) (45.9% of the sample). Table 2 shows the starting and follow-up values for percent interference at each time point for each class.

Table 2: Proportions and estimated means for % Interference (Top) and Pain Severity (Bottom) trajectory classes with 95% confidence intervals. Differences between classes were explored using Bonferroni-corrected post-hoc analyses for significant Class x Time interactions. 1: Mean % Interference in the *Rapid Recovery* group is significantly lower than the other two groups, with no difference between *Delayed* and *Minimal* recovery groups by virtue of overlapping confidence intervals. 2: Mean % Interference / mean pain severity is significantly different across all groups. 3: Mean % Interference is significantly higher in the *Minimal* recovery group than the other two groups, with no difference between the *Rapid* and *Delayed* groups.

Trajectory	N (%)	Baseline	1-month	3-month	6-12-month
Class 1: Low intercept, smooth recovery	34.9	21.9 (19.8, 24.1) ¹	3.2 (2.6, 3.8) ¹	0.1 (0.0, 0.1) ²	0.0 (0.0, 0.1)
Class 2: High intercept, rapid recovery	19.2	43.4 (38.8, 48.3)	32.0 (28.7, 35.4)	13.2 (11.4, 15.1) ²	0.3 (0.1, 0.6)
Class 3: High intercept, little or no recovery by 6 months	45.9	40.8 (38.7, 43.0)	28.5 (26.9, 30.3)	22.0 (20.5, 23.5) ²	19.7 (18.1, 21.4) ³

Summary

We have provided two different methods of describing recovery following acute MSK trauma, one based on a distal outcome when captured 6-12 months after the inciting event, and another based on the trajectories taken to reach that distal outcome. A 3-class approach appears most suitable to the data. The relative proportions in the distal outcomes groups are almost identical to those shown by prior authors in single-region trauma cohorts such as traumatic neck pain (e.g. Sterling et al. 2010). The trajectories are also similar to prior work, such as that of Ailliet and colleagues (2018) in a mixed sample of people with neck and low back pain. This reproduction of findings is encouraging as it provides confidence that subsequent work predicting these clinical outcomes is more likely to be generalizable outside of our cohort.

It is important to note that the trajectories and distal outcomes are not the same, as can clearly be seen by the differences in relative proportions across the 3 classes when interpreted by a single point or by a slope, intercept, and quadratic function. While trajectory class 1 included largely participants who were also classed as fully recovered by the distal outcome, there were still 8.6% of the group who scored between 5 and 20% disability at 6-12 months. Similarly, while Class 3 included the largest number of participants who still rated higher percent disability at 6-12 months, there were 59.6% of participants within the 5 to

20% disability category and 1.1% of participants under 5% disability. The distal outcomes can best be thought of as 'where people end up' while the trajectories can be thought of as 'how they get there', but these are not the same thing.

5 The next steps to be described are to better understand the differences between these groups at baseline in terms of biological, psychological, and social markers. These will be explored in the following example.

Example 2:

10 In this analyses we explored potential interactions between person-level variables (age, sex, body mass index, life stress, pre-existing health conditions) and blood markers in predicting pain and interference in the acute post-trauma period.

Bivariate correlations

15 Alone, none of the biomarkers demonstrated a significant linear correlation with either outcome. The Tables in this example also present the correlation coefficients when stratified by level of metadata variable. Bolded values are those that were explored through formal regression-based moderator analysis based on the difference in magnitude of correlation coefficient between the two levels of stratification.

Moderator analyses for Pain Severity

20 *Employment prior to trauma* (employed for pay/not employed for pay) fully moderated the association of TNF- α (interaction $\Delta R^2 = 4.4\%$ of total variance in pain severity, **Figure 2A**). *Pre-existing psychopathology* (yes/no) fully moderated the association between TGF- $\beta 1$ and pain severity (interaction $R^2 = 8.0\%$ of total variance, **Figure 2B**). *Sex* (male/female) fully moderated the association between CRP and pain severity ($\Delta R^2 = 6.3\%$, **Figure 2C**).

Moderator analyses for Pain Interference

25 A *pre-existing pain condition* (yes/no) was significantly associated with worse pain interference ($\Delta R^2 = 7.2\%$), and partially moderated the effect of IL-1 β on pain interference ($\Delta R^2 = 6.9\%$, **Figure 3A**). *Region of injury* fully moderated the effect of cortisol on pain interference ($\Delta R^2 = 4.5\%$, $p = 0.04$, **Figure 3B**), fully moderated the effect of TGF- $\beta 1$ on pain interference ($\Delta R^2 = 4.5\%$, **Figure 3C**), and fully moderated the effect of CRP on pain interference ($\Delta R^2 = 10\%$, $p < 0.01$, **Figure 3D**). Higher *peri-traumatic life stress* significantly explained 8.9% of variance in pain interference alone, and partially moderated the effect of TGF- $\beta 1$ on interference ($\Delta R^2 = 4.4\%$, **Figure 3E**).

30

Example 3:

In this example we explain the derivation of the blood panel, showing that 3 markers at minimum can be used to define clinically-relevant clusters but that 3 additional markers may be useful in some circumstances.

5 METHODS

Data from this observational cohort study were drawn from the longitudinal SYMBIOME (Systematic Merging of Biology, Mental Health and Environment) databanking study (clinicaltrials.gov ID no. NCT02711085). The study was approved by the office of Human Research Ethics at Western University and the Lawson Health Research Institute, and written, informed consent was obtained from all participants. Eligible participants were identified by emergency or acute-care clinicians from an urgent care centre in London, ON, Canada. After being medically discharged, a member of the research team described the study, answered questions, enrolled and screened potential participants prior to leaving the hospital. Two samples of antecubital blood were drawn into 4mL K2 EDTA BD vacutainer tubes by a trained phlebotomist and immediately stored on ice for transfer and storage at an immunity and proteomics lab. Prior to freezing the samples were centrifuged for 10 minutes at 2000 x g, had plasma pipetted into up to 6 x 50µL aliquots, and then both supernatant and pellet were stored at -80°C. Participants were concurrently provided a package of self-report questionnaires that included demographic metadata (age, sex, education level, work status, household income, pre-existing pathology, BMI, and region of injury) and pain intensity and functional interference through the Brief Pain Inventory. All participants provided informed, written consent prior to participation.

Follow-up occurred at 1, 2, 3, 6 and 12 months from injury, with the biological samples collected at baseline, 3, 6 and 12 months only. Participants were paid up to \$300 in total compensation for participation. For the purposes of this study, only the baseline blood samples were analysed and interpreted for biomarker classes, and owing to attrition recovery up to the 6-month follow-up was used as the final end point. Functional recovery was measured using the pain and interference subscales of the BPI. The BPI is one of the most widely used pain-interference scales globally and has adequate evidence of validity across many clinical populations including musculoskeletal pain.

Analysis of serum biomarkers

The target markers for this analysis were those shown previously to be associated with pain, distress, or inflammation. Through a collaborative consultative process, eight markers were specifically chosen: Brain-Derived Neurotrophic Factor (BDNF), Transforming Growth Factor-beta 1 (TGFβ1), C-reactive protein (CRP), Tumour Necrosis Factor-alpha

(TNF- α), Interleukins 1-beta (IL-1 β), 6 (IL-6) and 10 (IL-10), and the stress hormone cortisol. Analyte concentrations in plasma were assayed using multiplexed biomarker immunoassay kits according to manufacturers' instruction for Brain Derived Neurotrophic Factor (Human Premixed Multi-Analyte Kit, R&D Systems Inc. cat. no. LXSAHM), Transforming Growth

5 Factor-Beta 1 (TGFB1 Single Plex Magnetic Bead Kit, EMD Millipore cat. no. TGFB1MAG-64K-01), Interleukins 1- β , 6, and 10 and TNF- α (Human High Sensitivity T Cell Magnetic Bead Panel Multiplex Kit, EMD Millipore cat. no. HSTCMAG-28SK). A BioPlex™ 200 readout System was used (Bio-Rad Laboratories, Hercules, CA), that uses

10 Luminex® xMAP™ fluorescent bead-based technology (Luminex Corp., Austin, TX). Levels were automatically calculated from standard curves using Bio-Plex Manager software (v.4.1.1, Bio-Rad). Cortisol (Cortisol Enzyme Immunoassay Kit, Arbor Assays cat. no. K003-H1/H5), and C-Reactive Protein (C-Reactive Protein (human) ELISA Kit, Cayman Chemical Company cat. no. 10011236) were assayed following industry-standard approaches for

15 Enzyme-Linked Immunosorbant assay (ELISA). All assays were performed in duplicate with the value for analysis being the mean concentration of the two runs.

ANALYSIS

Participant characteristics were summarized descriptively (means and distributions or proportions).

Pre-analysis of analytes

20 Prior to primary analyses we explored the distribution of the data both qualitatively and statistically. Concentrations of all 8 analytes were significantly positively skewed and in violation of normality via Kolmogorov-Smirnov tests. High outliers (>4SD above the mean) or those for which the assay resulted in non-detectable (too low or too high) concentrations were first removed. All concentrations were then square-root transformed to reduce

25 skewness, and then Z-transformed to place all concentrations on the same scale with a mean of 0.0 and standard deviation of 1.0.

Bivariate associations

A matrix of all cross-product Pearson correlations between the 8 markers was created as an exploratory step and to identify potential problems with collinearity in cluster

30 analysis ($r > 0.80$). There was no statistical correction for multiple comparisons, accepting the potential for alpha error rather than prematurely rejecting potentially important findings at this exploratory stage.

Profile Analysis

Meaningful clusters in the data were identified with maximum likelihood estimation

35 (MLE)-based latent profile analysis (LPA) as previously described using MPlus software

v6.12 (Muthen and Muthen, Los Angeles, USA). Using all 8 target biomarkers, a series of models were constructed, starting with a single profile (termed 'class') and increasing until model fit no longer improved in a meaningful way, the LPA estimation could no longer derive a mathematically definable model, one of the latent classes possessed fewer than 10% of participants, or the class structure did not make clinical sense. The fit indicators of interest were the Aikake Information Criterion (AIC), the Bayesian Information Criterion (BIC), entropy, and the adjusted Lo-Mendell-Rubin likelihood ratio test (LMR-LRT) while considering solutions that provide generally strong posterior classification probabilities (ideally ≥ 0.85). While no set criteria exist for deeming model fit acceptable, the cluster solution that provides the lowest AIC and BIC and the highest entropy value (acceptably >0.70 , ideally >0.80) that also conforms to theory is generally considered optimal. The LMR-LRT is used to statistically compare the fit of the k cluster solution with that of the k-1 class solution. When fit no longer statistically improves ($p>0.05$) with the addition of a new class, the solution with the smaller number of classes is generally accepted.

In the interest of parsimony, once an overall class solution was determined biomarkers were then systematically eliminated to obtain the simplest discriminatory model. To start, mean differences in square-root transformed marker concentration were explored across the identified classes using one-way analysis of variance (ANOVA). The marker with the smallest interclass differences was eliminated first, followed by the next smallest, and so on until the simplest model remained that still showed good fit indicators in LPA. The intention was that each of the blood markers defining the final class solution should show a significant difference between the groups.

Recovery and outcome analysis

After LPA each participant was assigned to one of the identified classes based on relative blood marker concentration. From a prior study of derivation of recovery curves each participant was also assigned to one of 3 trajectory classes: Rapid, Delayed, or Minimal recovery. Both the Rapid and Delayed recovery groups were grouped together as a 'Recovery predicted' group and proportions of the blood marker clusters were statistically compared against the 'Minimal or No Recovery predicted' group using χ^2 analysis.

Sample size estimation

There is little guidance in the literature for optimal sample size in MLE-based LPA. Prior to the exploratory analyses described herein there was also no clear existing evidence to inform the likely number of clusters or the relative proportions or communalities to assist with sample estimation. Therefore we adopted the general position in the field that a minimum of 100 samples is a minimum for meaningful results, and continued to position the

analyses as discovery (exploratory) in nature, that is, hypothesis-generating rather than hypothesis-testing.

RESULTS

We note that the proportions described here in the recovery trajectories and distal outcomes will be different than those in Example 1 as these data include approximately half the sample used to derive the trajectories in that Example. Table 3 provides the characteristics of the study population. There were 109 participants in the SYMBIOME database who provided blood samples within 3 weeks of MSK trauma. After assay, data for 3 participants were removed as all analytes were not detectable or out of range of the kits. Mean age of the remaining $n=106$ was 44.6 years and 58.5% of the sample was female. The modal mechanism of injury was reported as 'other' and 74.3% of the sample reported the primary region of injury as the upper or lower extremity (vs. the axial spine). Pain severity and interference at inception was moderate (Mean Severity = 4.5/10, SD = 2.0; Mean Interference = 28.6/70, SD = 16.8).

Table 3: Characteristics and baseline values of SYMBIOME participants in this analysis.

N = 109	
Sex (% female)	58.5%
Age (mean, range)	44.6 years (18 to 66)
BMI (mean, range)	26.4 kg/m ² (14.4 to 51.5)
Primary Region of Injury (%)	
Axial	25.7%
Extremity	74.3%
Mechanism of injury (%)	
Motor vehicle injury	7.1%
Fall	28.6%
Hit by person or object	19.4%
Awkward lift or twist	14.3%
Other	30.6%
Brief Pain Inventory at Inception (mean, range)	
Pain Severity (/10)	4.5 (0 to 8)
Pain Interference (/70)	28.6 (0 to 67)

Table 4 is the cross-product correlation matrix between all biomarker pairs after removal of outliers and square root transformation. BDNF and TGF- β 1 demonstrated the strongest association ($r = 0.74$, $p < 0.01$). Cortisol and CRP did not appear to be associated with any other biomarker while IL-6 and IL-1 β were significantly correlated with all markers except those two.

Table 4: Cross-product correlation matrix of all 8 analytes (Pearson's r) after square-root transformation. *: correlation is significant at the $p < 0.05$ level, **: correlation is significant at the $p < 0.01$ level. Biomarkers: Brain-Derived Neurotrophic Factor (BDNF), Transforming Growth Factor-beta 1 (TGF- β 1), C-reactive protein (CRP), Tumour Necrosis Factor-alpha (TNF- α), Interleukins 1-beta (IL-1 β), 6 (IL-6) and 10 (IL-10), and cortisol.

	IL-6	IL-10	TNF- α	TGF- β 1	BDNF	CRP	Cortisol
IL-1 β	0.47**	0.53**	0.42**	0.34**	0.31**	0.03	-0.06
IL-6		0.47**	0.34**	0.25*	0.21*	-0.01	0.01
IL-10			0.42**	0.19*	0.17	-0.09	-0.10
TNF- α				-0.01	0.18	0.02	0.11
TGF- β 1					0.74**	-0.16	0.11
BDNF						-0.01	0.16
CRP							-0.05

Table 5 shows the results of the LPA models with associated fit indicators for the models tested. The final class solution was a 3-class model as it showed a meaningful improvement over a 2-class solution based on relevant fit indicators (AIC = 2257.31, BIC = 2348.82, Entropy = 0.83, LMR-LRT = 28.08, $p = 0.08$). Figure 1 show the relative concentrations of all 8 markers in the 3 class model. After settling on the 3-class model, analytes were removed in a systematic fashion based on total interclass differences. CRP ($F(2,108) = 0.14$, $p = 0.87$) and cortisol ($F(2,108) = 2.34$, $p = 0.10$) displayed the smallest interclass mean differences (Fig.1) and were eliminated first. Table 5 also shows the model fit adjustment of the 3-class latent profile solution with the sequential elimination of biomarkers. TNF- α ($F(2,108) = 10.65$, $p < 0.01$), IL-6 ($F(2,108) = 11.40$, $p < 0.01$), and IL-10 were also removed, in that order, each time retesting model fit and posterior classification probabilities. The remaining 3 markers were BDNF, TGF β 1 and IL-1 β . BDNF and TGF β 1 were both discriminative across the 3 classes, while IL-1 β provided improved discrimination between the two lower concentration classes. The decision to retain IL-1 β despite acceptable model fit is described in the discussion section. The final model indicated a 3-class solution that could be adequately described by 3 of the 8 markers (AIC = 827.41, BIC = 865.09, Entropy = 0.80, LMR-LRT = 34.08, $p = 0.03$). The 3 classes were labeled according to the relative concentrations of the 3 markers as: Class 1 = Low concentration of all markers (33.9% of the sample), Class 2 = Average Concentration of all markers (47.7%), and Class 3 = High concentration of BDNF and TGF β 1 (18.3%). Figure 2 shows relative (Z-transformed) concentrations graphically and Table 6 shows the raw (non-transformed) values with 95% confidence intervals.

Table 5: Fit Indicators for latent profile analysis and class assignment: Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), Entropy and Lo-Mendall-Rubin Adjusted Likelihood Ratio Test (LMR-LRT). Values highlighted in **BOLD** indicate the preferred class for analysis. Biomarkers: Brain-Derived Neurotrophic Factor (BDNF), Transforming Growth Factor-beta 1 (TGF- β 1), C-reactive protein (CRP), Tumour Necrosis Factor-alpha (TNF- α), Interleukins 1-beta (IL-1 β), 6 (IL-6) and 10 (IL-10), and cortisol.

	AIC	BIC	Entropy	LMR-LRT (p)
2 class	2298.77	2366.06	0.78	90.80 (0.07)
3 class	2257.31	2348.82	0.83	58.08 (0.08)
4 class	2231.06	2346.79	0.89	43.23 (0.30)
3 class (- CRP)	1986.71	2067.45	0.83	57.81 (0.058)
3 class (- cortisol)	1678.22	1748.20	0.82	56.22 (0.054)
3 class (- TNFα)	1384.94	1444.15	0.81	47.06 (0.053)
3 class (- IL6)	1121.54	1169.99	0.80	39.44 (0.029)
3 class (- IL10)	827.41	865.09	0.80	34.08 (0.033)
3 class (- IL1β)	539.24	566.16	0.81	27.44 (0.025)

Table 6: Mean (raw, untransformed) concentrations of the 3 retained analytes across the 3 classes identified through LPA. 1: The mean concentration was significantly lower in Class 1 compared to the other two groups. 2: The mean concentrations of both BDNF and TGF- β 1 were significantly different across all 3 groups. Statistical tests were one-way ANOVA with Tukey's post-hoc test using square-root transformed data to reduce deviations from normality. **BOLD** are the 3 markers retained in the final model solution. Biomarkers: Brain-Derived Neurotrophic Factor (BDNF), Transforming Growth Factor-beta 1 (TGF- β 1), C-reactive protein (CRP), Tumour Necrosis Factor-alpha (TNF- α), Interleukins 1-beta (IL-1 β), 6 (IL-6) and 10 (IL-10), and cortisol.

	Overall mean (95%CI)	Class 1 (n = 42)	Class 2 (n = 47)	Class 3 (n = 20)	F (p)
IL-1 β (pg/mL)	2.71 (2.43, 2.99)	1.32 (1.07, 1.58)	3.46 (3.14, 3.77)	3.19 (2.52, 3.87)	19.75 (<0.01) ₁
BDNF (ng/mL)	3.55 (3.00, 4.09)	1.78 (1.22, 2.34)	3.08 (2.71, 3.46)	8.65 (7.51, 9.80)	182.92 (<0.01) ₂
TGF- β 1 (ng/mL)	24.45 (21.11, 27.79)	16.96 (12.22, 21.70)	21.78 (19.02, 24.54)	46.67 (35.74, 57.60)	67.14 (<0.01) ₂
IL-10 (pg/mL)	21.12 (18.08, 24.16)	15.7 (11.9, 19.5)	23.1 (18.5, 27.7)	27.8 (18.2, 37.4)	6.06 (<0.01) ₁
IL-6 (pg/mL)	92.17 (80.05, 104.29)	70.1 (56.9, 83.2)	101.9 (81.5, 122.2)	115.6 (80.3, 150.8)	4.81 (0.01) ₁
TNF- α (pg/mL)	5.61 (5.08, 6.13)	4.9 (3.9, 5.8)	6.0 (5.4, 6.7)	6.1 (4.6, 7.5)	2.77 (0.07)
CRP (mg/L)	3.34 (2.65, 4.01)	3.22 (2.24, 4.21)	3.36 (2.29, 4.44)	3.48 (1.41, 5.54)	0.00 (1.00)
Cortisol (μ g/dL)	12.04 (10.58, 13.49)	10.44 (8.65, 12.22)	13.42 (10.75, 16.08)	12.05 (8.68, 15.43)	1.99 (0.14)

With each participant assigned to the most likely biomarker class based on posterior probabilities, the sample was split into 3 groups. BPI Pain Severity and Pain Interference scores captured both at inception (<3 weeks from injury) and at 6-month follow-up were compared across the 3 groups using one-way ANOVA. Significant main effects were present in each of the 6-month follow-up scores, and a strong trend ($p = 0.06$) was seen in the main

effect of class for the BPI Pain Interference score at inception (Table 7). The pattern of responses indicated that the scores of Class 3 (High BDNF/TGF β 1) were higher than those of the other two classes. As such, post-hoc tests were conducted with the scores of the first 2 classes grouped (Low or Average concentration of all markers) against those of Class 3, using a Mann-Whitney U-test due to skewed data at the 6-month follow-up. Mean scores were significantly higher in Class 3 for the BPI Interference subscale at inception [27.0 (SD 16.4) vs. 35.8 (SD 17.3), $p = 0.05$] and at 6-month follow-up [2.2 (SD 4.8) vs. 7.3 (SD 10.7), $p = 0.03$] compared to those of the other 2 classes, and BPI Pain Severity at 6 months showed a strong trend towards significance [0.3 (SD 0.7) vs. 1.4 (SD 1.8), $p = 0.07$].

Table 7: Mean scores on the Brief Pain Inventory (BPI) Pain Severity and Pain Interference scales, captured at inception (<3 weeks from injury) and at 6 month follow-up, separated by biomarker class. Biomarkers: Brain-Derived Neurotrophic Factor (BDNF) and Transforming Growth Factor β 1 (TGF- β 1)

	Class 1 (Low all markers)	Class 2 (Average all markers)	Class 3 (High BDNF/TGF β 1)	F (p)
BPI Pain Severity (Acute)	4.9 (4.2, 5.5)	4.1 (3.6, 4.7)	4.6 (3.6, 5.6)	1.52 (0.23)
BPI Pain Interference (Acute)	30.6 (24.7, 36.4)	23.9 (19.5, 28.3)	33.7 (25.0, 42.3)	2.9 (0.06)
BPI Pain Severity (6 months)	0.2 (0.1, 0.4)	0.4 (0.0, 0.8)	1.3 (0.0, 2.7) ¹	3.9 (0.03)
BPI Pain Interference (6 months)	1.8 (0.9, 2.8)	2.7 (0.9, 4.4)	6.1 (1.4, 10.8) ¹	3.4 (0.04)

The above disclosure generally describes the present invention. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

All publications, patents and patent applications cited above are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

Although preferred embodiments of the invention have been described herein in detail, it will be understood by those skilled in the art that variations may be made thereto without departing from the spirit of the invention or the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A panel of biomarkers for predicting functional recovery after musculoskeletal trauma, the panel comprising BDNF and TGF- β 1, wherein the levels of BDNF and TGF- β 1 are inversely correlated with recovery.
- 5 2. The panel of claim 1, further comprising IL-1 β .
3. The panel of claim 1 or 2, further comprising one or more of TNF- α , IL-6, IL-10, cortisol, and CRP.
4. The panel of claim 1, comprising all of BDNF, TGF- β 1, IL-1 β , TNF- α , IL-6, IL-10, cortisol, and CRP.
- 10 5. The panel of claim 1, consisting of BDNF, TGF- β 1, IL-1 β , TNF- α , IL-6, IL-10, cortisol, and CRP.
6. The panel of any one of claims 1 to 5, wherein the biomarkers are blood biomarkers.
7. The panel of any one of claims 1 to 6, wherein the biomarkers are detected as protein.
- 15 8. The panel of any one of claims 1 to 6, wherein the biomarkers are detected as mRNA.
9. The panel of any one of claims 1 to 6, wherein low levels of BDNF and TGF- β 1 as compared to control values assigns the subject to a some recovery group and high levels of BDNF and TGF- β 1 as compared to control values assigns the subject to a no or minimal recovery group.
- 20 10. The panel of any one of claims 2 to 6, wherein low or moderate levels of BDNF, TGF- β 1, and IL-1 β as compared to control values assigns the subject to a 'some recovery group' (rapid recovery group and delayed recovery group) and high levels of BDNF and TGF- β 1 as compared to control values assigns the subject to a no or minimal recovery group.
- 25 11. The panel of any one of claims 2 to 6, wherein low or average levels of BDNF, TGF- β 1, and IL-1 β as compared to control values assigns the subject to a rapid recovery group or a delayed recovery group and high levels of BDNF and TGF- β 1 as compared to control values assigns the subject to the no or minimal recovery group.
- 30 12. The panel of claim 10 or 11, wherein the control values are sample-specific control values.

13. An assay comprising probes for detecting the panel of biomarkers of any one of claims 1 to 6 for predicting functional recovery after musculoskeletal trauma.
14. A kit for detecting the biomarkers of any one of claims 1 to 6 for predicting functional recovery after musculoskeletal trauma.
- 5 15. A method for predicting functional recovery after musculoskeletal trauma in a subject, the method comprising measuring the levels of a panel of biomarkers comprising BDNF and TGF- β 1 in the subject, wherein the levels of BDNF and TGF- β 1 are inversely correlated with recovery.
16. The method of claim 15, wherein the panel of biomarkers further comprises IL-1 β .
- 10 17. The method of claim 15 or 16, wherein the panel of biomarkers further comprises one or more of TNF- α , IL-6, IL-10, and cortisol.
18. The method of claim 15, wherein the panel of biomarkers further comprises BDNF, TGF- β 1, IL-1 β , TNF- α , IL-6, IL-10, and cortisol.
- 15 19. The method of claim 15, wherein the panel of biomarkers consists of BDNF, TGF- β 1, IL-1 β , TNF- α , IL-6, IL-10, and cortisol.
20. The method of any one of claims 15 to 19, wherein the panel of biomarkers excludes CRP.
21. The method of any one of claims 15 to 20, wherein the biomarkers are blood biomarkers.
- 20 22. The method of any one of claims 15 to 21, wherein the biomarkers are detected as protein.
23. The method of any one of claims 15 to 21, wherein the biomarkers are detected as mRNA.
24. The method of any one of claims 15 to 21, wherein low levels of BDNF and TGF- β 1 as compared to control values assigns the subject to a some recovery group and high levels of BDNF and TGF- β 1 as compared to control values assigns the subject to a no or minimal recovery group.
- 25 25. The method of any one of claims 16 to 21, wherein low or moderate levels of BDNF, TGF- β 1, and IL-1 β as compared to control values assigns the subject to a 'some recovery group' (rapid recovery group and delayed recovery group) and high levels of BDNF and
- 30

TGF- β 1 as compared to control values assigns the subject to a no or minimal recovery group.

26. The method of any one of claims 16 to 21, wherein low or average levels of BDNF, TGF- β 1, and IL-1 β as compared to control values assigns the subject to a rapid recovery group or a delayed recovery group and high levels of BDNF and TGF- β 1 as compared to control values assigns the subject to the no or minimal recovery group.

27. The method of any one of claims 24 to 26, wherein the control values are sample-specific control values.

28. The method of any one of claims 15 to 27, further comprising assessing factors associated with the subject.

29. The method of claim 28, wherein the factors comprise one or more of sex, age, BMI, anatomical region of trauma, employment status, household income, educational attainment, post-traumatic distress, and pre-existing physical or psychological comorbidities.

30. The method of any one of claims 15 to 29, further comprising treating the subject based on the predicted functional recovery.

31. A prognostic phenotyping protocol for predicting rate of recovery in MSK trauma in a subject, the protocol comprising measuring the levels of a panel of biomarkers comprising BDNF and TGF- β 1 in the subject, wherein low levels of BDNF and TGF- β 1 as compared to control values assigns the subject to a 'some recovery group' (rapid recovery and delayed recovery) and high levels of BDNF and TGF- β 1 as compared to control values assigns the subject to a no or minimal recovery group.

32. The prognostic phenotyping protocol of claim 31, wherein the panel further comprises IL-1 β and wherein low or average levels of BDNF, TGF- β 1, and IL-1 β as compared to control values assigns the subject to a rapid recovery group or a delayed recovery group and high levels of BDNF and TGF- β 1 as compared to control values assigns the subject to the no or minimal recovery group.

33. A method for predicting risk of chronic pain in a subject, the method comprising measuring the levels of a panel of biomarkers comprising BDNF and TGF- β 1 in the subject, wherein the levels of BDNF and TGF- β 1 are positively correlated with the likelihood of the subject experiencing chronic pain.

34. A method for predicting and/or estimating the current severity of pain in a subject, the method comprising measuring the levels of a panel of biomarkers comprising IL-1 β and

TGF- β 1 in the subject, wherein the levels of IL-1 β and TGF- β 1 are negatively correlated with the predicted and/or estimated severity of pain that the subject is currently experiencing.

35. A method for predicting and/or estimating current pain interference in a subject, the method comprising measuring the levels of a panel of biomarkers comprising TNF- α and
5 CRP in the subject, wherein the levels of TNF- α and CRP are negatively correlated with the predicted and/or estimated pain interference that the subject is currently experiencing.

36. The method of claim 25, wherein the subject has an injury affecting the axial spine.

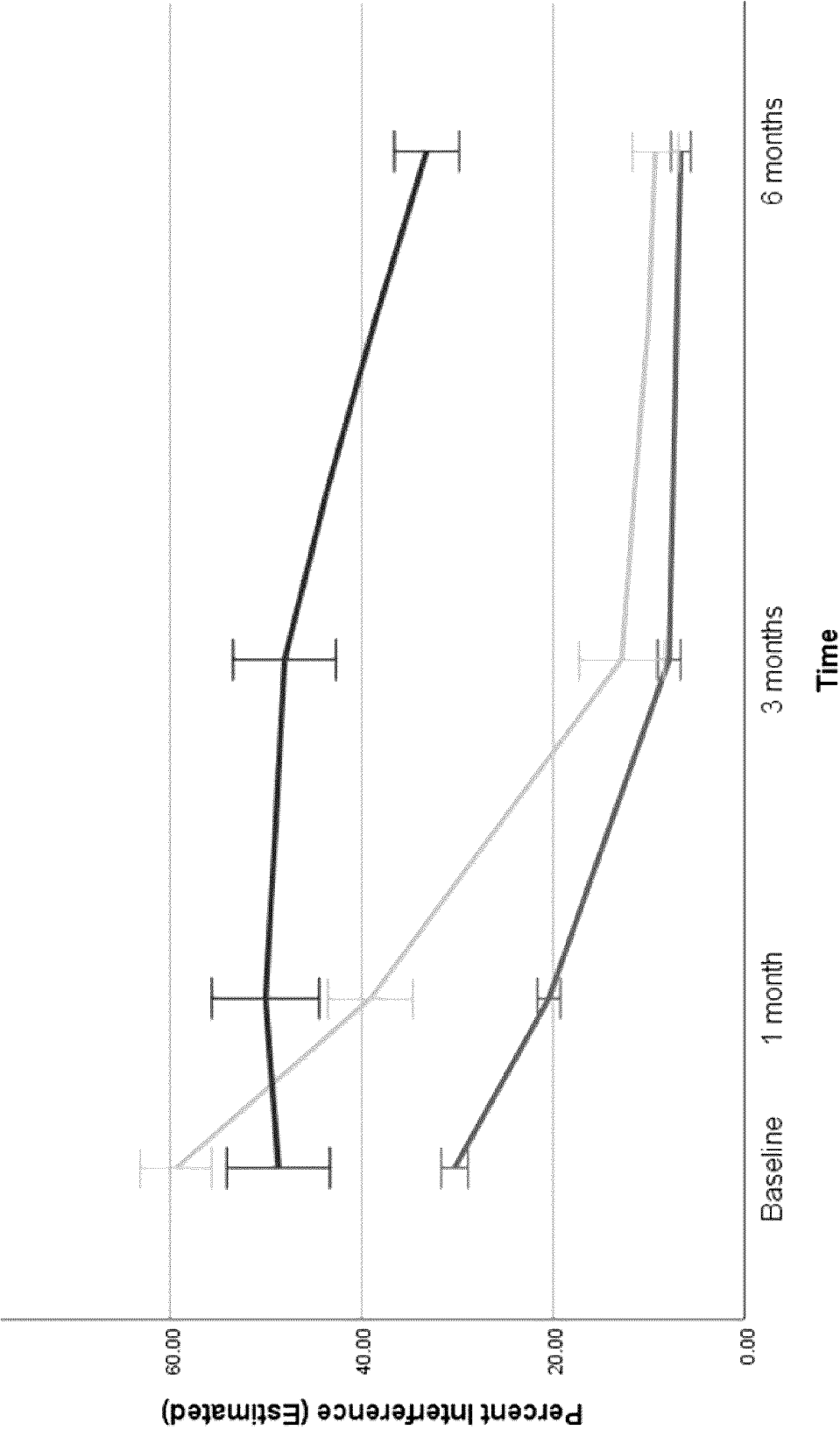


Figure 1

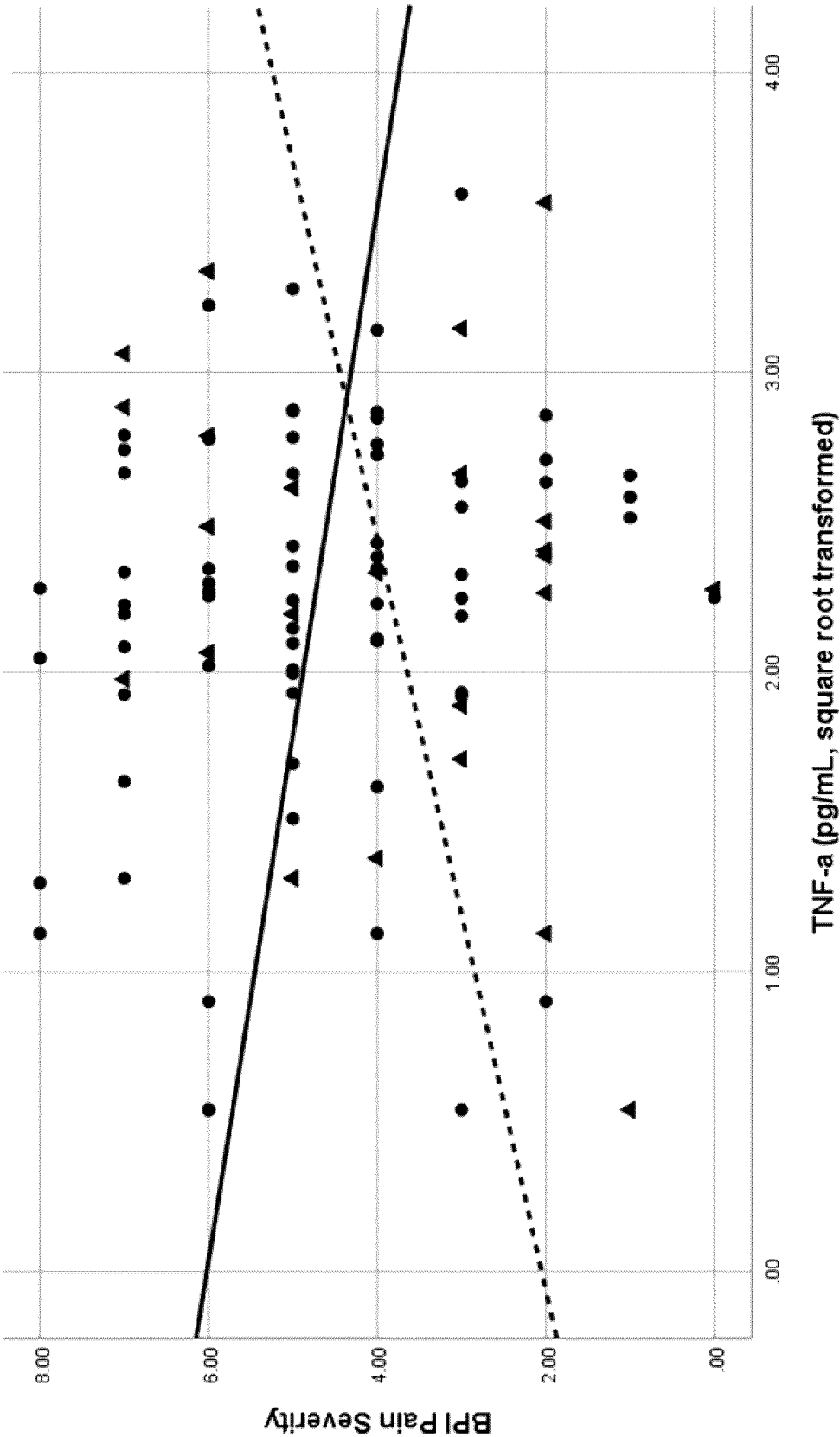


Figure 2A

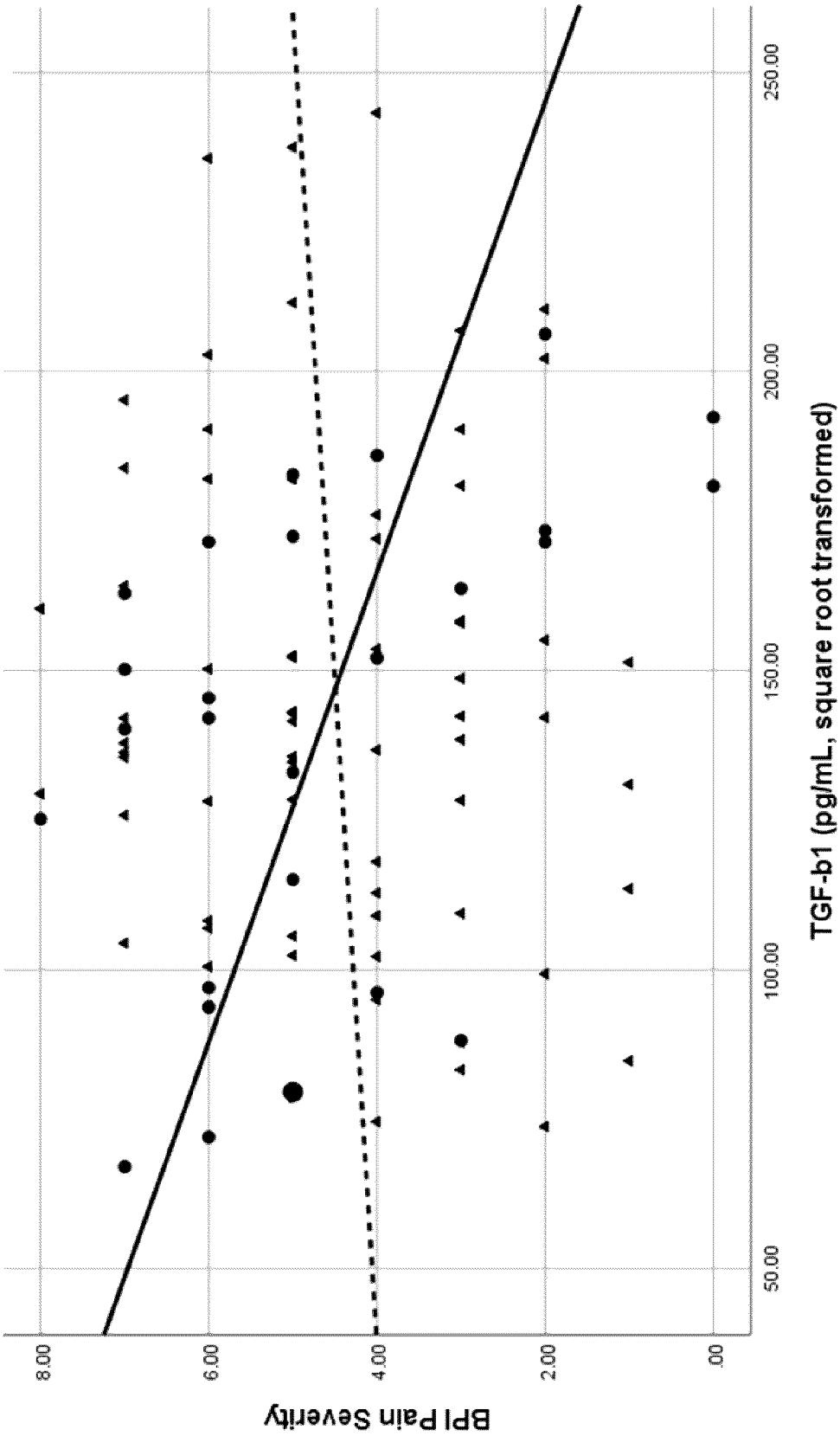


Figure 2B

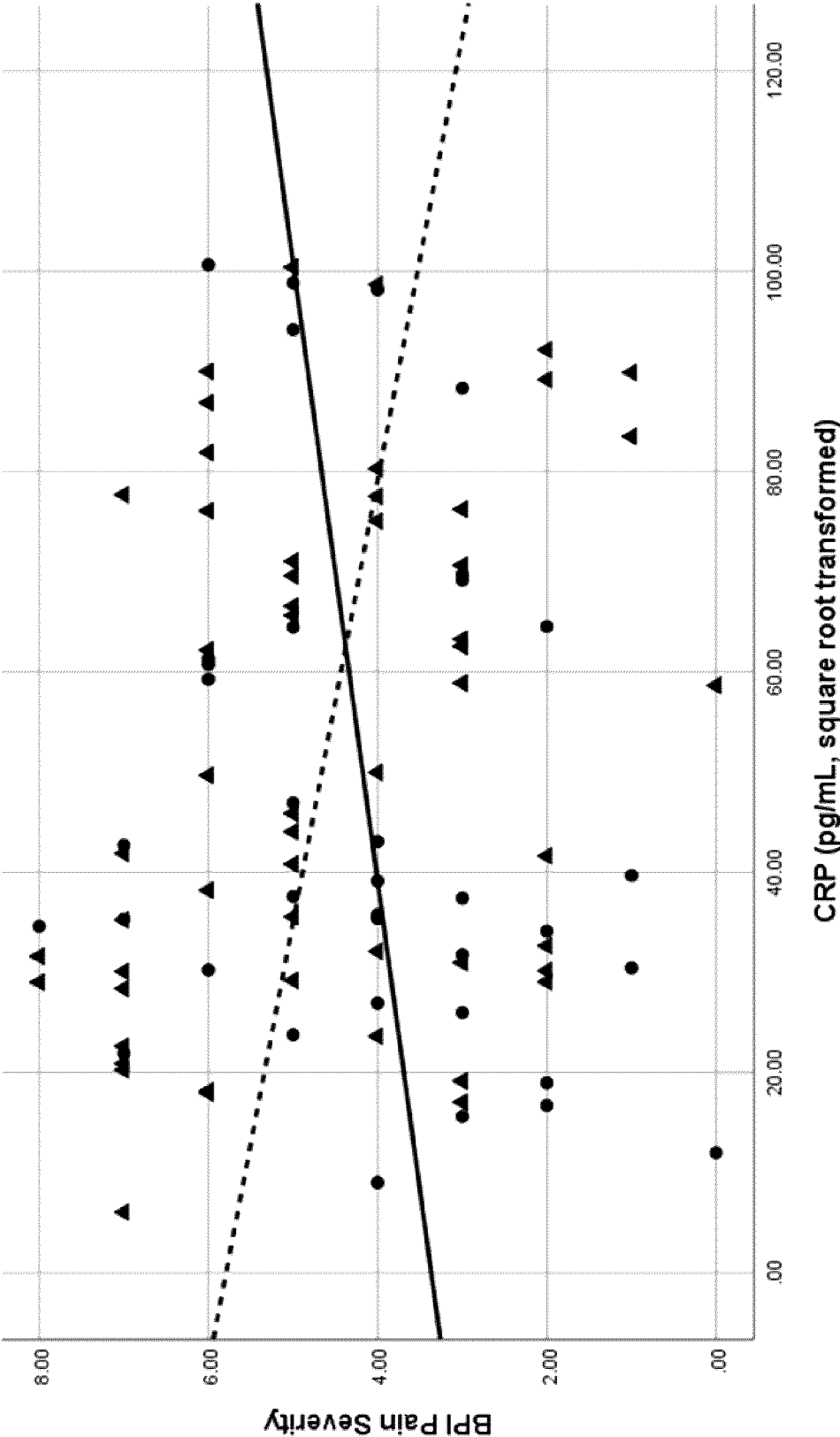


Figure 2C

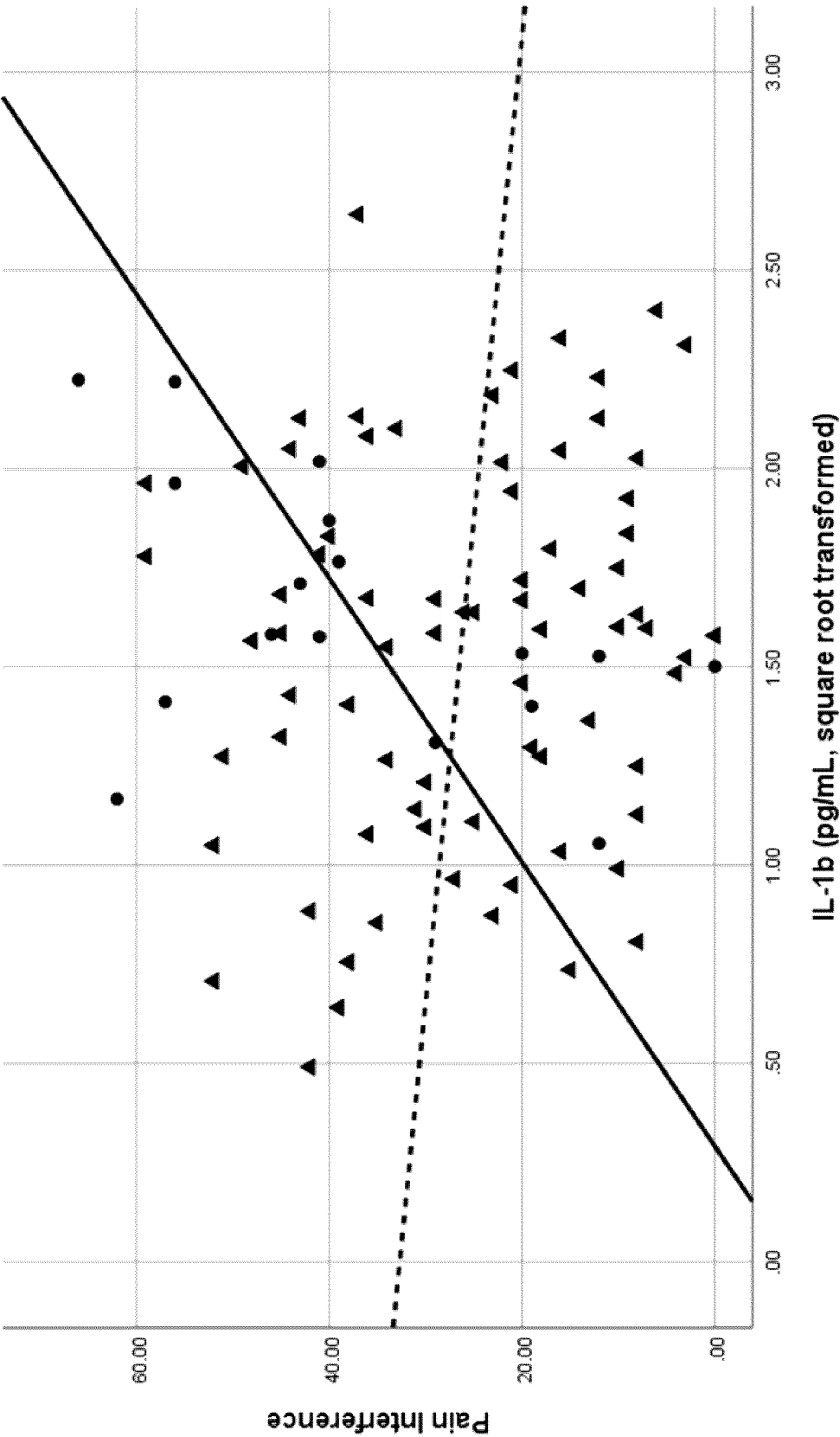


Figure 3A

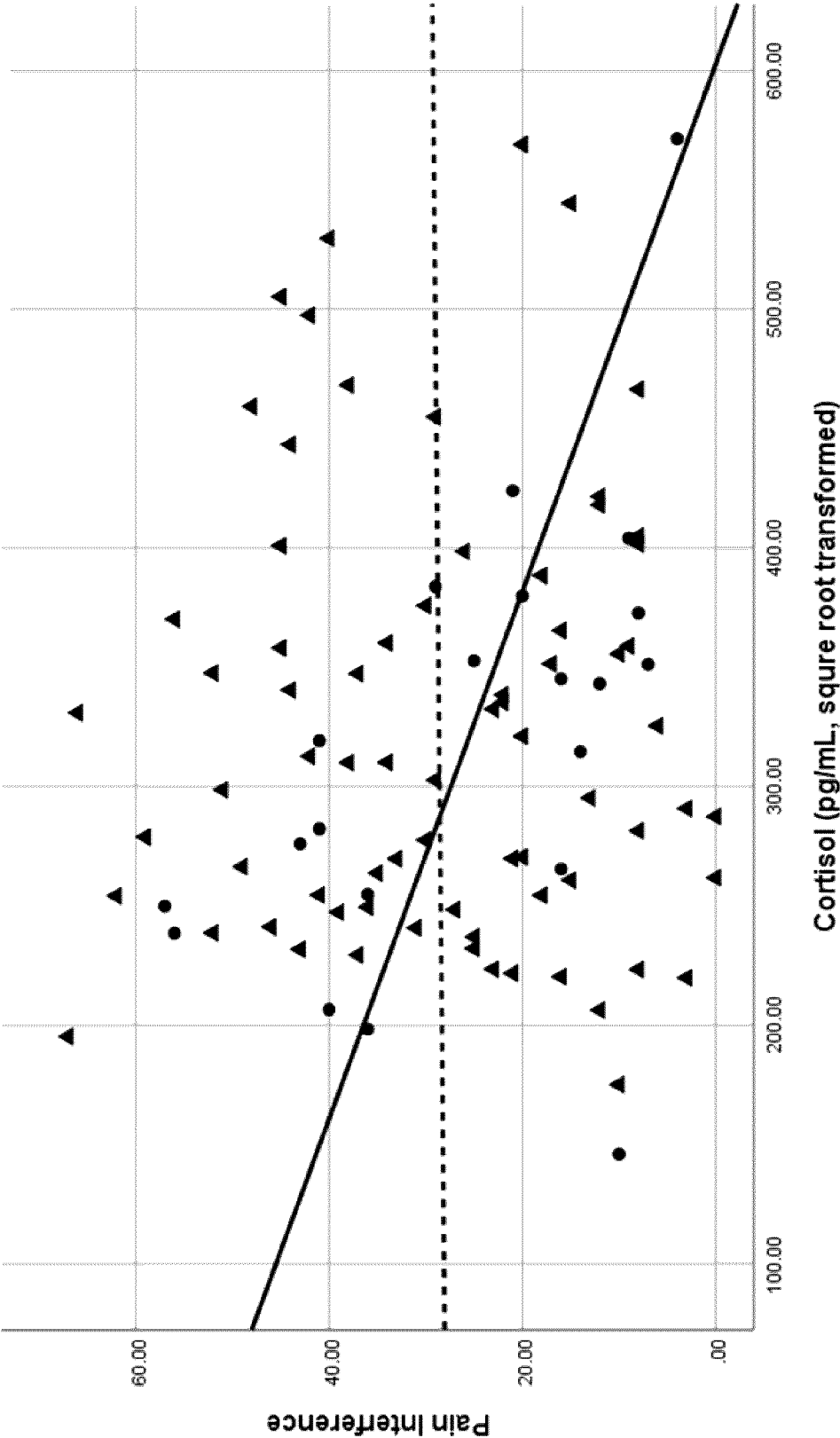


Figure 3B

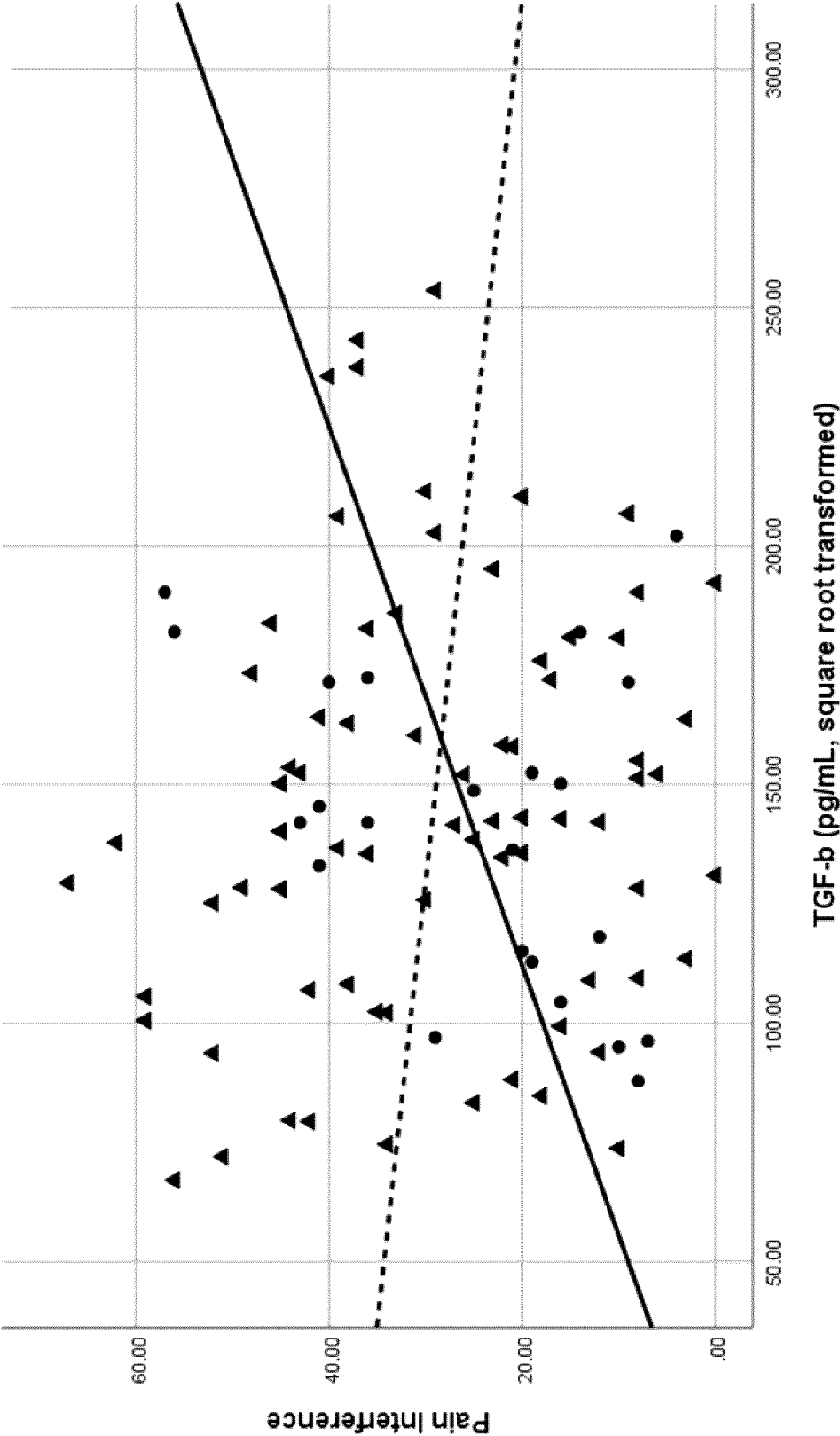


Figure 3C

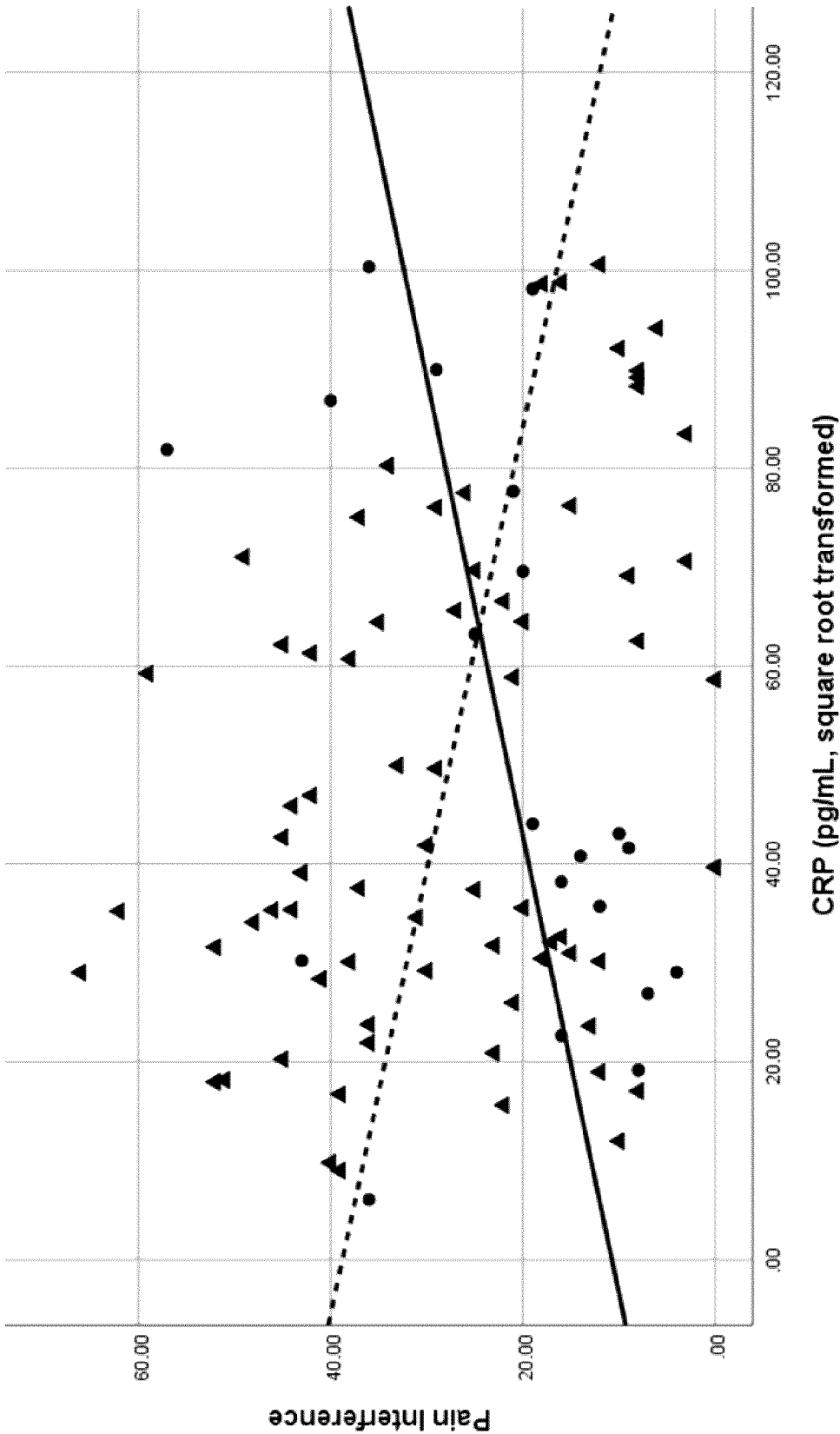


Figure 3D

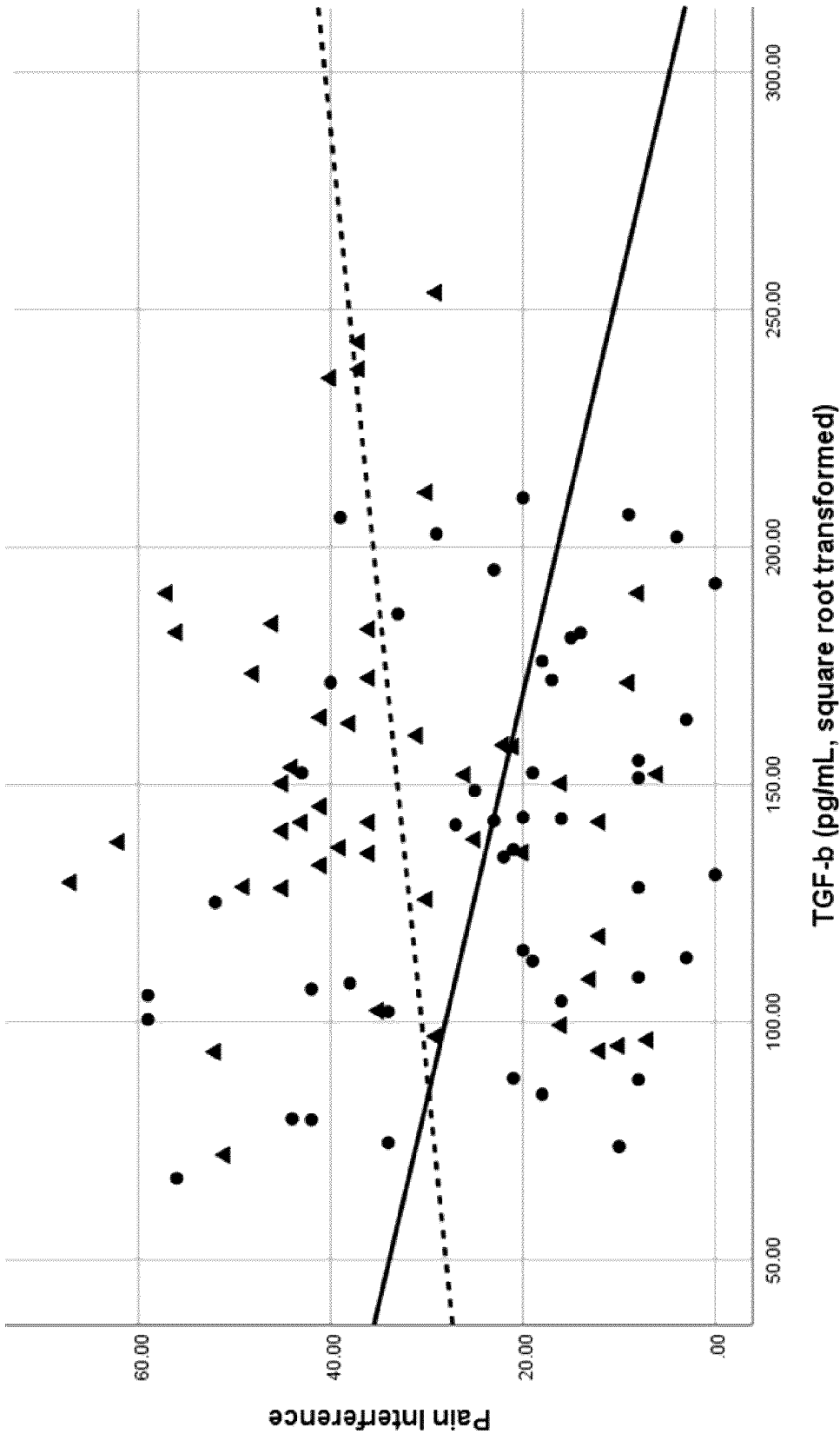


Figure 3E

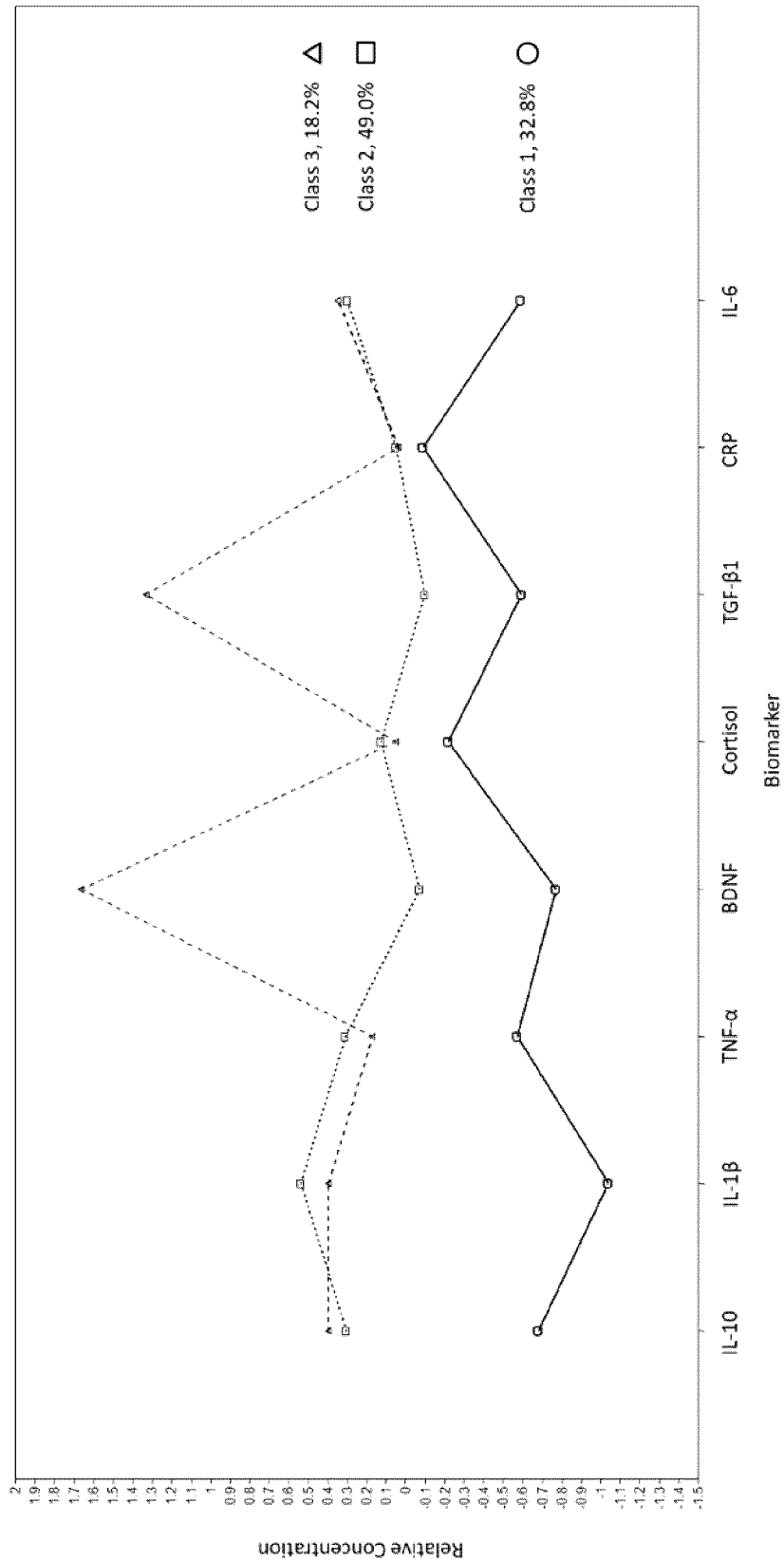


Figure 4

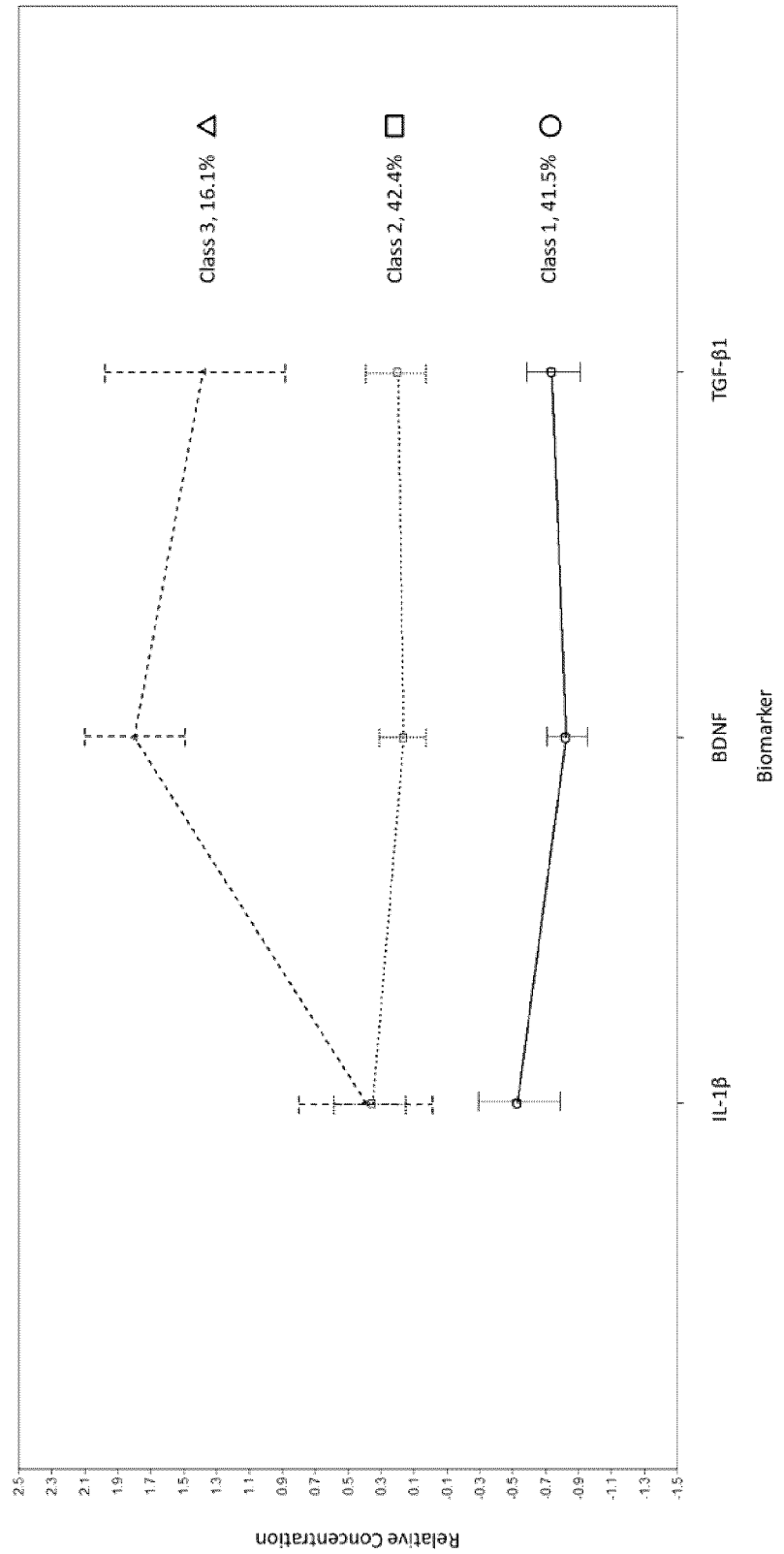


Figure 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2020/050433

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **C40B 40/08** (2006.01), **C12Q 1/6809** (2018.01), **C12Q 1/6837** (2018.01), **C12Q 1/6876** (2018.01),
C40B 30/04 (2006.01), **C40B 40/10** (2006.01), **G01N 33/48** (2006.01), **G16B 20/00** (2019.01), **G16B 25/10** (2019.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC:ALL

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Canadian Patent Database, Questel Orbit, Pubmed, Google Scholar, Google Patent, Google, SCOPUS

Keywords: BDNF, TGFβ1, musculoskeletal, pain, biomarker

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SIRAJ, S., " <i>A cross-sectional study of stress biomarkers and their associations with post-trauma complaints, and how those associations are moderated by early life adversity</i> ". University of Western Ontario Electronic Thesis and Dissertation Repository, 17 August 2017 (17-08-2017), Thesis 4856, [online] [retrieved on 23 June 2020 (23-06-2020)]. Retrieved from the Internet: https://ir.lib.uwo.ca/etd/4856	1-4, 6-18, 20-32, 36
A	MELEMEDJIAN, O.K. et al., " <i>BDNF regulates atypical PKC at spinal synapses to initiate and maintain a centralized chronic pain state</i> ". Mol. Pain, 20 March 2013 (20-03-2013), Vol. 9:12, pp. 1-14, [online] [retrieved on 23 June 2020 (23-06-2020)]. Retrieved from the Internet: https://doi.org/10.1186/1744-8069-9-12	1-32, 36

☒ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* "A" "D" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance document cited by the applicant in the international application earlier application or patent but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"I" "X" "Y" "&"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
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Date of the actual completion of the international search
 30 June 2020 (30-07-2020)

Date of mailing of the international search report
 06 July 2020 (06-07-2020)

Name and mailing address of the ISA/CA
 Canadian Intellectual Property Office
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 50 Victoria Street
 Gatineau, Quebec K1A 0C9
 Facsimile No.: 819-953-2476

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See supplemental sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Claims 1-32 and 36 corresponding to Group 1

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2020/050433

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZHANG, H. et al., " <i>TGF-β1/Smad2/3/Foxp3 signaling is required for chronic stress-induced immune suppression</i> ". J. Neuroimmunol., 15 January 2018 (15-01-2018), Vol. 314, pp. 30-41, ISSN 1550-6606	1-32,36

Continuation of Box III

The claims are directed to a plurality of inventive concepts as follows:

Group 1 - Claims 1-32 and 36 are directed to a method for predicting functional recovery after musculoskeletal trauma in a subject comprising measuring the levels of a panel of biomarkers comprising BDNF and TGF- β 1 in the subject, wherein the levels of BDNF and TGF- β 1 are inversely correlated with recovery, as well as corresponding biomarker panels, assays and kits.

Group 2 – Claim 33 is directed to a method for predicting risk of chronic pain in a subject comprising measuring the levels of a panel of biomarkers comprising BDNF and TGF- β 1 in the subject, wherein the levels of BDNF and TGF- β 1 are positively correlated with the likelihood of the subject experiencing chronic pain.

Group 3 – Claim 34 is directed to a method for predicting the current severity of pain in a subject comprising measuring the levels of a panel of biomarkers comprising IL-1 β and TGF- β 1 in the subject, wherein the levels of IL-1 β and TGF- β 1 are negatively correlated with the predicted severity of pain that the subject is currently experiencing.

Group 4 – Claim 35 is directed to a method for predicting current pain interference in a subject comprising measuring the levels of a panel of biomarkers comprising TNF- α and CRP in the subject, wherein the levels of TNF- α and CRP are negatively correlated with the predicted pain interference that the subject is currently experiencing.

There is no technical relationship among the above identified groups of inventions involving one or more of the same or corresponding special technical features. The claimed methods therefore do not meet the requirement of unity of invention. The claims must be limited to one inventive concept as set out in PCT Rule 13.