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Perturbations in Neuroinflammatory Pathways Are Associated With a Worst Pain Profile in Oncology Patients Receiving Chemotherapy

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Abstract

Unrelieved pain occurs in 55% of cancer patients. Identification of molecular mechanisms for pain may provide insights into therapeutic targets. Purpose was to evaluate for perturbations in neuroinflammatory pathways between oncology patients with and without severe pain. Worst pain severity was rated using a 0 to 10 numeric rating scale six times over two cycles of chemotherapy. Latent profile analysis was used to identify subgroups of patients with distinct pain profiles. Pathway impact analyses were performed in two independent samples using gene expression data obtained from RNA sequencing (n = 192) and microarray (n = 197) technologies. Fisher's combined probability test was used to identify significantly perturbed pathways between None versus the Severe pain classes. In the RNA sequencing and microarray samples, 62.5% and 56.3% of patients were in the Severe pain class, respectively. Nine perturbed pathways were related to neuroinflammatory mechanisms (i.e., retrograde endocannabinoid signaling, gamma-aminobutyric acid synapse, glutamatergic synapse, Janus kinase-signal transducer and activator of transcription signaling, phagosome, complement and coagulation cascades, cytokine-cytokine receptor interaction, chemokine signaling, calcium signaling). First study to identify perturbations in neuroinflammatory pathways associated with severe pain in oncology outpatients. Findings suggest that complex neuroimmune interactions are involved in the maintenance of chronic pain conditions.

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Supplementary data

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Perspective: In this study that compared oncology patients with none versus severe pain, nine perturbed neuroinflammatory pathways were identified. Findings suggest that complex neuroimmune interactions are involved in the maintenance of persistent pain conditions.

Keywords

Cancer; chemotherapy; cytokines; gene expression; gamma amino butyric acid; glutamine; neuro-immune interactions; neuroinflammation

Approximately 70% of patients experience moderate to severe pain during chemotherapy.[56,64] Pain can be related to the cancer itself, associated with treatment (e.g., mucositis, peripheral neuropathy), or be related to other chronic conditions (e.g., back pain, osteoarthritis). In our previous study of patients undergoing chemotherapy,[64] of the 926 patients evaluated, 20.8% reported only non-cancer pain, 37.7% reported only cancer pain, and 41.5% reported both types of pain. In the context of the opioid epidemic, recent evidence suggests that the undertreatment of pain in oncology patients remains a significant clinical problem.[43] One of the gaps in effective management of multiple pain problems in oncology patients is an incomplete understanding of the mechanisms that underlie chronic pain. While direct neuronal activation is involved in the development and maintenance of chronic pain,[22] emerging evidence suggests a role for neuroinflammation.[14,32,49] The bidirectional communication between the immune and the nervous systems may provide opportunities to develop more targeted interventions for pain.[27]

Neuroinflammation plays a fundamental role in mediating neuronal plasticity.[32] As part of this process, activation of cytokines results in peripheral and central sensitization and the development of chronic pain,[28] including chronic cancer and non-cancer pain.[71] As noted in one review,[86] the transition from acute to chronic pain involves prolonged innate and adaptive immune signaling that induces maladaptive neuronal plasticity within the peripheral and central nervous systems.[32,71] However, while in a study of breast cancer survivors,[52] we identified perturbations in neuroinflammatory pathways associated with chemotherapy-induced peripheral neuropathy (CIPN), no studies have evaluated for these types of perturbations in oncology patients with severe pain during chemotherapy. Therefore, the purpose of this study, using the results of a previous latent profile analysis (LPA) that identified four classes of patients with distinct pain profiles (i.e., None, Mild, Moderate, Severe),[74] was to use an extreme phenotype approach, to evaluate for differentially perturbed pathways associated with neuroinflammation between the None and the Severe pain classes.

Methods

Patients and Settings

This study is part of a larger, longitudinal study of the symptom experience of oncology outpatients receiving chemotherapy.[60,75] Eligible patients were ≥18 years of age; had a diagnosis of breast, gastrointestinal, gynecological, or lung cancer; had received chemotherapy within the preceding four weeks; were scheduled to receive at least two additional cycles of chemotherapy; were able to read, write, and understand English; and

gave written informed consent. Patients were recruited from two Comprehensive Cancer Centers, one Veteran's Affairs hospital, and 4 community-based oncology programs.

Study Procedures

The study was approved by the Institutional Review Board at each of the study sites. Of the 2234 patients approached, 1343 consented to participate (60.1% response rate). The major reason for refusal was being overwhelmed with their cancer treatment. Eligible patients were approached in the infusion unit during their first or second cycle of chemotherapy by a member of the research team to discuss study participation and obtain written informed consent. Blood for ribonucleic acid (RNA) isolation was collected at the enrollment assessment. Medical records were reviewed for disease and treatment information. For this study, a total of 717 patients provided a blood sample for the analyses (Supplemental Figure 1). Of these 717 patients, 357 patients had their samples processed using RNA sequencing (i.e., RNA-seq sample) and 360 patients had their samples processed using microarray (i.e., microarray sample) technologies.

Instruments

Demographic and Clinical Characteristics—Demographic information was obtained using a self-report questionnaire. Functional status was assessed using the Karnofsky Performance Status (KPS) scale.[37] The occurrence, treatment, and functional impact of 13 common medical conditions were assessed using the Self-Administered Comorbidity Questionnaire (SCQ).[70] Alcohol consumption, behaviors, and associated problems were measured using the Alcohol Use Disorders Identification test (AUDIT).[5] The toxicity of each patient's chemotherapy regimen was rated using the MAX2 index.[20,79] Medical records were reviewed for disease and treatment information.

Pain Measure—Worst pain severity was assessed using the Brief Pain Inventory (BPI).[16] Patients were asked to indicate whether they were generally bothered by pain (yes/no). If they were generally bothered by pain, patients rated their worst pain severity in the past 24 hours using a 0 (no pain) to 10 (worst pain imaginable) numeric rating scale (NRS). Additional information was collected on causes of pain, as well as its duration, locations, and interference.

Data Analysis

Latent Profile Analysis—In our previous analysis,[74] LPA was used to identify unobserved subgroups of patients (i.e., latent classes) with distinct worst pain profiles over the six assessments, using the patients' ratings of worst pain severity. Before performing the LPA, patients who reported the occurrence of pain for ≤1 of the six assessments were identified and label as the "None" class (n = 371, 28.4%). Then, the LPA was performed on the remaining 934 patients using MPlus™ Version 8.4.[58] Estimation was carried out with full information maximum likelihood with standard error and a Chi square test that are robust to non-normality and non-independence of observations ("estimator=MLR"). Model fit was evaluated to identify the solution that best characterized the observed latent class structure with the Bayesian Information Criterion,[36] Vuong-Lo-Mendell-Rubin likelihood ratio test, entropy, and latent class percentages that were large enough to be reliable.[58] Missing data

were accommodated for with the use of the Expectation-Maximization (EM) algorithm.[57] Three latent classes were identified based on clinically meaningful cutoff scores. For the current analysis, using an extreme phenotype approach, an evaluation of differentially perturbed pathways between patients in the None and Severe pain classes was performed.

Imputation Process—Missing data for demographic and clinical characteristics were imputed by the k-nearest-neighbors method, with k=9. For continuous variables the Euclidean distance was used to find the nearest neighbors. The imputed value was the weighted average of the nearest neighbors, with each weight originally exp(−dist(x,j)), after which the weights were scaled to one. For categorical variables, distance was 0 if the predictor and the neighbor had the same value and 1 if they did not. The imputed value was the mode of the nearest neighbors.

Demographic and Clinical Data—Demographic and clinical data from the two patient samples (i.e., RNA-seq, microarray) were analyzed separately. Differences in demographic and clinical characteristics between the patients in the None and Severe pain classes were evaluated using parametric and non-parametric tests. Significance corresponded to a p-value of <.05. Characteristics included in the final model were selected using a backwards stepwise logistic regression approach based on the likelihood ratio test (LRT). The area under the curve (AUC) of the receiver operating characteristic (ROC) curves was used to gauge the overall adequacy of the logistic regression model for each sample.[17] All these analyses were performed using R (version 4.1).[78]

Differential Expression and Pathway Impact Analyses (PIA)—Details on the methods of the gene expression and pathway impact analyses are provided in Supplemental File 1. In brief, differential expression was quantified using empirical Bayes models that were implemented separately for each sample (i.e., using edgeR[67] for the RNA-seq sample and limma[77] for the microarray sample). These analyses were adjusted for demographic and clinical characteristics that were significantly different between the None versus Severe pain classes. In addition, the models included surrogate variables not associated with class memberships to adjust for variations due to unmeasured sources.[44,45] Expression loci were annotated with Entrez gene identifiers. Gene symbols were derived from the HUGO Gene Nomenclature Committee resource database.[29] The differential expression results were summarized as the log fold-change and *p*-value for each gene. Only genes that had a common direction of expression (i.e., the same sign for the log fold-change) across the two samples were retained for subsequent analyses. Common genes were matched using gene symbol.

To interpret the results in the context of pain-related mechanisms, we used PIA that included potentially important biological factors (e.g., gene-gene interactions, flow signals in a pathway, pathway topologies), as well as the magnitude (i.e., log fold-change) and *P*-values from the differential expression analysis for each sample.[53] The PIA included the results of the differential expression analyses for all of the genes (i.e., cutoff free) that had a common direction of differential expression to determine probability of pathway perturbations (pPERT) using Pathway Express.[18] A total of 225 signaling pathways were defined using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.[3] For

each sample, a separate test was performed for each pathway. Then, we used Fisher's Combined Probability method to combine these test results to obtain a single test (global) of the null hypothesis.[24,25] The significance of the combined transcriptome-wide PIA was assessed using a false discovery rate (FDR) of 0.015.[19] Finally, we evaluated these results for perturbed neuroinflammatory signaling pathways.

Results

RNA-seq Performance

Of the 357 patients in the RNA-seq sample, 72 were in the None and 120 were in the Severe pain classes. Median library threshold size was 9,042,589 reads. Following the application of quality control filters, 10,881 genes were included in the final analysis. The common dispersion was estimated as 0.26493, yielding a biological coefficient of variation of 0.5147 well within the expected value for clinical samples.[41,51]

RNA Microarray Performance

Of the 360 patients in the microarray sample, 86 were in the None and 111 were in the Severe pain classes. All of these samples demonstrated good hybridization performance for biotin, background negative, and positive control assays on the arrays. Limma was used for background correction, quantile normalization, and log2 transformation.[76] Following quality control filters, 46,542 loci were included in the final analysis.

Differences in Demographic and Clinical Characteristics

Of 192 patients with phenotypic data in the RNA seq sample (Table 1), compared to the None class, the Severe class was more likely to be female; had fewer years of education; a higher body mass index; a lower performance status; a higher number of comorbidities; and a higher comorbidity burden; were more likely to have adult care responsibilities; had a lower annual income, and were more likely to self-report diagnoses of anemia or back pain.

Of 197 patients with phenotypic data in the microarray sample (Table 2), compared to the None class, the Severe class had fewer years of education; a lower performance status; a higher number of comorbidities, a higher comorbidity burden, and a higher number of prior cancer treatments; were more likely to be not married or partnered; had lower annual income; were less likely to be employed; were less likely to exercise regularly; were more likely to have a current or previous history of smoking; and were more likely to self-reported diagnoses of anemia, depression, osteoarthritis, or back pain.

Differences in Pain Characteristics

As summarized in Table 3, no differences were found between the two Severe pain classes in any of the pain characteristics evaluated. In brief, the majority of the patients had both cancer and non-cancer pain; were experiencing chronic pain; had worst pain scores in the severe range; and reported moderate levels of interference.

Logistic Regression Analyses

In the logistic regression analysis for the RNA-seq sample (Table 4), five variables were retained in the final model (i.e., gender, income, adult care responsibilities, KPS score, self-reported diagnosis of back pain) and were used as covariates in the gene expression analysis. Patients who were female, had a lower annual income, had adult care responsibilities, had a lower functional status, and self-reported a diagnosis of back pain were more likely to belong to the Severe class.

In the logistic regression analysis for the microarray sample (Table 4), eight variables were retained in the final model (i.e., married/partnered, exercise on a regular basis, current or history of smoking, KPS score, number of prior cancer treatments, self-reported diagnoses of anemia, depression, back pain) and were used as covariates in the gene expression analysis. Patients who were not married or partnered did not exercise on a regular basis, had a current or history of smoking, had a lower KPS score, had a higher number of prior cancer treatments, and self-reported a diagnosis of depression or back pain were more likely to belong to the Severe class.

Perturbed Signaling Pathways Associated With Worst Pain Severity

Of the 13 surrogate variables identified for the RNA-seq sample, none were associated with class membership. The final differential expression model for this sample included 13 surrogate variables and five phenotypic characteristics. Of the 15 surrogate variables identified for microarray sample, two were associated with class membership and were excluded from the final model. The final differential expression model for this sample included 13 surrogate variables and eight phenotypic characteristics. For both samples, a total of 3,868 genes were included in the PIA analyses. Using Fisher's Combined Probability method, across the two samples, 51 KEGG signaling pathways were significantly perturbed at an FDR of 0.015 (see Supplemental File 2). Of these, nine were related to neuroinflammatory mechanisms (Table 5).

Discussion

This study is the first to provide evidence that suggests that perturbations in several neuroinflammatory pathways are associated with severe pain in oncology patients receiving chemotherapy. As noted in one review,[63] a growing body of evidence suggests that both neurons and immune cells directly and indirectly detect and respond to painful stimuli and contribute to the initiation and maintenance of chronic pain. Our findings suggest that shared neuroinflammatory mechanisms may contribute to both cancer and non-cancer pain in oncology patients with severe pain. The remainder of this discussion focuses on the nine perturbed pathways identified in this study.

Complement and Coagulation Cascades Pathway

As noted in a recent review,[82] complement signaling is important in directing neuronal responses to tissue injury and nerve trauma. After an acute injury, a complex interplay occurs between nociceptive neurons and immune cells to promote healing and facilitate guarding of the site of injury. The complement works by activating immune cells and

stimulates these cells to release inflammatory mediators. However, in the setting of chronic pain, persistent or unbalanced signaling of complement factors occurs.[82] In fact elevated levels of several key complement factors (e.g., C3a, C5, C5a) were found in patients with rheumatoid arthritis[35,40,59] and osteoarthritis.[81,87]

Phagosome Pathway

While little is known about the interaction between phagocytes and nociceptor signaling, emerging evidence suggests that phagocytes (i.e., macrophage, dendritic cells) can contribute to the development and maintenance of pain.[27] For example, macrophages release a variety of immune mediators that bind to receptors on nociceptors. This binding induces neuronal hyperexcitability and hypersensitivity.[27] In addition, microglia play an essential role in the initiation of neuroinflammation by releasing the complement components C1q and C3 that induce phagocytosis by binding to neuronal surfaces.[9] For example, following high-frequency stimulation-induced spinal long-term potentiation in rats,[94] the number of activated microglia in the dorsal and ventral horn increased, which suggests an association between microglial activation, spinal plasticity, and chronic pain hypersensitivity. In addition, in a recent preclinical study,[93] blockade of spinal microglia function significantly attenuated neuropathic pain through the inhibition of neuroinflammation.

Cytokine-cytokine Receptor Interaction Pathway

As noted in one review,[49] in response to tissue injury, nociceptors induced a number of pro- and anti-inflammatory mediators that directly bind to and activate cytokine receptors. This bidirectional interaction between pain and inflammation leads to hyperexcitability and hypersensitivity of nociceptor neurons (i.e., peripheral sensitization).[33] For example, in both preclinical and clinical studies, interleukin (IL)-6, tumor necrosis factor-alpha (TNF-α), and IL-1β appear to be involved in the development and maintenance of pain associated with cancer,[21,72] rheumatoid arthritis,[1] and peripheral neuropathies.[83,92] In contrast, in a recent preclinical study,[42] IL-10 attenuated pain hypersensitivity following cisplatin administration, which suggests that IL-10 may decrease neuroinflammation. In another preclinical study,[90] IL-4 receptor knockout mice showed upregulation of pro-inflammatory mediators. Furthermore, cytokine signaling in the periphery is transmitted to the central terminals of the nociceptors and the brain.[28] As a result, pro-inflammatory cytokines activate microglia that contributes to the development and maintenance of central sensitization.[39,93] For example, following sciatic nerve chronic constriction injury, differentially perturbed cytokine-cytokine receptor interaction pathways were found in dorsal horn tissues.[13]

Chemokine Signaling Pathway

Chemokines are chemotactic cytokines that control the movement of circulating peripheral immune cells (e.g., T lymphocytes, natural killer cells, B cells, dendritic cells) by mediating cell-to-cell communication.[34] In addition, they activate G-protein-coupled receptors.[10,28] The chemokine (C-X-C motif) ligand-(CXCL) receptor pair serves as a mediator for glia-neuron communication[61,95] that when activated leads to persistent hyperexcitability and neuroplasticity in peripheral nociceptors.[28] Subsequently, this chemokine signaling alters nociceptive transduction through activation of chemokine receptors in dorsal root ganglia (DRG) cells.[47] As noted in one review of rodent models of neuropathic pain,[85] upregulation

of the expression of C-C motif chemokine ligand 2 (CCL2) and its receptor (CCR2) in DRG neurons was identified. In addition, in another preclinical study of autologous nucleus pulposus-induced pain,[95] increases in chemokine CCL2/CCR2 signaling in DRG and spinal cord were associated with the maintenance of lumbar disc herniation-induced pain. Of note, the administration of CCR2 antagonist decrease mechanical allodynia.

Additional preclinical studies provide evidence that chemokines and their receptors play a crucial role in cancer pain,[61,62,89] visceral pain,[4] and inflammatory pain.[11,88] For example, chemokines appear to be involved in the regulation of neuronal excitability, neurotransmitter release, and neuronal survival[84] by enhancing the activity of the N-methyl-D-aspartate (NMDA) receptors in dorsal horn neurons.[26] In a recent preclinical study of inflammatory pain,[12] CXCL1/CXCR2 signaling induced an enhancement of NMDA-induced currents in spinal cord neurons. The authors suggested that CXCL1/CXCR2 drives hyperactivity of NMDA receptors, which in turn mediates persistent inflammatory pain through the induction and maintenance of central sensitization.[12]

Janus Kinase-signal Transducer and Activator of Transcription (JAK-STAT) Signaling Pathway

As noted in one review on the association between the JAK-STAT signaling pathway and pain,[10] this pathway is involved in both pro-and anti-nociceptive mechanisms through numerous inflammatory responses. For example, IL-6 binding to the IL-6 receptor induces the activation of the JAK-STAT transduction pathway.[54] Phosphorylated JAK1 and 2 and STAT3 are translocated to the nucleus, leading to the expression of target genes and an increase in the release of proinflammatory cytokines.[55,92] In contrast, IL-4 binding to the IL-4 receptor results in the activation of JAKs 1 and 3 and consequently STAT6, which in turn leads to the inhibition of the production of proinflammatory cytokines.[10] In fact, a JAK-STAT inhibitor was approved by Food and Drug Administration and is used to treat rheumatoid arthritis.[31] Given the positive findings across other studies,[2,48,65] the use of a JAK-STAT inhibitor may warrant investigation in oncology patients with severe pain.

Calcium Signaling Pathway

Primary afferent neurons express multiple types of voltage-gated calcium channels (VGCCs), including N- and T-type channels.[8] Calcium signaling mediated by these VGCCs is involved in the development and maintenance of chronic pain, including neuropathic[80] and inflammatory[73] pain, through the induction of a multifaceted cascade of signaling molecules.[30] This calcium signaling-related cascade begins with an influx of calcium ions into the post-synaptic neuron through NMDA and a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors for glutamate and/or VGCCs.[8] Subsequently, modulation of the ion channel pool induces sensitization and hyperexcitability of sensory neurons by releasing glutamate, an excitatory neurotransmitter at central nerve endings.[30,50]

Of note, several preclinical studies suggest that N-type calcium channel (Cav2.2) knockout mice have attenuation of inflammatory and neuropathic pain.[69] Upregulation of N-type calcium channels in rat DRG neurons was associated with neuropathic pain.[46,91] In addition, the deletion of the T-type calcium channel genes (e.g., Cav3.2) in rats was associated

with major antinociceptive effects.[7,15] L-type calcium channels (Cav1.2 and Cav1.3) are primarily located on post-synaptic channels that are involved in dorsal horn hyperexcitability and short- and long-term neuronal plasticity.[68] As demonstrated in a preclinical study,[66] L-type calcium channels contribute to the integration of afferent inputs and the maintenance of hyperexcitability in DRG neurons by controlling plateau potentials.

Retrograde Endocannabinoid Signaling Pathway

Retrograde endocannabinoid signaling is implicated in several forms of short- and long-term synaptic plasticity.[6] The endocannabinoid system includes cannabinoid receptor subtypes 1 (CB1) and 2 (CB2), as well as their ligands, namely endocannabinoids.[6] The endocannabinoids synthesized in response to activation of post-synaptic metabotropic glutamate receptors (mGluRs) travel retrogradely to bind CB1 receptors and impede neurotransmitter release through inhibition of presynaptic VGCCs.[38] In addition, retrograde endocannabinoid signaling decreases presynaptic neurotransmitter release and balances glutamate/GABAergic transmission.[38] Interestingly, while CB2 receptors are not found in the healthy brain, upregulation of CB2 receptors on microglia appears to be induced by neuroinflammatory processes.[38] Therefore, increased endocannabinoid signaling may be associated with anti-inflammatory and neuroprotective phenotypes in microglia that suggests the therapeutic potential of targeting CB1 or CB2 receptors.[23]

Strengths and Limitations—While some limitations warrant consideration in that detailed information on the causes of cancer pain and analgesic use were not available for our patients, this study had a relatively large sample size and used LPA to identify distinct pain profiles. In addition, our sample represents the clinical reality in that oncology patients experience cancer and/or non-cancer pain. Additional strengths of this study include the performance of rigorous quality controls; utilization of two complementary methods to measure gene expression; the provision of results from independent tests across two samples; and the use of strict criteria for the selection of the perturbed neuroinflammatory pathways.

Conclusions

Our findings suggest that neuroimmune interactions are involved in the maintenance of chronic pain conditions in patients with cancer who are receiving chemotherapy. Our findings provide new evidence for potential therapeutic targets for the management of moderate to severe pain in oncology patients receiving chemotherapy. However, while no differences in cancer types and toxicity of the chemotherapy regimens was found between the None and Severe classes, additional research is warranted on the potential effects of the underlying tumor biology. Given that a growing body of evidence suggests a role for interactions between neuroimmune and endocrine systems in the maintenance of chronic pain, our subsequent study will evaluate for associations between pain in oncology patients receiving chemotherapy and perturbations in endocrine pathways.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1.

Differences in Demographic and Clinical Characteristics Between Patients in the None Versus the Severe Pain Classes in the RNA-sequencing Sample

Characteristic	None 37.5% (n = 72) Mean (SD)	Severe 62.5% (n = 120) Mean (SD)	Statistics
Age (years)	57.2 (12.3)	55.2 (12.2)	t = 1.09, p = 0.277
Education (years)	16.4 (3.5)	15.2 (2.9)	t = 2.53, p = 0.012
Body mass index (kg/m^2)	25.5 (5.6)	27.6 (6.7)	t =−2.22, p = 0.028
Karnofsky Performance Status score	82.4 (13.3)	72.9 (11.6)	t = 5.16, p < 0.001
Number of comorbidities	2.2 (1.6)	3.0 (1.6)	t = −3.49, p < 0.001
Self-administered Comorbidity Questionnaire score	5.0 (3.3)	7.2 (4.1)	t = −3.94, p < 0.001
Alcohol Use Disorders Identification Test score	2.8 (1.4)	2.7 (1.9)	t = 0.29, p = 0.774
Time since diagnosis (years)	2.1 (3.7)	1.2 (2.2)	U, p = 0.443
Time since diagnosis (years, median)	0.45	0.44	
Number of prior cancer treatments	1.5 (1.3)	1.5 (1.4)	t =−0.24, p = 0.811
Number of metastatic sites including lymph node involvement	1.2 (1.2)	1.2 (1.3)	t = 0.16, p = 0.871
Number of metastatic sites excluding lymph node involvement	0.7 (1.0)	0.7 (1.1)	t =−0.02, p = 0.986
Hemoglobin (g/dL)	11.6 (1.5)	11.3 (1.4)	t = 1.19, p = 0.234
Hematocrit (%)	34.7 (4.4)	34.1 (4.0)	t = 0.92, p = 0.361
MAX2 score	0.17 (0.08)	0.19 (0.08)	t = −1.43, p = 0.155
	% (n)	**% (n)**	
Gender			FE, p = 0.007
Female	68.1 (49)	85.0 (102)	
Male	31.9 (23)	15.0 (18)	
Ethnicity			X^2 = 3.67; p = 0.300
White	70.8 (51)	58.3 (70)	
Black	5.6 (4)	10.0 (12)	
Asian or Pacific Islander	11.1 (8)	11.7 (14)	
Hispanic mixed or other	12.5 (9)	20.0 (24)	
Married or partnered (% yes)	61.1 (44)	54.2 (65)	FE, p = 0.370
Lives alone (% yes)	23.6 (17)	31.7 (38)	FE, p = 0.253

Childcare responsibilities (% yes)	26.4 (19)	19.2 (23)	FE, p = 0.281
Adult care responsibilities (% yes)	1.4 (1)	11.7 (14)	FE, p = 0.011
History of premature birth (% yes)	2.8 (2)	5.8 (7)	FE, p = 0.488
Currently employed (% yes)	34.7 (25)	26.7 (32)	FE, p = 0.256
Income			U, p < 0.001
<$30,000	11.1 (8)	38.3 (46)	
$30,000 to <$70,000	15.3 (11)	23.3 (28)	
$70,000 to <$100,000	22.2 (16)	15.0 (18)	
≥$100,000	51.4 (37)	23.3 (28)	
Specific comorbidities (% yes)			
Heart disease	4.2 (3)	8.3 (10)	FE, p = 0.377
High blood pressure	29.2 (21)	36.7 (44)	FE, p = 0.345
Lung disease	11.1 (8)	12.5 (15)	FE, p = 0.823
Diabetes	11.1 (8)	15.8 (19)	FE, p = 0.400
Ulcer or stomach disease	2.8 (2)	7.5 (9)	FE, p = 0.214
Kidney disease	1.4 (1)	0.8 (1)	FE, p = 1.000
Liver disease	9.7 (7)	4.2 (5)	FE, p = 0.136
Anemia or blood disease	4.2 (3)	14.2 (17)	FE, p = 0.029
Depression	18.1 (13)	29.2 (35)	FE, p = 0.121
Osteoarthritis	12.5 (9)	17.5 (21)	FE, p = 0.416
Back pain	16.7 (12)	51.7 (62)	FE, p < 0.001
Rheumatoid arthritis	1.4 (1)	6.7 (8)	FE, p = 0.157
Exercise on a regular basis (% yes)	66.7 (48)	65.0 (78)	FE, p = 0.876
Smoking current or history of (% yes)	38.9 (28)	35.0 (42)	FE, p = 0.643
Cancer diagnosis			$X^2 = 7.84$, p = 0.049
Breast	36.1 (26)	40.0 (48)	NS
Gastrointestinal	43.1 (31)	25.0 (30)	NS
Gynecological	11.1 (8)	19.2 (23)	NS
Lung	9.7 (7)	15.8 (19)	NS
Type of prior cancer treatment			$X^2 = 0.54$, p = 0.909
No prior treatment	25.0 (18)	26.7 (32)	
Only surgery, CTX, or RT	41.7 (30)	45.0 (54)	

Surgery & CTX, or surgery & RT, or CTX & RT	15.3 (11)	13.3 (16)	
Surgery & CTX & RT	18.1 (13)	15.0 (18)	
CTX cycle length			$X^2 = 3.03$, p = 0.220
14 day cycle	47.2 (34)	42.5 (51)	
21 day cycle	40.3 (29)	50.8 (61)	
28 day cycle	12.5 (9)	6.7 (8)	
Emetogenicity of CTX			$X^2 = 0.73$, p = 0.695
Minimal/low	15.3 (11)	19.2 (23)	
Moderate	62.5 (45)	62.5 (75)	
High	22.2 (16)	18.3 (22)	
Antiemetic regimens			$X^2 = 0.92$, p = 0.821
None	4.2 (3)	2.5 (3)	
Steroid alone or serotonin receptor antagonist alone	18.1 (13)	18.3 (22)	
Serotonin receptor antagonist and steroid	52.8 (38)	49.2 (59)	
NK-1 receptor antagonist and two other antiemetics	25.0 (18)	30.0 (36)	

Abbreviations: CTX = chemotherapy; dL = deciliter; FE = Fisher's exact test; g = grams; kg = kilograms; m^2 = meter squared; NK-1 = neurokinin-1; NS = not significant; RNA = ribonucleic acid; RT = radiation therapy; SD = standard deviation; U = Mann-Whitney U test.

Table 2.

Differences in Demographic and Clinical Characteristics Between Patients in the None Versus Severe Pain Classes in the Microarray Sample

Characteristic	None 43.7% (n = 86) Mean (SD)	Severe 56.3% (n= 111) Mean (SD)	Statistics
Age (years)	57.2 (10.2)	54.3 (12.9)	t = 1.67, p = 0.096
Education (years)	16.8 (3.1)	15.8 (2.8)	t = 2.37, p = 0.019
Body mass index (kg/m^2)	26.3 (6.4)	27.2 (5.8)	t =−0.97, p = 0.333
Karnofsky Performance Status score	83.7 (10.6)	74.6 (11.5)	t = 5.65, p < 0.001
Number of comorbidities	1.9 (1.1)	3.0 (1.5)	t = −5.67, p < 0.001
Self-administered Comorbidity Questionnaire score	4.3 (2.3)	6.9 (3.4)	t = −6.10, p < 0.001
Alcohol Use Disorders Identification Test score	3.0 (1.7)	2.8 (2.8)	t = 0.56, p = 0.579
Time since diagnosis (years)	1.4 (2.7)	2.3 (3.7)	U, p = 0.202
Time since diagnosis (years, median)	0.40	0.45	
Number of prior cancer treatments	1.5 (1.5)	2.0 (1.7)	t =−2.18, p = 0.030
Number of metastatic sites including lymph node involvement	1.0 (1.2)	1.4 (1.4)	t = −1.71, p = 0.089
Number of metastatic sites excluding lymph node involvement	0.6 (1.1)	0.9 (1.2)	t = −1.64, p = 0.103
Hemoglobin (g/dL)	11.7 (1.4)	11.6 (1.3)	t = 0.52, p = 0.607
Hematocrit (%)	34.9 (4.0)	34.6 (3.7)	t = 0.43, p = 0.670
MAX2 score	0.18 (0.09)	0.17 (0.08)	t = 1.13, p = 0.261
	% (n)	**% (n)**	
Gender			FE, p = 0.182
Female	79.1 (68)	86.5 (96)	
Male	20.9 (18)	13.5 (15)	
Ethnicity			X^2 = 1.60, p = 0.659
White	72.1 (62)	64.0 (71)	
Black	11.6 (5)	9.0 (10)	
Asian or Pacific Islander	5.8 (10)	14.4 (16)	
Hispanic, Mixed, or Other	10.5 (9)	12.6 (14)	
Married or partnered (% yes)	80.2 (69)	54.1 (60)	FE, p < 0.001
Lives alone (% yes)	15.1 (13)	20.7 (23)	FE, p = 0.356

Childcare responsibilities (% yes)	23.3 (20)	27.0 (30)	FE, p = 0.622
Adult care responsibilities (% yes)	5.8 (5)	13.5 (15)	FE, p = 0.097
History of premature births (% yes)	4.7 (4)	5.4 (6)	FE, p = 1.000
Currently employed (% yes)	45.3 (39)	24.3 (27)	FE, p = 0.002
Income			U, p < 0.001
<$30,000	12.8 (11)	31.5 (35)	
$30,000 to <$70,000	14.0(12)	26.1 (29)	
$70,000 to <$100,000	16.3 (14)	14.4 (16)	
≥$100,000	57.0 (49)	27.9 (31)	
Specific comorbidities (% yes)			
Heart disease	3.5 (3)	8.1 (9)	FE, p = 0.236
High blood pressure	31.4 (27)	31.5 (35)	FE, p = 1.000
Lung disease	5.8 (5)	12.6 (14)	FE, p = 0.145
Diabetes	5.8 (5)	9.0 (10)	FE, p = 0.434
Ulcer or stomach disease	2.3 (2)	7.2 (8)	FE, p = 0.191
Kidney disease	1.2 (1)	1.8 (2)	FE, p = 1.000
Liver disease	5.8 (5)	6.3 (7)	FE, p = 1.000
Anemia or blood disease	8.1 (7)	22.5 (25)	FE, p = 0.007
Depression	10.5 (9)	34.2 (38)	FE, p < 0.001
Osteoarthritis	4.7 (4)	17.1 (19)	FE, p = 0.007
Back pain	5.8 (5)	44.1 (49)	FE, p < 0.001
Rheumatoid arthritis	1.2 (1)	3.6 (4)	FE, p = 0.389
Exercise on a regular basis (% yes)	79.1 (68)	61.3 (68)	FE, p = 0.008
Smoking current or history of (% yes)	20.9 (18)	40.5 (45)	FE, p = 0.004
Cancer diagnosis			$X^2 = 0.72$, p = 0.869
Breast	41.9 (36)	39.6 (44)	
Gastrointestinal	25.6 (22)	22.5 (25)	
Gynecological	19.8 (17)	24.3 (27)	
Lung	12.8 (11)	13.5 (15)	
Type of prior cancer treatment			$X^2 = 5.59$, p = 0.133
No prior treatment	24.4 (21)	17.1 (19)	
Only surgery, CTX, or RT	50.0 (43)	41.4 (46)	

Surgery & CTX, or surgery & RT, or CTX & RT	12.8 (11)	20.7 (23)	
Surgery & CTX & RT	12.8 (11)	20.7 (23)	
CTX cycle length			$X^2 = 1.34$, p = 0.512
14 day cycle	36.0 (31)	30.6 (34)	
21 day cycle	59.3 (51)	61.3 (68)	
28 day cycle	4.7 (4)	8.1 (9)	
Emetogenicity of CTX			$X^2 = 2.21$, p = 0.331
Minimal/low	18.6 (16)	27.0 (30)	
Moderate	58.1 (50)	55.0 (61)	
High	23.3 (20)	18.0 (20)	
Antiemetic regimens			$X^2 = 3.28$, p = 0.351
None	11.6 (10)	8.1 (9)	
Steroid alone or serotonin receptor antagonist alone	24.4 (21)	19.8 (22)	
Serotonin receptor antagonist and steroid	47.7 (41)	45.9 (51)	
NK-1 receptor antagonist and two other antiemetics	16.3 (14)	26.1 (29)	

Abbreviations: CTX = chemotherapy; dL = deciliter; FE = Fisher's exact test; g = grams; kg = kilograms; m^2 = meter squared; NK-1 = neurokinin-1; RT = radiation therapy; U = Mann-Whitney U test.

Table 3.

Differences in Pain Characteristics Between Patients in the RNA-Sequencing Sample Compared to the Microarray Sample

Characteristic	RNA-seq(n = 120) % (n)	Microarray(n =111) % (n)	Statistics
Sources of pain			
Type of pain			$X^2 = 0.29$, p = .866
Only non-cancer pain	15.8 (18)	14.2 (15)	
Only cancer pain	27.2 (31)	30.2 (32)	
Both cancer and non-cancer pain	57.0 (65)	55.7 (59)	
Causes of non-cancer pain			
Headache	33.7 (28)	41.9 (31)	FE, p= .324
Low back pain	57.8 (48)	48.7 (36)	FE, p = .266
Fibromyalgia	4.8 (4)	9.5 (7)	FE, p = .351
Diabetic neuropathy	3.6 (3)	1.4 (1)	FE, p = .623
Arthritis	33.7 (28)	27.0 (20)	FE, p = .390
Acute versus Chronic Pain			
Length of time with non-cancer pain			FE, p = .460
Less than 3 months	19.4 (14)	17.1 (12)	
≥3 months	80.6 (58)	82.9 (58)	
Length of time with cancer pain			FE, p = .829
Less than 3 months	56.4 (53)	50.0 (45)	
≥3 months	43.6 (41)	50.0 (45)	
Pain Characteristics			
	Mean (SD)	**Mean (SD)**	
Pain intensity			
Now	3.2 (2.6)	2.8 (2.3)	t = 1.10, p = .275
Average	4.4 (2.0)	4.2 (1.8)	t = 0.80, p = .422
Worst	8.1 (1.3)	8.4 (1.5)	t = −1.33, p = .185

Pain duration			
Number of days per week in pain	4.1 (2.3)	4.2 (2.2)	t =−0.34, p = .731
Number of hours per day in pain	9.7 (8.3)	10.3 (8.7)	t =−0.56, p = .575
Pain locations			
Number of pain locations	11.3 (9.1)	11.5 (9.7)	t =−0.14, p = .889
Pain interference			
General activity	4.9 (3.1)	4.9 (2.9)	t = 0.04, p = .972
Mood	4.4 (3.2)	4.8 (2.8)	t =−0.79, p = .431
Walking ability	4.6 (3.3)	4.4 (3.0)	t = 0.48, p = .633
Normal work	5.3 (3.3)	5.1 (2.9)	t = 0.48, p = .634
Relations with other people	3.4 (3.1)	3.5 (3.0)	t =−0.33, p = .741
Sleep	5.1 (3.2)	5.3 (2.9)	t =−0.59, p = .556
Enjoyment of life	5.0 (3.3)	5.0 (3.0)	t = −0.11, p = .912
Sexual activity	4.5 (4.2)	5.4 (4.1)	t = −1.51, p = .133
Mean pain interference score	4.6 (2.7)	4.8 (2.4)	t =−0.59, p = .558
Pain frequency	% (n)	% (n)	
1 to 4 times per month	15.5 (16)	14.9 (15)	U, p = .740
Several times per week	18.5 (19)	17.8 (18)	
Multiple times per day	42.7 (44)	40.6 (41)	
Continuously	23.3 (24)	26.7 (27)	

Abbreviations: FE = Fisher’s Exact test; RNA = ribonucleic acid; SD = standard deviation; seq = sequencing, U = Mann Whitney U test.

Table 4.

Multiple Logistic Regression Analyses Predicting Membership in the Severe Pain Class

RNA seq Sample (n = 192)			
Predictors	**Odds Ratio**	**95% CI**	**p-value**
Gender (male)	0.42	0.18, 0.98	0.047
Income			
<$30,000	1.00		
$30,000 to <$70,000	0.43	0.13, 1.33	0.147
$70,000 to <$100,000	0.22	0.07, 0.68	0.010
≥$100,000	0.17	0.06, 0.45	<0.001
Adult care responsibilities	10.96	1.75, 218.39	0.033
Karnofsky Performance Status score	0.96	0.93, 0.99	0.005
Self-reported diagnosis of back pain	2.98	1.36, 6.78	0.007
Overall model fit: AUC of the ROC = 0.820			
Microarray Sample (n = 197)			
Predictors	**Odds Ratio**	**95% CI**	**p-value**
Married or partnered	0.29	0.12, 0.66	0.003
Exercise on a regular basis	0.38	0.16, 0.87	0.022
Current or history of smoking	2.69	1.19, 6.32	0.018
Karnofsky Performance Status score	0.93	0.90, 0.96	< 0.001
Number of prior cancer treatments	1.37	1.07, 1.79	0.013
Self-reported diagnosis of anemia or blood disease	2.53	0.85, 8.17	0.096
Self-reported diagnosis of depression	3.60	1.34, 10.42	0.011
Self-reported diagnosis if back pain	9.15	3.21, 31.53	<0.001
Overall model fit: AUC of the ROC = 0.883			

Abbreviations: AUC = Area under curve; CI = confidence interval; ROC = receiver operating characteristic.

Table 5.

Perturbed Neuroinflammatory KEGG Pathways Between Patients in the None Versus the Severe PaSin Classes

Pathway ID	Pathway Name	Combined Analysis Statistics
Neuroinflammatory Pathways		
hsa04060	Cytokine-cytokine receptor interaction	$X^2 = 19.92$, pPert = 0.008
hsa04062	Chemokine signaling pathway	$X^2 = 21.85$, pPert = 0.005
hsa04145	Phagosome	$X^2 = 20.31$, pPert = 0.007
hsa04610	Complement and coagulation cascades	$X^2 = 19.33$, pPert = 0.008
hsa04630	JAK-STAT signaling pathway	$X^2 = 17.03$, pPert = 0.012
Signal Transduction Pathway		
hsa04020	Calcium signaling pathway	$X^2 = 22.84$, pPert = 0.005
Neurotransmitter Pathways		
hsa04723	Retrograde endocannabinoid signaling	$X^2 = 17.55$, pPert = 0.011
hsa04724	Glutamatergic synapse	$X^2 = 21.91$, pPert = 0.005
hsa04727	GABAergic synapse	$X^2 = 15.84$, pPert = 0.014

Abbreviations: GABA = gamma-aminobutyric acid; JAK-STAT = Janus kinase-signal transducer and activator of transcription; KEGG = Kyoto Encyclopedia of Genes and Genomes; pPert = Combined perturbation P-value using Fisher's Method adjusted using the Bonferroni method.