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Moving the Science of Symptom Cluster Research Forward: Phenotypic and Mechanistic Considerations

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Moving the Science of Symptom Cluster Research Forward: Phenotypic and Mechanistic Considerations

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Abstract

Moving the Science of Symptom Cluster Research Forward: Phenotypic and Mechanistic Considerations

Carolyn S. Harris

Oncology patients receiving chemotherapy report on average 14 concurrent symptoms. The co-occurrence of these symptoms is associated with poorer functional status, decrements in quality of life (QOL), and increased mortality. Given that symptoms rarely occur in isolation, the concept of a symptom cluster emerged in the literature in 2001. An increased understanding of how symptoms cluster together and the biological mechanism(s) that underlie them has the potential to lead to the development of targeted interventions to decrease symptom burden. Therefore, the overall aims of this dissertation research were to: 1) review the conceptual basis for using variable-centered versus patient-centered analytic approaches in symptom cluster research; 2) systematically review studies published since 2016 that evaluated for symptom clusters in patients receiving primary or adjuvant chemotherapy; 3) evaluate the stability and consistency of symptom clusters across time and across three symptom dimensions (i.e., occurrence, severity, and distress); 4) identify common and distinct symptom clusters across various types of cancer; and 5) evaluate for associations between psychological and gastrointestinal symptom clusters and epigenetic regulation of inflammatory genes in a heterogeneous sample of oncology patients.

In terms of aim 1, a theoretical paper described two conceptual approaches that are used to evaluate symptom clusters; namely: “clustering” symptoms (i.e., variable-centered analytic approach) and “clustering” patients (i.e., person-centered analytic approach). Findings suggest that while each approach has unique strengths and weaknesses, conceptual clarity is needed when a study is designed and the specific research question(s) should guide the selection of the appropriate analytic method. The application of newer analytic approaches (e.g., network analysis (NA), natural language processing (NLP)) to study symptom clusters were

reviewed. This paper summarized the paucity of research on the evaluation of the underlying mechanism(s) for symptom clusters.

In terms of aim 2, in a systematic review, 23 studies were identified that evaluated for symptom clusters in patients receiving chemotherapy. Across these studies, the Memorial Symptom Assessment Scale (MSAS) was the most common instrument and exploratory factor analysis (EFA) was the most common statistical method used to identify symptom clusters. While psychological, gastrointestinal, and nutritional clusters were the most common clusters identified across studies, only the psychological cluster remained relatively stable over time. A major conclusion from this review was that clear criteria are needed to evaluate the stability of symptom clusters across time and dimensions. In addition, only five studies evaluated for secondary outcomes (e.g., functional status, QOL). Additional research is needed to evaluate the biological mechanism(s) for symptom clusters.

In terms of aim 3, prior to the start of their second or third cycle of chemotherapy, outpatients reported an average of 13.9 (±7.2) concurrent symptoms. Lack of energy was both the most common and severe symptom while “I don’t look like myself” was the most distressing. Psychological, gastrointestinal, weight gain, respiratory, and hormonal clusters were the common symptom clusters identified across the three symptom dimensions. Our findings suggest that psychological, gastrointestinal, and weight gain clusters are common across various types of cancer while respiratory and hormonal clusters are cancer specific.

In terms of aim 4, across a cycle of chemotherapy, the number of symptoms remained relatively stable over time, with patients reporting 13.9 (±7.2) symptoms prior to, 14.0 (±7.0) at one week after, and 12.2 (±6.8) at two weeks after receipt of chemotherapy. While the psychological, weight gain, respiratory, and gastrointestinal clusters were stable over time and dimensions, only the psychological, weight gain, and respiratory clusters were consistent across time and dimensions.

In terms of aim 5, given the paucity of studies on the underlying mechanism(s) for the two most common symptom clusters (i.e., psychological, gastrointestinal), exploratory analyses were done to evaluate for associations between these clusters and epigenetic variation of inflammatory genes. Findings from both studies provide preliminary support for the hypothesis that epigenetic dysregulation of inflammatory processes contributes to the occurrence of psychological and gastrointestinal symptom clusters in patients receiving chemotherapy. For the psychological symptom cluster, cluster of differentiation (CD) 40 was differentially methylated across two independent samples (false discovery rate (FDR) = .017). Six expression-associated CpGs (i.e., eCpG; cg22232207, cg06571407, cg17929951, cg21601405, cg01943874, cg11841529) located in the promoter region of this gene were hypomethylated across both samples. For the gastrointestinal symptom cluster, one trans eCpG locus (i.e., cg03171795) that was associated with expression of the lymphotoxin beta (*LTB*) gene was associated with the occurrence of the gastrointestinal symptom cluster (FDR = 0.168). These findings warrant validation. This dissertation concludes with implications for clinical practice and future research.

Table of Contents

Chapter 1

Page

Chapter 2

Chapter 3

Page

Page

Page

Chapter 6

Chapter 7

Page

Chapter 8

List of Figures

Chapter 2

Page

Chapter 3

Chapter 6

Chapter 7

Page

List of Tables

Chapter 2

Page

Chapter 3

Chapter 4

Chapter 5

Page

Chapter 6

Chapter 7

List of Abbreviations

CD40 = cluster of differentiation 40

DNA = deoxyribonucleic acid

eCpG = expression-associated CpG

EFA = exploratory factor analysis

EHR = electronic health record

FDR = false discovery rate

HCA = hierarchical cluster analysis

IL = interleukin

LCA = latent class analysis

LTβ = lymphotoxin beta

MSAS = Memorial Symptom Assessment Scale

NA = network analysis

NCI = National Cancer Institute

NLP = natural language processing

NF-κB = nuclear factor kappa B

TNFα = tumor necrosis factor alpha

Chapter 1

Introduction to Dissertation

While receiving life-saving cancer treatment, patients experience multiple co-occurring symptoms. In one review,[1] patients reported experiencing an average of 10 unrelieved co-occurring symptoms during cancer treatment. This phenomenon is described as a symptom cluster or an individual's experience of two or more concurrent, related symptoms that may share underlying mechanisms and/or outcomes.[2] Because of the negative impact of symptom clusters on patients' QOL,[1] it is essential to develop and test targeted interventions to decrease symptom burden. One way that progress will be made in reducing the occurrence and severity of some of the most common co-occurring symptoms (e.g., anxiety, depression) is to determine the mechanism(s) that underlie them. This area of research is highly relevant for nursing who has at its core the assessment and management of symptoms and the enhancement of patients' functional status and QOL.

CONCEPTUAL AND METHODOLOGICAL ISSUES

Since 2001, when the University of California, San Francisco Symptom Management Research Group first described symptom clusters and challenged the scientific community to explore this emerging concept,[3] research on symptom clusters has increased steadily.[2, 4, 5] Despite tremendous growth, several important methodological questions remain unanswered. One essential question is how does the dimension of the symptom experience (i.e., occurrence, severity, distress) used to create a symptom cluster affect the number and types of symptom clusters that are identified.[2] In a review of studies that evaluated for symptom clusters in patients receiving chemotherapy,[6] the authors highlighted large differences in the instruments and dimensions used to identify the clusters. This methodological variability resulted in a wide range of symptom clusters, in terms of both the number and types of clusters identified. Additional research is warranted to compare the number and types of symptom clusters that are

created using the dimensions of occurrence, severity, and distress in the same sample of patients.

As described by Miaskowski,[2, 7] a potential outcome of symptom cluster research is to determine which symptom clusters can become “diagnostic entities” and be used to develop symptom-cluster-specific management strategies. The identification of symptom clusters “de novo” is an important step in determining which symptom clusters are found across oncology patients regardless of cancer diagnosis, stage of disease, or treatments. Multiple studies have identified that psychological[8-11] and gastrointestinal[8-13] symptom clusters are extremely common in patients receiving chemotherapy, as well as with other types of cancer treatments. However, numerous inconsistencies were found in the specific symptoms within each of these two common symptom clusters.[4, 14] These inconsistencies may be attributed to variations in the instruments used to assess the symptoms, as well as in the statistical analyses used to create the clusters. Additional research on these two common symptom clusters in a single study is warranted at the present time.

MOLECULAR MECHANISM(S) UNDERLYING SYMPTOM CLUSTERS

Symptoms are hypothesized to cluster together because they share common biological or behavioral mechanism(s).[4] At this time, research on the biological mechanisms that underlie symptom clusters is quite limited. In a review that summarized the relationships between various biomarkers and the co-occurrence of cancer-related symptoms,[15] the authors concluded that it was difficult to draw definitive conclusions because the symptom and biomarker data were limited and disparate. Additional investigations into the relationships between symptom clusters and biological mechanism(s) in cancer patients are needed.

One emerging area of oncology symptom management research is to evaluate the molecular mechanisms (e.g., changes in gene expression, changes in deoxyribonucleic acid (DNA) methylation) that are associated with single symptoms and/or symptom clusters.[16-21] DNA methylation is an epigenetic mechanism that regulates gene function. This mechanism acts

throughout an individual's lifespan and allows an individual to respond to the environment through changes in gene expression.[22] Specifically, DNA methylation regulates gene expression by adding or removing methyl groups at the 5'-position of cytosine residues of DNA.[23] This methylation of specific DNA regions (e.g., promoter, trans) can impact transcription (i.e., activation or repression) through the recruitment or blocking of transcription factors at binding sites. An increased understanding of the associations between the occurrence of symptom clusters and epigenetic regulation may provide insights into the underlying mechanism(s) for the cluster.

Cytokines are known to contribute to the development of symptom(s) in patients with cancer.[24] Specifically, numerous studies have found associations between the pre-specified symptom cluster of pain, depression, fatigue, and/or sleep disturbance and three proinflammatory cytokines (i.e., interleukin 6 (IL6), tumor necrosis factor alpha (TNFα), nuclear factor kappa B 1 (NF-κB1)) in patients with cancer.[18, 25-27] Most of these studies evaluated for associations between this pre-specified symptom cluster and changes in serum levels of these cytokines[27] or variations in single nucleotide polymorphisms for cytokine genes.[18, 25, 26] While this body of research is increasing, no study has evaluated for associations between symptom clusters and variation in DNA methylation. The identification of associations between the occurrence of symptom clusters and the methylation status of putative regulatory regions for proinflammatory cytokine genes (e.g., TNFα, NF-κB1) will provide novel information on how gene regulatory processes may contribute to an increased symptom burden.

FOCUS OF DISSERTATION RESEARCH

Therefore, the aims of this dissertation research were to: 1) review the conceptual basis for symptom cluster research and 2) conduct a systematic review of the literature to evaluate the progress in symptom clusters research in adults receiving primary or adjuvant chemotherapy since 2016. Following these two theoretical papers,[28, 29] the remaining aims utilized phenotypic and molecular data from a heterogeneous sample of oncology patients

(n=1329) who were followed over two cycles of chemotherapy. The additional study aims were to: 3) identify symptom clusters across a cycle of chemotherapy using three dimensions of the symptom experience (i.e., occurrence, severity, distress); 4) evaluate the stability and consistency of symptom clusters over time, across symptom dimensions, and across four distinct types of cancer (i.e., breast, gastrointestinal, gynecological, and lung); and 5) evaluate for associations between the occurrence of a psychological and a gastrointestinal symptom cluster and levels of methylation for inflammatory genes.

This dissertation consists of six papers. The first paper is a review of the conceptual basis for using variable-centered versus patient-centered analytic approaches in symptom cluster research.[28] The second paper is a systematic review of symptom clusters in adults receiving primary or adjuvant chemotherapy.[29] The third paper reports on the number and types of symptom clusters that were identified using three symptom dimensions (i.e., occurrence, severity, and distress) and identifies common and distinct clusters in oncology patients prior to their second or third cycle of chemotherapy.[30] The fourth paper reports on the stability and consistency of symptom clusters across a cycle of chemotherapy, three symptom dimensions, and four types of cancer (i.e., breast, gastrointestinal, gynecological, lung).[31] The fifth and sixth papers report on associations between psychological and gastrointestinal symptom clusters and epigenetic variation at putative regulatory sites for inflammatory genes in oncology patients receiving chemotherapy.

In the first paper (Chapter 2), a review of two conceptual approaches for evaluating symptom clusters was reported. In addition, we compared and contrasted the conceptual basis for using variable-centered versus patient-centered analytic approaches in symptom cluster research; reviewed their strengths and weaknesses; and compared their applications in symptom cluster research. Among studies that used a variable-centered approach, EFA was the most common statistical approach. For studies that used a patient-centered approach, latent variable modeling was the most common method. Findings suggest that while each approach

has unique strengths and weaknesses, conceptual clarity is needed when a study is designed and the research question(s) should inform the selection of the most appropriate method. The application of newer analytic approaches (e.g., NA, NLP) to study symptom clusters were reviewed. For both approaches, relatively few studies evaluated the underlying mechanisms for the symptom clusters. This chapter is a reprint of the original paper that was published in *Advances in Nursing Science*.[28]

The second paper (Chapter 3) reports on findings from a systematic review of 23 studies published since 2016 that evaluated for symptom clusters in patients receiving primary or adjuvant chemotherapy. Across these studies, the MSAS was the most common instrument (69.6%) and EFA was the most common statistical method used to identify symptom clusters (73.9%). Psychological, gastrointestinal, and nutritional clusters were the most commonly identified clusters, and were identified in 82.6%, 69.6%, and 56.5% of studies, respectively. Only the psychological cluster remained relatively stable over time. While the majority of the studies that evaluated for the stability of symptom clusters across dimensions or time used the method proposed by Kirkova and Walsh,[32] the criteria were applied with relative subjectivity. In addition, only five studies evaluated for secondary outcomes. Additional research is needed to evaluate the biological mechanism(s) for symptom clusters. This chapter is a reprint of the original paper that was published in *BMJ Supportive and Palliative Care*.[29]

In the third paper (Chapter 4), we describe ratings of occurrence, severity, and distress for 38 symptoms in a heterogeneous sample of oncology patients prior to their second or third cycle of chemotherapy and identify and compare the number and types of symptom clusters based on three symptom dimensions (i.e., occurrence, severity, and distress). In addition, an evaluation of common and distinct symptom clusters was done for the total sample compared to four distinct types of cancer (i.e., breast, gastrointestinal, gynecological, lung) and for two different methods (i.e., EFA, NA). A modified version of the MSAS was used to assess the

occurrence, severity, and distress ratings for 38 symptoms. For each dimension, symptom clusters were identified using EFA. This paper is in press in *Supportive Care in Cancer*.[30]

Patients reported an average of 13.9 (±7.2) concurrent symptoms. Lack of energy was both the most common and severe symptom while "I don't look like myself" was the most distressing. Psychological, gastrointestinal, weight gain, respiratory, and hormonal clusters were identified across all three dimensions. Our findings suggest that psychological, gastrointestinal, and weight gain clusters are common across various types of cancer. However, respiratory and hormonal clusters were associated with only gynecological and lung and only breast and gynecological cancer, respectively. Psychological, gastrointestinal, weight gain, hormonal, and respiratory clusters are stable across the dimensions of occurrence, severity, and distress in oncology patients receiving chemotherapy. Given the stability of these clusters and the consistency of the symptoms within the clusters across dimensions, the use of a single dimension to identify these clusters may be sufficient. However, comprehensive and disease-specific symptom inventories need to be used to identify distinct clusters.

In the fourth paper (Chapter 5), we report on the occurrence, severity, and distress of 38 symptoms; evaluate the stability and consistency of symptom clusters across a cycle of chemotherapy and three symptom dimensions; and identify common and distinct symptom clusters across four types of cancer (i.e., breast, gastrointestinal, gynecological, lung). Oncology outpatients (n=1329) completed the MSAS prior to their second or third cycle of chemotherapy (T1) and at one (T2) and two weeks after chemotherapy (T3). Clusters were stable if they were identified across each time point and/or dimension. Clusters were consistent if the same two or three symptoms with the highest factor loadings were identified across each time point and/or dimension. This paper is under review in *BMJ Supportive and Palliative Care*.[31]

Patients reported 13.9 (±7.2) symptoms at T1, 14.0 (±7.0) at T2, and 12.2 (±6.8) at T3. Psychological, weight gain, respiratory, and gastrointestinal clusters were stable over time and dimensions. Only the psychological, respiratory, and weight gain clusters were consistent

across time and dimensions. Given the stability of the psychological, weight gain, and gastrointestinal clusters across cancer diagnoses, symptoms within these clusters need to be routinely assessed. However, the hormonal and respiratory clusters were unique to specific cancer types and the symptoms within these clusters were variable.

The fifth paper (Chapter 6) reports on findings from an evaluation of the associations between the occurrence of a psychological symptom cluster and variation in levels of DNA methylation in putative regulatory regions of inflammatory genes. Prior to their second or third cycle of chemotherapy, 1071 patients reported the occurrence of 38 symptoms using the MSAS. EFA was used to identify the psychological symptom cluster. Differential methylation analyses were performed in two independent samples using 450K (n=146) and EPIC (n=925) microarrays. Expression-associated CpG (i.e., eCpG) loci in the promoter region of 114 inflammatory genes for the 450K microarray sample and 112 genes for the EPIC microarray sample were evaluated for associations with the psychological symptom cluster. Robust Rank Aggregation was used to identify genes that were differentially methylated across both samples.

Cluster of differentiation (CD) 40 was differentially methylated across both samples (FDR = .017). For this gene, six promoter eCpGs (i.e., cg22232207, cg06571407, cg17929951, cg21601405, cg01943874, cg11841529) were hypomethylated in a psychological symptom cluster group across both samples. This study is the first to identify associations between a psychological symptom cluster and differential methylation of a gene that is involved in tissue inflammation and cell-mediated immunity. Findings suggest that increased *CD40* expression by hypomethylation of promoter eCpG loci is associated with the occurrence of a psychological symptom cluster in patients receiving chemotherapy.

The sixth paper (Chapter 7) reports on the findings from a study that evaluated for associations between the occurrence of a gastrointestinal symptom cluster and levels of DNA methylation of trans eCpGs for genes within the NF-κB signaling pathway. Prior to their second or third cycle of chemotherapy, 1071 patients reported symptom occurrence using the MSAS.

EFA was used to identify a gastrointestinal symptom cluster. Differential methylation analyses were performed in patients using the EPIC microarray (n=925) and were validated in an independent sample using the 450K microarray (n=146). Trans eCpG loci on 56 genes in the NF-κB signaling pathway were evaluated. Significance of the candidate trans eCpG loci were assessed using a FDR of 25% under the Benjamini-Hochberg procedure.

For the EPIC microarray sample, one trans eCpG locus (i.e., cg03171795) that is associated with expression of the lymphotoxin beta (*LTB*) gene was significantly associated with the occurrence of the gastrointestinal symptom cluster (FDR = 0.168). However, this association was not confirmed in the 450K microarray sample. This study is the first to evaluate for associations between the gastrointestinal symptom cluster and markers of epigenetic regulation of inflammatory mechanisms. Findings suggest that increased *LTB* expression regulated by hypermethylation of a trans eCpG locus is involved in the occurrence of the gastrointestinal symptom cluster in patients receiving chemotherapy.

As research on symptom clusters increases, ongoing clarification and/or refinement of the definition of, conceptual basis for, and methods to evaluate symptom clusters are needed. To advance this area of scientific inquiry, new definitions and criteria for assessing the stability and consistency of symptom clusters were proposed in this dissertation. As reported in the theoretical and systematic reviews, relatively few studies have evaluated the underlying mechanism(s) of symptom clusters. Findings from this dissertation research support the hypothesis that two common symptom clusters (i.e., psychological, gastrointestinal) are associated with epigenetic regulation of inflammatory genes. Additional research is needed to validate these findings.

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Chapter 2

Advances in Conceptual and Methodological Issues in Symptom Cluster Research: A Twenty Year Perspective

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ABSTRACT

Two conceptual approaches are used to evaluate symptom clusters: “clustering” symptoms (i.e., variable-centered analytic approach) and “clustering” patients (i.e., person-centered analytic approach). However, these methods are not used consistently and conceptual clarity is needed. Given the emergence of novel methods to evaluate symptom clusters, a review of the conceptual basis for older and newer analytic methods is warranted. Therefore, this paper will review the conceptual basis for symptom cluster research; compare and contrast the conceptual basis for using variable-centered versus patient-centered analytic approaches in symptom cluster research; review their strengths and weaknesses; and compare their applications in symptom cluster research.

Keywords: cluster analysis; factor analysis; latent class analysis; latent variable modeling; natural language processing; network analysis; symptom clusters; symptom science

INTRODUCTION

Symptom science was transformed by two landmark papers that suggested the existence of "symptom clusters" in oncology patients.[1, 2] Prior to these papers, symptom research focused primarily on an evaluation of the prevalence and severity of single symptoms in patients with chronic conditions.[3] Building on the clinical reality that symptoms rarely occur alone, researchers and clinicians were challenged to evaluate for and manage co-occurring symptoms and/or symptom clusters.

Given that these two studies published in 2001 are credited with launching the field of "symptom cluster" research,[1, 2] they warrant careful evaluation twenty years later. In the first study,[2] the relationships between pain and fatigue and the co-occurrence of 20 other symptoms were evaluated in a heterogeneous sample of newly diagnosed oncology patients over one year. In the second study,[1] the effect of a pre-specified symptom cluster (i.e., pain, fatigue, sleep disturbance) on oncology patients' functional status was evaluated over three cycles of chemotherapy. Of note, in this paper,[1] the first definition of a symptom cluster was proposed to be "three or more concurrent symptoms" that "are related to each other….The symptoms within a cluster are not required to share the same etiology," (pp465).

While these studies provided a stimulus and new directions for symptom science research, several limitations warrant consideration. First, only two symptoms (i.e., pain, fatigue) were evaluated in one study[2] and three symptoms (i.e., pain, fatigue, sleep insufficiency) in the other study.[1] In both studies, the symptom cluster was pre-specified, not created "de novo". Third, both studies evaluated for associations between single symptoms and a distal outcome, not with the "symptom cluster" as a whole.

While symptom cluster research has grown considerably since the publication of these two relatively "simplistic" studies,[1, 2] as noted in the most recent expert panel report,[4] this field is relatively new and ongoing conceptual issues warrant consideration. One key question is a rather simple one, namely: "What constitutes symptom cluster research?" As noted by

Miaskowski and colleagues in 2007,[5] two conceptual approaches to evaluate symptom clusters evolved over a period of five years, namely: “clustering” symptoms (equates with a variable-centered analytic approach) and “clustering” patients (equates with a person-centered analytic approach) (Figure 2.1). The use of the word “clustering” for both approaches has led to confusion in the literature on symptom cluster research. For example, it is not uncommon to find publications that have described “symptom clusters” when patients were grouped based on an evaluation of a pre-specified symptom cluster that consisted of two or more symptoms.[6, 7] Given this confusion, it is imperative to use the correct terminology as outlined below.

As noted in Figure 2.1A, variable-centered approaches (e.g., EFA) identify *symptoms* that cluster together empirically through the use of an analytic approach that creates distinct groups of related symptoms (i.e., symptom clusters).[5] These approaches are based on the hypothesis that symptoms cluster together because they may share a common underlying mechanism(s).[8, 9]

Patient-centered approaches (Figure 2.1B; e.g., latent class analysis (LCA)) identify subgroups of *patients* with distinct symptom profiles using one or more symptoms or a pre-specified symptom cluster (e.g., pain, fatigue, depression, sleep disturbance[10]). With these approaches, it is important to note that in the context of symptom clusters research, a symptom cluster must be pre-specified. These patient-centered analyses can be used to identify subgroups of patients with distinct symptom(s) profiles (i.e., lower versus higher symptom burden) and associated risk factors (e.g., demographic, clinical, biomarkers).[5]

Previous reviews have evaluated the conceptual, methodological, and clinical basis for symptom clusters research.[5, 11-15] In a concept analysis that included a review of symptom cluster research across psychiatry, medicine, and nursing, Kim and colleagues[14] identified five key attributes of a symptom cluster (e.g., co-occurrence of symptoms within a cluster, stability, shared or common etiology). Based on research findings and clinical evidence, both Kim and colleagues[14] and Aktas[11] argued for the definition of a symptom cluster to be modified to include

a minimum of two symptoms. Kim and Abraham[13] and Skerman and colleagues[15] examined the application of various statistical methods to identify symptom clusters and reviewed the conceptual and methodological challenges of each method. Building on a previous paper by Miaskowski and colleagues[5] that described the two conceptual approaches for symptom clusters research, Barsevick[12] examined the application of qualitative approaches to symptom clusters research and expanded on the concept of stability in symptom cluster research.

In the most recent state of the science report,[4] an expert panel called for the identification of symptom clusters using newer analytic techniques and for an investigation of the underlying mechanisms for symptom clusters. In addition, they suggested that additional research is warranted to clarify the "de novo" approach to the identification of symptom clusters versus the grouping of patients with distinct symptom profiles based on a "pre-specified" symptom cluster. Given the recent application of newer methods to symptom cluster research (e.g., NA,[16] NLP[17]), a review of the conceptual basis for these older and newer methods in the context of symptom cluster research is warranted. Therefore, the purposes of this paper are to review the conceptual basis for symptom cluster research; compare and contrast the conceptual basis for using variable-centered versus patient-centered analytic approaches in symptom clusters research; review the strengths and weaknesses of the most common variable-centered and patient-centered analytic approaches for symptom clusters research; and compare the various applications of each approach in symptom cluster research.

DEFINITION OF A SYMPTOM CLUSTER

As the science of symptom cluster research has advanced over the past 20 years, the definition of a symptom cluster has gone through multiple revisions.[1, 12, 14] In the most recent revision by an expert panel,[4] several characteristics of both a symptom and a symptom cluster were identified (Table 2.1). While some debate continues on the minimum number of symptoms that constitutes a symptom cluster,[11, 12] a minimum of two symptoms in a cluster is generally accepted. However, clarification and/or refinement of the other characteristics are needed. For

example, in terms of “stability,” neither the definition of nor the methods to assess stability exist. This issue is particularly important when one considers the temporal dimension of symptom clusters. Does stability refer to whether or not the various types of symptom clusters (e.g., psychological, gastrointestinal) remain “stable” or whether or not the symptoms within each cluster (e.g., sad, irritable, angry) remain “stable” over time? We propose that the term “stable” be used to describe whether the symptom clusters change over time and/or across symptom dimensions. Alternatively, the term “consistent” should be used to describe whether the specific symptoms within a cluster remain the same over time and/or across symptom dimensions. For both stability and consistency, the assessment methods and numeric criteria need to be determined.[18]

Equally important is the question of whether or not symptom clusters need to be independent of other clusters. Given the recent use of NA, that demonstrates that symptoms within one cluster are related to symptoms in other clusters,[16] this criterion may need to be reconsidered. Equally important, research is needed to support the criteria that symptom clusters may share common underlying mechanisms and may have shared outcomes.

TWO BROAD APPROACHES TO SYMPTOM CLUSTER RESEARCH

De Novo Identification of Symptom Clusters

Variable-centered approaches explore the relationships among symptoms using either regression-based techniques[19] or measures of similarity[13] and create symptom clusters “de novo.” As a first step, participants need to complete one or more symptom assessment instruments or a symptom inventory (Figure 2.1A).[5] Then, a variable-centered analytic approach is used to identify the symptom clusters. Historically, four statistical approaches were used to identify symptom clusters, namely: cluster analysis, EFA, confirmatory factor analysis (CFA), and principal components analysis (PCA).[14]

Following the recommendations of Skerman and colleagues,[15] EFA is the most common approach used to identify symptom clusters in oncology research, followed by hierarchical

cluster analysis (HCA).[14, 18, 20] In contrast, PCA is the most common approach used to identify symptom clusters in other chronic conditions (e.g., chronic obstructive pulmonary disease (COPD),[21] human immunodeficiency virus[22]). However, PCA uses a data-reduction approach to analyze symptoms and does not assume any causal relationship between the symptoms within a cluster.[15, 23] Given that one hypothesis underlying symptom cluster research is that symptoms cluster together due to a shared, underlying mechanism,[8, 9] the use of PCA is not consistent with this hypothesis.

A non-exhaustive search of the Cumulative Index of Nursing and Allied Health Literature (CINAHL) and PubMed databases was conducted to explore the use of different variable-centered approaches for studying symptom clusters. Exemplars for each statistical method are described in Supplemental Table 2.1. As noted below, compared to studies of oncology patients, research on symptom clusters in patients with other chronic conditions is much less common. Therefore, exemplar studies conducted in samples with other chronic conditions are highlighted in Supplemental Table 2.1 to stimulate growth in symptom clusters research within these patient populations.

Hierarchical cluster analysis. HCA is one type of cluster analysis that has been used in symptom cluster research across a variety of chronic conditions.[20, 22, 24] It is important to note that depending on the research question, HCA can be used to group symptoms or patients.[13]

Two types of HCA can be used: agglomerative or divisive.[25] Starting with all of the symptoms in individual clusters, agglomerative HCA is used to identify and successively group pairs or groups of similar symptoms into mutually exclusive clusters of related symptoms.[26] In contrast, divisive HCA starts with all of the symptoms in a single cluster. Then, it systematically partitions the cluster into smaller groups of similar symptoms.[25] The hierarchical clustering of symptoms continues in a stepwise fashion until a certain level of groupings that have clinical meaning and interpretability are selected.[15] These steps are displayed graphically on a

dendrogram. Measures of similarity for interval data include correlation coefficients or squared Euclidean distances,[13] while coefficients of association can be used for binary data.[15]

HCA has several limitations.[13, 15] First, it is important to note that cluster analytic methods are not based on the underlying assumption of shared causality. Rather, they seek to identify groupings based on statistical measures of similarity.[13] Second, because cluster analytic methods strive to identify mutually exclusive groups of similar symptoms, a symptom can belong to only one cluster.[15] Given that a single symptom may be related to multiple symptoms that associate into different clusters, this limitation does not allow for an examination of symptoms that cross-load on other clusters. In addition, it impedes our ability to identify common and distinct underlying mechanisms. Third, using HCA, the determination of the final number of clusters is highly subjective. This subjectivity may lead to bias, as well as variability in both the number and types of symptom clusters identified across studies.

Thirty-nine studies were identified that evaluated for symptom clusters “de novo” using HCA. While 74.4% of these studies were conducted in patients with cancer, exemplars of studies that used HCA to identify symptom clusters in patients with other chronic conditions are provided in Supplemental Table 2.1.

Exploratory factor analysis. The common factor model consists of two factor analytic methods: EFA and CFA. Factor analytic methods are used to discover unobserved or latent factors (i.e., symptom clusters) that account for the common variance among multiple, observed variables (i.e., symptoms).[27] The underlying conceptual framework for factor analytic methods is that variables within a latent factor covary due to a common, underlying cause. The “strength and direction of the influence”[23](pp10) of the latent factors on the variables in the common factor model are estimated with factor loadings. Because of the exploratory nature of EFA, no assumptions are made a priori about the nature of the relationships between the observed variables.[23]

A unique feature of EFA is that symptoms can load on more than one factor (i.e., symptom cluster).[23] Given the possibility that one symptom can influence symptoms on different clusters, the ability for a symptom to load on more than one cluster has conceptual utility. For example, in a study that evaluated for symptom clusters in patients with lung cancer,[28] difficulty concentrating and feeling nervous cross-loaded on a sickness behavior and a psychological cluster. However, a lack of consensus exists on whether a symptom can load on multiple factors. For example, in a recent review of studies that evaluated for symptom clusters in patients receiving adjuvant chemotherapy,[18] only 58.8% of the studies that used EFA allowed for symptoms to cross-load.

Compared to HCA where 39 studies were identified, 89 studies used EFA to identify symptom clusters “de novo.” Of these studies, 66.3% were conducted in patients with cancer. This pattern is consistent with previous reviews that identified EFA as the most common statistical approach for identifying symptom clusters in oncology patients.[15, 18, 20] Exemplars of studies that used EFA to identify symptom clusters in patients with other chronic conditions are provided in Supplemental Table 2.1.

Confirmatory factor analysis. This approach is used to test hypotheses on the relationships between latent factors and observed variables.[27] More specifically, all of the model’s assumptions (e.g., number of factors, pattern of variable to factor loadings) must be specified a priori. These hypotheses must be rooted in theory and/or empirical evidence.

Given that the conceptual basis for CFA is to confirm hypotheses, it can be used to confirm the number and types of symptom clusters previously identified using another variable-centered approach (e.g., EFA).[15] For example, in a study that evaluated for symptom clusters in children and adolescents receiving myelosuppressive therapy,[29] EFA was used to identify symptom clusters. Then, CFA was used to confirm the structure of the findings. Given the continued need to evaluate and compare different statistical methods to identify symptom

clusters “de novo,”[4] CFA may be one approach to validate the stability and/or consistency of symptom clusters.

Use of variable-centered approaches to investigate underlying biological mechanisms. Relatively few studies have used a variable-centered approach to evaluate the underlying biological mechanisms of symptom clusters.[30, 31] In one study,[31] EFA was used to identify symptom clusters in oncology patients using the severity dimension. Then, a factor severity score was calculated for each of the three symptom clusters that were identified (i.e., mood-cognitive, sickness-behavior, and treatment-related symptom). These scores were used in regression analyses to identify associations between each symptom cluster and polymorphisms in cytokine genes.

Another study used EFA to identify two symptom clusters in patients with COPD.[30] Next, symptom cluster severity scores were calculated for each cluster. Subgroups of patients were identified based on their average symptom cluster severity score. Inflammatory biomarkers were used in logistic regression analyses to identify associations between subgroup membership and levels of C-reactive protein.

A Priori Identification of Symptom Clusters and Associated Symptom Cluster Profiles

Patient-centered analytic approaches evaluate for relationships among individuals using the principles of structural equation modeling[19] or measures of similarity.[13] Similar to variable-centered approaches, participants complete one or more symptom assessment instruments or a symptom inventory (Figure 2.1B).[5] In the context of symptom cluster research, a symptom cluster must be identified a priori (e.g., pain, fatigue, sleep disturbance, and depression). Then, with this pre-specified symptom cluster, groups of patients with distinct symptom cluster profiles are identified using patient-centered analytic approaches. Because these methods allow for the identification of subgroups of patients based on their experiences with a pre-specified symptom cluster, a variety of phenotypic and molecular risk factors can be identified that distinguish the various patient subgroups.

A search of the CINAHL and PubMed databases identified 31 studies that evaluated the symptom profiles of patients experiencing a pre-specified symptom cluster. Exemplars for each statistical method are provided in Supplemental Table 2.2.

Hierarchical cluster analysis. As mentioned previously, cluster analysis methods like HCA can be used to "cluster" symptoms or patients. With the latter approach, subgroups of patients are identified based on similar symptom cluster profiles using a pre-specified symptom cluster.[13] Eight studies were identified that used HCA to evaluate for subgroups of patients based on a clearly defined pre-specified symptom cluster. While the majority of these studies were conducted in patients with cancer (75%), exemplar studies that used HCA to identify subgroups of patients with a distinct symptom cluster profile in other chronic conditions are provided in Supplemental Table 2.2.

Latent variable modeling (LVM). LVM is used to identify subgroups or classes of individuals within a sample or population who have similar attributes or symptom experiences.[19] The underlying conceptual framework for LVM is that subgroup membership is based on an unobserved, latent variable (i.e., pre-specified symptom cluster) whose "value indicates what group the individual belongs to"[25](pp819). Common types of LVM include LCA for categorical data (e.g., symptom occurrence) and latent profile analysis for continuous data (e.g., symptom severity). In addition, latent transition analysis can be used to evaluate for changes in subgroup membership over time.[19]

The identification of subgroups of patients based on their distinct symptom cluster profiles using LVM has multiple advantages. First, differences in salient characteristics (e.g., demographics, stress, resilience) between the subgroups can be identified. Second, LVM can be used to evaluate how patient outcomes (e.g., functional status, QOL) differ by class membership.[25]

While the use of both HCA and LVM results in the identification of subgroups of patients with distinct symptom cluster profiles, the methods differ in a few key ways. First, with LVM,

multiple models are evaluated using fit indices prior to selecting the final model.[25] In contrast, selection of the final solution for HCA is highly subjective. Second, because LVM tends to be computationally more challenging than HCA,[25] fewer variables may be included in the LVM analysis.

Twenty-three studies have used a form of LVM to identify subgroups of patients with a distinct symptom cluster profile. While most of these studies were conducted in oncology patients (56.5%), exemplar studies that used LVM to identify subgroups of patients with a distinct symptom cluster profile in other chronic conditions are provided in Supplemental Table 2.2.

Use of patient-centered analytic approaches to investigate underlying biological mechanisms. Ten studies have used a patient-centered analytic approach to evaluate the underlying biological mechanism(s) for a pre-specified symptom cluster (exemplars in Supplemental Table 2.2). In one study,[32] latent profile analysis was used to identify three distinct subgroups of breast cancer patients based on their experience with a pain, fatigue, sleep disturbance, and depression cluster. Multiple associations were found between latent class membership and cytokine gene polymorphisms. Another study used HCA to identify subgroups of patients with advanced cancer based on their experience with the symptom cluster of pain, fatigue, depression, and sleep disturbance.[10] Higher serum levels of IL-6 were associated with an increased risk for membership in the moderate-to-high symptom subgroup.

EMERGING METHODS IN SYMPTOM CLUSTER RESEARCH

Network Analysis

One novel approach that can be used to identify symptom clusters “de novo” is NA. Based on the principles of graph theory,[33] NA is used to evaluate the relationships between a set of variables (i.e., symptoms). The structure of these relationships is presented in graphs. Within these graphs, symptoms are represented as nodes and the relationship(s) between symptoms are represented as edges (Figure 2.2A). The presence (i.e., a relationship between

the symptoms) and strength (e.g., correlation, conditional association) of these edges are calculated from the data. While firmly based in mathematical and statistical methods, a strength of NA is that it allows for a qualitative (i.e., visual) appraisal of the data.

One challenge with NA is the determination of the importance of nodes or groups of nodes within a network. Various types of centrality indices are used to aid in the interpretation of which nodes (i.e., symptoms) may have the largest influence on a network.[33-35] These highly influential nodes are sometimes referred to as "core" or "sentinel" nodes[16] and have the potential to serve as targets for therapeutic interventions.

Following the network's construction, community detection algorithms are used to identify clusters of symptoms (i.e., nodes) that are closely connected relative to other symptoms or clusters.[36] Various types of community detection algorithms are available and selection of the appropriate algorithm depends on multiple factors, including the network's size.[37]

One of the advantages of NA over other analytic approaches is that you can visualize the relationships between symptom clusters and how symptoms within one cluster relate to symptoms in another cluster. In addition, this approach allows for the identification of core or sentinel symptoms. However, a variety of approaches exist to create the networks and selection of the appropriate algorithms to estimate and evaluate the networks warrant consideration.

Three studies were identified that used NA to evaluate symptoms and/or symptom clusters in patients with cancer.[16, 38, 39] In one study,[16] NA was used to identify symptom clusters using multiple dimensions of the symptom experience (i.e., occurrence, severity, distress) in a heterogeneous sample of oncology patients. While five symptom clusters were identified across all three symptom dimensions (i.e., psychological, hormonal, respiratory, nutritional, chemotherapy-related), two additional symptom clusters (i.e., gastrointestinal, epithelial) were identified using distress (Figure 2.2B). The authors hypothesized that these results suggest that distress is a unique dimension of the patients' symptom experience. Because nausea and lack

of appetite had the highest centrality index scores, the authors suggested that targeting these symptoms may decrease the other symptoms within the network.

In another study,[39] a network was constructed using severity scores for eight symptoms and serum concentrations for 13 cytokines. Two communities were identified: a symptom cluster with five symptoms and another cluster with all 13 cytokines. While an evaluation of the associations between symptoms and biomarkers warrants additional research, findings from this study illustrate the challenges with incorporating heterogenous types of data (i.e., symptom severity scores and cytokine levels) into a NA.

A third study used HCA and PCA to identify symptom clusters in a sample of patients receiving chemotherapy.[38] Three common symptom clusters were identified over five assessments. Then, using only the 12 symptoms that were identified in the initial analyses, NA identified comparable symptom clusters that were found using PCA only at one timepoint. Fatigue, anxiety, and depression were identified as the most central symptoms in the network.

Bayesian Networks Analysis

Bayesian NA incorporates Bayesian statistics with NA to allow for an evaluation of the strength and direction of the relationships among symptoms.[40] While both types of networks contain nodes (i.e., symptoms) and edges (i.e., relationships between the symptoms), Bayesian NA graphically displays these relationships in a causal model (i.e., directed acyclic graph). Conditional dependencies are estimated for each node (i.e., symptom). The strength and direction of these relationships are calculated with joint probability distributions.[41]

Bayesian NA approaches offer many advantages for symptom cluster research. First, in addition to identifying “sentinel” symptoms, Bayesian NA can be used to elucidate the direction and the flow of a symptom’s influence on other symptoms within a network.[41] Second, similar to EFA and LVM, Bayesian NA can identify latent variables.[42, 43] However, given the complexity of the relationships between symptoms, interpretation of these relationships on an acyclic graph

may be challenging. In addition, Bayesian NA methods are computationally expensive,[44] particularly with large sample sizes or with large symptom inventories.

While Bayesian NA is used extensively in bioinformatics[45] and health sciences[46] research, only one study was identified that used Bayesian NA to examine the relationships between symptoms within a pre-specified cluster (i.e., sleep disturbance, fatigue, depressive symptoms) and their effect on cognitive performance and quality of life in breast cancer patients receiving chemotherapy.[47] Findings from this analysis suggest that the relationships among symptoms changed across time. For example, while mood directly impacted fatigue prior to the start of treatment and at the end of chemotherapy, previous levels of fatigue and sleep disturbance and current QOL directly impacted the severity of fatigue one year after the start of chemotherapy.

Application of NLP to Symptom Cluster Research

An ongoing issue in symptom cluster research is to determine the optimal number of common symptoms that need to be assessed across chronic conditions.[18] The determination of a consistent, comprehensive, and clinically meaningful list of symptoms would enable the identification of common symptom clusters across chronic conditions, as well as their common underlying mechanisms. Because of this lack of consensus, inventories with a large number of symptoms are administered to patients to evaluate for symptom clusters, with a potential for increased burden. A variety of new and emerging data science approaches (e.g., machine learning, NLP) have the potential to resolve this issue. The application of one of these approaches in symptom clusters research is described below.

NLP is a data extraction method that uses computer-based algorithms to acquire, process, and modify natural language obtained from “Big Data” (e.g., electronic health record (EHR)) for computational analyses.[48] Systematic extraction of “real world” symptom data from EHRs and its subsequent evaluation has the potential to not only lessen the burden on patients with chronic conditions, but provide researchers with the “most comprehensive, longitudinal,

population-wide dataset"[17](pp907) available. NLP methodologies have the potential to provide novel information on symptoms and symptom management throughout and beyond treatment of chronic conditions.[49]

Two recent publications describe the use of NLP in symptom science research. In the first publication,[50] the authors used a free and open-source NLP software (i.e., NimbleMiner) to find and extract data on five symptoms (i.e., constipation, depressed mood, disturbed sleep, fatigue, palpitations) from the EHR. While this method was piloted using only five symptoms, it can be expanded to include a larger symptom "vocabulary."

In the second study,[17] Koleck and colleagues used NLP to extract 56 symptoms from the EHR nursing notes of 22,647 patients across four common chronic conditions (i.e., cancer, COPD, heart failure, type II diabetes). Then, HCA was used to identify subgroups of patients with distinct symptom profiles for each chronic condition. While condition-specific symptom profiles were identified (e.g., gastrointestinal symptoms and fatigue for cancer, mental health symptoms for COPD), multiple symptom profiles were identified across two or more chronic conditions (e.g., cognitive and neurological). Given the strength of their results and the ability of NLP software tools to accurately identify and obtain specific symptom data, ongoing development of these methods has the potential to advance symptom science.

FUTURE DIRECTIONS

In their report,[4] the expert panel called for an examination of symptom clusters across various chronic conditions. These types of comparative studies are needed to determine whether or not "generic" symptom clusters occur across chronic conditions. To accomplish this goal, a comprehensive symptom assessment, as well as consistent methods, need to be used. Equally important, with the emergence of NA and NLP, studies are needed that compare symptom clusters that are created "de novo" using various analytic approaches.

Based on the literature reviews for each analytic approach, notable gaps in symptom cluster research were identified. In general, the study samples were homogeneous in terms of

race or ethnicity, gender identity, socioeconomic status, and educational attainment. Given that each of these characteristics can impact an individual's symptom experience, health outcomes, and QOL, this lack of diversity and evaluation of a limited number of social determinants of health limits our understanding of how these factors may influence the relationships with and among symptoms and symptom clusters. Future research that evaluates for symptom clusters in diverse and/or underserved samples, across a variety of acute and chronic conditions, is needed. Exemplars of studies that evaluated for differences in symptom clusters in relationship to age, gender, socioeconomic status, or ethnicity are provided in Supplemental Tables 1 and 2.

While the definition of a symptom cluster has evolved over the past 20 years, multiple issues remain that warrant careful consideration to move this area of scientific inquiry forward (Table 2.1). Specifically, clear criteria need to be developed to determine the stability and consistency of symptom clusters. The establishment of these criteria will allow researchers to determine within studies whether symptom clusters change over time and/or across dimensions of the symptom experience. In addition, they can be used to evaluate stability and consistency of symptom clusters across studies of patients with similar and different chronic conditions. Additional research is needed to determine whether symptoms in a cluster must be independent or can cross-load on more than one cluster. Given that previous studies that used EFA and NA demonstrated that symptoms may load on multiple clusters, or that symptoms within clusters and the clusters themselves are related, this characteristic of a symptom cluster may need to be revised. One way to resolve this issue would be to evaluate common and distinct mechanisms that underlie various symptom clusters that include symptoms that cross-load on more than one cluster.

CONCLUSION

As symptom cluster research continues to evolve, the use of both variable-centered and patient-centered analytic approaches are needed to move the science forward. While each approach has unique strengths and weaknesses, conceptual clarity is needed when a study is

designed and the research question should inform the selection of the appropriate method. The conceptual approaches illustrated in Figure 2.1 can serve as a guide for future studies. Variable-centered approaches identify symptom clusters and are based on the hypothesis that symptoms cluster together because they may share a common underlying mechanism(s). The terminology “symptom clusters” should be used when symptom clusters are created with this approach (Figure 2.1A). Patient-centered analyses identify subgroups of patients with distinct symptom cluster profiles and associated risk factors. Researchers should clearly specify when they are “clustering” patients (Figure 2.1B) that they have used a pre-specified symptom cluster and identified “subgroups of patients with distinct symptom cluster profiles.”

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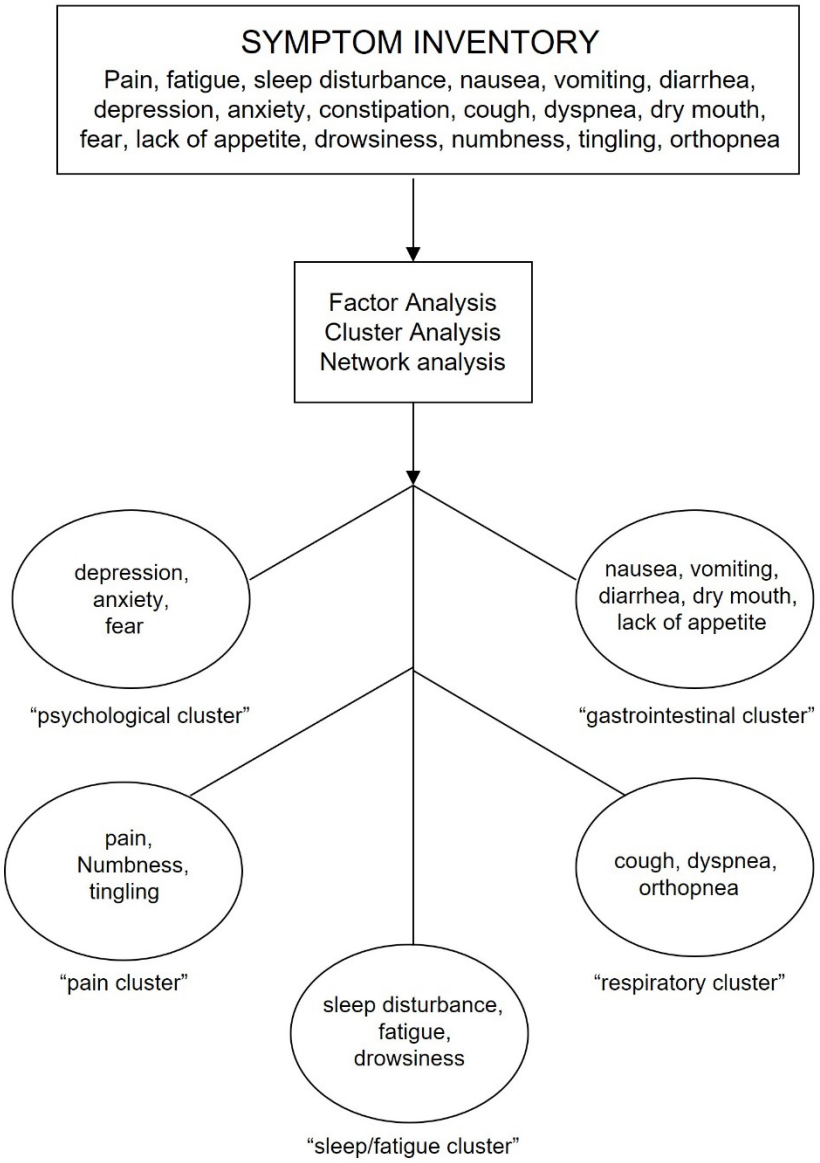
A.


SYMPTOM INVENTORY
Pain, fatigue, sleep disturbance, nausea, vomiting, diarrhea, depression, anxiety, constipation, cough, dyspnea, dry mouth, fear, lack of appetite, drowsiness, numbness, tingling, orthopnea
Factor Analysis
Cluster Analysis
Network analysis
depression, anxiety, fear
"psychological cluster"
nausea, vomiting, diarrhea, dry mouth, lack of appetite
"gastrointestinal cluster"
pain, Numbness, tingling
"pain cluster"
cough, dyspnea, orthopnea
"respiratory cluster"
sleep disturbance, fatigue, drowsiness
"sleep/fatigue cluster"

B.

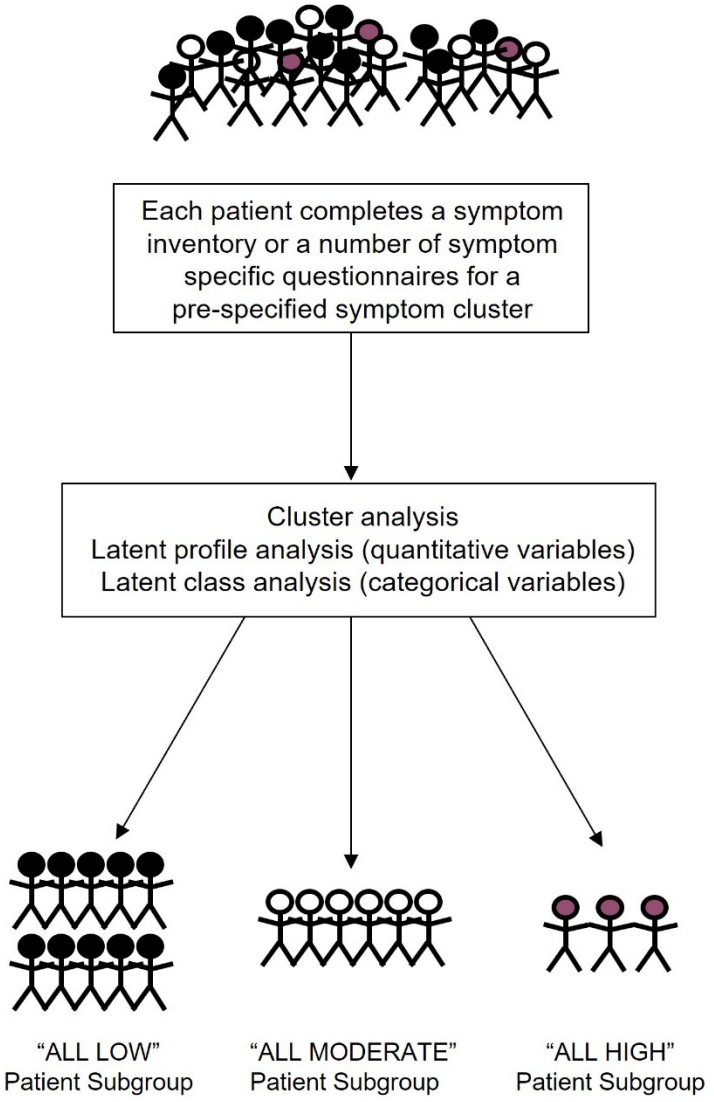

Each patient completes a symptom inventory or a number of symptom specific questionnaires for a pre-specified symptom cluster
Cluster analysis
Latent profile analysis (quantitative variables)
Latent class analysis (categorical variables)
"ALL LOW" Patient Subgroup
"ALL MODERATE" Patient Subgroup
"ALL HIGH" Patient Subgroup

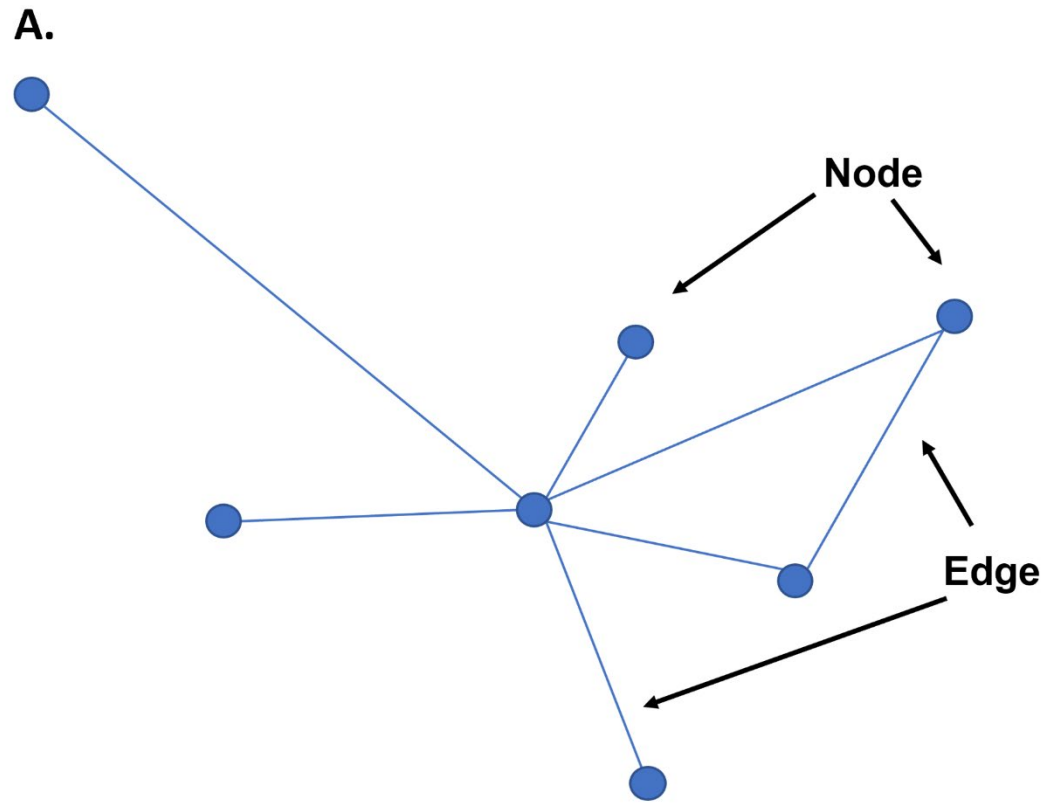
Figure 2.1. Two conceptual approaches to symptom cluster research. A) Illustrates the identification of symptom clusters using a variable-centered approach. B) Illustrates the identification of subgroups of patients based on their experience with a pre-specified symptom cluster (e.g., pain, fatigue, sleep disturbance, depression). Adapted from Miaskowski C, Aouizerat BE, Dodd M, Cooper B. (2007). Conceptual issues in symptom clusters research and their implications for quality-of-life assessment in patients with cancer. J Natl Cancer Inst Monogr (37), 39-46. Reprinted with permission from the Journal of the National Cancer Institute Monographs.

Table 2.1. Areas of ongoing development in the definition of a symptom cluster

Symptom*	Symptom cluster Same characteristics as a symptom – plus:	Exemplars of areas for future research and development:
Subjective perception	Two or more concurrent symptoms	Consensus is needed on the specific characteristics that encompass the definition of a symptom cluster within and across acute and chronic conditions
May vary over time	Stable group of symptoms	The definition of and criteria for stability and consistency need to be established and evaluated. In addition, the conditions or circumstances when symptom clusters may or may not be stable warrants additional research (e.g., across symptom dimensions, within and across symptom dimensions over time)
Has antecedents	Independent of other clusters	The inter-relationships between and among symptoms and symptom clusters warrant detailed evaluation
Influences outcomes	May have shared underlying mechanism(s)	How do the mechanisms that underlie single symptoms within a cluster differ from mechanisms that underlie the entire cluster?
May be influenced by an intervention	May have shared outcome(s)	Do symptom clusters influence patient outcomes similarly or differently?
Has an underlying mechanism	Temporal dimension	When and how do symptom clusters change over time?

*Symptoms are subjective sensations. Signs are objective indications of some medical characteristics.

Adapted from Miaskowski C, Barsevick A, Berger A, Casagrande R, Grady PA, Jacobsen P, Kutner J, Patrick D, Zimmerman L, Xiao C, Matocha M, Marden S. (2017). Advancing symptom science through symptom cluster research: Expert panel proceedings and recommendations. *J Natl Cancer Inst*, 109(4). Reprinted with permission from Oxford University Press.


A.
Node
Edge

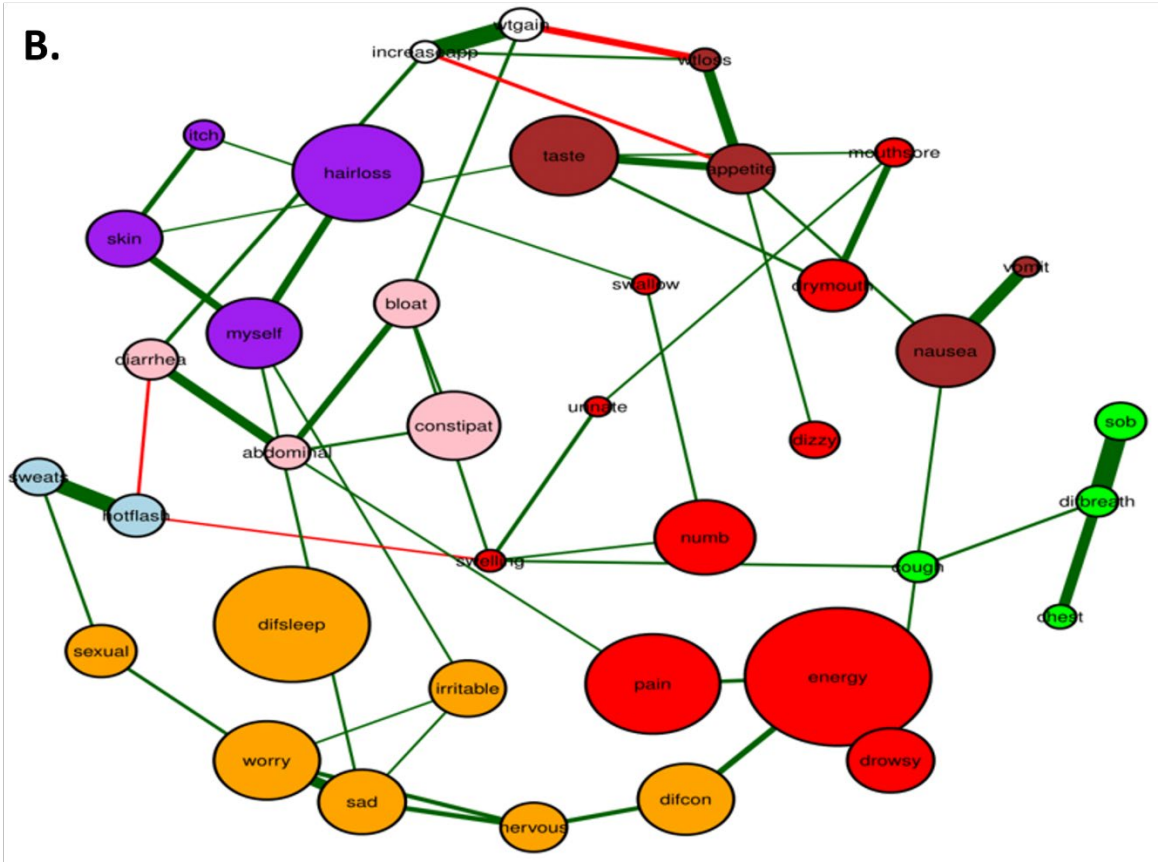

B.
itch
hairloss
taste
skin
bloat
myself
diarrhea
constipat
abdominal
sweats
hotflash
difsleep
sexual
irritable
worry
sad
nervous
pain
energy
drowsy
difcon
numb
dizzy
nausea
vomit
drymouth
sob
dibreath
chest
cough
bloat

Figure 2.2. A) An undirected graphical model with seven nodes. Each node represents a symptom. The presence of an edge between two nodes indicates a relationship between them. B) This figure represents the estimated network of 38 cancer symptoms across the “distress” symptom dimension. In this figure, the node size corresponds to the symptom distress scores and the strength of the relationship between nodes is illustrated by the thickness of the edges. Green edges indicate positive relationships and red edges indicate negative relationships. Symptom clusters were identified using a community detection algorithm and are identified by the color of the symptoms within each cluster. Adapted from Papachristou N, Barnaghi P, Cooper B, Kober KM, Maguire R, Paul SM, Hammer M, Wright F, Armes J, Furlong EP, McCann L, Conley YP, Patiraki E, Katsaragakis S, Levine JD, Miaskowski C. (2019). Network analysis of the multidimensional symptom experience of oncology. *Sci Rep*, *9*(1), 2258.

Chapter 3

Symptom Clusters in Patients Receiving Chemotherapy: A Systematic Review

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ABSTRACT

Background and purpose: Since 2001, symptom cluster research has grown considerably. However, because multiple methodological considerations remain, ongoing synthesis of the literature is needed to identify gaps in this area of symptom science. This systematic review evaluated the progress in symptom clusters research in adults receiving primary or adjuvant chemotherapy since 2016.

Methods: Eligible studies were published in English between January 1, 2017 and May 17, 2021; evaluated for and identified symptom clusters “de novo;” and included only adults being treated with primary or adjuvant chemotherapy. Studies were excluded if patients had advanced cancer or were receiving palliative chemotherapy; symptoms were measured after treatment; symptom clusters were pre-specified; or a patient-centered analytic approach was used. For each study, symptom instrument(s); statistical methods and symptom dimension(s) used to create the clusters; whether symptoms were allowed to load on more than one factor; method used to assess for stability of symptom clusters; and associations with secondary outcomes and biomarkers were extracted.

Results: Twenty-three studies were included. Memorial Symptom Assessment Scale was the most common instrument and exploratory factor analysis was the most common statistical method used to identify symptom clusters. Psychological, gastrointestinal, and nutritional clusters were the most commonly identified clusters. Only the psychological cluster remained relatively stable over time. Only five studies evaluated for secondary outcomes.

Discussion: While symptom cluster research has evolved, clear criteria to evaluate the stability of symptom clusters and standardized nomenclature for naming clusters are needed. Additional research is needed to evaluate the biological mechanism(s) for symptom clusters.

Keywords: chemotherapy, oncology, symptom clusters, biomarkers, patient-reported outcomes, symptom science

INTRODUCTION

As the incidence of new cancer cases and mortality rates increase globally,[1] the symptom burden of oncology patients remains high. For example, in one study,[2] 50% of patients receiving chemotherapy experienced an average of 13 symptoms. Equally important, co-occurring symptoms and/or symptom clusters result in increased distress,[3] decreased functional status,[4] poorer QOL,[5] and increased mortality.[6,7] Given that 50% of oncology patients may experience these negative effects, research on how and why symptoms co-occur is vital to the development of effective interventions.

In 2001, Dodd and colleagues[8] were the first to introduce the concept of a symptom cluster into oncology symptom science. Since then, symptom cluster research has increased dramatically.[9-12] While the definition of a symptom cluster has evolved,[8,13] most recently, it was defined as the co-occurrence of two or more symptoms that are stable and independent of other clusters, and may share underlying mechanisms and/or outcomes.[9] This research has grown to include the identification of symptom clusters in children[14] and adolescents;[15] in patients with advanced cancer;[16, 17] and in patients receiving active treatment.[11] An emerging area of research is the evaluation of biomarkers[18] and molecular mechanisms[19-21] associated with symptom clusters.

While this research provides important foundations in our understanding of cancer-related symptom clusters, two key methodological issues remain unresolved; namely: which statistical approach provides the most consistent identification of symptom clusters (e.g., cluster analysis, EFA) and how the dimension(s) of the symptom experience that are used to create the clusters (i.e., occurrence, severity, frequency, distress) influence the number and types of symptom clusters identified. Resolution of these issues is key to the development of effective interventions for symptom clusters.[9] In addition, consistent identification of symptom clusters will facilitate the investigation of their underlying mechanisms.

While Skerman and colleagues suggested that factor analysis methods were the optimal approach to create symptom clusters,[22] cluster analysis,[23] and more recently NA[24] have been used. Factor analysis methods, like EFA, are used to identify latent constructs or factors (i.e., symptom clusters) that account for the strength of the relationships between variables (i.e., symptoms).[25] This type of factor analysis is exploratory in nature as it does not test hypotheses on the nature of the relationships among the variables. Cluster analysis methods, (e.g., HCA), use measures of correlation or distance to group related variables (i.e., symptoms).[22] An emerging analytical approach for identifying symptom clusters is NA. With this approach, relationships between multiple variables or nodes (i.e., symptoms) are quantified and illustrated graphically.[26] Unique strengths of NA are its potential to identify “core” symptoms (i.e., symptoms that have a high impact on the network or cluster) and relationships among symptom clusters.[27-29]

Consensus is lacking on which dimension(s) (i.e., occurrence, severity, frequency, distress) of the symptom experience should be used to identify symptom clusters.[9] For example, in one review,[11] a significant amount of variability was found in the dimensions used to identify symptom clusters. This type of evaluation is important because the specific dimension used may influence the number, types, and composition of the symptom clusters that are identified, making comparisons across studies difficult. While each symptom dimension provides unique information, little is known about how the symptom clusters identified using different dimensions may affect various patient outcomes or the mechanisms that underlie various symptom clusters.

In the most recent review of symptom clusters research in oncology patients receiving chemotherapy,[11] findings from studies published between 2000 and 2016 were synthesized. However, the impact of symptom clusters on outcomes (e.g., QOL, functional status) and associations with underlying mechanisms were not evaluated. As noted in an expert panel report,[9] ongoing synthesis of symptom clusters research is warranted to identify gaps in this area of scientific inquiry. Therefore, the purpose of this systematic review was to evaluate the

progress in symptom clusters research in adult patients receiving primary or adjuvant chemotherapy since 2016. Specifically, this paper will: (1) describe the most common instrument(s), statistical approaches, and symptom dimensions used to evaluate symptom clusters; (2) describe the number and types of symptom clusters identified using different dimensions of the symptom experience; (3) determine whether symptom clusters change over time; and (4) describe associations between symptom clusters and patient-reported outcomes (PROs) and biological mechanisms.

METHODS

Search strategy

This review was performed using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[30] The protocol for this review was registered with PROSPERO (registration number CRD42021240216). Studies that were published between January 1, 2017 and May 17, 2021 were retrieved from the Cochrane Library, Cumulative Index to Nursing and Allied Health Literature, Embase, PubMed, and Web of Science databases. The search strategy for each database is listed in Table 3.1.

Study selection

Identified studies were downloaded into a pre-specified Endnote Library for review and duplicates were removed. Studies were retained for review if they met the following eligibility criteria: (1) evaluated for and identified at least one symptom cluster; (2) included only adults (aged ≥18 years); (3) included only oncology patients who were being treated with primary or adjuvant chemotherapy; (4) were published in English; (5) had a cohort, case-control, cross-sectional, or longitudinal design; and (6) identified symptom clusters “de novo” (i.e., used a statistical method to identify clusters). Studies were excluded if they: (1) were published prior to January 1, 2017; (2) included patients with advanced cancer (i.e., stage IV) or those receiving palliative chemotherapy; (3) measured symptoms after the completion of treatment; (4) used pre-specified symptom clusters (i.e., did not use a statistical method to identify clusters); (5)

used a patient-centered analytic approach (e.g., latent class analysis); or (6) were a systematic review, meta-analysis, conference abstract, dissertation work, case-report, or qualitative study. The title and abstract of each study were reviewed by a single author (CH) for eligibility based on our pre-specified inclusion and exclusion criteria. The first (CH) and senior (CM) authors reviewed the full text of the remaining articles against the inclusion and exclusion criteria.

Data extraction

The pre-specified study characteristics that were extracted are detailed in Box 3.1. Separate evaluations were done for cross-sectional (Supplemental Table 3.1) and longitudinal (Supplemental Table 3.2) studies. Two reviewers (CH, CM) independently reviewed each study and consensus was reached on the data included in the tables.

Box 3.1. Pre-specified Study Characteristics for Extraction

Study characteristics: author(s), year published, purpose(s), study design, country, sample size
Patient characteristics: age, gender, ethnicity, race, employment status, inpatient/outpatient status, cancer diagnosis, cancer treatment, timing of symptom assessment(s)
Methods: symptom instrument(s), statistical methods used to create the clusters, symptom dimension(s), whether symptoms were allowed to load on more than one factor, and method used to assess for stability of symptom clusters
Associations with other patient-reported outcomes (PROs) and biomarkers
Study findings: symptom clusters identified, specific symptoms within each cluster, PROs, biomarkers
Strengths and limitations

Assessment of methodological quality

Each study's methodological quality was assessed using the National Heart, Lung, and Blood Institute's (NHLBI) National Institute of Health Quality Assessment Tool for Observational and Cross-Sectional Studies.[31] Questions on this tool were designed to enable researchers to critically appraise the internal validity of research studies. Each question is answered with "yes," "no," or "cannot determine, not reported, or not applicable." Items that receive a "no" or indeterminable response are considered a study weakness that may introduce bias. As recommended by the NHLBI tool guidelines, this potential risk of bias must be further evaluated

by a reviewer and is factored into the final rating of “good,” “fair” or “poor”. Two reviewers (CH, CM) independently assessed the quality of each study and combined their results in a shared Excel spreadsheet. All studies that met the inclusion and exclusion criteria were included in this review regardless of the methodological quality assessment rating.

RESULTS

Study selection

The initial search resulted in 574 articles. Following the removal of duplicates, 319 articles remained. Next, the title and abstract of each study were reviewed against our inclusion and exclusion criteria and 283 studies were excluded. The first (CH) and senior (CM) authors reviewed the full text of the remaining 36 articles against the inclusion and exclusion criteria. Following these steps, 23 articles were retained for data extraction and are included in this systematic review (Figure 3.1).

Methodological quality of studies

Nine of the 13 cross-sectional studies received a “good” quality rating, four received a “fair” rating, and none received a poor rating (Table 3.2). Across the four studies that received a “fair” rating, two sources of bias were: lack of reporting of whether the participation rate of eligible persons was at least 50% (item 3) and lack of clarity on whether the timing of the symptom assessment around the receipt of chemotherapy was sufficient in order to see an effect (item 7). All of the longitudinal studies received a “good” rating. Of note, seven of the 10 longitudinal studies either lost >20% of patients to follow-up or did not report this information.

Cross-sectional study results

Study characteristics. Of the 23 studies included in this review, 13 used a cross-sectional design to identify symptom clusters in oncology patients receiving chemotherapy (Supplemental Table 3.1). Seven studies were conducted in the United States,[29, 32-37] two in China,[38, 39] two in Thailand,[40, 41] one in Austria,[42] and one in Turkey.[43] Sample sizes ranged from 96[41] to 1328.[29] Across these studies, the majority of patients were female (weighted grand mean

76.8%), outpatients, not working, had a weighted grand mean age of 55.0 years, and were relatively homogeneous in terms of ethnicity and race.

Five studies evaluated for symptom clusters in patients with heterogeneous types of cancer.[29, 32, 33, 35, 42] Of the eight studies that evaluated for clusters in patients with homogeneous types of cancer, four evaluated patients with breast cancer,[34, 36, 40, 41] one with bladder cancer,[39] one with leukemia,[38] one with lymphoma,[43] and one with lung cancer.[37]

Symptom instrument(s). In terms of the instruments, nine of the 13 studies used the MSAS.[29, 32, 33, 35-38, 40, 43] Of these nine studies, six used a modified version of the MSAS[29, 32, 33, 35-37] and one used a condensed version.[38] One study used multiple symptom assessment tools to assess for clusters;[34] specifically, the Breast Cancer Prevention Trial Symptom Checklist, the Beck Depression Inventory-II, the Brief Pain Inventory, the Patient's Assessment of Own Functioning, and the Profile of Mood States. One study each used the Edmonton Symptom Assessment Scale,[41] the MD Anderson Symptom Inventory (MDASI),[39] and the Rotterdam Symptom Checklist.[42]

Statistical approach. Nine of the 13 studies used EFA to identify symptom clusters.[32-37, 39, 41, 42] Of the remaining studies, two used principal component analysis (PCA),[38, 40] one used HCA,[43] and one used NA.[29]

Symptom dimension(s). In terms of the symptom dimension(s), three of the 13 studies used only severity[34, 39, 41] and three used only distress.[32, 38, 42] Of the seven remaining studies, two used both occurrence and severity;[36, 37] one used severity and distress;[40] one used frequency, severity, and distress;[43] and three used occurrence, severity, and distress.[29, 33, 35]

Occurrence – Across the five studies that used occurrence,[29, 33, 35-37] a psychological cluster was identified. The number of symptoms ranged from five to 12. Worrying, feeling nervous, feeling sad, and feeling irritable were common across the five studies. A respiratory or lung cancer-specific cluster was identified across three of the five studies.[29, 35, 37] The number of

symptoms ranged from four to nine. Shortness of breath, difficulty breathing, and cough were common across the three studies.

A nutritional or weight change cluster was identified across all five studies.[29, 33, 35-37] The number of symptoms ranged from two to seven. While no common symptoms were identified across the five studies, increased appetite,[29, 33, 35, 37] weight gain,[29, 33, 35, 37] and weight loss[29, 35-37] were found in four of them. A gastrointestinal cluster was identified in three studies.[33, 35, 36] However, no common symptoms were identified across the three studies.

Severity - Ten studies used severity to evaluate for clusters.[29, 33-37, 39-41, 43] Of the eight studies that named the clusters, all identified a psychological cluster (i.e., emotion-related, psychological, psycho-urinary).[29, 33-37, 39, 40] The number of symptoms ranged from two to nine. Feeling sad, sadness, or depression was the only symptom that was identified across all of the studies.

Six studies identified a cluster related to nutritional status or weight (i.e., nutritional, weight, weight change).[29, 33-37] The number of symptoms ranged from two to six. While no symptoms were comon across all six studies, weight loss[29, 34-37] and weight gain[29, 33, 35-37] were each identified in five of them.

A gastrointestinal or gastrointestinal and energy related cluster was identified in five of the eight studies.[33, 34, 36, 39, 40] The number of symptoms ranged from two to eight. While no symptoms were common across all of the studies, nausea was identified in four of the five studies.[33, 34, 39, 40]

Distress - Eight studies evaluated for clusters using the distress dimension.[29, 32, 33, 35, 38, 40, 42, 43] Similar to occurrence and severity, a type of psychological cluster (i.e., anxiety and depression, emotion, energy, and pain related, emotions, psychological, psychological/gastrointestinal) was identified in seven of the studies that named the clusters.[29, 32, 33, 35, 38, 40, 42] The symptoms within this cluster ranged from three to 12. Feeling nervous or anxious and feeling sad or depressed mood were common symptoms across all seven studies.

Five studies identified a type of nutritional cluster (i.e., appetite, nutritional, nutrition impaired, weight change).[29, 32, 33, 35, 38] The symptoms ranged from two to seven. Lack of appetite was common across four of the five studies.[29, 32, 35, 38]

Multiple dimensions – Seven studies evaluated for differences in clusters across two or more symptom dimensions.[29, 33, 35-37, 40, 43] Of the six studies that named the clusters,[29, 33, 35-37, 40] a type of psychological cluster (i.e., emotion related, emotion, energy, and pain related, psychological/gastrointestinal, psychological) was common across all six studies and dimensions. Feeling irritable, feeling nervous, feeling sad, and worrying were the common symptoms across the six studies and dimensions.

A type of nutritional cluster (i.e., nutritional, image and nutrition, discomfort and nutrition, weight change) was identified across all six studies and dimensions. Weight loss was the common symptom across all symptom dimensions in five of the six studies.[29, 35-37, 40]

Evaluation of the stability of symptom clusters across symptom dimensions - Of the six studies that named the clusters and evaluated for clusters using two or more dimensions,[29, 33, 35-37, 40] all of them evaluated the stability of the clusters across dimensions. Five studies[33, 35-37, 40] used the method described by Kirkova and Walsh.[44] The sixth study[29] evaluated for stability through visualization of differences in the network's structures.

Analysis of secondary outcomes. In the four studies that evaluated for associations between clusters and other PROs,[32, 38, 39, 42] all of them used QOL. In addition, one evaluated for associations with patients' functional status.[38] None of the cross-sectional studies evaluated for associations between symptom clusters and biological mechanisms.

Longitudinal study results

Study characteristics. Of the 23 studies included in this review, 10 used a longitudinal design to evaluate for symptom clusters in oncology patients receiving chemotherapy (Supplemental Table 3.2). Six studies were conducted in the United States,[45-50] two in Sweden,[51, 52] one in China,[53] and one in South Korea.[54] Sample sizes ranged from 51[54] to 540.[50]

Across these studies, the majority of the patients were female (weighted grand mean 84.4%), currently employed, had a weighted grand mean age of 55.1 years, and were relatively homogeneous in terms of ethnicity and race.

Only one study evaluated for symptom clusters in a sample of patients with heterogeneous cancer diagnoses.[47] Of the nine studies that evaluated for clusters in patients with homogeneous diagnoses, six evaluated patients with breast cancer,[45, 46, 48, 50-52] one with acute myelogenous leukemia,[53] one with brain cancer,[54] and one with lung cancer.[49]

Symptom instrument(s). In terms of the instruments, seven of the 10 studies used the MSAS.[47, 49-54] Of these seven studies, three used a modified version of the MSAS.[47, 49, 50] Two studies used the Hospital Anxiety and Depression Scale, the Symptom Experience Scale, and the Medical Outcomes Study Short-Form Survey v2.[45, 46] One study used the Breast Cancer Prevention Trial Symptom Checklist, the Beck Depression Inventory-II, the Brief Pain Inventory, the Patient's Assessment of Own Functioning, and the Profile of Mood States.[48]

Statistical approach. In terms of the statistical methods, eight of the 10 studies used EFA.[45-50, 53, 54] The remaining two studies used PCA.[51, 52]

Symptom dimension(s). In terms of the symptom dimension(s), four studies used only the severity dimension.[45, 46, 48, 54] While two studies evaluated for clusters using both occurrence and severity,[49, 50] two used occurrence, severity, and distress.[47, 53] The remaining two studies created a symptom burden score (i.e., the average of the frequency, severity, and distress scores for each symptom on the MSAS).[51, 52]

Occurrence – Four studies used occurrence to identify clusters across three timepoints.[47, 49, 50, 53] For three of these studies,[47, 49, 50] these timepoints were: approximately one week before the second or third cycle of chemotherapy (T1), approximately one week after chemotherapy administration (T2), and approximately two weeks after chemotherapy administration (T3). For the fourth study,[53] these timepoints were: within six days of the start of

induction chemotherapy (T1a), one to seven days during induction chemotherapy (T2a), and one to seven days after induction chemotherapy (T3a).

A psychological cluster was identified across all four studies and all three timepoints, except for one study where the cluster was not identified until T2a.[53] Feeling nervous and feeling sad were common across each study and timepoint. In addition, difficulty concentrating, feeling irritable, and worrying were common to the three studies that identified a psychological cluster at T1.[47, 49, 50] Across these four studies, the symptoms within this cluster remained relatively consistent across time.

While a nutritional or weight change cluster was identified across all four studies, it was not identified at each timepoint. For three of the studies,[47, 49, 50] lack of appetite was present at T2 and lack of appetite and weight gain were present at T3. Except for one study,[53] the symptoms identified within this cluster were relatively consistent across timepoints within each study.

While an epithelial, epithelial/gastrointestinal, or body image cluster was identified across all four studies, it was not identified at each timepoint and the symptoms within this cluster changed over time. Hair loss was identified at T2 in three studies.[47, 49, 50] Itching was identified at T3 and T3a in three studies.[47, 50, 53] Changes in skin was identified across all four studies at T3 and T3a.[47, 49, 50, 53]

A gastrointestinal cluster was identified across three studies at one or more timepoints.[47, 50, 53] However, this cluster was not identified at each timepoint and no common symptoms were consistent across each of the three studies. Abdominal cramps appeared across two of the studies that identified this cluster at T1.[47, 50]

Severity – Eight studies used severity to identify clusters across three or four timepoints.[45-50, 53, 54] Of the two studies that evaluated for clusters over four timepoints, one evaluated for clusters throughout all cycles of chemotherapy (i.e., prior to the first cycle to post-chemotherapy)[45] and the other evaluated for clusters from prior to and at 18 months post-

chemotherapy.[48] Five of the remaining six studies evaluated for clusters over three timepoints around the receipt of active treatment (e.g., prior to and post-chemotherapy).[47, 49, 50, 53, 54] The sixth study evaluated for clusters after the completion of chemotherapy (i.e., prior to chemotherapy to one year after initial chemotherapy treatment).[46]

While no single cluster was common across the eight studies, a gastrointestinal cluster was identified across seven of them.[45-48, 50, 53, 54] This cluster was not identified across all timepoints and no common symptoms were identified. In addition, a type of psychological cluster (i.e., negative emotion, negative emotion and decreased vitality, psychological, psychoneurocognitive) was identified in six of the eight studies.[47-50, 53, 54] This cluster was not identified across all of the timepoints. However, when the cluster was identified, feeling sad or depression was consistent across all of the studies.

Distress – Only two studies evaluated for clusters using distress across three timepoints.[47, 53] A psychological cluster was identified across both studies and at two of the three timepoints. Across these timepoints, feeling nervous and feeling sad were consistent. While an epithelial or body image cluster was identified across both studies, it was not present across all three timepoints. When the cluster did occur, itching was identified across both studies and timepoints.

Burden score – In the two studies that used a symptom burden score to identify clusters, one evaluated for clusters over four timepoints across multiple cycles of chemotherapy[51] and the other evaluated for clusters over three timepoints prior to the start of the second cycle of chemotherapy to 12 months post cycle two.[52] An emotional cluster was identified across both studies and timepoints. Feeling sad was common across both studies and all timepoints. While a physical cluster was identified across both studies and timepoints, no common symptoms were identified.

Multiple dimensions – Four studies evaluated for clusters using two or more dimensions over three timepoints.[47, 49, 50, 53] In three of these studies,[47, 49, 50] a psychological cluster was

identified across all of these studies, dimensions, and timepoints. In the fourth study,[53] this cluster occurred with some variability across timepoints and dimensions. Feeling nervous and feeling sad occurred consistently across studies, dimensions, and timepoints.

While an epithelial, epithelial/gastrointestinal, or body image cluster was identified across all four studies, it was not stable across dimensions or timepoints. Only changes in skin appeared across dimensions and studies at the third timepoint (i.e., two weeks post cycle two or three, one to seven days after induction).[47, 49, 50, 53] In addition, gastrointestinal and nutritional or weight change clusters were identified across three of the four studies.[47, 50, 53] No common symptoms were identified consistently across studies, dimensions, and/or timepoints for either cluster.

Evaluation of the stability of symptom clusters across symptom dimensions and/or timepoints. Six studies[47-50, 53, 54] used the method described by Kirkova and Walsh[44] to evaluate the stability of symptom clusters across dimensions and timepoints. Two studies[45, 46] relied on an investigator's appraisal of the stability. The remaining two studies[51, 52] did not report on a method to evaluate stability.

Analysis of secondary outcome(s). In the only longitudinal study that evaluated for associations between symptom clusters and a PRO,[46] measures of QOL were used. In the only study that evaluated for associations between symptom clusters and biological mechanisms,[54] levels of lipid peroxidation were examined in patients with primary brain tumors.

DISCUSSION

This systematic review evaluated the progress of symptom clusters research in adult patients receiving primary or adjuvant chemotherapy from 2017 through 2021. Given the relative infancy of symptom cluster research, this type of ongoing review and synthesis is needed to advance this area of scientific inquiry. This discussion focuses on how the science has evolved since the previous review.[11]

Symptom assessment instruments

The MSAS was the most common instrument used in 69.6% of the studies. While it was found to be one of the most commonly used instruments in the previous review,[11] its use grew from 26.3% to 69.6%. This growth may be due to the multiple strengths of the MSAS. First, because it evaluates 32 common symptoms, it is cited as one of the most comprehensive instruments to use in research and clinical practice.[55] In addition, the MSAS evaluates multiple dimensions of the symptom experience (i.e.,occurrence, severity, frequency, and distress); has well established validity and reliability;[56] and is available in more than eight languages (e.g., Arabic,[57] Chinese,[58] Spanish[59]).

In contrast with the previous review that noted that the MDASI was used in 26.3% of the studies,[11] it was used in only 4.3% of the studies in this review. This change may be due to a shift among researchers to use more comprehensive symptom instruments. Instruments like the MDASI (13 symptoms) and the Edmonton Symptom Assessment Scale (nine symptoms) are limited because they assess a relatively small number of symptoms using only severity ratings. Given that oncology patients receiving active treatment report an average of 13 unrelieved symptoms,[2] and the optimal symptom dimension to evaluate for symptom clusters has yet to be determined, use of a comprehensive, multidimensional instrument is warranted.

Statistical approaches

EFA was the most common method used in 73.9% of the studies,[32-37, 39, 41, 42, 45-50, 53, 54] followed by PCA in 17.4%.[38, 40, 51, 52] These findings are consistent with the previous review that reported that 68.4% of the studies used a factor analytic approach.[11] Given that one conceptual basis for the use of EFA is that symptoms cluster together because they share common underlying mechanism(s),[22, 60] EFA is preferred over HCA or PCA.

One of the key strengths of EFA is that it allows symptoms to load on more than one factor. As a result, the authors of the previous review recommended that the most common symptoms that load on more than one cluster be identified.[11] Of the studies that used EFA, 10

allowed for symptoms to load on multiple factors.[33, 35-37, 42, 46, 47, 49, 50, 53] While the symptoms that loaded on more than one factor were not specified in most studies, in the two studies that evaluated for symptom clusters in patients with lung cancer,[37, 49] difficulty concentrating, feeling nervous, feeling sad, swelling of the arms and legs, and worrying cross-loaded on multiple clusters. For the four studies that evaluated for clusters in patients with breast cancer,[36, 46, 50, 53] change in the way food tastes cross-loaded in three studies[36, 50, 53] and difficulty concentrating cross-loaded in two.[46, 53]

Symptom dimensions

While severity was the most common dimension used to create the clusters (78.3%),[29, 33-37, 39-41, 43, 45-50, 53, 54] 43.5% used distress,[29, 32, 33, 35, 38, 40, 42, 43, 47, 53] 39.1% used occurrence,[29, 33, 35-37, 47, 49, 50, 53] 8.7% used a burden score,[51, 52] and 4.3% used frequency.[43] Only 47.8% of the studies evaluated for symptom clusters using two or more symptom dimensions.[29, 33, 35-37, 40, 43, 47, 49, 50, 53]

Among the 10 studies that evaluated for clusters using two or more dimensions and named the clusters,[29, 33, 35-37, 40, 47, 49, 50, 53] psychological and nutritional clusters were the two common clusters identified across all of the studies and dimensions. However, none of the symptoms within these clusters were constistent across studies. This finding may be partially explained by the variability in cancer diagnoses across the studies. In the previous review,[11] the authors were unable to compare the number and types of clusters identified across dimensions due to the fact that only 15.8% (n=3) of the studies used two or more dimensions. The growth in the number of studies from 15.8% to 47.8% may be a result of multiple reports recommending that research be done on the stability of symptom clusters across the different dimensions.[9-11]

Number and types of symptom clusters

Across the 23 studies included in this review, the number of clusters identified ranged from two to eight. A psychological cluster was the most common cluster identified in 82.6% of the 23 studies in this review.[29, 32-40, 42, 47-54] Similar to the previous review,[11] feeling sad or

depressed was common across 18 of the 19 studies, while feeling anxious or nervous was common across 16.

Consistent with the previous review,[11] a gastrointestinal cluster was another common cluster identified in 69.6% of the studies.[29, 33-36, 39, 40, 42, 45-48, 50, 51, 53, 54] Nausea was the most common symptom in this cluster that occurred in 13 of the 16 studies, followed by diarrhea in eight. This finding is similar to the previous review[11] that identified nausea as one of the most common symptoms across 10 of the 13 studies.

In a departure from the previous review that identified a nutrition or nutritional cluster in only 15.8% of the studies,[11] a nutritional or weight change cluster was identified across 56.5% of the studies in this review.[29, 32-38, 47-50, 53] Lack of appetite was the most common symptom in 12 of the 13 studies,[29, 32, 34-38, 47-50, 53] followed by weight loss in 11.[29, 34-38, 47-50, 53]

The emergence of a nutritional or weight cluster may be due to the inclusion of an increased number of symptoms related to these two problems. For example, in nine of the 13 studies that identified a nutritional or weight change cluster, the MSAS was modified to include additional symptoms (e.g., abdominal cramps, increased appetite, weight gain).[29, 32, 33, 35-37, 47, 49, 50] Weight gain was common across nine studies[29, 33, 35-37, 47-50] and increased appetite was common across six.[29, 33, 35, 37, 47, 49] Additional research is needed to determine the optimal number, as well as the most common and disease and treatment-specific symptoms, to assess in order to obtain more specific and mechanistically-based symptom clusters.

In factor analytic methods, factor loading scores are standardized partial regression coefficients that provide an estimate of the strength of the association between a variable (i.e., symptom) and a factor (i.e., symptom cluster) while controlling for the impact of other factors.[25] This score is used to determine which symptoms load on which factors using a pre-determined cutoff that indicates a meaningful relationship. While factor loadings of ≥0.30 or ≥0.40 are commonly accepted,[61] it is not clear what the optimal minimum factor loading score should be to include a symptom within a cluster.

In this review, ≥0.40 was the most common minimum factor loading score (n=11),[33, 34, 36-38, 40, 47-50, 53] followed by ≥0.30 (n=3),[35, 45, 46] and ≥0.50 (n=1).[52] Of note, seven studies did not report this score. In the studies that used a minimum factor loading score of 0.40, two to eight symptom clusters were identified. While no clear pattern emerged in terms of sample size, this wide gap may be due to differences in the instruments used (e.g., disease specific vs. cancer specific); the type of treatment (e.g., adjuvant vs. induction chemotherapy); or the timing of the symptom assessments (e.g., during chemotherapy, post-chemotherapy). Two of the three studies that used a factor loading of 0.30 identified only two clusters (n=219,[45] n=219[46]) and the third identified five (n=232).[35] This difference may be due to the fact that two of these studies[45, 46] used only 10 symptoms to evaluate for clusters.

Unique symptom clusters

While it is important to identify which clusters are consistent across cancer types and treatments, it is equally important to identify clusters that are unique to a specific cancer and/or treatment. A hormonal or vasomotor cluster was identified in 26.1% of the studies.[29, 34-36, 48, 50] Of note, four of these studies evaluated for clusters in women with breast cancer[34, 36, 48, 50] and one in women with a gynecological cancer.[35] In the sixth study,[29] the majority of women had either breast (40.2%) or gynecological cancer (17.3%).

Changes in symptom clusters over time

Ten studies evaluated for changes in clusters over three[46, 47, 49, 50, 52-54] or four timepoints.[45, 48, 51] While three studies evaluated for clusters beyond the completion of chemotherapy (e.g., six months post-chemotherapy),[46, 48, 52] the other seven studies evaluated for clusters around and during active treatment.[45, 47, 49-51, 53, 54] Of these studies, six reported a psychological or emotional cluster that remained relatively stable over time.[47-52] In contrast, six studies identified a gastrointestinal cluster that varied over time.[45-48, 51, 53]

Methods to evaluate the stability of symptom clusters across dimensions and/or over time

Stability was evaluated using the method proposed by Kirkova and Walsh[44] in 81.1% of the studies that evaluated for differences in symptom clusters across two or more dimensions,[33, 35-37, 40, 47, 49, 50, 53] and in 60% of the longitudinal studies[47-50, 53, 54] that evaluated for the stability across dimensions and timepoints. The method proposed by Kirkova and Walsh[44] specifies that 75% of the symptoms in a cluster should be in agreement in order for a symptom cluster to be stable across timepoints or dimensions. In addition, the most "prominent or important symptom(s)" needs to be present.[44, p.1012] While the majority of studies that evaluated for stability of symptom clusters across dimensions or time used Kirkova and Walsh's method, the criteria were applied with relative subjectivity (e.g., described clusters as "relatively stable;" [50, p. 47] described symptoms within clusters as "relatively stable"[53, p. 787]).

This subjectivity may be due in part to a lack of clarity and consensus on the definition of "stability." Similar to Kirkova and Walsh,[44] in their definition of a symptom cluster, Kim and colleagues[13] used stability as a characteristic to describe the group of symptoms within the cluster. In contrast, other researchers have described stability in terms of the type of cluster that is identified. Skerman and colleagues[22] suggested that for a cluster to be stable, it must be "reproducible" (i.e., replicated in a similar sample) or appear reliably over time. Barsevick[12] went further to describe stability as how consistently clusters appeared across statistical methods, within homogeneous populations, or over time. From these descriptions, it is unclear if stability refers to the the stability of a specific cluster itself (e.g., gastrointestinal, nutritional) across time and/or symptom dimensions or the symptoms within the cluster. Adding to this confusion, only one of these reports provided criteria to evaluate stability.[44]

Building on Barsevick's description, we suggest that the term *stability* should be used to describe whether or not the same clusters are identified across study samples, dimensions, and/or over time. While *consistency* should be used to describe whether the symptoms within a

cluster remain the same across these conditions. The use of separate terms to describe these characteristics of symptom clusters may provide clarity and move the science forward. In addition, consensus on how stability is used in the definition of a symptom cluster research warrants consideration.

Secondary outcomes and biomarker evaluation

Of the five studies that evaluated for associations between symptom clusters and other PROs,[32, 38, 39, 42, 46] all used measures of QOL. In addition, Chen and colleagues[38] examined the relationships between symptom clusters and functional performance. Cherwin and Perkhounkova[32] examined how symptom clusters impact symptom interference with daily life and QOL. Of the 23 studies included in this review, only one[54] evaluated for associations between symptom clusters and a biological mechanism.

Limitations

Despite the strict criteria that were employed to ensure a comprehensive review of the literature, only one author made the initial study selection and only two authors did the data extraction. Therefore it is possible that some studies and/or information were missed. Because the majority of the studies in this review included patients who were homogeneous in terms of gender, race, ethnicity, and cancer diagnosis, our findings may not generalize to all patients with cancer. In addition, because this review focused on adults with stage I to III cancer, our findings may not generalize to patients with advanced cancer or cancer survivors. Finally, 34.8% of the studies came from a single, large study of patients undergoing chemotherapy and may influence the findings of this review.

CONCLUSIONS

This review highlighted numerous areas of growth within symptom clusters research, and identified multiple areas that warrant consideration. One ongoing issue in symptom cluster research is the lack of consistent methods for naming the clusters. In 2016,[10] Miaskowski stressed that a standardized nomenclature needed to be developed in order to facilitate

comparisons of clusters across studies. However, as demonstrated in this review, a large amount of variability exists in how clusters were named. For example, the psychological cluster had 10 different names. In addition, researchers must name their clusters to allow for comparisons. In this review, symptom clusters were unnamed in 8.7% of the studies[41, 43] compared to 26.3% in the previous review.[11]

We identified only one study that evaluated for symptom clusters using NA.[29] An advantage of NA is that it allows for an examination of the strengths of the relationships among the symptoms within a cluster and how symptom clusters relate to each other within the network. Additional research using NA is needed to explore the inter-relationships among symptoms within clusters and whether these relationships differ based on the dimension used.

One of the aims of this review was to describe associations between symptom clusters and biological mechanisms. Of the 23 studies included in this review, only one study evaluated for associations between symptom clusters and a biological mechanism.[54] Investigation of the mechanisms that underlie symptoms and symptom clusters is a key priority set by the National Institute of Nursing Research.[62] Future research needs to incorporate the evaluation of biological mechanisms that may underlie symptom clusters in order to better understand why these symptoms cluster and to develop interventions to target clusters of symptoms rather than single symptoms.

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Table 3.1. Summary of Search Strategy

Database	Search Terms
Cochrane Library	"symptom cluster" OR "symptom clusters" OR ("symptom" AND "cluster") OR ("symptom" AND "clusters") OR ("symptoms" AND "clusters") in All Text AND cancer OR neoplasm in All Text AND chemotherapy OR CTX in All Text NOT reviews NOT protocols. Restricted to 01/01/2017 to 05/17/2017
Cumulative Index to Nursing and Allied Health Literature	("symptom cluster" or "symptom clusters" or "symptom" AND "cluster" or "symptom" AND "clusters" or "symptoms" AND "clusters") AND (cancer OR neoplasm) AND (chemotherapy OR CTX). Limiters: Published date: 20170101-20210531; Language: English
Embase	('symptom cluster' OR 'symptom clusters' OR ('symptoms' AND 'clusters') OR ('symptom' AND 'clusters') OR ('symptom' AND 'cluster')) AND (cancer OR neoplasm) AND (chemotherapy OR ctx). Search limited to 2017/1/1-2021/5/17; Language: English
PubMed	((((("symptom cluster"[All Fields]) OR ("symptom clusters"[All Fields])))) OR ((((("symptom"[All Fields])) AND ("cluster"[All Fields])))) OR ((("symptom"[All Fields])) AND ("clusters"[All Fields])))) OR ((("symptoms"[All Fields])) AND ("clusters"[All Fields])))))) AND ((cancer[All Fields])) OR (neoplasm[All Fields])))) AND ((chemotherapy[All Fields])) OR (CTX[All Fields]))). Filter applied: 2017/1/1-2021/5/17; Language: English
Web of Science	Topic=(symptom cluster* OR *symptom clusters*) OR Topic=(symptom* AND cluster*) OR Topic=(symptom* AND clusters*) OR Topic=(symptoms* AND clusters*) AND Topic=(cancer* OR neoplasm*) AND (chemotherapy* OR CTX*) AND Topic=(chemotherapy* OR CTX*). Restricted to: 2017/1/1-2021/5/17; Language: English

Table 3.2. Quality Assessment by the National Heart, Lung, and Blood Institute (NHLBI) of the National Institute of Health Quality Assessment Tool for Observational and Cross-Sectional Studies

Author(s), Year	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10	Item 11	Item 12	Item 13	Item 14	Final Quality
Cross-sectional studies (n=13)															
Chen et al., 2020	Y	Y	NR	Y	N	Y	CD	NA	Y	N	Y	NA	NA	NA	Fair
Cherwin & Perkhounkova, 2017	Y	Y	Y	Y	N	Y	Y	NA	Y	N	Y	NA	NA	NA	Good
Chongkham-ang et al., 2018	Y	Y	Y	Y	Y	Y	Y	NA	Y	N	Y	NA	NA	NA	Good
Han et al., 2019	Y	Y	Y	Y	N	Y	Y	NA	Y	N	Y	NA	NA	NA	Good
Li et al., 2019	Y	Y	NR	Y	N	Y	Y	NA	Y	N	Y	NA	NA	NA	Good
Matzka et al., 2018	Y	Y	NR	Y	N	Y	CD	NA	Y	N	Y	NA	NA	NA	Fair
Papachristou et al., 2019	Y	Y	Y	Y	N	Y	Y	NA	Y	N	Y	NA	NA	NA	Good
Pozzar et al., 2021	Y	Y	Y	Y	N	Y	Y	NA	Y	N	Y	NA	NA	NA	Good
Ren et al., 2017	Y	Y	Y	Y	N	CD	CD	NA	Y	N	Y	NA	NA	NA	Fair
Sezgin & Bektas, 2020	Y	Y	NR	Y	Y	Y	CD	NA	Y	N	Y	NA	NA	NA	Fair
Sullivan et al., 2017	Y	Y	Y	Y	N	Y	Y	NA	Y	N	Y	NA	NA	NA	Good
Vuttanon et al., 2019	Y	Y	Y	Y	Y	Y	Y	NA	Y	N	Y	NA	NA	NA	Good
Wong et al., 2017	Y	Y	Y	Y	N	Y	Y	NA	Y	N	Y	NA	NA	NA	Good
Longitudinal studies (n=10)															
Albusoul et al., 2017	Y	Y	Y	Y	Y	Y	Y	NA	Y	Y	Y	NA	Y	NA	Good
Berger et al., 2020	Y	Y	Y	Y	Y	Y	Y	NA	Y	Y	Y	NA	Y	NA	Good
Browall et al., 2017	Y	Y	Y	Y	N	Y	Y	NA	Y	Y	Y	NA	NR	NA	Good
Han et al., 2019	Y	Y	Y	Y	N	Y	Y	NA	Y	Y	Y	NA	NR	NA	Good
Kim, 2018	Y	Y	NR	Y	N	Y	Y	NA	Y	Y	Y	NA	Y	NA	Good
Li et al., 2020	Y	Y	NR	Y	N	Y	Y	NA	Y	Y	Y	NA	N	NA	Good
Lin et al., 2019	Y	Y	Y	Y	N	Y	Y	NA	Y	Y	Y	NA	N	NA	Good
Russell et al., 2019	Y	Y	Y	Y	N	Y	Y	NA	Y	Y	Y	NA	NR	NA	Good
Sullivan et al., 2018	Y	Y	Y	Y	N	Y	Y	NA	Y	Y	Y	NA	NR	NA	Good
Wiggenraad et al., 2020	Y	Y	Y	Y	Y	Y	Y	NA	Y	Y	Y	NA	N	NA	Good

Abbreviations: CD = cannot determine; N = no; NA = not applicable; NR = not reported; Y = yes
Study methodological quality ratings: Good, Fair, Poor

NHLBI of the National Institute of Health Quality Assessment Tool for Observational and Cross-Sectional Studies Criteria: Item 1 (Clear research question); Item 2 (Define study population); Item 3 (Participation rate at least 50%); Item 4 (Uniform eligibility criteria); Item 5 (Sample size justification); Item 6 (Exposure assessed prior to outcome measurement); Item 7 (Sufficient timeframe to see an effect); Item 8 (Examine different levels of exposure); Item 9 (Clearly defined exposure measures); Item 10 (Exposure assessed more than once over time); Item 11 (Clearly defined outcome measures); Item 12 (Outcome assessors were blinded to exposure status of participants); Item 13 (Loss to follow-up less than 20%); Item 14 (Key confounding variables measured and adjusted statistically)

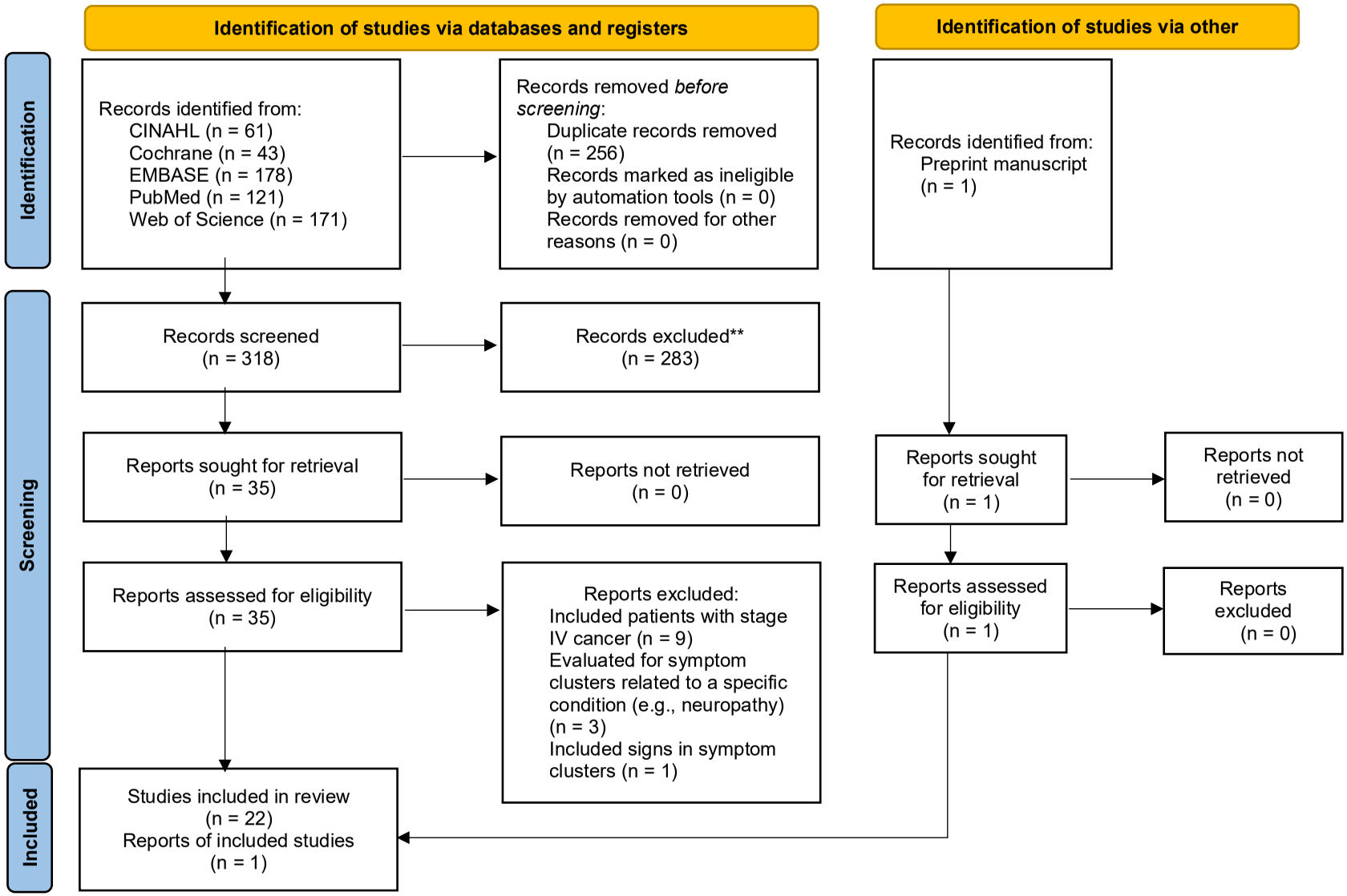

Figure 3.1. Preferred Reporting Items for Systematic Reviews and Meta-Analysis flow diagram to determine the final selection of studies that evaluated for symptom clusters in patients receiving adjuvant chemotherapy, 2017-2021. From Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ 2021;372:n71.

Chapter 4

Symptom Clusters in Outpatients with Cancer Using Different Dimensions of the Symptom Experience

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ABSTRACT

Purpose: Relatively few studies have evaluated for symptom clusters across multiple dimensions. It is unknown whether the symptom dimension used to create symptom clusters influences the number and types of clusters that are identified. Study aims were to describe ratings of occurrence, severity, and distress for 38 symptoms in a heterogeneous sample of oncology patients (n=1329) undergoing chemotherapy; identify and compare the number and types of symptom clusters based on three dimensions (i.e., occurrence, severity, and distress); and identify common and distinct clusters.

Methods: A modified version of the Memorial Symptom Assessment Scale was used to assess the occurrence, severity, and distress ratings of 38 symptoms in the week prior to patients' next cycle of chemotherapy. Symptom clusters for each dimension were identified using exploratory factor analysis.

Results: Patients reported an average of 13.9 (±7.2) concurrent symptoms. Lack of energy was both the most common and severe symptom while "I don't look like myself" was the most distressing. Psychological, gastrointestinal, weight gain, respiratory, and hormonal clusters were identified across all three dimensions. Findings suggest that psychological, gastrointestinal, and weight gain clusters are common while respiratory and hormonal clusters are distinct.

Conclusions: Psychological, gastrointestinal, weight gain, hormonal, and respiratory clusters are stable across occurrence, severity, and distress in oncology patients receiving chemotherapy. Given the stability of these clusters and the consistency of the symptoms across dimensions, use of a single dimension to identify these clusters may be sufficient. However, comprehensive and disease-specific inventories need to be used to identify distinct clusters.

Keywords: cancer; chemotherapy; exploratory factor analysis; network analysis; symptoms; symptom clusters

INTRODUCTION

Patients receiving chemotherapy report between 10[1] to 14.5[2] concurrent symptoms. While these data fostered symptom clusters' research,[3, 4] progress in this area of scientific inquiry is limited by multiple unanswered questions.[5-7] One question is whether the symptom dimension (i.e., occurrence, severity, distress) impacts the number and types of symptom clusters that are identified. As highlighted in one systematic review of symptom clusters in patients receiving adjuvant chemotherapy,[7] less than half of the 23 studies evaluated for symptom clusters across two or more symptom dimensions. A second question that warrants investigation is the determination of which clusters are common and distinct across various types of cancer.[5] The answers to these questions will guide clinical assessments and inform mechanistic-based studies.

Nine cross-sectional studies evaluated for symptom clusters in heterogeneous samples receiving chemotherapy.[8-16] Six studies used a single symptom dimension to identify the clusters,[8-10, 12, 15, 16] two used two or more dimensions,[11, 13] and one did not report the dimension used in the analysis.[14] Across these nine studies, the number of clusters varied from three to eight. While a psychological cluster was the only common one across seven of these studies,[8-10, 12, 13, 15, 16] none of them contained the same symptoms. This variability in both the types of clusters and symptoms within the clusters is related to heterogeneity in the symptom inventories used; number of symptoms evaluated; timing of the assessments; and statistical methods used. Because of these differences, one cannot determine if the number and types of symptom clusters vary based on the dimensions used to create the clusters. In addition, these data suggest that the only common cluster, in samples with heterogeneous types of cancer, is a psychological one.

While we previously evaluated for symptom clusters across two or more symptom dimensions in patients with breast,[17] gastrointestinal,[18] gynecological,[19] or lung[20] cancer using EFA, we have not used EFA to evaluate for symptom clusters in the entire sample. In addition,

we recently reported on the results of a NA of symptom clusters in the combined sample.[13] A comparison of the number and types of symptom clusters that were identified for each type of cancer diagnosis to those that are identified for the combined sample, as well as a comparison of findings using different analytic approaches,[5] will allow for the generation of hypotheses related to common and unique symptom clusters in oncology patients.

Therefore, the purposes of this study were to describe ratings of occurrence, severity, and distress for 38 symptoms in a heterogeneous sample of oncology patients undergoing chemotherapy and identify and compare the number and types of symptom clusters based on three symptom dimensions (i.e., occurrence, severity, and distress). In addition, an evaluation of common and distinct symptom clusters was done for the total sample compared to four distinct types of cancer (i.e., breast,[17] gastrointestinal,[18] gynecological,[19] lung[20]) and for two different methods (i.e., EFA, NA[13]).

METHODS

Patients and Settings

This analysis is part of a larger study that evaluated symptom clusters in oncology outpatients receiving chemotherapy.[13, 17-20] Eligible patients were ≥18 years of age; had a diagnosis of breast, lung, gastrointestinal, or gynecologic 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 four community-based oncology programs. Of the 1343 patients enrolled, 1329 patients had complete MSAS data.

Procedures

Eligible patients were approached during their first or second cycle of chemotherapy and provided written informed consent. Patients completed questionnaires in their home and returned them in a postage paid envelope, six times over two cycles of chemotherapy. Data

from the enrollment assessment (symptoms in the week before the patient's second or third cycle of chemotherapy) were used in these analyses. Medical records were reviewed for disease and treatment information. This study was approved by the Committee on Human Research at the University of California, San Francisco.

Instruments

Patients completed a demographic questionnaire, Karnofsky Performance Status (KPS) scale,[21] and Self-Administered Comorbidity Questionnaire.[22] Toxicity of each patient's chemotherapy regimen was rated using the MAX2 index.[23, 24]

A modified version of the 32-item MSAS was used to evaluate the occurrence, severity, and distress of 38 common symptoms associated with cancer and its treatment.[25] Six common symptoms were added: hot flashes, chest tightness, difficulty breathing, abdominal cramps, increased appetite, and weight gain. Using the MSAS, patients reported whether they had experienced each symptom in the past week. If they had experienced the symptom, they were asked to rate its severity and distress. Severity was measured using a four-point Likert scale (i.e., 1 = slight, 2 = moderate, 3 = severe, 4 = very severe). Distress was measured using a five-point Likert scale (i.e., 0 = not at all, 1 = a little bit, 2 = somewhat, 3 = quite a bit, 4 = very much). The validity and reliability of the MSAS are well established.[25]

Data Analysis

Descriptive statistics and frequency distributions were calculated for the demographic and clinical characteristics, as well as symptom occurrence rates and severity and distress ratings using the Statistical Package for the Social Sciences Version 27 (IBM Corporation, Armonk, NY). EFA was used to identify symptom clusters using Mplus Version 8.6.[26]

For the EFA, factor loadings were considered meaningful if the loading was ≥0.40.[26] In addition, factors were considered to be adequately defined if at least two items (i.e., symptoms) had loadings of ≥0.40.[27] Items were allowed to load on two factors (i.e., cross-load) if they fell within our preset criteria of ≥0.40. For the EFA of the occurrence items, tetrachoric correlations

were used to create the matrix of associations.[26] For the EFAs of the severity and distress ratings, polychoric correlations were used to create the matrix of associations. The simple structure for the occurrence, severity, and distress EFAs were estimated using the method of unweighted least squares with geomin (i.e., oblique) rotation. The unweighted least squares estimator was selected to achieve more reliable results with the dichotomous (i.e., occurrence) and ordinal (i.e., severity, distress) items.[26]

The EFA for severity was done using severity ratings that included a zero (i.e., 0, 1, 2, 3, 4). If the patient indicated that they did not have the symptom, a severity score of zero was assigned. The EFA for distress was done using distress ratings that included a zero (did not have the symptom) and the original ratings shifted from 1 (not at all) to 5 (very much). The initial EFA analyses were done using severity and distress ratings that did not include zero (i.e., 1, 2, 3, 4, 5). However, the pairwise missingness (i.e., 1-covariance coverage for each of the item pairs) was over 90% and the estimation failed to converge.

Factor solutions were estimated for two through five factors. The factor solution with the greatest interpretability and clinical meaningfulness was selected given that it met the criteria set for evaluating simple structure (i.e., size of item loadings, number of items on a factor). Then, each factor solution was examined to determine a clinically appropriate name for the symptom cluster. Clusters were named based on the symptoms with the highest factor loadings and the majority of the symptoms within the cluster.

Differences in Number and Types of Clusters

To evaluate percent agreement among the symptoms within the same cluster using occurrence, severity, and distress ratings, previous studies by our group[17-20, 28-31] and others[32, 33] used the criteria proposed by Kirkova and Walsh.[34] They suggested that to be in agreement with each other, at least 75% of the symptoms in the cluster should be present including the prominent and most important symptom (i.e., symptom with the largest factor loading).

While Kirkova and Walsh[34] used the term “stability” to describe these criteria, the definition and use of stability within symptom cluster research is inconsistent[7] and has led to the subjective application of these criteria. Therefore, in this study, the term *stability* is used to describe whether or not the same clusters are identified across dimensions and/or studies. In contrast, *consistency* is used to describe whether the specific symptoms within a cluster remain the same across symptom dimensions (i.e., percent agreement among the symptoms within the cluster).

RESULTS

Demographic and Clinical Characteristics

Of the 1329 patients in this study, 77.8% were female, 69.9% were White, 64.4% were married or partnered, and had a mean age of 57.3 (±12.3) years (Table 4.1). While the majority (60.4%) reported a mean household annual income of ≥$70,000, only 35.1% were currently employed. Most patients were well-educated (16.2 ±3.0 years), exercised on a regular basis (70.9%), and had never smoked (64.7%). Patients had 2.4 (±1.4) comorbid conditions and an average KPS score of 80.1 (±12.4). On average, patients reported 13.9 (±7.2) concurrent symptoms before their second or third cycle of chemotherapy.

Symptom Prevalence

Lack of energy was the most common symptom (Table 4.2). Mean severity ratings were calculated in two ways (i.e., with and without zeros). When zeros were included in the calculation, lack of energy was the most severe symptom. In the “without zeros” analyses, hair loss was rated as the most severe symptom. “I don’t look like myself” was the most distressing symptom.

Occurrence Clusters

Five-factor solution was selected for the occurrence EFA (Table 4.3). Psychological cluster had six symptoms and worrying had the highest factor loading. Gastrointestinal cluster had 11 symptoms and lack of appetite had the highest factor loading. Weight gain cluster had

two symptoms and weight gain had the highest factor loading. Hormonal cluster had two symptoms and hot flashes had the highest factor loading. Respiratory cluster had four symptoms and difficulty breathing had the highest factor loading.

Severity Clusters

Five-factor solution was selected for the severity EFA (Table 4.3). Psychological cluster had five symptoms and worrying had the highest factor loading. Gastrointestinal cluster had 10 symptoms and lack of appetite had the highest factor loading. Weight gain cluster had two symptoms and weight gain had the highest factor loading. Hormonal cluster had two symptoms and hot flashes had the highest factor loading. Respiratory cluster had four symptoms and difficulty breathing had the highest factor loading.

Distress Clusters

Five-factor solution was selected for the distress EFA (Table 4.3). Psychological cluster had six symptoms and worrying had the highest factor loading. Gastrointestinal cluster had nine symptoms and lack of appetite had the highest factor loading. Weight gain cluster had two symptoms and weight gain had the highest factor loading. Hormonal cluster had two symptoms and hot flashes had the highest factor loading. Respiratory cluster had four symptoms and difficulty breathing had the highest factor loading.

Stability and Consistency

Five stable clusters were identified across all three symptom dimensions (Table 4.3). Across all five clusters, the symptom with the highest factor loading was the same across all three dimensions. In terms of consistency, for psychological cluster, consistency ranged from 83.3% (severity) to 100% (occurrence, distress). For gastrointestinal cluster, consistency ranged from 75.0% (distress) to 91.7% (occurrence). For weight gain, hormonal, and respiratory clusters, consistency was 100% across the three dimensions.

DISCUSSION

Findings from this study provide new information on the occurrence, severity, and distress of 38 symptoms in a large, heterogeneous sample of oncology patients. In the week prior to their second or third cycle of chemotherapy, patients reported on average 13.9 symptoms. Consistent with previous studies of patients receiving chemotherapy, lack of energy was the most common and severe symptom.[8, 9, 15] However, as noted previously,[18, 19, 35] the most common symptoms are not always the most distressing. Hair loss was rated as the most severe symptom when zeros were not included in the mean severity scores, while "I don't look like myself" was the most distressing. Based on these findings, to have a more complete picture of the impact of individual symptoms, multiple dimensions of the symptom experience warrant evaluation.

Using findings from the literature, as well as our previous EFAs for breast,[17] gastrointestinal,[18] gynecological,[19] and lung[20] cancers, and our NA for the entire sample,[13] the remainder of this discussion describes the common and distinct symptom clusters (Table 4.4).

Psychological Cluster

Consistent with two reviews that reported that a psychological cluster was one of the most common clusters in patients receiving chemotherapy,[6, 7] this cluster was identified across all three symptom dimensions. Therefore, it is not surprising that a psychological cluster was identified in our previous studies of four types of cancer[17-20] as well as in our NA.[13] In this cluster, the most consistent symptoms across dimensions, cancer types, and analytic methods were: worrying, feeling sad, feeling nervous, and feeling irritable. Taken together, these findings suggest that a psychological cluster is stable across various cancer types and can be identified using any symptom dimension. Given its stability, psychological symptoms need to be routinely assessed in all oncology patients.

Gastrointestinal Cluster

Across studies of patients receiving chemotherapy,[6, 7] a gastrointestinal cluster was identified repeatedly using ratings of occurrence, severity, and distress. Given chemotherapy affects rapidly dividing cells, its impact on the gastrointestinal tract results in a constellation of symptoms.[36] While nausea, vomiting, and diarrhea are the most consistent symptoms within this cluster,[6, 7] in the current study, lack of appetite, weight loss, nausea, change in the way food tastes, vomiting, difficulty swallowing, diarrhea, abdominal cramps, and dry mouth were consistent across the three dimensions.

When compared with our previous studies of individual types of cancer,[17-20] as well as the NA of the total sample,[13] the names of this cluster, as well as the specific symptoms were not consistent. For example, abdominal cramps was the only symptom that was consistent across these studies and dimensions. In addition, the "gastrointestinal" cluster identified in patients with gynecological or lung cancer included multiple symptoms related to the epithelium (e.g., changes in skin, itching). This variability has a number of plausible explanations, including: differential effects of specific chemotherapy regimens on the gastrointestinal mucosa; differential effects of the cancer itself (e.g., colon cancer versus breast cancer) on the gastrointestinal tract; differential perceptions of a specific symptom in terms of its severity versus its distress; and/or variations in the relationships among various symptoms that are associated with specific types of cancer (e.g., feeling bloated in gastrointestinal cancers). Despite these variations, given the identification of a gastrointestinal cluster across multiple independent samples,[8, 9, 12, 32, 33, 37-39] this cluster can be considered stable. Additional research is warranted to determine the specific factors that contribute to subtle variations in the consistency of symptoms within this cluster.

Weight Gain Cluster

In the current study, a weight gain cluster was identified that included weight gain and increased appetite across all three symptom dimensions. However, across previous studies with

heterogeneous cancer types,[10, 13, 15, 16] as well as in our own studies with specific cancer diagnoses,[17-20] this cluster was highly variable both in terms of stability and consistency. For example, in a study of patients with hematologic malignancies,[10] lack of appetite, taste changes, and nausea were included in an appetite cluster. In another study of older cancer patients with a variety of solid tumors,[15] lack of appetite, change in the way food tastes, constipation, weight loss, and "I do not look like myself" were identified as a nutrition cluster. In our work,[13, 17-20] weight gain was the only consistent symptom across cancer types, analytic methods, and dimensions.

Variability, in both stability and consistency, across studies may be due to differences in the types of chemotherapy received, medications patients are taking, and/or the location of tumors in or near the digestive system. Another factor that may contribute to variability is the symptom assessment instrument that was used. In our[13, 17-20] and one of the aforementioned studies,[10] modified versions of the MSAS were used that included multiple symptoms related to appetite and nutrition. Studies that use an instrument with fewer symptoms will not be able to identify a weight- or nutrition-related cluster. Given that changes in nutritional status can lead to a variety of comorbidities (e.g., diabetes),[40] comprehensive nutritional assessments are a vital component of cancer care.

Respiratory Cluster

Respiratory cluster, that included difficulty breathing, shortness of breath, chest tightness, and cough, was found across all three dimensions. In our previous studies, a respiratory cluster was identified in the total sample using NA[13] and in patients with gynecological[19] and lung[20] cancer across two or more dimensions; but not in patients with breast[17] or gastrointestinal[18] cancers. In addition, across two studies that evaluated for symptom clusters in a heterogeneous sample,[15, 38] only one identified a respiratory cluster.[38] The inconsistent identification of this cluster suggests that it may be unique to certain cancer types.

These differences may be related to tumor locations and/or conditions that are more common to specific diagnoses (e.g., ascites, pleural effusion).

Hormonal Cluster

Hormonal cluster was identified that included hot flashes and sweats across all three symptom dimensions. In another study that compared symptom clusters that were identified in younger (<60 years) and older (≥60 years) patients receiving chemotherapy,[15] a hormonal cluster was identified in only the younger group. The identification of this cluster in younger patients supports the hypothesis that this cluster may emerge during/following cancer treatments that induce menopause.[41, 42]

In addition, this cluster may be unique to specific cancer diagnoses. For example, a type of hormonal cluster (i.e., menopausal, vasomotor) was identified in women with breast[39] and ovarian[43] cancer. In addition, among our previous analyses,[13, 17-20] a hormonal cluster was identified in the total sample using NA, and in women with breast[17] and gynecological[19] cancer across two or more symptom dimensions. Across all symptom dimensions within these three studies,[13, 17, 19] hot flashes and sweats were consistent. Of note, studies that do not use disease-specific or comprehensive symptom inventories will not be able to identify this distinct cluster in patients with breast or gynecological cancers, and perhaps in men with prostate cancer.

Comparison with Network Analysis

Identification of psychological, gastrointestinal, weight gain or nutritional, hormonal, and respiratory clusters using EFA is consistent with our previous NA of the total sample.[13] For both analyses, the symptoms within the psychological, hormonal, and respiratory clusters were relatively consistent across all three symptom dimensions. While both studies identified a gastrointestinal cluster, this cluster was only identified using distress in the NA. While both analytic approaches use measures of correlation to identify clusters, they differ in key ways. In our previous NA,[13] symptom clusters were identified using the Walktrap algorithm and all symptoms within the network were retained regardless of the strength of the relationship

between and among symptoms. For the EFAs, because the symptoms needed to have a factor loading ≥0.40, 13 to 15 symptoms did not load on one or more clusters. The advantages and disadvantages of various analytic methods need to be explored in future studies with large samples.

A number of limitations warrant consideration. Because our previous studies of patients with breast[17] and lung[20] cancer used only two symptom dimensions (i.e., occurrence, severity) to identify symptom clusters, our evaluation of the stability and consistency of clusters using distress warrants additional research. Given the study's cross-sectional design, additional research needs to determine which clusters remain stable across dimensions, cancer diagnoses, and/or time. While these findings suggest that respiratory and hormonal clusters are distinct clusters that occur with specific types of cancer, the proportions of patients with a gynecological (i.e., 17.5%) or lung (i.e., 11.7%) cancer were relatively small. In addition, our sample was primarily White and well-educated, which limits the generalizability of our findings.

CONCLUSION

Our findings suggest that psychological, gastrointestinal, weight gain, hormonal, and respiratory clusters are stable across occurrence, severity, and distress prior to the start of the next cycle of chemotherapy. Given the stability of these clusters across dimensions and the consistency of the symptoms within the clusters, they can be identified using any dimension of the symptom experience. However, for any single symptom, multiple dimensions of the symptom experience warrant evaluation to assess its full impact on a patient.

In addition, these findings suggest that gastrointestinal, psychological, and nutrition or weight change clusters are common across cancer types. Given the stability of these clusters across diagnoses, future research should explore whether these clusters share common biological mechanisms. Furthermore, additional research is needed to evaluate whether these clusters remain stable over time and across other cancer treatments (e.g., radiation therapy, surgery). Conversely, hormonal and respiratory clusters may be unique to specific cancer types.

Symptoms within these distinct clusters need to be assessed in patients with breast, gynecological, or lung cancer in the clinical and research settings.

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Table 4.1. Demographic and Clinical Characteristics of the Patients (n=1329)

Characteristic	Mean	SD
Age (years)	57.3	12.3
Education (years)	16.2	3.0
Body mass index (kilograms/meters squared)	26.2	5.7
Karnofsky Performance Status score	80.1	12.4
Number of comorbidities out of 13	2.4	1.4
Self-administered Comorbidity Questionnaire score	5.5	3.2
Time since cancer diagnosis (years)	2.0	3.9
Time since diagnosis (median)	0.42	
Number of prior cancer treatments (out of 9)	1.6	1.5
Number of metastatic sites including lymph node involvement (out of 9)	1.2	1.2
Number of metastatic sites excluding lymph node involvement (out of 8)	0.8	1.0
MAX2 Index of Chemotherapy Toxicity score (0 to 1)	0.17	0.08
Mean number of MSAS symptoms (out of 38)	13.9	7.2
	n	(%)
Gender		
Female	1033	77.8
Male	295	22.2
Self-Reported Ethnicity		
Asian or Pacific Islander	161	12.3
Black	95	7.2
Hispanic, Mixed, or Other	139	10.6
White	917	69.9
Married or partnered (% yes)	843	64.4
Lives alone (% yes)	283	21.6
Child care responsibilities (% yes)	286	22.0
Care of adult responsibilities (% yes)	95	7.9
Currently employed (% yes)	462	35.1
Income		
< $30,000	219	18.4
$30,000 to < $70,000	252	21.2
$70,000 to < $100,000	199	16.7
≥ $100,000	520	43.7
Exercise on a regular basis (% yes)	922	70.9
Current or history of smoking (% yes)	462	35.3
Type of cancer		
Breast	534	40.2
Gastrointestinal	407	30.6
Gynecological	233	17.5
Lung	155	11.7
Type of prior cancer treatment		
No prior treatment	323	25.0
Only CTX, surgery, or RT	543	42.0
CTX and surgery, or CTX and RT, or surgery and RT	257	19.9
CTX and surgery and RT	169	13.1

Characteristic	n	(%)
Cycle length		
14 days	558	42.1
21 days	671	50.6
28 days	97	7.3
Emetogenicity of the chemotherapy regimen		
Minimal/low	259	19.5
Moderate	810	61.0
High	258	19.4
Antiemetic regimen		
None	92	7.1
Steroid alone or serotonin receptor antagonist alone	265	20.4
Serotonin receptor antagonist and steroid	618	47.7
NK-1 receptor antagonist and two other antiemetics	321	24.8

Abbreviations: CTX, chemotherapy; MSAS, Memorial Symptom Assessment Scale; NK-1, neurokinin 1; RT, radiation therapy; SD, standard deviation

Table 4.2. Occurrence Rates and Severity and Distress Ratings for Symptoms Prior to Chemotherapy

Symptoms[a]	Occurrence Rates[b]		Severity Ratings with Zeros[c]		Severity Ratings without Zeros[d]		Distress Ratings[e]	
	n	%	Mean	SD	Mean	SD	Mean	SD
Lack of energy	1106	83.2	1.67	1.01	2.02	0.72	1.79	1.14
Difficulty sleeping	918	69.1	1.38	1.13	2.01	0.76	1.79	1.11
Pain	803	60.4	1.14	1.10	1.92	0.73	1.77	1.10
Feeling drowsy	801	60.3	1.04	1.01	1.75	0.70	1.16	1.05
Hair loss	728	54.8	1.35	1.49	2.49	1.12	1.88	1.34
Numbness/tingling in hands/feet	694	52.2	0.94	1.09	1.84	0.81	1.52	1.18
Worrying	692	52.1	0.94	1.06	1.85	0.74	1.63	1.04
Difficulty concentrating	690	51.9	0.79	0.90	1.55	0.64	1.48	1.07
Change in the way food tastes	656	49.4	1.04	1.23	2.12	0.89	1.72	1.26
Nausea	631	47.5	0.82	1.04	1.76	0.81	1.65	1.12
Feeling sad	612	46.0	0.77	0.97	1.71	0.71	1.50	1.06
Dry mouth	603	45.4	0.77	1.00	1.73	0.75	1.23	1.12
Constipation	578	43.5	0.84	1.12	1.98	0.83	1.70	1.17
Feeling irritable	549	41.3	0.69	0.95	1.70	0.72	1.46	1.03
Lack of appetite	549	41.3	0.78	1.07	1.92	0.79	1.28	1.11
Feeling nervous	505	38.0	0.59	0.88	1.62	0.68	1.41	0.98
“I don’t look like myself”	503	37.8	0.80	1.18	2.15	0.93	1.98	1.22
Changes in skin	482	36.3	0.68	1.03	1.91	0.81	1.64	1.19
Feeling bloated	440	33.1	0.58	0.93	1.79	0.73	1.54	1.07
Cough	433	32.6	0.45	0.75	1.42	0.62	1.02	1.08
Hot flashes	423	31.8	0.58	0.98	1.87	0.81	1.42	1.16
Dizziness	416	31.3	0.46	0.79	1.51	0.69	1.24	0.98
Sweats	415	31.2	0.53	0.92	1.77	0.78	1.29	1.09
Problems with sexual interest or activity	397	29.9	0.71	1.24	2.47	0.98	1.87	1.28
Diarrhea	393	29.6	0.54	0.95	1.87	0.81	1.46	1.13
Shortness of breath	357	26.9	0.44	0.82	1.67	0.71	1.51	1.04
Increased appetite	344	25.9	0.44	0.83	1.75	0.68	0.91	1.11
Weight gain	337	25.4	0.39	0.76	1.58	0.70	1.37	1.33
Weight loss	335	25.2	0.38	0.76	1.56	0.71	0.96	1.17
Itching	330	24.8	0.41	0.82	1.71	0.74	1.28	1.07

Symptoms[a]	Occurrence Rates[b]		Severity Ratings with Zeros[c]		Severity Ratings without Zeros[d]		Distress Ratings[e]	
	n	%	Mean	SD	Mean	SD	Mean	SD
Abdominal cramps	299	22.5	0.40	0.84	1.87	0.75	1.61	1.08
Mouth sores	278	20.9	0.34	0.76	1.70	0.74	1.46	1.06
Difficulty breathing	265	19.9	0.32	0.72	1.64	0.72	1.63	1.13
Chest tightness	237	17.8	0.27	0.64	1.54	0.67	1.42	1.00
Swelling of arms or legs	194	14.6	0.27	0.74	1.91	0.83	1.62	1.16
Problems with urination	187	14.1	0.24	0.68	1.79	0.80	1.51	1.21
Difficulty swallowing	183	13.8	0.23	0.66	1.73	0.82	1.64	1.15
Vomiting	164	12.3	0.21	0.66	1.80	0.90	1.74	1.18

Abbreviation: SD, standard deviation

[a]Symptoms are from the Memorial Symptom Assessment Scale with the addition of the following six symptoms: chest tightness, difficulty breathing, increased appetite, hot flashes, abdominal cramps, weight gain.

[b]Symptoms are listed in descending order of occurrence.

[c]Severity ratings with zeros: 0 = did not have the symptoms, 1 = slight, 2 = moderate, 3 = severe, 4 = very severe

[d]Severity ratings without zeros: 1 = slight, 2 = moderate, 3 = severe, 4 = very severe

[e]Distress ratings: 0 = not at all, 1 = a little bit, 2 = somewhat, 3 = quite a bit, 4 = very much

Table 4.3. Comparison of Symptom Clusters Prior to Initiation of Chemotherapy Using Ratings of Occurrence, Severity, and Distress[a]

Cluster	Symptoms	Occurrence	Severity	Distress
Psychological symptom cluster	Worrying	0.864	0.866	0.875
	Feeling sad	0.855	0.850	0.872
	Feeling nervous	0.744	0.750	0.760
	Feeling irritable	0.626	0.569	0.574
	Difficulty concentrating	0.549	0.517	0.560
	"I don't look like myself"	0.458	−	0.427
	Total number of symptoms in this cluster	6/6	5/6	6/6
Gastrointestinal symptom cluster	Lack of appetite	0.784	0.774	0.770
	Weight loss	0.679	0.658	0.680
	Nausea	0.663	0.624	0.612
	Change in the way food tastes	0.612	0.690	0.677
	Vomiting	0.546	0.538	0.525
	Difficulty swallowing	0.513	0.517	0.503
	Abdominal cramps	0.455	0.472	0.444
	Diarrhea	0.433	0.483	0.455
	Dry mouth	0.431	0.472	0.474
	Constipation	0.430	−	−
	Dizziness	0.404	−	−
	Mouth sores	−	0.420	−
	Total number of symptoms in this cluster	11/12	10/12	9/12
Weight gain symptom cluster	Weight gain	0.921	0.875	0.914
	Increased appetite	0.785	0.746	0.736
	Total number of symptoms in this cluster	2/2	2/2	2/2
Hormonal symptom cluster	Hot flashes	0.883	0.907	0.920
	Sweats	0.670	0.728	0.647
	Total number of symptoms in this cluster	2/2	2/2	2/2
Respiratory symptom cluster	Difficulty breathing	1.037	1.032	1.035
	Shortness of breath	0.716	0.763	0.741
	Chest tightness	0.689	0.614	0.628
	Cough	0.457	0.430	0.427
	Total number of symptoms in this cluster	4/4	4/4	4/4

[a]Extraction method: unweighted least squares. Rotation method: Geomin (oblique) rotation.
− = Factor loadings for these symptoms were <0.40.

Table 4.4. Comparison of Symptom Clusters Across Cancer Types and Analytic Methods Using Ratings of Occurrence, Severity, and Distress

Symptom dimension	Symptom cluster	EFA n=1329	NA[a] n=1328	Breast[b] n=534	GI[c] n=399	GYN[d] n=232	Lung[e] n=145
Occurrence	Psychological	●	●	●	●	●	●
	GI	●		●	●	●	
	Epithelial/GI						●
	Epithelial			●			
	Nutritional		●				●
	Weight change			●	●	●	
	Weight gain	●					
	Hormonal	●	●	●		●	
	Respiratory	●	●			●	
	Lung CA-specific						●
	CTX related		●		●		
	Sickness behavior			●			●
	Pain and abdominal		●				
Severity	Psychological	●	●	●	●	●	●
	GI	●		●	●		
	GI/epithelial					●	
	Epithelial/GI						●
	Epithelial			●			
	Nutritional		●				●
	Weight change			●	●	●	
	Weight gain	●					
	Hormonal	●	●	●		●	
	Respiratory	●	●			●	
	Lung CA-specific						●
	CTX related		●		●		
	Sickness behavior			●			
Distress	Psychological	●	●	Not assessed	●		Not assessed
	Psychological/GI					●	
	GI	●	●		●		
	GI/epithelial					●	
	Epithelial		●				
	Nutritional		●				
	Weight change				●	●	
	Weight gain	●					
	Hormonal	●	●			●	
	Respiratory	●	●			●	
	CTX related		●		●		

Abbreviations: CA, cancer; CTX, chemotherapy; EFA, exploratory factor analysis; GI, gastrointestinal; GYN, gynecological; NA, network analysis

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[c]Han CJ, Reding K, Cooper BA, et al (2019) Symptom clusters in patients with gastrointestinal cancers using different dimensions of the symptom experience. J Pain Symptom Manage 58(2):224-234.
[d]Pozzar RA, Hammer MJ, Cooper BA, et al (2021) Symptom clusters in patients with gynecologic cancer receiving chemotherapy. Oncol Nurs Forum 48(4):441-452.
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Chapter 5

Stability and Consistency of Symptom Clusters in Oncology Outpatients Across a Cycle of Chemotherapy

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ABSTRACT

Background and purpose: Improved understanding of the stability and consistency of symptom clusters over time, across symptom dimensions, and cancer diagnoses will lead to refinements in symptom assessments and management, as well as provide direction for mechanistic studies. Study purposes were to describe the occurrence, severity, and distress of 38 symptoms; evaluate the stability and consistency of symptom clusters across a cycle of chemotherapy, three symptom dimensions, and four distinct cancer types; and identify common and distinct symptom clusters.

Methods: Oncology outpatients (n=1329) completed the Memorial Symptom Assessment Scale prior to their next cycle of chemotherapy (T1), one week after chemotherapy (T2), and two weeks after chemotherapy (T3). Symptom clusters were identified using exploratory factor analysis using unweighted least squares. GEOMIN rotated factor loadings with absolute values ≥0.40 were considered meaningful. Clusters were stable if they were identified across each time point and/or dimension. Clusters were consistent if the same two or three symptoms with the highest factor loadings were identified across each time point and/or dimension.

Results: Patients reported 13.9 (±7.2) symptoms at T1, 14.0 (±7.0) at T2, and 12.2 (±6.8) at T3. Psychological, weight gain, respiratory, and gastrointestinal clusters were stable over time and dimensions. Only the psychological, respiratory, and weight gain clusters were consistent across time and dimensions.

Conclusions: Given the stability of the psychological, weight gain, and gastrointestinal clusters across cancer diagnoses, symptoms within these clusters need to be routinely assessed. However, hormonal and respiratory clusters are unique to specific cancer types and the symptoms within these clusters are variable.

Keywords: cancer; chemotherapy; exploratory factor analysis; oncology; symptoms; symptom

INTRODUCTION

Over the past 20 years, research on symptom clusters in oncology patients has increased exponentially.[1] However, whether symptom clusters change over time or differ based on the dimension of the symptom experience (i.e., occurrence, severity, distress) warrant additional consideration. In a systematic review of 23 studies that evaluated for symptom clusters in patients receiving chemotherapy,[1] 43.5% were longitudinal. Only four of these studies evaluated for symptom clusters across two or more symptom dimensions.[2-5] An improved understanding of the stability and consistency of symptom clusters will lead to refinements in symptom assessments and management, as well as provide direction for mechanistic studies.

Of the five longitudinal studies that evaluated for symptom clusters in patients with various types of cancer receiving chemotherapy,[6-10] three used severity to identify the clusters,[8-10] one used distress,[6] and one did not report on the dimension.[7] Across these five studies, the number of clusters ranged from three to seven. While a gastrointestinal cluster was identified across four studies,[6-9] no symptoms were consistent across studies and time points. Of the four studies that identified a psychological cluster,[7-10] anxiety- and depression-related symptoms (e.g., worry, feeling sad) were consistently identified across studies and time points. These inconsistencies are due to variability in the number of symptoms evaluated; symptom dimensions used; timing of symptom assessments; and statistical methods used. Because of these differences, the stability and consistency of clusters requires additional investigation.

In our cross-sectional study of symptom clusters in patients with heterogeneous types of cancer,[11] we identified five symptom clusters that were stable across occurrence, severity, and distress in the week prior to chemotherapy. Based on comparisons with our previous analyses of specific types of cancer (i.e., breast,[5] gastrointestinal,[12] gynecological,[13] lung[4]), we identified three symptom clusters that were common across all four cancer diagnoses (i.e., psychological, gastrointestinal, weight gain or change) and two clusters that were unique to specific types of

cancer (i.e., hormonal for breast[5] and gynecological,[13] respiratory for gynecological[13] and lung[4]). Given the stability of these five clusters across three symptom dimensions, we suggested that a single dimension can be used to identify these clusters.

However, an unanswered question is whether these common and distinct clusters remain stable over time. While we previously reported on the stability of symptom clusters across a single cycle of chemotherapy in patients with breast,[5] gastrointestinal,[2] gynecological,[14] and lung[4] cancer using two or more symptom dimensions, we have not evaluated for symptom clusters over time using the total sample. A comparison of the stability and consistency of symptom clusters across the specific cancer diagnoses to the total sample may provide additional evidence for the existence of common and distinct symptom clusters in oncology patients.

Therefore, the study purposes were to describe the occurrence, severity, and distress of 38 symptoms across a cycle of chemotherapy and evaluate the stability and consistency of symptom clusters over time and across symptom dimensions. In addition, an evaluation of common and distinct symptom clusters across the total sample and the four distinct types of cancer (i.e., breast,[5] gastrointestinal,[2] gynecological,[14] lung[4]) was done.

METHODS

Patients and settings

This analysis was planned as part of a larger study funded by the NCI.[2, 4, 5, 14] Eligible patients were ≥18 years of age; had a diagnosis of breast, lung, gastrointestinal, or gynecologic 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 four community-based oncology programs. Of the 1343 patients enrolled, 1329 patients had complete MSAS data.

Procedures

Eligible patients were approached during their first or second cycle of chemotherapy and provided written informed consent. Patients completed questionnaires six times over two cycles of chemotherapy. Data from the first three assessments were used in these analyses. Assessments took place in the week prior to patients' second or third cycle of chemotherapy (T1), approximately one week after chemotherapy (T2), and approximately two weeks after chemotherapy (T3). Medical records were reviewed for disease and treatment information. The study was approved by the Committee on Human Research at the University of California, San Francisco and Institutional Review Board at each of the study sites.

Instruments

Patients completed a demographic questionnaire, Karnofsky Performance Status (KPS) scale,[15] and Self-Administered Comorbidity Questionnaire.[16] Toxicity of each patient's chemotherapy regimen was rated using the MAX2 index.[17, 18]

A modified version of the 32-item MSAS was used to evaluate the occurrence, severity, and distress of 38 common symptoms associated with cancer and its treatment.[19] Six common symptoms were added: hot flashes, chest tightness, difficulty breathing, abdominal cramps, increased appetite, and weight gain. Using the valid and reliable MSAS,[19] patients reported whether they had experienced each symptom in the past week. If they had experienced the symptom, they were asked to rate its severity and distress. Severity and distress were rated using four- and five-point Likert scales, respectively.

Data analysis

Descriptive statistics and frequency distributions were calculated using Statistical Package for Social Sciences Version 27 (IBM Corporation, Armonk, NY). To identify the symptom clusters, EFA was done using MPlus Version 8.6.[20]

Factor loadings were considered meaningful if the loading was ≥0.40.[20] Factors were adequately defined if at least two symptoms had loadings of ≥0.40.[21] Items were allowed to

cross-load if they fell within our preset criteria of ≥0.40. Tetrachoric correlations were used to create the matrix of associations for the occurrence items, while polychoric correlations were used for the severity and distress ratings.[20] Simple structure for the EFAs were estimated using the method of unweighted least squares with geomin (i.e., oblique) rotation.[20]

EFA for severity was done using severity ratings that included a zero (i.e., 0, 1, 2, 3, 4). If the patient indicated that they did not have the symptom, a severity score of zero was assigned. The EFA for distress was done using distress ratings that included a zero (did not have the symptom) and the original ratings shifted from 1 (not at all) to 5 (very much). Initial EFA analyses were done using severity and distress ratings that did not include zero (i.e., 1, 2, 3, 4, 5). However, the pairwise missingness was over 90% and the estimation failed to converge.

Factor solutions were estimated for two through five factors. Factor solution with the greatest interpretability and clinical meaningfulness was selected given that it met the criteria set for evaluating simple structure. Clusters were named based on the symptoms with the highest factor loadings and the majority of the symptoms in the cluster.

Evaluation of stability and consistency

To evaluate the stability of symptom clusters across time and/or dimensions, previous work by our group[2, 4, 5, 11-14, 22, 23] and others[3, 6, 24] used the Kirkova and Walsh criteria.[25] They suggested that for a cluster to be considered stable, at least 75% of the symptoms in the cluster should be present including the prominent and most important symptom (i.e., symptom with the highest factor loading). This method has some limitations. First, while the term “stability” was used to describe these criteria, its definition and use within symptom cluster research are inconsistent.[1] This lack of consensus has led to the subjective application of these criteria. Second, a cutoff of 75% agreement is somewhat arbitrary and is applied inconsistently. Finally, in order to assess percent agreement, multiple calculations are needed. These considerations make the interpretation of results, within and across studies, challenging.

Given these limitations, we propose the following terminology and criteria to clarify this component of symptom cluster research. The term *stability* is used to describe whether or not the same clusters are identified over time, across symptom dimensions, and/or study samples.[11] In contrast, *consistency* is used to describe whether the specific symptoms within a cluster remain the same across these conditions. For a cluster to be considered consistent, the two or three symptoms with the highest factor loadings must be present across all time points and/or symptom dimensions. This evaluation of consistency builds on previous work that evaluated for "core sets of symptoms" that occurred consistently over time (p.98).[6] Given that a symptom cluster must contain a minimum of two symptoms,[26] a minimum of the same two symptoms with the highest factor loadings should be applied to clusters with only two or three symptoms. For clusters with four or more symptoms, a minimum of the same three symptoms with the highest factor loadings must present across all time points and/or dimensions to be considered consistent.

This appraisal of consistency has multiple strengths. First, by requiring the symptoms with the highest factor loadings to be consistent across each assessment, a rank-based method is utilized to prioritize symptoms with the highest factor loading. Given that the threshold for a minimum factor loading is still being determined and that symptoms with a lower score may negatively skew the results, this method improves upon the previous method. Second, these criteria can be rapidly applied and easily interpreted.

RESULTS

Demographic and clinical characteristics

Characteristics of the patients were reported previously.[11] In brief, of the 1329 patients in the total sample, 77.8% were female, 69.9% were White, 60.4% reported a mean household annual income of ≥$70,000, and had a mean age of 57.3 (±12.3) years (Table 1). Most patients were well-educated (16.2 ±3.0 years), exercised on a regular basis (70.9%), and had never

smoked (64.7%). Patients had 2.4 (±1.4) comorbid conditions and an average KPS score of 80.1 (±12.4).

Symptom prevalence and characteristics

Mean number of symptoms was 13.9 (±7.2) at T1, 14.0 (±7.0) at T2, and 12.2 (±6.8) at T3. Across the three assessments, lack of energy had the highest occurrence rate (Table 2). The most severe symptoms were hair loss at T1 and problems with sexual interest or activity at T2 and T3. The most distressing symptoms were: “I don’t look like myself” at T1, “I don’t look like myself” and problems with sexual interest or activity at T2, and problems with sexual interest or activity at T3.

Symptom clusters over time

At T1, a five-factor solution was selected for the occurrence, severity, and distress EFAs (Table 3). Psychological, weight gain, respiratory, gastrointestinal, and hormonal clusters were identified across all three dimensions. At T2, a four-factor solution was selected for the occurrence, severity, and distress EFAs. Psychological, weight gain, respiratory, and gastrointestinal clusters were identified across all three dimensions. At T3, a five-factor solution was selected for the occurrence, severity, and distress EFAs. Psychological, weight gain, respiratory, gastrointestinal, and body image clusters were identified using occurrence and severity. Using distress, psychological, weight gain, respiratory, gastrointestinal, and hormonal clusters were identified. The stability (Table 4) and consistency (Table 5) of each of these clusters is reported next.

Psychological cluster

Psychological cluster, comprised of five (T1 for severity) to nine (T2 and T3 for occurrence) symptoms, was stable across all three times and dimensions. For all three dimensions, worrying had the highest factor loading across all three times.

Symptoms within the psychological cluster were consistent across times and dimensions. Worrying, feeling sad, and feeling nervous had the highest factor loadings across time and dimensions.

Weight gain cluster

Weight gain cluster, comprised of two (T1 for occurrence, severity, and distress; T3 for severity and distress) to three (T2 for occurrence, severity, and distress) symptoms, was stable across all three times and dimensions. For all three dimensions, weight gain had the highest factor loading across all three times.

Weight gain cluster was comprised of two or three symptoms. Given that only two symptoms with the highest factor loadings needed to be present and weight gain and increased appetite had the highest factors loadings across times and dimensions, this cluster is consistent.

Gastrointestinal cluster

Gastrointestinal cluster, comprised of six (T3 for occurrence and severity) to 11 (T1 for occurrence) symptoms, was stable across all three times and dimensions. While lack of appetite had the highest factor loading at T1 for occurrence, severity, and distress and at T2 and T3 for distress, nausea had the highest factor loading at T2 and T3 for occurrence and severity.

Regarding the consistency of symptoms over time, none of the clusters met the criteria for consistency. For occurrence, only two symptoms were consistent across times. None of the symptoms were consistent across time for severity. For distress, only one symptom was consistent over time.

Regarding the consistency of symptoms across dimensions, this cluster met the criteria for consistency only at T2. At T1, only two symptoms were consistent across dimensions. At T3, only one symptom was consistent.

Respiratory cluster

Respiratory cluster, comprised of four symptoms, was stable across all three times and dimensions. For all three dimensions, difficulty breathing had the highest factor loading across all three times.

Symptoms within the respiratory cluster were consistent across all three times and dimensions. Difficulty breathing, shortness of breath, and chest tightness had the highest factor loadings across times and dimensions.

Hormonal cluster

Hormonal cluster was stable across all three dimensions at T1 and was identified using distress at T3. It was comprised of two symptoms. When this cluster was identified, hot flashes had the highest factor loading. Symptoms within the hormonal cluster were consistent across dimensions only at T1.

Body image cluster

Body image cluster was identified at T3 using severity and distress. It was comprised of three symptoms. When this cluster was identified, changes in skin had the highest factor loading. Given the lack of stability of the body image cluster across times and dimensions, its consistency was not evaluated.

DISCUSSION

This study is the first to provide a detailed characterization of the symptom burden of oncology patients across a cycle of chemotherapy and present an approach to characterize both the stability and consistency of symptom clusters across time and dimensions. In terms of symptom burden, patients reported an average of 13 symptoms across the three assessments. This finding suggests that symptoms persist across an entire cycle of chemotherapy and patients enter the next cycle with a high symptom burden.

The remainder of the Discussion describes the stability (Table 4) and consistency (Table 5) of each cluster, compares these clusters with our previous findings in patients with breast,[5]

gastrointestinal,[2] gynecological,[14] and lung[4] cancers, and places our findings in the context of the extant literature.

Psychological cluster

Consistent with our previous studies of patients with breast,[5] gastrointestinal,[2] gynecological,[14] and lung[4] cancers, in the current study, a psychological cluster was stable and consistent over time and symptom dimensions. Of note, across all five studies, worrying and feeling sad were the consistent symptoms for the majority of the EFAs. Because worrying and feeling sad are two of the most common symptoms associated with a psychological cluster,[1, 27] one can hypothesize that these two symptoms may represent core or sentinel symptoms within this cluster. Given that anxiety and depressive symptoms occurred in 38% and 46% of patients undergoing chemotherapy, respectively, it is imperative to routinely assess for these symptoms and initiate interventions and/or referrals to psychological support services.

Weight gain cluster

Named nutrition or weight change clusters in our patients with gastrointestinal,[2] gynecological,[14] and lung[4] cancers, and weight gain in the total sample, this cluster was stable across times and dimensions. However, across these four studies, the symptoms in this cluster were not consistent. Furthermore, in our patients with breast cancer,[5] this cluster was neither stable nor consistent. Similarly, in two studies of patients with acute myelogenous leukemia[3] and breast cancer,[24] while a nutritional or weight cluster was stable across time, the cluster was not consistent.

These findings suggest that the relationships among symptoms associated with nutritional status are dynamic. Differences in chemotherapy regimens, specific types of cancer and/or disease stage, comorbid conditions, and/or concurrent medications may contribute to this variability. An additional consideration is the specific nutritional symptoms on the symptom assessment instrument. For example, while the MSAS includes the items “weight loss” and “lack of appetite,” for our studies, weight gain and increased appetite were added. This cluster is an

example of how the specific symptoms on an inventory may allow for the identification of different symptom clusters based on the type of cancer (e.g., weight gain in women with breast cancer[24]) and/or stage of disease (e.g., cachexia in patients with lung cancer[28]).

Gastrointestinal cluster

Because a gastrointestinal cluster is one of the most common symptom clusters,[1, 27] it is not surprising that it was identified across each cancer type and the total sample.[2, 4, 5, 14] However, its stability and consistency were highly variable across time, dimensions, and cancer types. For example, in the total sample, across dimensions at T1, lack of appetite and weight loss were the two consistent symptoms. However, across dimensions at T2, weight loss, nausea, and vomiting were the consistent symptoms. Across dimensions at T3, only nausea was consistent.

The dynamic nature of this cluster is consistent with previous reports. For example, in three studies[6, 8, 9] that evaluated for symptom clusters across two or more cycles of chemotherapy, while stable, the gastrointestinal cluster was not consistent. Additional research is warranted to examine how the gastrointestinal cluster evolves during chemotherapy.

Respiratory cluster

In the total sample, the respiratory cluster was stable and consistent across times and dimensions. However, this cluster was identified only in patients with gynecological[14] and lung[4] cancers which suggests it may be cancer-specific. Across the breast,[5] lung,[4] and total samples, difficulty breathing was the only consistent symptom. Given that respiratory symptoms may arise from different mechanisms (e.g., bronchial lesions in lung cancer, ascites in gynecological cancer), this inconsistency has some clinical validity. Given that 26.9% of the entire sample reported shortness of breath at enrollment and that it persisted over time, suggests that it warrants evaluation and management across all cancer types.

Hormonal cluster

While the hormonal cluster was identified in the entire sample, it was only identified in our previous studies of women with breast[5] and gynecological[14] cancers. While this cluster was stable across times and dimensions in these previous studies,[5, 14] for the entire sample, it was only stable across dimensions at T1. When this cluster was identified, hot flashes and sweats were the consistent symptoms. These findings suggest that a hormonal cluster is unique to specific cancer types. Evidence from studies of women with breast cancer receiving chemotherapy support our findings. For example, in three studies,[6, 24, 29] a vasomotor cluster was stable over time and hot flashes and sweats were the consistent symptoms.

Body image cluster

While a body image cluster was not identified across our previous studies of individual cancer types,[2, 4, 5, 14] the symptoms in this cluster were found in an epithelial cluster. However, the stability and consistency of this cluster varied across times, dimensions, and cancer types. For example, in the entire sample, changes in skin, “I don’t look like myself,” and change in the way food tastes comprised the body image cluster. In our other studies, symptoms unique to specific cancer types were: hair loss and itching for breast[5] and gastrointestinal[2] cancers, and mouth sores for breast[5] and lung[4] cancers. This variability may be due to differences in the type of chemotherapy received, cycle length, and/or prior treatments. Despite these differences, a body image or epithelial cluster is stable across cancer types. Of note, change in the way food tastes and “I don’t look like myself” were two of the most common, severe, and distressing symptoms reported by patients across a cycle of chemotherapy. By providing education and management strategies prior to and throughout chemotherapy,[30] clinicians can help patients manage and cope with these symptoms.

These findings are limited by several considerations. Among our previous studies of patients with breast[5] and lung[4] cancer, only two symptom dimensions (i.e., occurrence, severity) were used to identify symptom clusters. Therefore, an evaluation of the stability and consistency

of clusters using distress ratings are needed. In addition, our sample was primarily White and well-educated, which limits the generalizability of our findings. Finally, given that this study was the first to evaluate the consistency of symptoms within clusters using a new approach, this method warrants evaluation in future studies.

CONCLUSION

In the most recent state of the science report,[26] an expert panel identified stability of symptoms within a cluster as one of the key characteristics of a symptom cluster. However, our findings suggest that while a specific cluster may be stable across time, dimensions, and/or cancer type, its consistency may vary. These findings support our hypothesis that stability and consistency are two distinct but related characteristics of symptom clusters. While various terms have been used to describe the stability of symptom clusters and the symptoms within them (e.g., stable,[26] prominent,[25] core sets of symptoms[6]), these terms were applied inconsistently. Our proposed method to evaluate the stability and consistency of clusters has the potential to advance symptom cluster research and provide direction for mechanistic studies.

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Table 5.1. Demographic and Clinical Characteristics of the Patients (n=1329)

Characteristic	Mean	SD
Age (years)	57.3	12.3
Education (years)	16.2	3.0
Body mass index (kilograms/meters squared)	26.2	5.7
Karnofsky Performance Status score	80.1	12.4
Number of comorbidities out of 13	2.4	1.4
Self-administered Comorbidity Questionnaire score	5.5	3.2
Time since cancer diagnosis (years)	2.0	3.9
Time since diagnosis (median)	0.42	
Number of prior cancer treatments (out of 9)	1.6	1.5
Number of metastatic sites including lymph node involvement (out of 9)	1.2	1.2
Number of metastatic sites excluding lymph node involvement (out of 8)	0.8	1.0
MAX2 Index of Chemotherapy Toxicity score (0 to 1)	0.17	0.08
Mean number of MSAS symptoms (out of 38)	13.9	7.2
	n	(%)
Gender		
Female	1033	77.8
Male	295	22.2
Self-Reported Ethnicity		
Asian or Pacific Islander	161	12.3
Black	95	7.2
Hispanic, Mixed, or Other	139	10.6
White	917	69.9
Married or partnered (% yes)	843	64.4
Lives alone (% yes)	283	21.6
Child care responsibilities (% yes)	286	22.0
Care of adult responsibilities (% yes)	95	7.9
Currently employed (% yes)	462	35.1
Income		
< $30,000	219	18.4
$30,000 to < $70,000	252	21.2
$70,000 to < $100,000	199	16.7
≥ $100,000	520	43.7
Exercise on a regular basis (% yes)	922	70.9
Current or history of smoking (% yes)	462	35.3
Type of cancer		
Breast	534	40.2
Gastrointestinal	407	30.6
Gynecological	233	17.5
Lung	155	11.7
Type of prior cancer treatment		
No prior treatment	323	25.0
Only CTX, surgery, or RT	543	42.0
CTX and surgery, or CTX and RT, or surgery and RT	257	19.9
CTX and surgery and RT	169	13.1

Characteristic	n	(%)
Cycle length		
14 days	558	42.1
21 days	671	50.6
28 days	97	7.3
Emetogenicity of the chemotherapy regimen		
Minimal/low	259	19.5
Moderate	810	61.0
High	258	19.4
Antiemetic regimen		
None	92	7.1
Steroid alone or serotonin receptor antagonist alone	265	20.4
Serotonin receptor antagonist and steroid	618	47.7
NK-1 receptor antagonist and two other antiemetics	321	24.8

Abbreviations: CTX, chemotherapy; MSAS, Memorial Symptom Assessment Scale; NK-1, neurokinin 1; RT, radiation therapy; SD, standard deviation

Table 5.2. Occurrence Rates and Severity and Distress Ratings for Symptoms Over One Cycle of Chemotherapy in Patients with Cancer

Symptoms[a]	Occurrence Rates % (n)			Severity Ratings with Zeros[b] Mean (SD)			Severity Ratings without Zeros[c] Mean (SD)			Distress Ratings[d] Mean (SD)		
	Time 1*	Time 2	Time 3	Time 1	Time 2	Time 3	Time 1	Time 2	Time 3	Time 1	Time 2	Time 3
Lack of energy	83.2 (1106)	86.2 (1091)	81.0 (1000)	1.67 (1.0)	1.91 (1.1)	1.64 (1.1)	2.02 (0.7)	2.23 (0.8)	2.04 (0.7)	1.79 (1.1)	1.98 (1.1)	1.75 (1.1)
Difficulty sleeping	69.1 (918)	68.2 (864)	64.3 (793)	1.38 (1.1)	1.37 (1.1)	1.25 (1.2)	2.01 (0.8)	2.04 (0.8)	1.99 (0.8)	1.79 (1.1)	1.76 (1.1)	1.72 (1.1)
Pain	60.4 (803)	65.9 (834)	60.9 (751)	1.14 (1.1)	1.30 (1.1)	1.15 (1.1)	1.92 (0.7)	2.00 (0.8)	1.94 (0.7)	1.77 (1.1)	1.92 (1.1)	1.74 (1.1)
Feeling drowsy	60.3 (801)	65.2 (825)	53.4 (659)	1.04 (1.0)	1.17 (1.1)	0.90 (1.0)	1.75 (0.7)	1.84 (0.7)	1.74 (0.7)	1.16 (1.1)	1.28 (1.1)	1.14 (1.0)
Hair loss	54.8 (728)	49.6 (628)	47.3 (584)	1.35 (1.5)	1.13 (1.4)	1.04 (1.4)	2.49 (1.1)	2.34 (1.1)	2.27 (1.1)	1.88 (1.3)	1.89 (1.4)	1.81 (1.3)
Numbness/tingling in hands/feet	52.2 (694)	53.9 (682)	50.3 (621)	0.94 (1.1)	0.99 (1.1)	0.91 (1.1)	1.84 (0.8)	1.89 (0.8)	1.87 (0.8)	1.52 (1.2)	1.57 (1.2)	1.56 (1.2)
Worrying	52.1 (692)	47.5 (601)	43.5 (537)	0.94 (1.1)	0.85 (1.0)	0.77 (1.0)	1.85 (0.7)	1.82 (0.7)	1.81 (0.8)	1.63 (1.0)	1.61 (1.0)	1.57 (1.1)
Difficulty concentrating	51.9 (690)	56.0 (709)	51.7 (638)	0.79 (0.9)	0.91 (1.0)	0.80 (0.9)	1.55 (0.6)	1.64 (0.7)	1.60 (0.7)	1.48 (1.1)	1.48 (1.1)	1.36 (1.0)
Change in the way food tastes	49.4 (656)	55.4 (701)	45.9 (567)	1.04 (1.2)	1.19 (1.3)	0.89 (1.2)	2.12 (0.9)	2.19 (0.9)	2.00 (0.9)	1.72 (1.3)	1.86 (1.2)	1.61 (1.2)
Nausea	47.5 (631)	58.5 (741)	41.0 (506)	0.82 (1.0)	1.06 (1.1)	0.69 (1.0)	1.76 (0.8)	1.86 (0.8)	1.74 (0.8)	1.65 (1.1)	1.84 (1.1)	1.61 (1.1)
Feeling sad	46.0 (612)	45.3 (573)	39.0 (481)	0.77 (1.0)	0.76 (1.0)	0.66 (1.0)	1.71 (0.7)	1.73 (0.7)	1.74 (0.7)	1.50 (1.1)	1.59 (1.0)	1.51 (1.0)
Dry mouth	45.4 (603)	44.3 (561)	33.7 (416)	0.77 (1.0)	0.76 (1.0)	0.58 (0.9)	1.73 (0.8)	1.77 (0.8)	1.77 (0.8)	1.23 (1.1)	1.24 (1.1)	1.25 (1.1)
Constipation	43.5 (578)	46.3 (586)	33.5 (414)	0.84 (1.1)	0.89 (1.1)	0.63 (1.0)	1.98 (0.8)	1.97 (0.8)	1.94 (0.8)	1.70 (1.2)	1.70 (1.1)	1.65 (1.1)
Feeling irritable	41.3 (549)	43.8 (554)	40.9 (505)	0.69 (1.0)	0.73 (1.0)	0.66 (0.9)	1.70 (0.7)	1.71 (0.7)	1.66 (0.7)	1.46 (1.0)	1.50 (1.0)	1.43 (1.0)
Lack of appetite	41.3 (549)	50.1 (634)	37.0 (456)	0.78 (1.1)	0.98 (1.1)	0.67 (1.0)	1.92 (0.8)	2.00 (0.8)	1.87 (0.8)	1.28 (1.1)	1.39 (1.1)	1.28 (1.2)
Feeling nervous	38.0 (505)	31.4 (397)	26.3 (324)	0.59 (0.9)	0.49 (0.8)	0.42 (0.8)	1.62 (0.7)	1.63 (0.7)	1.65 (0.7)	1.41 (1.0)	1.48 (1.0)	1.46 (1.1)
“I don't look like myself”	37.9 (503)	40.2 (509)	38.5 (475)	0.80 (1.2)	0.84 (1.2)	0.77 (1.1)	2.15 (0.9)	2.16 (1.0)	2.04 (0.9)	1.98 (1.2)	1.98 (1.2)	1.90 (1.2)
Changes in skin	36.3 (482)	37.8 (478)	32.7 (403)	0.68 (1.0)	0.69 (1.0)	0.59 (1.0)	1.91 (0.8)	1.87 (0.8)	1.86 (0.8)	1.64 (1.2)	1.60 (1.2)	1.58 (1.2)
Feeling bloated	33.1 (440)	31.1 (394)	27.1 (334)	0.58 (0.9)	0.56 (0.9)	0.47 (0.9)	1.79 (0.7)	1.85 (0.8)	1.79 (0.7)	1.54 (1.1)	1.60 (1.1)	1.55 (1.0)
Cough	32.6 (433)	28.9 (366)	28.8 (355)	0.45 (0.8)	0.42 (0.8)	0.44 (0.8)	1.42 (0.6)	1.53 (0.7)	1.59 (0.7)	1.02 (1.1)	1.17 (1.1)	1.28 (1.1)
Hot flashes	31.8 (423)	29.7 (376)	26.7 (329)	0.58 (1.0)	0.54 (1.0)	0.48 (0.9)	1.87 (0.8)	1.89 (0.8)	1.89 (0.8)	1.42 (1.2)	1.49 (1.2)	1.49 (1.1)
Dizziness	31.3 (416)	32.7 (414)	24.2 (299)	0.46 (0.8)	0.49 (0.8)	0.36 (0.7)	1.51 (0.7)	1.54 (0.7)	1.53 (0.7)	1.24 (1.0)	1.34 (1.0)	1.34 (0.9)
Sweats	31.2 (415)	28.6 (362)	22.8 (281)	0.53 (0.9)	0.49 (0.9)	0.40 (0.9)	1.77 (0.8)	1.77 (0.8)	1.84 (0.8)	1.29 (1.1)	1.30 (1.1)	1.42 (1.1)
Problems with sexual interest/activity	29.9 (397)	26.7 (338)	25.7 (317)	0.71 (1.2)	0.64 (1.2)	0.62 (1.2)	2.47 (1.0)	2.49 (0.9)	2.49 (1.0)	1.87 (1.3)	1.98 (1.3)	2.00 (1.3)
Diarrhea	29.6 (393)	28.4 (360)	25.8 (318)	0.54 (1.0)	0.52 (0.9)	0.46 (0.9)	1.87 (0.8)	1.88 (0.8)	1.84 (0.8)	1.46 (1.1)	1.53 (1.1)	1.51 (1.1)
Shortness of breath	26.9 (357)	24.9 (315)	21.7 (268)	0.44 (0.8)	0.41 (0.8)	0.36 (0.8)	1.67 (0.7)	1.71 (0.7)	1.70 (0.7)	1.51 (1.0)	1.51 (1.1)	1.50 (1.0)
Increased appetite	25.9 (344)	20.5 (260)	23.0 (284)	0.44 (0.8)	0.35 (0.8)	0.38 (0.8)	1.75 (0.7)	1.78 (0.7)	1.72 (0.7)	0.91 (1.1)	1.05 (1.2)	0.98 (1.2)
Weight gain	25.4 (337)	20.6 (260)	20.9 (258)	0.39 (0.8)	0.31 (0.7)	0.32 (0.7)	1.58 (0.7)	1.58 (0.7)	1.64 (0.8)	1.37 (1.3)	1.51 (1.4)	1.55 (1.4)
Weight loss	25.2 (335)	26.2 (332)	21.1 (260)	0.38 (0.8)	0.39 (0.7)	0.31 (0.7)	1.56 (0.7)	1.53 (0.7)	1.56 (0.8)	0.96 (1.2)	1.02 (1.1)	1.05 (1.2)
Itching	24.8 (330)	21.1 (267)	19.7 (243)	0.41 (0.8)	0.35 (0.8)	0.32 (0.7)	1.71 (0.7)	1.72 (0.7)	1.72 (0.7)	1.28 (1.1)	1.40 (1.1)	1.31 (1.0)
Abdominal cramps	22.5 (299)	26.9 (340)	19.0 (235)	0.40 (0.8)	0.49 (0.9)	0.34 (0.8)	1.87 (0.8)	1.91 (0.8)	1.89 (0.8)	1.61 (1.1)	1.67 (1.1)	1.64 (1.1)
Mouth sores	20.9 (278)	21.1 (267)	20.3 (251)	0.34 (0.8)	0.35 (0.8)	0.34 (0.8)	1.70 (0.7)	1.74 (0.8)	1.69 (0.8)	1.46 (1.1)	1.47 (1.1)	1.46 (1.1)

Symptoms[a]	Occurrence Rates % (n)			Severity Ratings with Zeros[b] Mean (SD)			Severity Ratings without Zeros[c] Mean (SD)			Distress Ratings[d] Mean (SD)		
	Time 1*	Time 2	Time 3	Time 1	Time 2	Time 3	Time 1	Time 2	Time 3	Time 1	Time 2	Time 3
Difficulty breathing	19.9 (265)	17.4 (220)	14.9 (184)	0.32 (0.7)	0.28 (0.7)	0.23 (0.6)	1.64 (0.7)	1.74 (0.7)	1.68 (0.7)	1.63 (1.1)	1.63 (1.1)	1.56 (1.1)
Chest tightness	17.8 (237)	16.5 (209)	11.8 (145)	0.27 (0.6)	0.25 (0.7)	0.18 (0.6)	1.54 (0.7)	1.61 (0.7)	1.65 (0.6)	1.42 (1.0)	1.50 (1.0)	1.54 (1.0)
Swelling of arms or legs	14.6 (194)	13.3 (168)	13.8 (170)	0.27 (0.7)	0.23 (0.7)	0.24 (0.7)	1.91 (0.8)	1.82 (0.9)	1.82 (0.8)	1.62 (1.2)	1.56 (1.2)	1.59 (1.2)
Problems with urination	14.1 (187)	14.8 (187)	11.8 (145)	0.24 (0.7)	0.25 (0.7)	0.20 (0.6)	1.79 (0.8)	1.73 (0.8)	1.79 (0.8)	1.51 (1.2)	1.53 (1.2)	1.71 (1.2)
Difficulty swallowing	13.8 (183)	15.6 (198)	12.2 (151)	0.23 (0.7)	0.26 (0.7)	0.21 (0.6)	1.73 (0.8)	1.75 (0.8)	1.76 (0.8)	1.64 (1.2)	1.60 (1.1)	1.63 (1.2)
Vomiting	12.3 (164)	14.5 (184)	9.0 (111)	0.21 (0.7)	0.25 (0.7)	0.15 (0.5)	1.80 (0.9)	1.82 (0.8)	1.79 (0.8)	1.74 (1.2)	1.68 (1.3)	1.76 (1.2)

Abbreviation: SD, standard deviation; *Orientation column in rank order

Timing of symptom assessments: Time 1 = prior to the initiation of next cycle of chemotherapy (i.e., recovery from the first or second cycle of chemotherapy), Time 2 = approximately one week after chemotherapy (i.e., acute symptoms), Time 3 = approximately two weeks after chemotherapy (i.e., potential nadir).

[a]Symptoms from the Memorial Symptom Assessment Scale with the addition of the following six symptoms: chest tightness, difficulty breathing, increased appetite, hot flashes, abdominal cramps, weight gain.

[b]Severity ratings with zeros: 0 = did not have the symptoms, 1 = slight, 2 = moderate, 3 = severe, 4 = very severe

[c]Severity ratings without zeros: 1 = slight, 2 = moderate, 3 = severe, 4 = very severe

[d]Distress ratings: 0 = not at all, 1 = a little bit, 2 = somewhat, 3 = quite a bit, 4 = very much

Table 5.3. Number and Types of Symptoms within Each Symptom Cluster Over a Cycle of Chemotherapy Using Ratings of Occurrence, Severity, and Distress[a]

Symptom Cluster	Symptoms	Time 1			Time 2			Time 3		
		Occurrence	Severity	Distress	Occurrence	Severity	Distress	Occurrence	Severity	Distress
Psychological	Worrying	**0.864**	**0.866**	**0.875**	**0.859**	**0.858**	**0.874**	**0.906**	**0.856**	**0.874**
	Feeling sad	**0.855**	**0.850**	**0.872**	**0.831**	**0.848**	**0.816**	**0.823**	**0.845**	**0.868**
	Feeling nervous	**0.744**	**0.750**	**0.760**	**0.682**	**0.682**	**0.701**	**0.788**	**0.749**	**0.766**
	Feeling irritable	0.626	0.569	0.574	0.670	0.680	0.646	0.652	0.650	0.682
	Difficulty concentrating	0.549	0.517	0.560	0.621	0.545	0.558	0.659	0.596	0.587
	"I don't look like myself"	0.458	–	0.427	0.591	0.627	0.553	0.422	–	0.590
	Problems with sexual interest or activity	–	–	–	0.510	0.413	0.456	0.467	–	–
	Difficulty sleeping	–	–	–	0.444	–	–	0.436	0.425	–
	Lack of energy	–	–	–	0.403	–	0.429	0.574	0.441	0.469
	Sweats	–	–	–	–	–	0.416	–	–	–
	Total number of symptoms	6/10	5/10	6/10	9/10	7/10	9/10	9/10	7/10	7/10
Weight gain	Weight gain	**0.921**	**0.875**	**0.914**	**0.875**	**0.858**	**0.923**	**0.824**	**0.818**	**0.912**
	Increased appetite	**0.785**	**0.746**	**0.736**	**0.711**	**0.695**	**0.708**	**0.666**	**0.664**	**0.724**
	Lack of appetite	–	–	–	-0.494	-0.498	-0.443	–	–	–
	Feeling bloated	–	–	–	–	–	–	0.416	–	–
	Total number of symptoms	2/4	2/4	2/4	3/4	3/4	3/4	3/4	2/4	2/4
Respiratory	Difficulty breathing	**1.037**	**1.032**	**1.035**	**0.971**	**0.972**	**0.958**	**0.965**	**0.974**	**0.964**
	Shortness of breath	**0.716**	**0.763**	**0.741**	**0.821**	**0.846**	**0.843**	**0.856**	**0.845**	**0.863**
	Chest tightness	**0.689**	**0.614**	**0.628**	**0.677**	**0.618**	**0.617**	**0.710**	**0.610**	**0.644**
	Cough	0.457	0.430	0.427	0.466	0.438	0.429	0.483	0.440	0.437
	Total number of symptoms	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4
Gastrointestinal	Lack of appetite	**0.784**	**0.774**	**0.770**	**0.691**	**0.687**	**0.691**	**0.669**	0.622	**0.762**
	Weight loss	**0.679**	**0.658**	**0.680**	0.443	0.459	0.537	0.579	0.592	**0.690**
	Nausea	**0.663**	0.624	0.612	**0.867**	**0.766**	**0.610**	**0.873**	**0.735**	**0.693**
	Change in the way food tastes	0.612	**0.690**	**0.677**	–	–	0.498	–	–	0.481
	Vomiting	0.546	0.538	0.525	**0.735**	**0.738**	**0.638**	**0.777**	0.639	0.686
	Difficulty swallowing	0.513	0.517	0.503	0.411	0.478	0.566	–	–	0.442
	Abdominal cramps	0.455	0.472	0.444	0.465	0.531	0.511	0.569	**0.666**	0.583
	Diarrhea	0.433	0.483	0.455	0.479	0.549	0.501	0.634	**0.688**	0.634
	Dry mouth	0.431	0.472	0.474	0.492	0.424	0.483	–	–	–
	Constipation	0.430	–	–	–	–	–	–	–	0.401
	Dizziness	0.404	–	–	–	0.421	0.407	–	–	–
	Mouth sores	–	0.420	–	–	–	–	–	–	–
	Lack of energy	–	–	–	0.454	–	–	–	–	–
	Total number of symptoms	11/13	10/13	9/13	9/13	9/13	10/13	6/13	6/13	9/13
Hormonal	Hot flashes	**0.883**	**0.907**	**0.920**						0.843
	Sweats	**0.670**	**0.728**	**0.647**			Not identified			0.799
	Total number of symptoms	2/2	2/2	2/2						2/2
Body image	Changes in skin							0.595	0.582	
	"I don't look like myself"			Not identified				0.512	0.558	Not identified
	Change in the way food tastes							0.461	0.496	
	Total number of symptoms							3/3	3/3	

[a]Extraction method: unweighted least squares. Rotation method: Geomin (oblique) rotation.
Timing of symptom assessments: Time 1 = prior to the initiation of next cycle of chemotherapy (i.e., recovery from the first or second cycle of chemotherapy), Time 2 = approximately one week after chemotherapy (i.e., acute symptoms), Time 3 = approximately two weeks after chemotherapy (i.e., potential nadir).
For total number of symptoms, the numerator represents the number of symptoms identified at the corresponding time point according to the corresponding dimension of the symptom experience. The denominator represents the total number of symptoms identified across all time points and according to all dimensions of the symptom experience.
Not identified = This symptom cluster was not identified at the corresponding time point according to the corresponding dimension of the symptom experience.
− = Factor loadings for these symptoms were <0.40.
Bold font indicates the highest factor loading.

Table 5.4. Comparison of Stability of Symptom Clusters Across the Total Sample and Individual Cancer Types Using Ratings of Occurrence, Severity, and Distress

Symptom dimension	Symptom cluster	Total Sample (n=1329)			Breast[a] (n=534)			GI[b] (n=399)			GYN[c] (n=232)			Lung[d] (n=145)		
		T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
Occurrence	Psychological	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	GI	●	●	●	●	●	●	●			●	●	●			
	Epithelial/GI													●		●
	Epithelial				●	●	●		●	●					●	
	Body image			●												
	Nutritional					●	●							●	●	●
	Weight change				●			●	●	●	●	●	●			
	Weight gain	●	●	●												
	Respiratory	●	●	●							●	●	●			
	Lung CA-specific													●	●	●
	Hormonal	●			●	●	●				●	●	●			
	CTX related							●	●	●						
	Sickness behavior				●									●	●	●
Severity	Psychological	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
	GI	●	●	●	●	●	●	●				●	●			
	GI/epithelial										●					
	Epithelial/GI													●		●
	Epithelial				●	●	●		●	●					●	
	Body image			●												
	Nutritional					●	●							●	●	●
	Weight change				●			●	●	●	●	●	●			
	Weight gain	●	●	●												
	Respiratory	●	●	●							●	●	●			
	Lung CA-specific													●	●	●
	Hormonal	●			●	●	●				●	●	●			
	CTX neuropathy					●										
	CTX related							●	●	●						
	Sickness behavior				●										●	●
Distress	Psychological	●	●	●	NA			●	●	●		●	●	NA		
	Psychological/GI										●					
	GI	●	●	●				●				●	●			
	GI/epithelial										●					
	Epithelial								●	●						
	Weight change							●	●	●	●	●	●			
	Weight gain	●	●	●												
	Respiratory	●	●	●							●	●	●			
	Hormonal	●		●							●	●	●			
	CTX related							●	●	●						

Abbreviations: CA, cancer; CTX, chemotherapy; GI, gastrointestinal; GYN, gynecological; NA, dimension not assessed

[a]Sullivan CW, Leutwyler H, Dunn LB, et al. Stability of symptom clusters in patients with breast cancer receiving chemotherapy. J Pain Symptom Manage. 2018;55(1):39-55. doi:10.1016/j.jpainsymman.2017.08.008

[b]Han CJ, Reding K, Cooper BA, et al. Stability of symptom clusters in patients with gastrointestinal cancers receiving chemotherapy. *J Pain Symptom Manage*2019;58(6):989-1001. doi:10.1016/j.jpainsymman.2019.07.029

[c]Pozzar RA, Hammer MJ, Cooper BA, et al. Stability of symptom clusters in patients with gynecologic cancer receiving chemotherapy. *Cancer Nurs*[Preprint]. September 23, 2021. doi:10.1097/NCC.0000000000000988

[d]Russell J, Wong ML, Mackin L, et al. Stability of symptom clusters in patients with lung cancer receiving chemotherapy. *J Pain Symptom Manage*2019;57(5):909-922. doi:10.1016/j.jpainsymman.2019.02.002

Table 5.5. Consistency of Symptoms within Each Symptom Cluster Over Time and Across Dimensions of the Symptom Experience for the Total Sample

Symptom cluster	Time point	Occurrence	Severity	Distress	Symptom agreement over time[a]
Psychological	Time 1	Worrying	Worrying	Worrying	3 of 3
		Feeling sad	Feeling sad	Feeling sad	
		Feeling nervous	Feeing nervous	Feeling nervous	
	Time 2	Worrying	Worrying	Worrying	3 of 3
		Feeling sad	Feeling sad	Feeling sad	
		Feeling nervous	Feeling nervous	Feeling nervous	
	Time 3	Worrying	Worrying	Worrying	3 of 3
		Feeling sad	Feeling sad	Feeling sad	
		Feeling nervous	Feeling nervous	Feeling nervous	
Symptom agreement across dimensions[b]		3 of 3	3 of 3	3 of 3	
Weight gain	Time 1	Weight gain	Weight gain	Weight gain	2 of 2
		Increased appetite	Increased appetite	Increased appetite	
		-	-	-	
	Time 2	Weight gain	Weight gain	Weight gain	2 of 2
		Increased appetite	Increased appetite	Increased appetite	
		Lack of appetite	Lack of appetite	Lack of appetite	
	Time 3	Weight gain	Weight gain	Weight gain	2 of 2
		Increased appetite	Increased appetite	Increased appetite	
		Feeling bloated	-	-	
Symptom agreement across dimensions		2 of 2	2 of 2	2 of 2	
Respiratory	Time 1	Difficulty breathing	Difficulty breathing	Difficulty breathing	3 of 3
		Shortness of breath	Shortness of breath	Shortness of breath	
		Chest tightness	Chest tightness	Chest tightness	
	Time 2	Difficulty breathing	Difficulty breathing	Difficulty breathing	3 of 3
		Shortness of breath	Shortness of breath	Shortness of breath	
		Chest tightness	Chest tightness	Chest tightness	
	Time 3	Difficulty breathing	Difficulty breathing	Difficulty breathing	3 of 3
		Shortness of breath	Shortness of breath	Shortness of breath	
		Chest tightness	Chest tightness	Chest tightness	
Symptom agreement across dimensions		3 of 3	3 of 3	3 of 3	
Gastrointestinal	Time 1	Lack of appetite	Lack of appetite	Lack of appetite	2 of 3
		Weight loss	Change in the way food tastes	Weight loss	
		Nausea	Weight loss	Change in the way food tastes	
	Time 2	Nausea	Nausea	Lack of appetite	3 of 3
		Vomiting	Vomiting	Vomiting	
		Lack of appetite	Lack of appetite	Nausea	
	Time 3	Nausea	Nausea	Lack of appetite	1 of 3
		Vomiting	Diarrhea	Nausea	
		Lack of appetite	Abdominal cramps	Weight loss	
Symptom agreement across dimensions		2 of 3	0 of 3	1 of 3	
Hormonal	Time 1	Hot flashes	Hot flashes	Hot flashes	2 of 2
		Sweats	Sweats	Sweats	
		-	-	-	
	Time 2	NI	NI	NI	NA
	Time 3	NI	NI	Hot flashes	NA
				Sweats	
Symptom agreement across dimensions		NA	NA	NA	

Symptom cluster	Time point	Occurrence	Severity	Distress	Symptom agreement over time[a]
Body image	Time 1	NI	NI	NI	NA
	Time 2	NI	NI	NI	NA
	Time 3	Changes in skin	Changes in skin	NI	NA
		"I don't look like myself"	"I don't look like myself"		
		Change in the way food tastes	Change in the way food tastes		
Symptom agreement across dimensions		NA	NA	NA	

Timing of symptom assessments: Time 1 = prior to the initiation of next cycle of chemotherapy (i.e., recovery from the first or second cycle of chemotherapy), Time 2 = approximately one week after chemotherapy (i.e., acute symptoms), Time 3 = approximately two weeks after chemotherapy (i.e., potential nadir).
[a]Calculated as the number of symptoms out of two or three that were identified across the three time points
[b]Calculated as the number of symptoms out of two or three that were identified across the three symptom dimensions (i.e., occurrence, severity, distress)
NA = Symptom agreement was not assessed.
NI = This symptom cluster was not identified.
− = Only two symptoms were identified at a dimension and/or time point.

Chapter 6

Evaluation of Epigenetic Regulation of Inflammatory Mechanisms Associated with a Psychological Symptom Cluster in Patients Receiving Chemotherapy

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ABSTRACT

Introduction: A psychological symptom cluster is the most common cluster identified in oncology patients. While inflammatory mechanisms are hypothesized to underlie this cluster, epigenetic contributions are unknown. Study purpose was to evaluate for associations between the occurrence of a psychological symptom cluster and levels of DNA methylation for inflammatory genes.

Methods: Prior to their second or third cycle of chemotherapy, 1071 patients reported on the occurrence of 38 symptoms using the Memorial Symptom Assessment Scale. A psychological cluster was identified using exploratory factor analysis. Differential methylation analyses were performed in two independent samples using Illumina Infinium 450K (n=146) and EPIC (n=925) microarrays. Expression-associated CpG (eCpG) loci in the promoter region of 114 inflammatory genes on the 450K and 112 genes on the EPIC microarray were evaluated for associations with the psychological cluster. Robust Rank Aggregation was used to identify genes that were differentially methylated across both samples.

Results: Cluster of differentiation 40 (*CD40*) was differentially methylated across both samples (false discovery rate=.017). All six promoter eCpGs for *CD40* (i.e., cg22232207, cg06571407, cg17929951, cg21601405, cg01943874, cg11841529) that were identified across both samples were hypomethylated in the psychological cluster group.

Conclusions: This study is the first to suggest associations between a psychological symptom cluster and differential DNA methylation of a gene that is involved in tissue inflammation and cell mediated immunity. Findings suggest that increased *CD40* expression through hypomethylation of promoter eCpG loci is involved in the occurrence of a psychological symptom cluster in patients receiving chemotherapy.

Keywords: anxiety; cancer; chemotherapy; depression; DNA methylation; inflammation; psychological symptom cluster

INTRODUCTION

As noted in two reviews,[1, 2] a psychological symptom cluster is the most common cluster identified in patients receiving chemotherapy. This cluster is observed across cancer types, persists over time,[3] and is stable across various dimensions of the symptom experience.[1, 2, 4] In addition, the psychological symptom cluster (referred to as psychological cluster in the remainder of this manuscript) is associated with decrements in functional status[5-7] and quality of life.[5, 8, 9] In our previous study,[4] this cluster consisted of primarily anxious (i.e., worrying, feeling nervous, feeling irritable) and depressive (i.e., feeling sad, difficulty concentrating, "I don't look like myself")[10] symptoms (Figure 1). Individually, clinical or subclinical levels of anxiety and depression occur in 41.6% and 29.4% of oncology patients, respectively.[11] However, as noted in one study,[12] 34.0% of oncology patients experience both symptoms. This finding suggests a strong association between these two symptoms and explains their inclusion in a psychological cluster.

Given the strong relationship between these two symptoms and the ubiquitous nature and negative impact of the psychological cluster on patients with cancer, investigation into the mechanism(s) that underlie this cluster is warranted. In the psychiatric literature, both anxiety[13, 14] and depressive disorders[15, 16] are associated with inflammatory processes. However, studies on the associations between concurrent anxiety and depressive symptoms and inflammatory markers in oncology patients are limited. In a series of two studies that used the Hospital Anxiety and Depression Scale (HADS) to identify colorectal cancer patients with concurrent anxiety and depression (i.e., HADS total score of >19),[17, 18] associations between group membership and serum levels of a number of cytokines (i.e., interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-12, tumor necrosis factor (TNF)-α, transforming growth factor (TFG)-β) were evaluated. Across both studies, in the patients with concurrent anxiety and depression, higher HADS scores were associated with increased levels of IL-1β, Il-6, IL-8, and TNF-α and lower serum levels of IL-10. No associations were found with IL-12 and TGF-β. While these findings support

an association between the co-occurrence of anxiety and depression and inflammatory mechanisms in oncology patients, the sample sizes were very small (n=20 per group); only patients with colorectal cancer were included; and only seven cytokines were evaluated. Given these limitations, additional research is warranted on the relationships between psychological symptoms and inflammatory mechanisms.

Deoxyribonucleic acid (DNA) methylation is an epigenetic mechanism that regulates gene expression by adding or removing methyl groups at the 5'-position of cytosine residues.[19] DNA methylation can be used to evaluate for changes in gene regulation that occur in response to environmental stimuli and stressors.[20] While the physiologic and psychological stress associated with a cancer diagnosis and its treatments can impact the epigenome,[21] less is known about its impact on symptom burden. Previous research in oncology patients found that cognitive impairment[22] and fatigue[23] were associated with epigenetic changes in genes involved in inflammatory processes or immune function. An increased understanding of the associations between a psychological cluster and epigenetic regulation of inflammatory processes may provide insights into its underlying mechanism(s). In addition, DNA methylation is potentially modifiable,[24] making it a potential target for therapeutic interventions.[25]

In patients without cancer, recent evidence suggests that anxiety[26, 27] and depressive disorders[28-32] are associated with methylation of inflammatory genes. For example, in a population-based cohort study that compared individuals with no or minimal anxiety to those with severe anxiety,[27] increased methylation of a single CpG locus in the promoter region of the ankyrin repeat and suppressor of cytokine signaling box containing 1 (*ASB1*) gene was associated with being in the severe anxiety group. This finding was confirmed in an independent sample of patients with anxiety disorders. Of note, the product of this gene is involved in the regulation of cytokine signaling.

In terms of depressive symptoms, a cohort study of elderly men evaluated for associations between depression scores and levels of methylation in CpG-rich promoter regions

of seven genes involved in immune or inflammatory processes.[32] Higher depression scores were associated with higher average promoter methylation of coagulation factor III (*F3*) and intercellular adhesion molecule-1 (*ICAM-1*). However, no associations were found with serum levels of ICAM-1.

While in previous reviews,[21, 33] epigenetic modifications were hypothesized to play a role in the development of psychological symptoms in oncology patients, no study has evaluated for associations between a psychological cluster and epigenetic regulation of inflammatory mechanisms. Therefore, in a sample of outpatients receiving chemotherapy, the purpose of this study was to evaluate for associations between a psychological cluster and levels of DNA methylation using a panel of inflammatory genes.

METHODS

Patients and settings

This analysis is part of a larger study that evaluated symptom clusters in oncology outpatients receiving chemotherapy.[4] Eligible patients were ≥18 years of age; had a diagnosis of breast, lung, gastrointestinal, or gynecologic 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 four 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. Data from the enrollment assessment (i.e., symptoms in the week prior to the patient's second or

third cycle of chemotherapy) were used in this analysis. At enrollment, a total of 1071 patients provided a blood sample for the DNA methylation analyses. Medical records were reviewed for disease and treatment information.

Instruments

Patients completed a demographic questionnaire, Karnofsky Performance Status (KPS) scale,[34] and Self-Administered Comorbidity Questionnaire.[35] Toxicity of each patient's chemotherapy regimen was rated using the MAX2 index.[36, 37]

A modified version of the 32-item MSAS was used to evaluate the occurrence, severity, and distress of 38 common symptoms associated with cancer and its treatment.[38] Six additional symptoms were added: hot flashes, chest tightness, difficulty breathing, abdominal cramps, increased appetite, and weight gain. Using the MSAS, patients were asked to indicate whether they had experienced each symptom in the past week (i.e., symptom occurrence). The patients' responses to the occurrence items were used to create the symptom clusters. The validity and reliability of the MSAS are well-established.[38]

Phenotypic analyses

Descriptive statistics and frequency distributions were calculated for the demographic and clinical characteristics, using the Statistical Package for the Social Sciences Version 27 (IBM Corporation, Armonk, NY). Exploratory factor analysis (EFA) was used to identify symptom clusters using Mplus Version 8.6.[39]

Methods for the EFA were reported elsewhere.[4] In brief, for the EFA using the dichotomous occurrence items, tetrachoric correlations were used to create the matrix of associations.[39] The simple structure for the occurrence EFA was estimated using the method of unweighted least squares with geomin (i.e., oblique) rotation.[39] Factor loadings were considered meaningful if the loading was ≥0.40.[39] Factors (i.e., symptom clusters) were adequately defined if at least two items (i.e., symptoms) had loadings of ≥0.40.[40] Clusters were named based on the symptoms with the highest factor loadings and the majority of the symptoms within the cluster.

With these methods, a psychological cluster (Figure 1) was identified in our previous analysis.[4] A factor score was calculated as the sum of the occurrence rates for the six symptoms in this cluster (range of 0 to 6). Initially, the DNA methylation analyses were conducted using the patients' symptom cluster factor scores as continuous values. However, the p-value distribution for the differential methylation tests across the genome was severely conservative (i.e., underabundance of low p-values; data not shown). Therefore, for the current analyses, the total factor score was dichotomized into two groups (i.e., 0 symptoms = no psychological cluster group versus 1 to 6 symptoms = psychological cluster group).

Selection of DNA methylation loci

To evaluate the hypothesis that inflammatory mechanisms may underlie a psychological cluster, a comprehensive list of 1,027 genes involved in immune and inflammatory processes (e.g., cytokine signaling, nuclear factor kappa B (NF-κB) signaling) was identified from the literature.[41] Then, CpG sites that reside in the promoter region[42] of these genes and are known to have methylation values associated with changes in gene expression[43] (i.e., expression-associated CpG (eCpG)) were used in our analyses.

Biospecimen processing, quantification of methylation status, and quality control

Methods for the methylation analyses are described in detail elsewhere.[44] In brief, DNA was extracted from archived buffy coats using the PUREGene DNA isolation kit (Invitrogen, Carlsbad, CA); quantified using a NanoDrop UV spectrophotometer (Thermo Fisher Scientific, Waltham, MA); and normalized to a concentration of 50 ng/μL. DNA was bisulfite converted using the Zymo EZ-96 DNA Methylation Kit (Catalog #D5004) Deep-Well Format (Zymo Research, Irvine, CA) and used as input for the Illumina Infinium HD Methylation Assay (Illumina, San Diego, CA).

Of the 1071 patients in this study, DNA methylation was measured for 146 patients using the Infinium HumanMethylation 450 BeadChip (i.e., 450K microarray sample) and for 925 patients using the Infinium MethylationEPIC BeadChip (i.e., EPIC microarray sample; Illumina,

Inc., San Diego, CA). All of the samples were scanned on the Illumina iScan (Illumina, Inc., San Diego, CA). Preliminary analysis and quality control procedures were performed using GenomeStudio (Illumina, Inc., San Diego, CA). Samples that had <90% of their targets detected at a p-value of ≤0.01 were flagged for review. Sample replicates and Jurkat control replicates were checked to ensure an r^2 value of >0.99.

Subsequent analyses were done using well-established protocols in R (version 4.1.0).[45] Corrections for Infinium I and II probes, balance correction, background correction, and quantile normalization were performed using the minfi package in R (version 1.40.0).[46, 47] Probes that contained a single nucleotide polymorphism at a CpG or flanking site and probes that aligned with multiple places on the genome were excluded.[48] Methylation scores were quantified as M-values.[49]

DNA methylation analyses

Given that DNA methylation levels differ among blood cell types,[50] cell types were estimated using the *estimateCellCounts2()* function in the FlowSorted.Blood.EPIC R package (version 1.10.1).[51] Cell type deconvolution was performed using the IDOL L-DMR library for cluster of differentiation 8 (CD8) and CD4 T-cells, natural killer (NK) cells, B cells, monocytes, and neutrophils.[52] Differences in estimates of cell type composition between the psychological cluster groups were evaluated using Welch two sample t-tests and assessed for significance at a p-value of <0.05. Any cell type composition estimates that were significantly associated with membership in the psychological cluster group were included as covariates in the final model. Given that methylation status changes over the lifespan,[53] age was included as a covariate in the final regression models. Surrogate variable analysis, using the Leek method (R package version 3.4.0),[54] was used to estimate surrogate variables for technical and non-technical variations that contributed to heterogeneity in the sample that were not due to the psychological cluster group, age, or cell type.

To evaluate for associations between the psychological cluster group and methylation status of regulatory regions of inflammatory genes, tests for differentially methylated probes (DMPs) were done using a generalized linear model implemented in the limma R package using the “ls” method (version 3.48.3).[55] For genes with multiple eCpG loci, in order to examine them as a region,[56] Fisher’s Combined Probability test was used to combine the DMP tests using their uncorrected p-values (Supplemental Figure 1).[57, 58] Using this approach, all tests for differential methylation of loci within the promoter region of a given gene were represented by a single, uncorrected p-value.

In order to identify findings across the 450K and EPIC microarrays, we used RobustRankAggreg (version 1.1).[59, 60] Rank aggregation meta-analytic approaches are used with information retrieval, marketing, and high-throughput data sets to integrate data from multiple ranked lists.[61] In addition, rank aggregation techniques are invariant to transformation and normalization and robust to outliers.[60] First, the gene lists from each sample were individually ranked using the uncorrected p-values from the differential methylation analyses. Then, the genes from both samples were integrated and evaluated based on their individual rankings on the combined gene list. Finally, a single p-value was assigned to each gene based on how “better it was positioned in the ranked lists than was expected by chance” (p.574).[59] The significance of this ranked set of genes was assessed using a false discovery rate (FDR) of 0.05 under the Benjamini-Hochberg procedure.[62]

To characterize the potential functional roles for these eCpGs, we identified the direction of expression associated with methylation levels as quantified by Kennedy and colleagues[43] in their eCpG dataset. In addition, we evaluated for evidence of regulatory elements in the region surrounding the loci using annotation data from the Encyclopedia of DNA Elements (ENCODE)[63] obtained from the University of California Santa Cruz Genome Browser.[64] Finally, we identified predicted functional partners of genes with differentially methylated promoter

eCpGs from a protein-protein interaction network that was created using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database.[65]

RESULTS

Demographic and clinical characteristics

Of the 146 patients in the 450K microarray sample, 100% were female, 65.5% were White, 67.6% were married or partnered, and had a mean age of 52.7 (±11.7) years (Table 1). Most patients were well-educated (16.3 ±2.9 years), exercised on a regular basis (75.7%), and had never smoked (72.4%). Patients had an average 2.4 (±1.4) comorbid conditions and a KPS score of 79.1 (±11.6). The most common type of cancer was breast (99.3%) followed by gastrointestinal (0.7%). Majority of patients (76.7%) had received either chemotherapy, surgery, and/or radiation therapy. Patients reported 16.0 (±7.8) concurrent symptoms before their second or third cycle of chemotherapy.

Of the 925 patients in the EPIC microarray sample, one was excluded for insufficient phenotypic data and one for poor sample quantification. Of the remaining 923 patients, 76.2% were female, 69.4% were White, 64.1% were married or partnered, and had a mean age of 57.5 (±12.2) years (Table 2). Most patients were well-educated (16.1 ±3.0 years), exercised on a regular basis (71.6%), and had never smoked (66.4%). Patients had an average of 2.4 (±1.4) comorbid conditions and a KPS score of 80.4 (±12.6). The most common type of cancer was breast (39.5%), followed by gastrointestinal (34.0%), gynecological (15.9%), and lung (10.5%). Majority of patients (73.3%) had received either chemotherapy, surgery, and/or radiation therapy. Patients reported 13.5 (±7.1) concurrent symptoms before their second or third cycle of chemotherapy.

DNA methylation analyses

For the 450K microarray sample, the NK cell type composition estimate was associated with the psychological cluster group and it was included with age and one surrogate variable as covariates in the final model. For this sample, of the 1027 inflammation-related genes that were

identified as candidates,[41] 283 eCpG loci across 114 genes were evaluated for differential methylation. Of note, three genes were unique to the 450K microarray (i.e., Fc gamma receptor IIa, Janus kinase 2, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein eta).

For the EPIC microarray sample, no cell type compositions were associated with the psychological cluster group. Therefore, age and the two surrogate variables were included as covariates in the final model. For this sample, 267 eCpG loci across 112 genes were tested for differential methylation. For this sample, 267 eCpG loci across 112 genes were tested for differential methylation. Of note, one gene was unique to the EPIC microarray (i.e., major histocompatibility complex, class I, A).

The robust rank aggregation method identified one differentially methylated gene across the 450K and EPIC microarray samples (i.e., *CD40*, FDR = 0.017; Table 3). All six eCpGs for *CD40* (i.e., cg22232207, cg06571407, cg17929951, cg21601405, cg01943874, cg11841529) that were identified across both the 450K and EPIC microarray samples were hypomethylated in the psychological cluster group.

DISCUSSION

This study is the first to evaluate for changes in epigenetic regulation of inflammatory mechanisms underlying a psychological cluster in patients receiving chemotherapy. Our findings suggest that membership in the psychological cluster group is linked to increased expression of the *CD40* gene through hypomethylation of multiple promoter loci. These findings build on previous research that suggests that dysregulation of a variety of inflammatory processes contributes to the development of psychological symptoms.[13, 14, 16-18, 27, 28, 31, 32]

Regulatory role of eCpGs in CD40 expression

One of the major challenges for methylation association studies is the establishment of a functional role for the epigenetic variation that is identified.[66] Without evidence of a functional role, it is difficult to distinguish between epigenetic variation as a cause or consequence of the

phenotype (i.e., symptom cluster). In this study, multiple lines of evidence support a regulatory role for the eCpG loci that were associated with the psychological cluster group. First, our results suggest that these six loci function together as a region. As illustrated in Figure 2, all six eCpG loci are located in the promoter region of *CD40* and are within 250 base pairs of each other. This finding is notable because previous findings suggest that multiple CpG sites that show similar patterns of methylation in a small region have shared regulatory functions.[66] Second, given that all six eCpG loci share the same direction of expression (i.e., hypomethylation), it suggests that they act together. Third, levels of methylation for all six loci were positively associated with increased expression of *CD40* in two previously reported independent samples,[43] which suggests a direct functional role in the expression of *CD40*. Furthermore, all six loci are located within putative regulatory regions as evidenced by independent ENCODE experiments that identified multiple types of regulatory elements. These elements include: histone protein marks that are associated with promoters or enhancers;[67] DNase I hypersensitivity clusters that are characteristic of *cis*-regulatory elements[68] that make DNA more accessible to transcription;[69] and clusters of transcription factor binding.[69] Taken together, these lines of evidence provide strong support for the hypothesis that these six loci that are associated with psychological cluster group membership act together in the regulation of *CD40* expression.

Role of CD40 in inflammatory processes

CD40 is a costimulatory protein receptor and a member of the tumor necrosis factor receptor (TNFR) superfamily.[70] Expression of *CD40* is stimulated by a variety of cytokines, including IL-3 and interferon (IFN)-γ.[71] CD40 signaling plays a central role in tissue inflammation, humoral and cell-mediated immunity,[71] and various autoimmune (e.g., irritable bowel disease, multiple sclerosis) and malignant conditions.[72] Together with its ligand CD40LG, membrane-bound CD40 forms a stimulatory immune checkpoint that is involved in T cell-dependent B cell differentiation and activation.[71] Specifically, the interaction between CD40 and

CD40LG is needed for fundamental B cell functions, including cellular proliferation, apoptosis, immunoglobulin production, and isotype switching.[73]

Situated at the beginning of the NF-κB signaling pathway (Supplemental Figure 3), CD40 signaling induces the production of NF-κB, a family of transcription factors involved in inflammatory responses as well as cell proliferation and survival.[74] Various TNFR associated factors (TRAFs) bind to the cytoplasmic domain of CD40 intracellularly and mediate its signaling to activate the canonical and non-canonical pathways within the NF-κB signaling pathway.[71] As illustrated in Figure 3, the protein product of *CD40* interacts directly with five TRAFs (i.e., TRAF1, 2, 3, 5, 6) and baculoviral inhibitor of apoptosis repeat containing 2 (BIRC2), a regulator of apoptosis and inflammatory signaling.[75] These TRAFs activate or inhibit various signaling pathways (e.g., NF-κB, mitogen activated protein kinase) and trigger the production of various inflammatory cytokines (e.g., IL-6, TNF-α).[70]

Role of CD40 in psychological disorders and/or symptoms

No studies have examined the relationships between anxiety and/or depressive symptoms and epigenetic regulation of *CD40* in oncology patients. However, multiple clinical[76-78] and pre-clinical[79, 80] studies provide evidence to support associations between depression and changes in *CD40* expression and inflammatory responses. In two studies that evaluated for associations between major depressive disorder (MDD) and inflammatory markers, platelet expression of CD40 was higher in patients newly diagnosed with MDD compared to healthy controls.[76, 78] In another study that evaluated for differences in plasma levels of pro-inflammatory cytokines and circulating monocytes in patients with MDD and suicidal ideation compared to healthy controls,[77] patients with MDD had significantly higher levels of activated CD40 expressing monocytes. In addition, these patients had increased plasma levels of IL-6 and IL-12.

Findings from two pre-clinical studies provide additional evidence to suggest that increased CD40 signaling is involved in the development of depressive symptoms and

inflammation.[79, 80] In these studies,[79, 80] the presence of depressive symptomatology was identified in mice by evaluating for specific behaviors (i.e., reduced saccharin preference or consumption indicated decreased interest or pleasure in activities; decreased weight indicated decreased appetite; decreased classical conditioning indicated cognitive impairment; decreased locomotor activity indicated sleep impairment). In the first study,[79] compared to untreated controls, mice treated with a CD40 agonist antibody exhibited symptoms characteristic of depressive symptomatology or "sickness-behavior syndrome" (i.e., reduced saccharin preference and consumption, decreased body weight, decreased classical conditioning). In the second study,[80] mice treated with this antibody exhibited weight loss, decreased activity, and had increased serum levels of TNF, IL-6, IL-10, IL-18, and IFN-γ. Taken together, these findings support the role of increased CD40 signaling in depression and inflammatory processes.

Findings from two pre-clinical studies suggest that antidepressant treatment may decrease expression of *Cd40* and other inflammatory markers.[81, 82] Using a lipopolysaccharide (LPS)-induced model of inflammation, the effects of two noradrenaline reuptake inhibitors (i.e., atomoxetine, desipramine) on the expression of inflammatory genes in the cortex of rats were evaluated.[81] Compared to controls, rats treated with LPS had increased cortical expression of *Cd40*, *Nfkb*, *Tnf*, and *Il1b*. In the rats treated with atomoxetine or desipramine prior to the administration of LPS, cortical expression of *Cd40*, *Nfkb, Tnf, Il1b*, and inducible nitric oxide synthase decreased. In another study,[82] the effect of tianeptine treatment on rat microglial cells stimulated with LPS was evaluated. While these microglial cells exhibited increased expression of *Cd40* compared to control cells, *Cd40* expression was moderated in cells treated with tianeptine. In addition, tianeptine treatment prevented the upregulation of *Tnf, Il1b, Il6*, and *Il18*.

These pre-clinical studies provide new insights into the mechanisms of action of antidepressants. In addition, they support the associations between LPS-induced inflammatory responses and depressive symptoms in humans.[16] While we do not know if the patients in our psychological cluster group were on antidepressants, our findings are consistent with previous

studies that suggest increased expression of *CD40* is associated with depressive symptoms.[81, 82]

Limitations and future directions

Several limitations warrant consideration. First, given our study's cross-sectional design, future research needs to determine whether associations between psychological cluster group membership and methylation levels change over time. Second, because the two samples were heterogeneous in terms of gender, cancer type, and sample sizes, confirmation of these findings is warranted. Third, given that we did not evaluate for antidepressant use, future research needs to evaluate the effect of antidepressants on psychological symptom cluster group membership. Fourth, due to the statistical challenges encountered with the distribution of the psychological cluster factor scores, we were unable to use these scores as a continuous value. Additional research is warranted to evaluate how to use symptom cluster factor scores in epigenetic analyses. Finally, while *CD40* is expressed as two isoforms (i.e., membrane-bound, soluble), this analysis examined only transcriptional regulatory mechanisms (i.e., total expression levels of *CD40*) and not post-transcriptional regulatory mechanisms (i.e., alternative splicing). Future research is needed to evaluate the role of the splice variants of *CD40* in the development of a psychological cluster.

CONCLUSION

This study is the first to evaluate for epigenetic regulation of inflammatory processes that underlie a psychological cluster in patients receiving chemotherapy. Our findings provide new evidence to support the hypothesis that inflammatory processes underlie the occurrence of a psychological cluster in these patients. By using a rank aggregation method to identify genes across two samples, multiple lines of evidence were integrated to identify the role of CD40 in the occurrence of the psychological cluster. These findings provide preliminary evidence to suggest that epigenetic regulation of *CD40* may be involved in the occurrence of a psychological symptom cluster and suggest a direction for mechanistic studies.

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Table 6.1. Demographic and Clinical Characteristics of the Patients in the 450K Microarray Sample (n=146)

Characteristic	Mean	SD
Age (years)	52.7	11.7
Education (years)	16.3	2.9
Body mass index (kilograms/meters squared)	26.3	6.4
Karnofsky Performance Status score	79.1	11.6
Number of comorbidities out of 13	2.4	1.4
Self-administered Comorbidity Questionnaire score	5.5	3.1
Time since cancer diagnosis (years)	3.0	4.7
Time since diagnosis (median)	0.43	
Number of prior cancer treatments (out of 9)	2.0	1.9
Number of metastatic sites including lymph node involvement (out of 9)	1.0	1.3
Number of metastatic sites excluding lymph node involvement (out of 8)	0.6	1.1
MAX2 Index of Chemotherapy Toxicity score (0 to 1)	0.20	0.09
Mean number of MSAS symptoms (out of 38)	16.0	7.8
	n	(%)
Gender		
Female	146	100.0
Self-Reported Ethnicity		
Asian or Pacific Islander	24	16.6
Black	10	6.9
Hispanic, Mixed, or Other	16	11.0
White	95	65.5
Married or partnered (% yes)	98	67.6
Lives alone (% yes)	25	17.2
Child care responsibilities (% yes)	45	31.0
Care of adult responsibilities (% yes)	14	10.5
Currently employed (% yes)	49	33.8
Income		
< $30,000	32	24.4
$30,000 to < $70,000	22	16.8
$70,000 to < $100,000	19	14.5
≥ $100,000	58	44.3
Exercise on a regular basis (% yes)	109	75.7
Current or history of smoking (% yes)	40	27.6
Type of cancer		
Breast	145	99.3
Gastrointestinal	1	0.7
Type of prior cancer treatment		
No prior treatment	34	23.3
Only CTX, surgery, or RT	62	42.5
CTX and surgery, or CTX and RT, or surgery and RT	18	12.3
CTX and surgery and RT	32	21.9
Cycle length		
14 days	48	32.9
21 days	86	58.9
28 days	12	8.2

Characteristic	n	(%)
Emetogenicity of the chemotherapy regimen		
Minimal/low	43	29.5
Moderate	57	39.0
High	46	31.5
Antiemetic regimen		
None	21	15.1
Steroid alone or serotonin receptor antagonist alone	30	21.6
Serotonin receptor antagonist and steroid	51	36.7
NK-1 receptor antagonist and two other antiemetics	37	26.6

Abbreviations: CTX, chemotherapy; MSAS, Memorial Symptom Assessment Scale; NK-1, neurokinin 1; RT, radiation therapy; SD, standard deviation

Table 6.2. Demographic and Clinical Characteristics of the Patients in the EPIC Microarray Sample (n=923)

Characteristic	Mean	SD
Age (years)	57.5	12.2
Education (years)	16.1	3.0
Body mass index (kilograms/metered squared)	26.1	5.6
Karnofsky Performance Status score	80.4	12.6
Number of comorbidities out of 13	2.4	1.4
Self-administered Comorbidity Questionnaire score	5.4	3.2
Time since cancer diagnosis (years)	1.9	3.9
Time since diagnosis (median)	0.42	
Number of prior cancer treatments (out of 9)	1.5	1.5
Number of metastatic sites including lymph node involvement (out of 9)	1.2	1.2
Number of metastatic sites excluding lymph node involvement (out of 8)	0.8	1.0
MAX2 Index of Chemotherapy Toxicity score (0 to 1)	0.17	0.08
Mean number of MSAS symptoms (out of 38)	13.5	7.1
	n	(%)
Gender		
Female	703	76.2
Male	220	23.8
Self-Reported Ethnicity		
Asian or Pacific Islander	114	12.4
Black	71	7.8
Hispanic, Mixed, or Other	95	10.4
White	636	69.4
Married or partnered (% yes)	581	64.1
Lives alone (% yes)	196	21.6
Child care responsibilities (% yes)	188	21.0
Care of adult responsibilities (% yes)	61	7.4
Currently employed (% yes)	327	35.9
Income		
< $30,000	142	17.3
$30,000 to < $70,000	172	20.9
$70,000 to < $100,000	143	17.4
≥ $100,000	365	44.4
Exercise on a regular basis (% yes)	643	71.6
Current or history of smoking (% yes)	305	33.6
Type of cancer		
Breast	365	39.5
Gastrointestinal	314	34.0
Gynecological	147	15.9
Lung	97	10.5
Type of prior cancer treatment		
No prior treatment	238	26.7
Only CTX, surgery, or RT	375	42.0
CTX and surgery, or CTX and RT, or surgery and RT	175	19.6
CTX and surgery and RT	104	11.7

Characteristic	n	(%)
Cycle length		
14 days	417	45.3
21 days	438	47.6
28 days	65	7.1
Emetogenicity of the chemotherapy regimen		
Minimal/low	161	17.5
Moderate	580	63.0
High	180	19.5
Antiemetic regimen		
None	56	6.2
Steroid alone or serotonin receptor antagonist alone	185	20.4
Serotonin receptor antagonist and steroid	436	48.2
NK-1 receptor antagonist and two other antiemetics	228	25.2

Abbreviations: CTX, chemotherapy; MSAS, Memorial Symptom Assessment Scale; NK-1, neurokinin 1; RT, radiation therapy; SD, standard deviation

Table 6.3. Five Highest Ranked Inflammation-Related Genes Using the Robust Rank Aggregation Method

Rank	Gene symbol[a]	Gene name[b]	Rank 450K	Rank EPIC	FDR[c]
1	*CD40*	Cluster of Differentiation 40 molecule	1	1	0.017
2	*PPP3CC*	Protein Phosphatase 3 Catalytic Subunit Gamma	19	2	1.000
3	*CAT*	Catalase	2	51	1.000
4	*IRF5*	Interferon Regulatory Factor 5	23	4	1.000
5	*PRF1*	Perforin 1	24	20	1.000

[a]HUGO Gene Nomenclature Committee-approved symbol
[b]HUGO Gene Nomenclature Committee-approved name
[c]Benjamini-Hochberg procedure
Abbreviations: FDR, false discovery rate

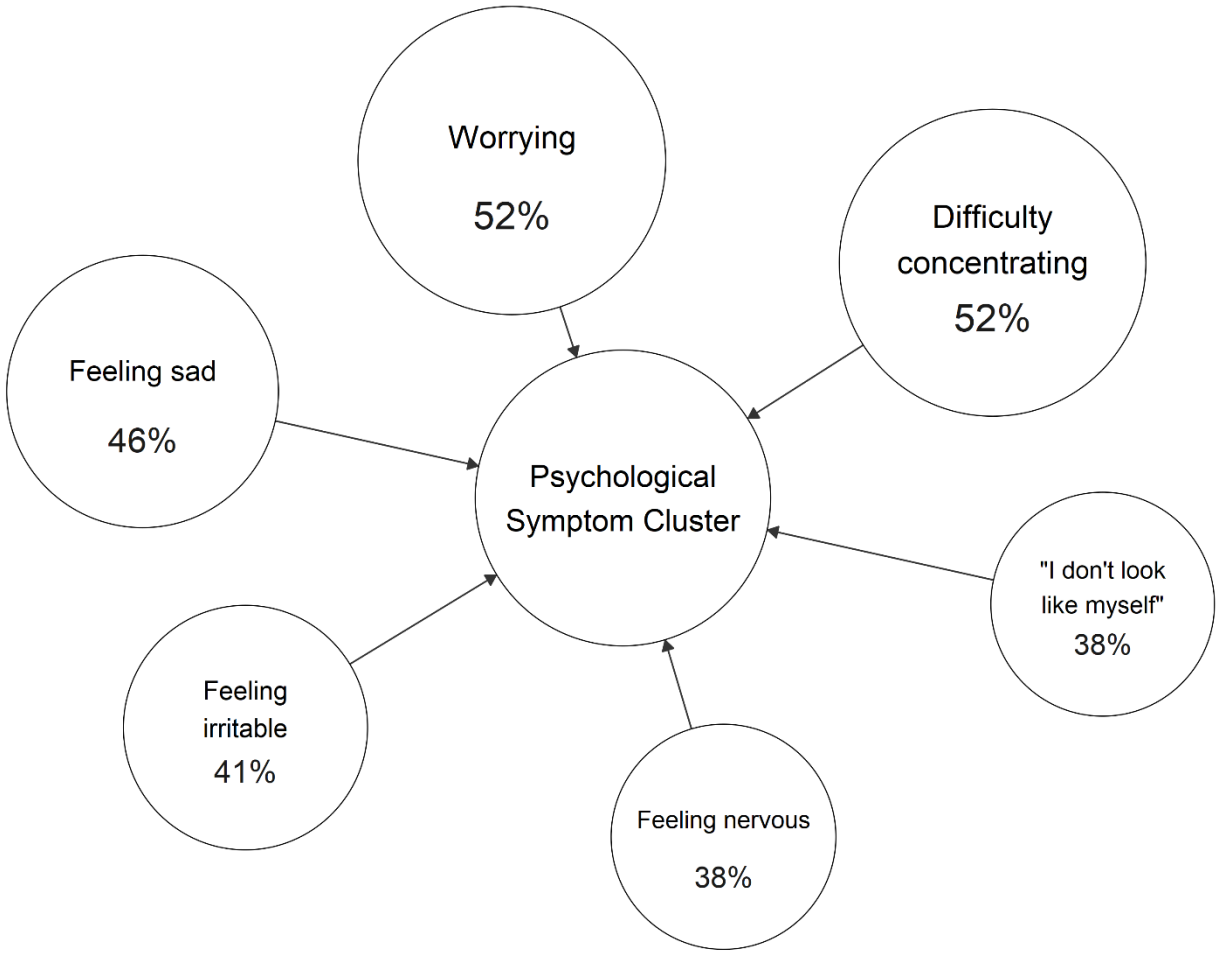


Figure 6.1. Symptoms within the psychological symptom cluster. The size of each node represents the occurrence rate for that symptom in oncology patients in the week prior to their second or third cycle of chemotherapy.[1]

[1]Harris CS, Kober KM, Cooper B, Conley YP, Dhruva AA, Hammer MJ, Paul S, Levine JD, Miaskowski CA. Symptom clusters in outpatients with cancer using different dimensions of the symptom experience. Support Care Cancer. 2022 May 11. Epub ahead of print. PMID: 35543816.

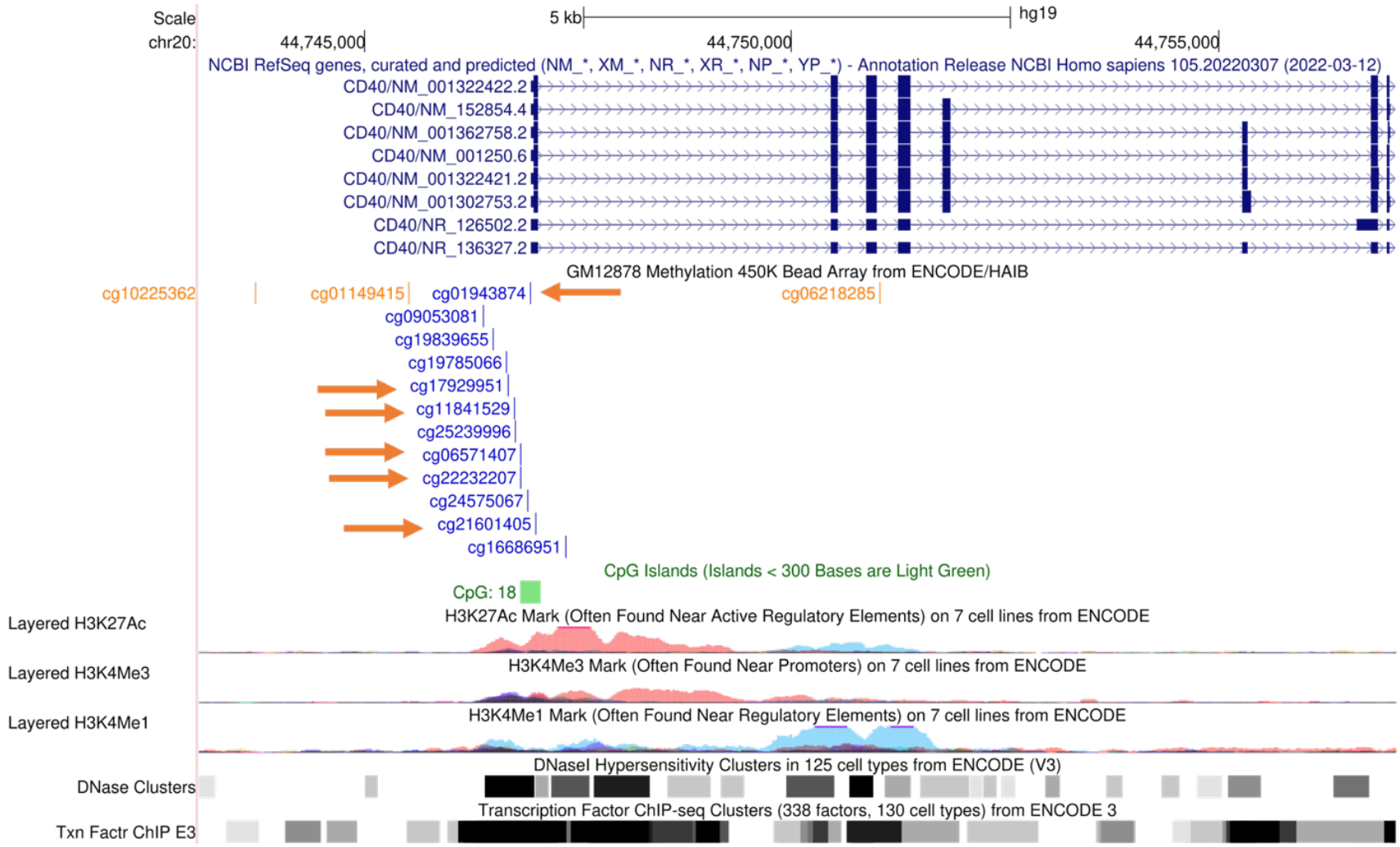

Scale
chr20:
5 kb
hg19
44,745,000
44,750,000
44,755,000
NCBI RefSeq genes, curated and predicted (NM_*, XM_*, NR_*, XR_*, NP_*, YP_*) - Annotation Release NCBI Homo sapiens 105.20220307 (2022-03-12)
CD40/NM_001322422.2
CD40/NM_152854.4
CD40/NM_001362758.2
CD40/NM_001250.6
CD40/NM_001322421.2
CD40/NM_001302753.2
CD40/NR_126502.2
CD40/NR_136327.2
GM12878 Methylation 450K Bead Array from ENCODE/HAIB
cg10225362
cg01149415
cg01943874
cg06218285
cg09053081
cg19839655
cg19785066
cg17929951
cg11841529
cg25239996
cg06571407
cg22232207
cg24575067
cg21601405
cg16686951
CpG Islands (Islands < 300 Bases are Light Green)
CpG: 18
Layered H3K27Ac
H3K27Ac Mark (Often Found Near Active Regulatory Elements) on 7 cell lines from ENCODE
Layered H3K4Me3
H3K4Me3 Mark (Often Found Near Promoters) on 7 cell lines from ENCODE
Layered H3K4Me1
H3K4Me1 Mark (Often Found Near Regulatory Elements) on 7 cell lines from ENCODE
DNaseI Hypersensitivity Clusters in 125 cell types from ENCODE (V3)
DNase Clusters
Transcription Factor ChIP-seq Clusters (338 factors, 130 cell types) from ENCODE 3
Txn Factr ChIP E3

Figure 6.2. Screenshot of the University of California Santa Cruz Genome browser displaying the promoter region of CD40 (i.e., 2500 bp upstream and downstream of the transcription start site) on chromosome 20 of the hg19 (genome reference consortium Version 37) assembly of the human genome. Assembly tracks show scale, chromosome, the genomic position of the six eCpG loci associated with the psychological cluster (orange arrows), and their unmethylated status as reported by the HAIB. The CD40 gene models are provided by the NCBI RefSeq.[1] The gene models depict exons as solid blocks connected by lines in introns with arrows showing the direction of transcription. Tracks denoting putative regulatory regions identified by ENCODE include: a CpG island (i.e., 5'-C-phosphate-G-3' linear DNA sequence); levels of enrichment for the layered H3K27Ac, H3K4Me3, and H3K4Me1 histone marks; DNase I hypersensitivity clusters; and transcription factor ChIP-seq clusters. For the H3K27Ac, H3K4Me3, and H3K4Me1 marks, the coloring indicates a different signal intensity from one of seven cell lines. For the DNase I hypersensitivity and transcription factor ChIP-seq clusters, the darkness of the shading corresponds to the strength of the signal intensity indicating the presence of cis-regulatory elements or transcription factors.

Abbreviations: bp, base pairs; CD40, cluster of differentiation 40; ChIP-seq, chromatin immunoprecipitation sequencing; chr, chromosome; eCpG, expression-associated CpG; ENCODE, Encyclopedia of DNA elements; GM12878, B-lymphoblastoid cell line; H3K4me1, histone H3 lysine 4 mono-methylation; H3K4me3, histone H3 lysine 4 trimethylation; H3K27Ac, histone H3 lysine 27 acetylation; HAIB, Hudson Alpha Institute for Biotechnology; hg, human genome; RefSeq, National Center for Biotechnology Information Reference Sequence

[1]Pruitt, K. D., Tatusova, T., & Maglott, D. R. (2005). NCBI Reference Sequence (RefSeq): A curated non-redundant sequence database of genomes, transcripts and proteins. Nucleic Acids Res, 33(Database issue), D501–D504. doi: 10.1093/nar/gki025

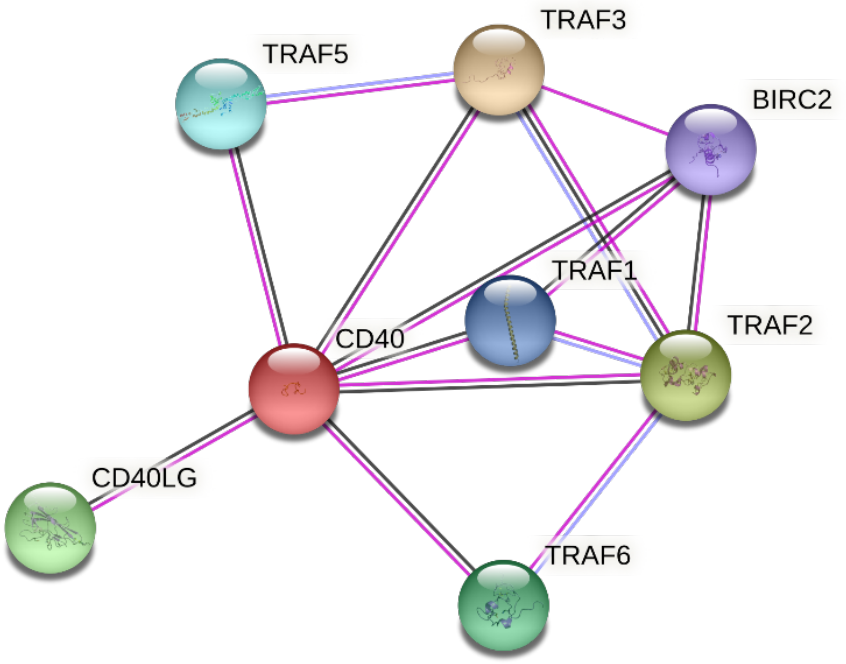
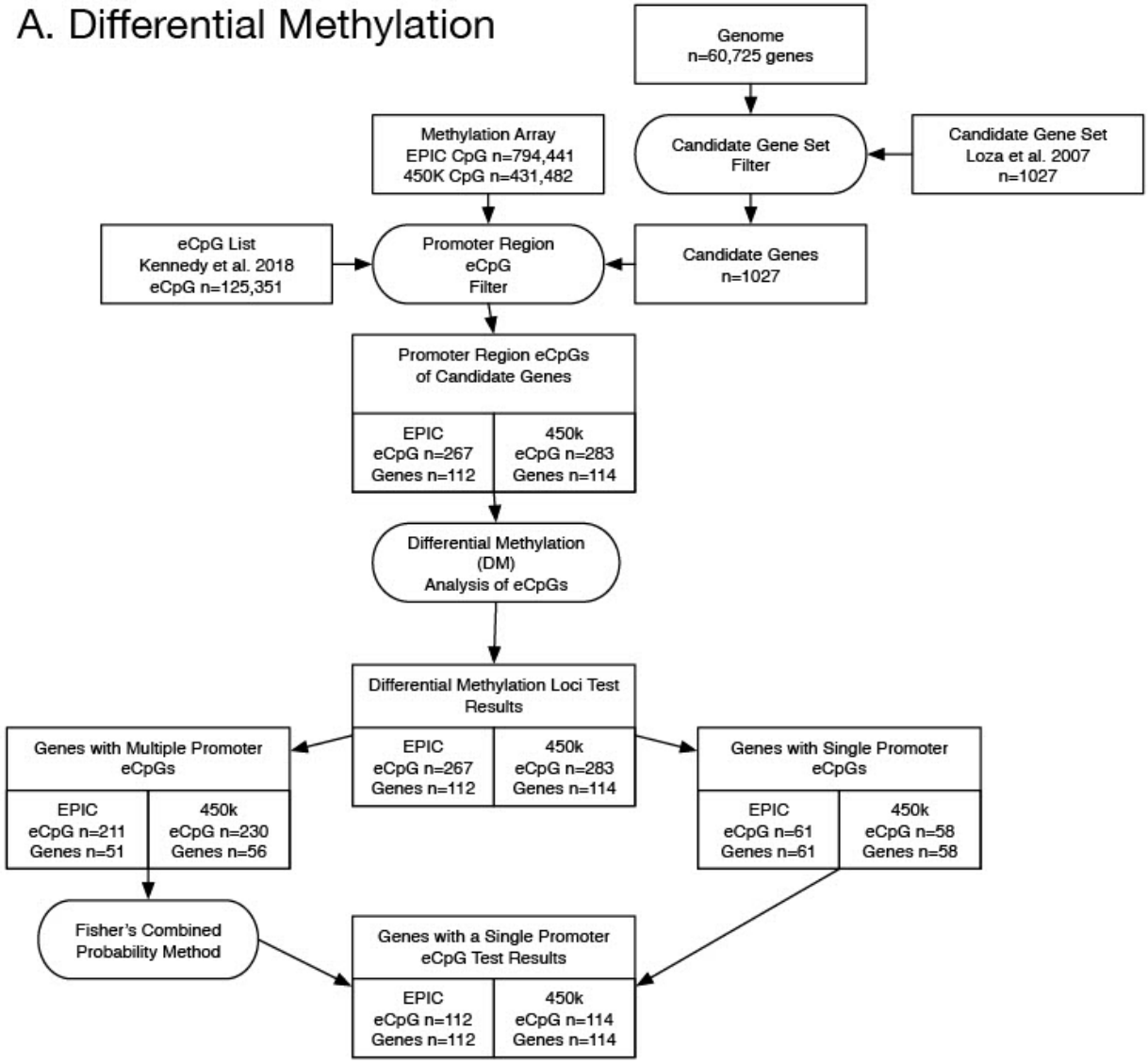


Figure 6.3. Protein-protein interaction network of predicted functional partners for the CD40 gene. Network interaction representation for CD40 was generated using the STRING database.[1]

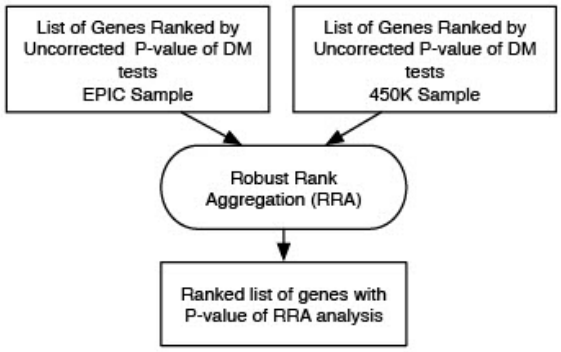
Edges represent specific or meaningful associations. The colors of the edges connecting the nodes represent the types of evidence supporting the connections, namely: known interactions from experimental evidence (pink), predicted gene co-occurrence (blue), and co-expression (black).
Abbreviations: BIRC2, baculoviral inhibitor of apoptosis repeat containing 2; CD40, cluster of differentiation 40; CD40LG, cluster of differentiation 40 ligand; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; TRAF1, tumor necrosis factor receptor associated factor

[1]Szklarczyk, D., Gable, A. L., Lyon, D., et al. (2019). STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res, 47(D1), D607–D613. doi: 10.1093/nar/gky1131

A. Differential Methylation

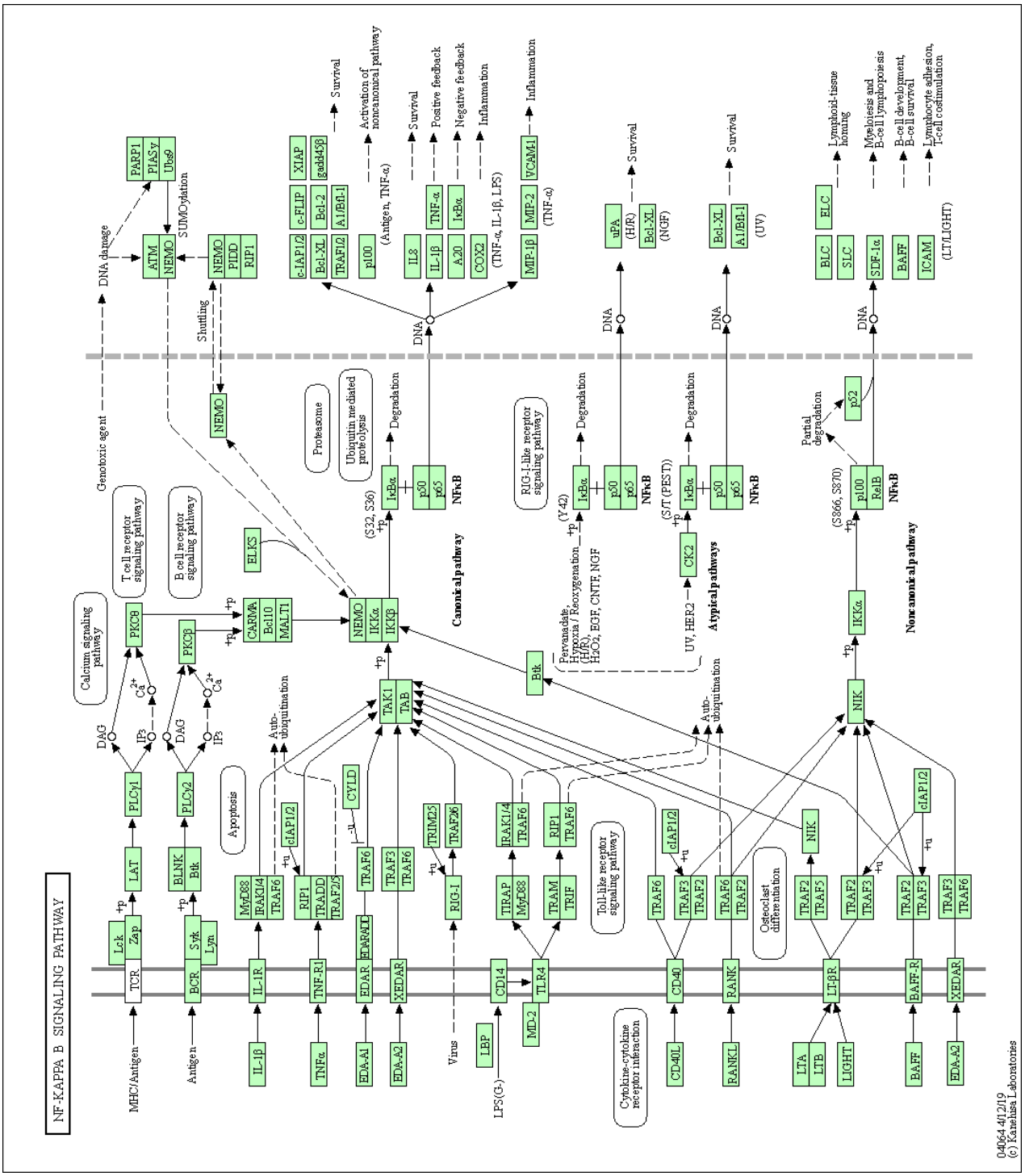


B. Meta-analysis



Supplemental Figure 6.1**.** Flow chart illustrating the analysis workflow for the differential methylation (A) and the meta-analysis (B). Oval shapes indicate an analysis or filter step. For the differential methylation analyses (A), of the 60,725 genes in the genome, we first narrowed our evaluation to a candidate list of 1,027 genes compiled by Loza and colleagues.[1] Then, using the Illumina EPIC and 450K microarrays, we only evaluated for expression-associated CpGs (eCpGs) located in the promoter region of the initial 1,027 genes. Differential methylation analyses were done for the resulting 267 eCpGs on 112 genes for the EPIC sample and 283 eCpGs on 114 genes for the 450K sample. For both samples, genes with multiple promoter eCpGs were combined using Fisher's Combined Probability Method. Results of the differential methylation tests for the EPIC and 450K samples were ranked by uncorrected p-values and evaluated using Robust Rank Aggregation (B).

[1]Kennedy EM, Goehring GN, Nichols MH, Robins C, Mehta D, Klengel T, Eskin E, Smith AK, Conneely KN. An integrated -omics analysis of the epigenetic landscape of gene expression in human blood cells. BMC Genomics. 2018 Jun 19;19(1):476. doi:10.1186/s12864-018-4842-3. Loza MJ, McCall CE, Li L, Isaacs WB, Xu J, Chang BL. Assembly of inflammation-related genes for pathway-focused genetic analysis. PLoS One. 2007 Oct 17;2(10):e1035. doi:10.1371/journal.pone.0001035.



Supplemental Figure 6.2. Nuclear factor kappa B (NF-κB) signaling pathway. Cluster of differentiation (CD)40 is identified by the circle on the left of the figure. Figure used with permission from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.[1]

[1]Kanehisa M, Goto S. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res. 2000;28(1):27-30.

Chapter 7

Gastrointestinal Symptom Cluster is Associated with Epigenetic Regulation of Lymphotoxin Beta in Oncology Patients Receiving Chemotherapy

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ABSTRACT

Objectives: Evidence suggests inflammatory processes underlie the gastrointestinal symptom cluster (GISC) through the actions of the nuclear factor kappa B (NF-κB) signaling pathway. This pilot study evaluated for associations between a GISC and levels of DNA methylation for genes within this pathway.

Sample and setting: 1071 outpatients.

Methods and variables: Prior to their next cycle of chemotherapy, patients reported symptom occurrence using the Memorial Symptom Assessment Scale. GISC was identified using exploratory factor analysis. Differential methylation analyses were performed in two independent samples (S1, n=925; S2, n=146). Trans expression-associated CpG (eCpG) loci for 56 NF-κB signaling pathway genes were evaluated. Loci significance were assessed using an exploratory false discovery rate (FDR; 25%) under the Benjamini-Hochberg procedure for S1 and at an unadjusted p-value of 0.05 for S2.

Results: For S1, increased expression of *LTB* by one differentially methylated trans eCpG locus (cg03171795) was associated with the GISC (FDR=0.168). Association was not validated in S2.

Conclusions: This study is the first to identify an association between a GISC and epigenetic regulation of a gene that is involved in initiating gastrointestinal immune responses. Findings suggest that increased *LTB* expression by hypermethylation of a trans eCpG locus is involved in the occurrence of this cluster in patients receiving chemotherapy. Findings warrant confirmation.

Relevance: Findings provide a potential therapeutic target for this common cluster.

Keywords: cancer; chemotherapy; constipation; diarrhea; DNA methylation; gastrointestinal symptom cluster; inflammation; nausea

INTRODUCTION

A gastrointestinal symptom cluster is one of the most common clusters in patients receiving chemotherapy.[1, 2] While this cluster is stable across dimensions of the symptom experience regardless of cancer types,[3] the consistency of the symptoms within this cluster are variable across dimensions and time.[4, 5] These findings are not surprising given the relatively high occurrence rates for individual gastrointestinal symptoms. For example, in a heterogenous sample of oncology patients,[3] 49.4% reported change in the way food tastes, 47.5% reported nausea, and 43.5% reported constipation prior to the start of their second or third cycle of chemotherapy. In addition, patients identified these symptoms as some of the most severe and distressing symptoms. Of note, the gastrointestinal symptom cluster (referred to as gastrointestinal cluster in the remainder of the manuscript) is associated with lower functional status[6, 7] and poorer quality of life.[8-10] In addition, poor management of the symptoms within this cluster are associated with increased economic burden.[11]

While no study has investigated the underlying mechanism(s) for a gastrointestinal cluster, a growing body of evidence suggests that inflammatory mechanisms play a role in the development of gastrointestinal symptoms in oncology patients.[12] Following the administration of chemotherapy, a cascade of biological processes are triggered that result in mucosal inflammation of the entire alimentary tract.[13] In the first stage, gastrointestinal mucositis is initiated by increases in oxidative stress, production of reactive oxygen species (ROS), deoxyribonucleic acid (DNA) damage, and activation of innate immunity. Next, ROS and the innate immune system accelerate the inflammatory response through macrophage stimulation and transcription factor activation. Both of these processes lead to the production of multiple proinflammatory cytokines (e.g., interleukin (IL)-6, tumor necrosis factor (TNF)-α). These cytokines in turn activate multiple signaling pathways (e.g., mitogen-activated protein kinase, nuclear factor kappa B (NF-κB)); increase the production of proinflammatory cytokines; and culminate in tissue ulceration. Mucositis of the gastrointestinal tract is associated with multiple

symptoms, including abdominal bloating, constipation, diarrhea, mouth sores, nausea, vomiting, and pain.[14]

Of the various transcription factors that are activated as part of this inflammatory cascade, NF-κB is hypothesized to play a central role.[15] Three studies have evaluated for associations between mucositis or a gastrointestinal symptom and differences in NF-κB signaling. In the first study that evaluated for differential expression in the stomach, jejunum, and colon of rats treated with irinotecan,[16] multiple genes within the NF-κB signaling pathway were upregulated. In another study of patients undergoing chemoradiation,[17] more severe mucositis was associated with perturbations in the NF-κB signaling pathway. In a third study that compared patients with and without chemotherapy-induced nausea,[18] perturbations were identified in a number of inflammatory pathways, including the NF-κB signaling pathway.

While these findings support the hypothesis that NF-κB signaling is involved in the development of a variety of symptoms associated with chemotherapy-induced injury of the gastrointestinal mucosa, the specific processes involved in its regulation warrant additional research. One approach is to evaluate epigenetic regulation of the genes in this pathway. While DNA methylation allows the body to adapt to external and internal stimuli, dysregulation of epigenetic processes may influence the development or severity of symptoms. For example, in women with breast cancer whose cognitive function was assessed prior to and following the receipt of chemotherapy,[19] lack of improvement in the memory domain was associated with 56 differentially methylated loci one year after chemotherapy initiation.

While previous research has focused primarily on promoter associated epigenetic regulation of gene expression,[20] emerging evidence suggests that methylation of a CpG locus on one chromosome can regulate the transcription of a gene on another chromosome (i.e., trans CpG).[21, 22] For example, methylation of trans CpGs can influence gene expression (i.e., trans expression-associated CpG (eCpG)) by binding to enhancer elements or transcription factor binding sites.[21] Given preliminary evidence of associations between chemotherapy-

induced gastrointestinal symptoms and NF-κB, an exploratory analysis was done to evaluate for associations between the occurrence of a gastrointestinal cluster and levels of DNA methylation on trans CpG loci for genes within the NF-κB pathway.

METHODS

Patients and settings

This analysis is part of a larger study that evaluated symptom clusters in oncology outpatients receiving chemotherapy.[3] Eligible patients were ≥18 years of age; had a diagnosis of breast, lung, gastrointestinal, or gynecologic 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 four 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. Data from the enrollment assessment (i.e., symptoms in the week prior to the patient's second or third cycle of chemotherapy) were used in this analysis. At enrollment, a total of 1071 patients provided a blood sample for the DNA methylation analyses. Medical records were reviewed for disease and treatment information.

Instruments

Patients completed a demographic questionnaire, Karnofsky Performance Status (KPS) scale,[23] and Self-Administered Comorbidity Questionnaire.[24] Toxicity of each patient's chemotherapy regimen was rated using the MAX2 index.[25, 26]

A modified version of the 32-item Memorial Symptom Assessment Scale (MSAS) was used to evaluate the occurrence, severity, and distress of 38 common symptoms associated with cancer and its treatment.[27] Six additional symptoms were added: hot flashes, chest tightness, difficulty breathing, abdominal cramps, increased appetite, and weight gain. Using the MSAS, patients were asked to indicate whether they had experienced each symptom in the past week (i.e., symptom occurrence). The patients' responses to the occurrence items were used to create the symptom clusters. The validity and reliability of the MSAS are well-established.[27]

Data analyses

Descriptive statistics and frequency distributions were calculated for the demographic and clinical characteristics, using the Statistical Package for the Social Sciences Version 27 (IBM Corporation, Armonk, NY). Exploratory factor analysis (EFA) was used to identify symptom clusters using Mplus Version 8.6.[28]

Methods for the EFA were reported elsewhere.[3] In brief, for the EFA using the dichotomous occurrence items, tetrachoric correlations were used to create the matrix of associations.[28] The simple structure for the occurrence EFA was estimated using the method of unweighted least squares with geomin (i.e., oblique) rotation.[28] Factor loadings were considered meaningful if the loading was ≥0.40.[28] Factors (i.e., symptom clusters) were adequately defined if at least two items (i.e., symptoms) had loadings of ≥0.40.[29] Clusters were named based on the symptoms with the highest factor loadings and the majority of the symptoms within the cluster.

With these methods, a gastrointestinal cluster (Figure 1) was identified in our previous analysis.[3] A factor score was calculated as the sum of the occurrence ratings for the 11 symptoms within the cluster (range of 0 to 11). Initially, the DNA methylation analyses were conducted using the patients' symptom cluster factor scores as a continuous value. However, the p-value distribution for the differential methylation tests across the genome was severely conservative (i.e., overabundance of low p-values; data not shown). Therefore, for the current

analyses, the total factor score was dichotomized into two groups (i.e., 0 symptoms = no gastrointestinal cluster group versus 1 to 11 symptoms = gastrointestinal cluster group).

Selection of trans DNA methylation loci

Candidate genes in the NF-κB signaling pathway were identified using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database[30] (Supplemental Figure 1). Then, methylated loci for these genes that were associated with changes in gene expression[21] (i.e., eCpGs) on another chromosome (i.e., trans eCpG) were selected. These trans eCpGs for genes within the NF-κB signaling pathway were evaluated for association with gastrointestinal group membership.

Biospecimen processing, quantification of methylation status, and quality control

Methods for the DNA methylation analyses are described in more detail elsewhere.[31] In brief, DNA was extracted from archived buffy coats using the PUREGene DNA isolation kit (Invitrogen, Carlsbad, CA); quantified using a NanoDrop UV spectrophotometer (Thermo Fisher Scientific, Waltham, MA); and normalized to a concentration of 50 ng/μL. DNA was bisulfite converted using the Zymo EZ-96 DNA Methylation Kit (Catalog #D5004) Deep-Well Format (Zymo Research, Irvine, CA) and used as input for the Infinium HD Methylation Assay (Illumina Inc., San Diego, CA).

Of the 1071 patients in this study, DNA methylation was measured for 925 patients using the Infinium MethylationEPIC BeadChip (i.e., EPIC microarray sample) and for 146 patients using the Infinium HumanMethylation 450 BeadChip (i.e., 450K microarray sample; Illumina, Inc., San Diego, CA). The EPIC microarray sample was used as the discovery sample while the 450K microarray sample was used as a validation sample. All of the samples were scanned on the Illumina iScan (Illumina, Inc., San Diego, CA). Preliminary analysis and quality control procedures were performed using GenomeStudio (Illumina, Inc., San Diego, CA). Samples that had <90% of their targets detected at a p-value of ≤0.01 were flagged for review. Sample replicates and Jurkat control replicates were checked to ensure an r^2 value of >0.99.

Subsequent analyses were done using well-established protocols in R (version 4.1.0).[32] Corrections for Infinium I and II probes, balance correction, background correction, and quantile normalization were performed using the minfi package in R (version 1.38.0).[33, 34] Probes that contained a single nucleotide polymorphism at a CpG or flanking site and probes that aligned with multiple places on the genome were excluded.[35] Methylation scores were quantified as M-values.[36]

DNA methylation analyses

Given that DNA methylation levels differ among blood cell types,[37] cell types were estimated using the *estimateCellCounts2()* function in the FlowSorted.Blood.EPIC R package (version 1.10.1).[38] Cell type deconvolution was performed using the IDOL L-DMR library for cluster of differentiation 8 (CD8) and CD4 T-cells, natural killer cells, B cells, monocytes, and neutrophils.[39] Differences in estimates of cell type composition between the gastrointestinal cluster groups were evaluated using Welch two sample t-tests and assessed for significance at a p-value of <0.05. Any cell type composition estimates that were significantly associated with membership in the gastrointestinal cluster group were included as covariates in the final model. Given that methylation status changes over the lifespan,[40] age was included as a covariate in the final regression model. Surrogate variable analysis, using the Leek method (R package version 3.4.0),[41] was used to estimate surrogate variables for technical and non-technical variations that contributed to heterogeneity in the sample that were not due to the gastrointestinal cluster group, age, or cell type.

To evaluate for associations between the gastrointestinal cluster groups and methylation status of trans eCpG loci for the NF-κB candidate genes, tests for differentially methylated probes (DMPs) were done using a generalized linear model implemented in the limma R package using the “ls” method (version 3.48.3).[42] The significance of the DMPs for the NF-κB candidate genes was assessed using an exploratory false discovery rate (FDR) of 25% under the Benjamini-Hochberg procedure for the EPIC microarray sample.[43] Then, candidate trans

eCpG loci identified as differentially methylated in the EPIC microarray sample were evaluated for differential methylation in the 450K microarray sample (Supplemental Figure 1B). For the validation assessment using the 450K microarray sample, significance of the candidate trans eCpG loci was assessed at an unadjusted p-value of 0.05. Finally, in order to characterize potential functional roles of the eCpGs of differentially methylated genes, we evaluated for evidence of regulatory elements in regions surrounding the loci using annotation data from the Encyclopedia of DNA Elements (ENCODE)[44] obtained from the University of California Santa Cruz Genome Browser.[45] Finally, we identified predicted functional partners of genes with differentially methylated trans eCpGs from a protein-protein interaction network that was created using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database.[46]

RESULTS

Demographic and clinical characteristics

Of the 925 patients in the EPIC microarray sample, one was excluded for insufficient phenotypic data and one for poor sample quantification. Of the remaining 923 patients, 76.2% were female, 69.4% were White, 64.1% were married or partnered, and had a mean age of 57.5 (±12.2) years (Table 1). Most patients were well-educated (16.1 ±3.0 years), exercised on a regular basis (71.6%), and had never smoked (66.4%). Patients had an average of 2.4 (±1.4) comorbid conditions and a KPS score of 80.4 (±12.6). Most common type of cancer was breast (39.5%), followed by gastrointestinal (34.0%), gynecological (15.9%), and lung (10.5%). Majority of patients (73.3%) had received either chemotherapy, surgery, and/or radiation therapy. Patients reported 13.5 (±7.1) concurrent symptoms before their second or third cycle of chemotherapy.

Of the 146 patients in the 450K microarray sample, 100% were female, 65.5% were White, 67.6% were married or partnered, and had a mean age of 52.7 (±11.7) years (Table 2). Most patients were well-educated (16.3 ±2.9 years), exercised on a regular basis (75.7%), and had never smoked (72.4%). Patients had an average of 2.4 (±1.4) comorbid conditions and a

KPS score of 79.1 (±11.6). Most common type of cancer was breast (99.3%) followed by gastrointestinal (0.7%). Majority of patients (76.7%) had previously received either chemotherapy, surgery, and/or radiation therapy. Patients reported 16.0 (±7.8) concurrent symptoms before their second or third cycle of chemotherapy.

DNA methylation analyses

For the EPIC microarray sample, of the 90 candidate genes that were identified in the NF-κB signaling pathway, 3785 trans eCpG loci across 56 genes were evaluated for differential methylation. Because cell type compositions were not associated with gastrointestinal cluster group membership, only age and 22 surrogate variables were included as covariates in the final model. For the 450K microarray sample, because cell type compositions were not associated with gastrointestinal cluster group membership, only age and one surrogate variable were included as covariates in the final model. The 450K microarray sample was used as the validation sample.

For the EPIC microarray sample, hypermethylation of the trans eCpG locus (i.e., cg03171795) for the lymphotoxin beta (LTβ) gene was found to be significantly associated with the occurrence of the gastrointestinal cluster (FDR = 0.168). For the 450K microarray sample, no association was found (p = 0.664).

DISCUSSION

This study is the first to evaluate for an association between a gastrointestinal symptom cluster and a specific inflammatory mechanism; namely epigenetic regulation of the NF-κB pathway. This cluster is associated with hypermethylation of one trans eCpG locus (i.e., cg03171795). While located on chromosome 3, this trans eCpG locus regulates the expression of *LTB* which is a gene located within the major histocompatibility complex of chromosome 6. In other studies,[21] hypermethylation of this eCpG is associated with increased expression of *LTB*. Notably, LTβ is situated at the beginning of the NF-κB signaling pathway and can induce NF-κB signaling. This finding supports previous research that suggests that signaling within the NF-κB

pathway is involved in chemotherapy-induced inflammation along the entire gastrointestinal tract.[16-18]

Regulatory role of trans eCpG locus

Given that epigenetic modifications are dynamic and multiple factors influence gene expression, the establishment of a functional role for epigenetic changes that are identified is challenging for methylation association studies.[47] However, it is particularly challenging when examining trans regulatory relationships because the regulatory role of DNA methylation in this region is unclear.[21] As illustrated in Figure 2, multiple sources of independent and complementary data show that the trans eCpG locus, cg03171795, is located within a putative regulatory region of chromosome 3. Specifically, evidence of regulatory elements compiled by ENCODE suggests that this locus is situated within a region of enhancer activity.[48, 49] Enhancer regions are areas of non-coding DNA that enhance gene transcription by recruiting transcription factors and RNA polymerase II and modifying chromatin accessibility.[50] Located distal to their target genes, enhancers form loops to move in closer proximity to gene promoters.[51]

Our findings are consistent with a study that sought to identify and characterize genome-wide eCpGs across two independent datasets and found that eCpGs were enriched for enhancer annotations and transcription factor binding sites, particularly among trans eCpGs.[21] The authors suggested that secondary regulation of transcription by trans eCpGs is an important but understudied area of epigenomic research.

Role of LTβ in inflammatory processes

LTβ is a member of the TNF super family of ligands. Along with LTα, it forms a heterotrimer complex (i.e., $LT\alpha_1\beta_2$) that exclusively binds to the LTβ receptor (LTβR).[52] $LT\alpha_1\beta_2$ is expressed on the surface of lymphoid cells (e.g., B cells, natural killer cells, T cells) while LTβR is expressed by stromal and myeloid cells (e.g., dendritic cells, macrophages). Signaling between these distinct cell types is important for the formation and maintenance of lymphoid tissue. While notable for its role in embryonic lymph node and Peyer's patch formation, splenic

structure maintenance, and lymph node homeostasis,[53] LTβ may play a role in regulating the mucosal immune responses of the gastrointestinal tract.[54]

From its position at the beginning of the NF-κB signaling pathway, LTβ can induce NF-κB signaling and the inflammatory response. As illustrated in Figure 3, the protein products of LTβ, LTα, and LTβR form a close, interacting network with three TNF receptor associated factors (TRAFs; i.e., TRAF 2, TRAF3, TRAF 5) and two major receptors for TNF-α (i.e., TNF receptor super family (TNFRSF)1A, TNFRSF1B). These TRAFs are intracellular signaling molecules that regulate the canonical and non-canonical pathways that lead to NF-κB activation.[55] In addition, TNFRSF1A and TNFRSF1B are receptors for $LT\alpha_3$ and are involved in the canonical NF-κB signaling pathway and mediation of apoptosis.[56]

Role of LTβ in intestinal inflammation

While no pre-clinical or clinical study has evaluated for associations between LTβ and gastrointestinal symptoms associated with chemotherapy administration, LTα, LTβ, and LTβR appear to be involved in the mechanisms that underlie inflammatory bowel disease.[57] For example, in one study that investigated the mechanisms by which activation of LTβR signaling influences acute inflammation in a mouse model of acute colitis induced by dextrose sulfate sodium (DSS),[58] comparisons of inflammatory responses in colonic tissue were performed using three models of LTβR signaling ablation (i.e., antibody binding to the receptor, LTβR-deficient mice, and LTαβ-deficient mice). All three ablation models resulted in aggravation of the colitis and release of inflammatory cytokines. In addition, all of the mice lost weight. In a second study of chronic DSS-induced colitis,[59] the expression of *Ltb* in colonic tissue was increased.

Interestingly, inhibition of LTβR resulted in *decreases* in the development of inflammation as well as in the production of TNF, IL-1β, and IL-6 in colonic tissue. Taken together, these findings from animal models provide evidence of an association between LTβ and inflammatory states in the bowel. In addition, these results suggest that the effects of LTβ signaling may be different in the settings of acute versus chronic inflammation. Additional

research is needed to understand the role of LTβ signaling in the development and manifestation of chemotherapy-induced inflammatory responses in the gastrointestinal mucosa.

Two clinical studies have evaluated for differences in *LTB* expression in patients with and without inflammatory bowel disease.[60, 61] In the first study,[60] compared to healthy controls, the expression of LTβ on lymphocytes and plasma cells was increased in the mucosa of the colonic tissue of patients with ulcerative colitis and in the ileum of patients with Crohn's disease. In the second study,[61] compared to healthy controls, *LTB*, C-C motif chemokine ligand (*CCL*)*19*, and *CCL21* were differentially expressed and upregulated in the colonic tissue of patients with microscopic colitis. These findings were confirmed in intestinal tissue from an independent sample of patients with microscopic colitis. These findings support the role of LTβ signaling in gastrointestinal inflammation.

Gastrointestinal cluster and future directions for research

While additional research is needed to evaluate the role of LTβ in a gastrointestinal cluster in oncology patients receiving chemotherapy, our findings build on previous studies of patients who overlapped with the samples used in this analysis.[18, 62-64] Two studies evaluated for differentially perturbed pathways between oncology patients with and without chemotherapy-induced nausea.[18, 63] In addition to the NF-κB signaling pathway,[18] two additional pathways that are implicated in gastrointestinal inflammation[12] were differentially perturbed between patients with and without nausea (i.e., apoptosis,[63] cytokine-cytokine signaling[18]).

While these previous studies focused on a single symptom,[18, 63] nausea is associated with the co-occurrence of several other gastrointestinal symptoms. For example, in another study conducted by our team,[64] compared to patients without nausea, patients with nausea were more likely to report the occurrence of 11 additional gastrointestinal symptoms. Of note, nine of these symptoms (i.e., change in the way food tastes, dry mouth, constipation, lack of appetite, diarrhea, weight loss, abdominal cramps, difficulty swallowing, vomiting) were identified in our gastrointestinal cluster using EFA (Figure 1). While these findings are not

surprising given that patients overlapped across the different analyses, similar findings were reported elsewhere. For example, in a study that evaluated the severity of 22 symptoms in patients with ovarian cancer receiving chemotherapy,[65] nausea was associated with five symptoms that were identified in our gastrointestinal cluster (i.e., bowel disturbances, dizziness, lack of appetite, vomiting, weight loss).

In another study by our team that identified a gastrointestinal cluster using NA,[62] nausea was identified as the most important symptom within the network (i.e., theoretically has the greatest impact on other symptoms). We suggested that alleviating nausea may reduce the occurrence of the other symptoms within the network. Taken together, these findings suggest that nausea may be a sentinel symptom that drives the occurrence of other gastrointestinal symptoms.

Strengths and limitations

While these data provide evidence to support the hypothesis that the trans eCpG locus cg03171795 is involved in regulatory processes, it is not entirely clear how hypermethylation of this trans eCpG locus regulates the expression of *LTB*. An association between the methylation state of cg03171795 and expression of *LTB* was identified by Kennedy and colleagues[21] in their study that tested for associations between CpG methylation and gene expression across two independent data sets (i.e., Grady Trauma Project, Multi-Ethnic Study of Atherosclerosis). Notably, trans eCpGs comprised 39% and 69% of the total eCpGs identified in the two data sets, while cis and distal eCpGs comprised the remaining eCpGs. Given these findings, the authors suggested that methylation regulates gene expression largely through secondary regulatory mechanisms, such as enhancer CpGs rather than promoter CpGs.[21] Therefore, ongoing research is warranted to evaluate the regulatory role of trans eCpGs. In vitro analyses may shed light on the indirect regulatory role that hypermethylation of cg03171795 has on the expression of *LTB*.[47]

While an association between the trans eCpG locus cg03171795 and *LTB* was identified in the EPIC sample, this association was not found in our 450K sample. This lack of validation may be due to heterogeneity between the samples (e.g., gender, cancer type); the relatively small sample size (n=146); and/or too small an effect size to identify a relationship. Additional research is needed to validate this association in patients receiving chemotherapy. In addition, given the study's cross-sectional design, evaluation of changes in levels of methylation for cg03171795 and *LTB* expression throughout chemotherapy is warranted. Given the statistical challenges with the distribution of the gastrointestinal cluster factor scores, the best methods to incorporate the use of symptom cluster factor scores in methylation analyses need to be determined.

CONCLUSION

This exploratory study is the first to evaluate for changes in epigenetic regulation of an inflammatory mechanism underlying a gastrointestinal cluster in patients receiving chemotherapy. This finding provides new evidence to support the hypothesis that NF-κB signaling results in a variety of gastrointestinal symptoms.[12, 13, 15] Our findings suggest that the occurrence of a gastrointestinal cluster is associated with increased expression of *LTB* through hypermethylation of one trans eCpG locus.

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Table 7.1. Demographic and Clinical Characteristics of the Patients in the EPIC Microarray Sample (n=923)

Characteristic	Mean	SD
Age (years)	57.5	12.2
Education (years)	16.1	3.0
Body mass index (kilograms/metered squared)	26.1	5.6
Karnofsky Performance Status score	80.4	12.6
Number of comorbidities out of 13	2.4	1.4
Self-administered Comorbidity Questionnaire score	5.4	3.2
Time since cancer diagnosis (years)	1.9	3.9
Time since diagnosis (median)	0.42	
Number of prior cancer treatments (out of 9)	1.5	1.5
Number of metastatic sites including lymph node involvement (out of 9)	1.2	1.2
Number of metastatic sites excluding lymph node involvement (out of 8)	0.8	1.0
MAX2 Index of Chemotherapy Toxicity score (0 to 1)	0.17	0.08
Mean number of MSAS symptoms (out of 38)	13.5	7.1
	n	(%)
Gender		
Female	703	76.2
Male	220	23.8
Self-Reported Ethnicity		
Asian or Pacific Islander	114	12.4
Black	71	7.8
Hispanic, Mixed, or Other	95	10.4
White	636	69.4
Married or partnered (% yes)	581	64.1
Lives alone (% yes)	196	21.6
Child care responsibilities (% yes)	188	21.0
Care of adult responsibilities (% yes)	61	7.4
Currently employed (% yes)	327	35.9
Income		
< $30,000	142	17.3
$30,000 to < $70,000	172	20.9
$70,000 to < $100,000	143	17.4
≥ $100,000	365	44.4
Exercise on a regular basis (% yes)	643	71.6
Current or history of smoking (% yes)	305	33.6
Type of cancer		
Breast	365	39.5
Gastrointestinal	314	34.0
Gynecological	147	15.9
Lung	97	10.5
Type of prior cancer treatment		
No prior treatment	238	26.7
Only CTX, surgery, or RT	375	42.0
CTX and surgery, or CTX and RT, or surgery and RT	175	19.6
CTX and surgery and RT	104	11.7

Characteristic	n	(%)
Cycle length		
14 days	417	45.3
21 days	438	47.6
28 days	65	7.1
Emetogenicity of the chemotherapy regimen		
Minimal/low	161	17.5
Moderate	580	63.0
High	180	19.5
Antiemetic regimen		
None	56	6.2
Steroid alone or serotonin receptor antagonist alone	185	20.4
Serotonin receptor antagonist and steroid	436	48.2
NK-1 receptor antagonist and two other antiemetics	228	25.2

Abbreviations: CTX, chemotherapy; MSAS, Memorial Symptom Assessment Scale; NK-1, neurokinin 1; RT, radiation therapy; SD, standard deviation

Table 7.2. Demographic and Clinical Characteristics of the Patients in the 450K Microarray Sample (n=146)

Characteristic	Mean	SD
Age (years)	52.7	11.7
Education (years)	16.3	2.9
Body mass index (kilograms/meters squared)	26.3	6.4
Karnofsky Performance Status score	79.1	11.6
Number of comorbidities out of 13	2.4	1.4
Self-administered Comorbidity Questionnaire score	5.5	3.1
Time since cancer diagnosis (years)	3.0	4.7
Time since diagnosis (median)	0.43	
Number of prior cancer treatments (out of 9)	2.0	1.9
Number of metastatic sites including lymph node involvement (out of 9)	1.0	1.3
Number of metastatic sites excluding lymph node involvement (out of 8)	0.6	1.1
MAX2 Index of Chemotherapy Toxicity score (0 to 1)	0.20	0.09
Mean number of MSAS symptoms (out of 38)	16.0	7.8
	n	(%)
Gender		
Female	146	100.0
Self-Reported Ethnicity		
Asian or Pacific Islander	24	16.6
Black	10	6.9
Hispanic, Mixed, or Other	16	11.0
White	95	65.5
Married or partnered (% yes)	98	67.6
Lives alone (% yes)	25	17.2
Child care responsibilities (% yes)	45	31.0
Care of adult responsibilities (% yes)	14	10.5
Currently employed (% yes)	49	33.8
Income		
< $30,000	32	24.4
$30,000 to < $70,000	22	16.8
$70,000 to < $100,000	19	14.5
≥ $100,000	58	44.3
Exercise on a regular basis (% yes)	109	75.7
Current or history of smoking (% yes)	40	27.6
Type of cancer		
Breast	145	99.3
Gastrointestinal	1	0.7
Type of prior cancer treatment		
No prior treatment	34	23.3
Only CTX, surgery, or RT	62	42.5
CTX and surgery, or CTX and RT, or surgery and RT	18	12.3
CTX and surgery and RT	32	21.9
Cycle length		
14 days	48	32.9
21 days	86	58.9
28 days	12	8.2

Characteristic	n	(%)
Emetogenicity of the chemotherapy regimen		
Minimal/low	43	29.5
Moderate	57	39.0
High	46	31.5
Antiemetic regimen		
None	21	15.1
Steroid alone or serotonin receptor antagonist alone	30	21.6
Serotonin receptor antagonist and steroid	51	36.7
NK-1 receptor antagonist and two other antiemetics	37	26.6

Abbreviations: CTX, chemotherapy; MSAS, Memorial Symptom Assessment Scale; NK-1, neurokinin 1; RT, radiation therapy; SD, standard deviation

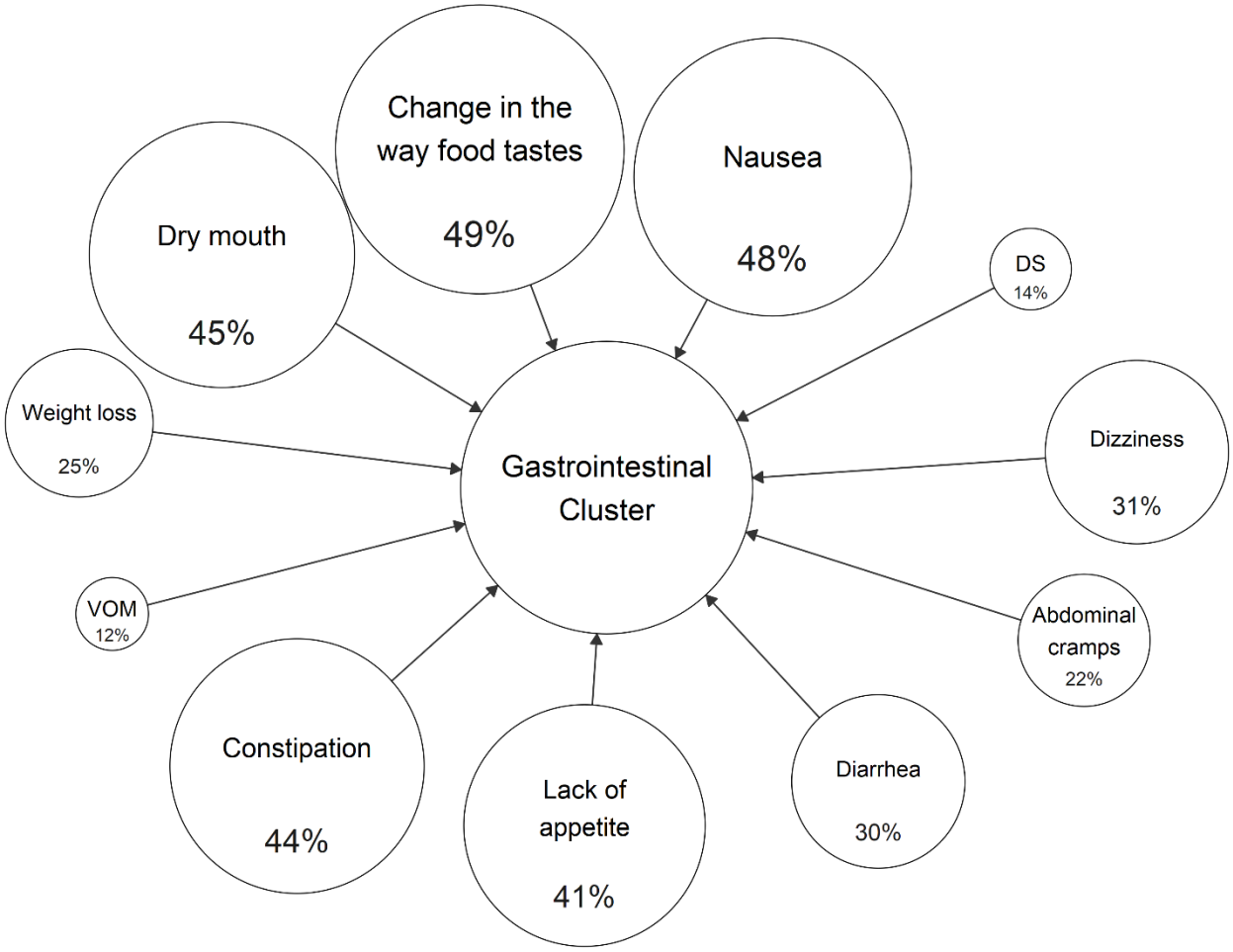


Figure 7.1. Symptoms within the gastrointestinal symptom cluster. The size of each node represents the occurrence rate for that symptom in oncology patients in the week prior to their second or third cycle of chemotherapy.[1]

Abbreviations: DS, difficulty swallowing; VOM, vomiting

[1]Harris CS, Kober KM, Cooper B, Conley YP, Dhruva AA, Hammer MJ, Paul S, Levine JD, Miaskowski CA. Symptom clusters in outpatients with cancer using different dimensions of the symptom experience. Support Care Cancer. 2022 May 11. doi: 10.1007/s00520-022-07125-z. Epub ahead of print. PMID: 35543816.

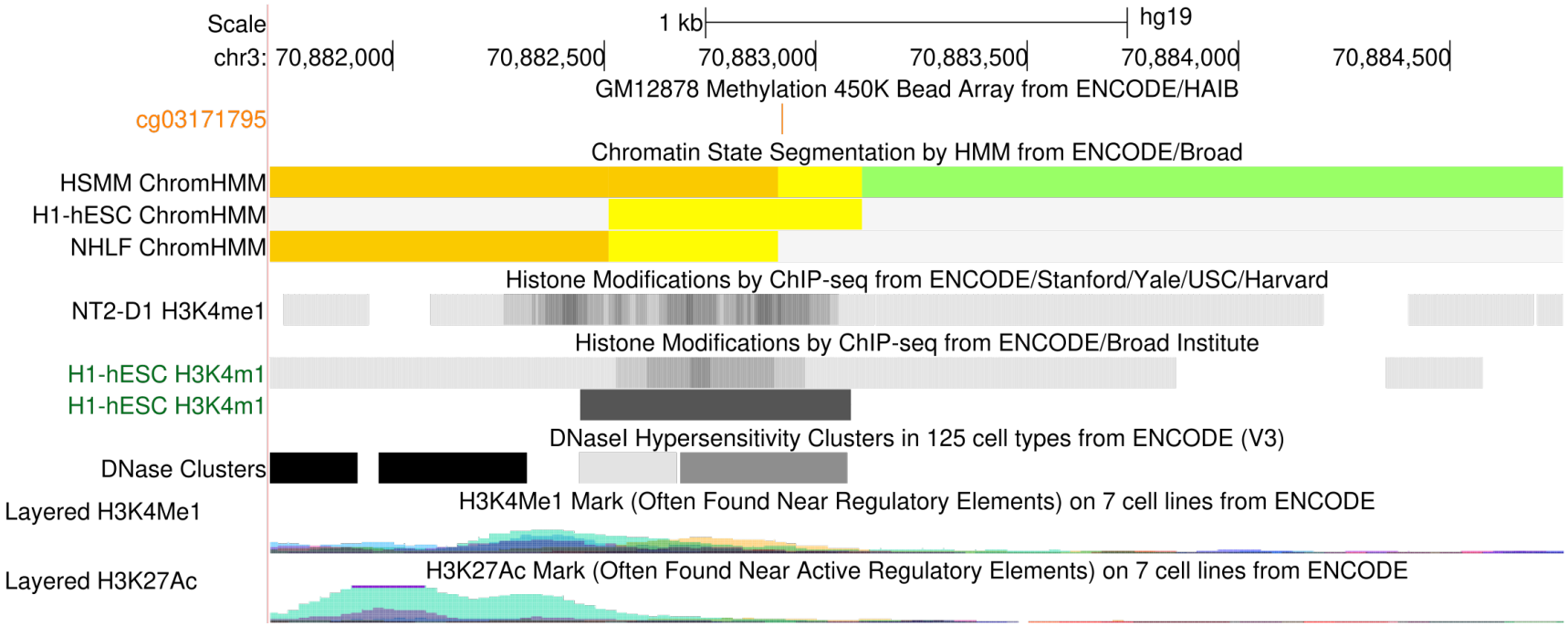


Figure 7.2. Screenshot of the University of California Santa Cruz Genome browser displaying cg03171795 on chromosome 3 of the hg19 (genome reference consortium Version 37; http://genome.ucsc.edu) assembly of the human genome. Assembly tracks show scale, chromosome, and the hypermethylated status of cg03171795 and its genomic position as reported by the HAIB. Tracks denoting putative regulatory regions across multiple cell lines that were identified by ENCODE include: predicted chromatin state using a HMM; histone modifications for H3K4me1 and H3K4m1; DNase I hypersensitivity clusters; and levels of enrichment for the layered H3K4Me1 and H3K27Ac histone marks. For the three tracks that illustrate the ChromHMM for three cell lines, the orange color indicates a “strong enhancer” predicted chromatin state; yellow color indicates a “weak/poised enhancer” state; and light grey color indicates heterochromatin or low signal. For the H3K4Me1 and H3K27Ac marks, the coloring indicates the signal intensity from one of seven cell lines.

Abbreviations: ChromHMM, chromatin state segmentation by multivariate Hidden Markov Modeling; ENCODE, Encyclopedia of DNA elements; GM12878, B-lymphoblastoid cell line; H1-hESC, embryonic stem cells, line H1; H3K4me1, histone H3 mono methyl K4; HAIB, Hudson Alpha Institute for Biotechnology; hg, human genome; HMM, Hidden Markov Model; HSMM, human skeletal muscle myoblasts; NT2-D1, clonally derived, pluripotent human embryonal carcinoma cell line

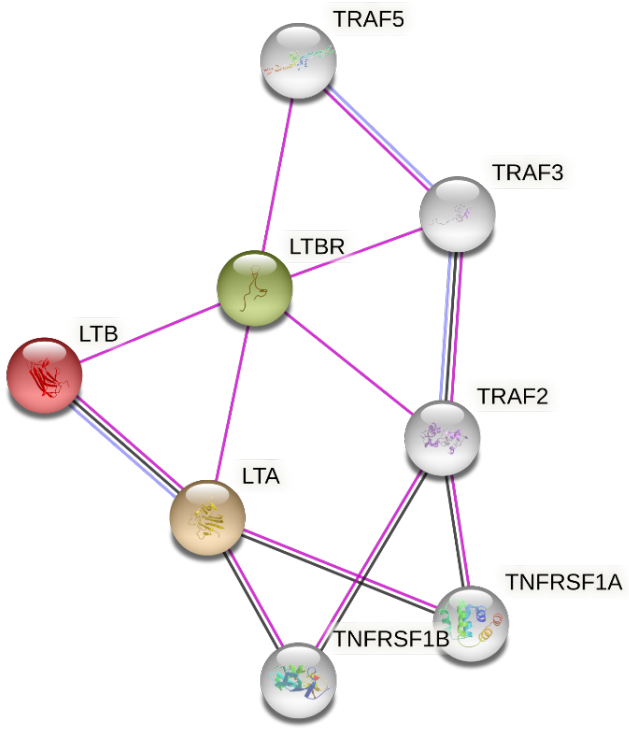
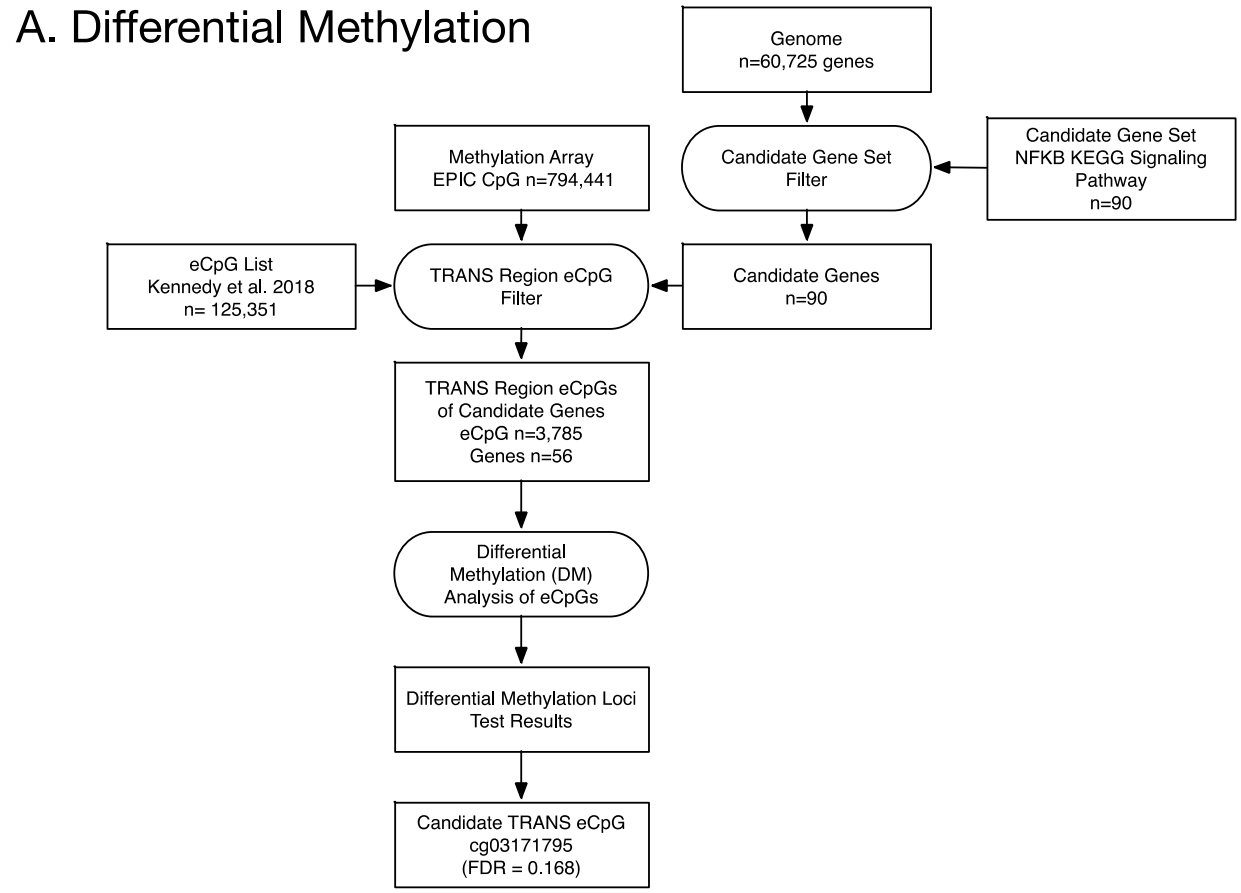


Figure 7.3. Protein-protein interaction network of predicted functional proteins for LTB. Network interaction representation for LTB was generated by the STRING database.[1] Edges represent specific or meaningful associations. Color of the edges connecting the nodes represents the types of evidence supporting the connections: known interactions from experimental evidence (pink); predicted gene co-occurrence (blue); and co-expression (black).

Abbreviations: LTA, lymphotoxin alpha; LTB, lymphotoxin beta; LTBR, lymphotoxin beta receptor; TNFRSF1A, tumor necrosis factor receptor super family 1 A; TNFRSF1B, tumor necrosis factor receptor super family 1 B; TRAF2, tumor necrosis factor receptor associated factor 2; TRAF3, tumor necrosis factor receptor associated factor 3; TRAF5, tumor necrosis factor receptor associated factor 5

[1]Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P, Jensen LJ, Mering CV. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 2019;47(D1):D607-D613.



Supplemental Figure 7.1. Flow chart illustrating the analysis workflow for the differential methylation (A) and independent evaluation of the candidate locus (B). Oval shapes indicate an analysis or filter step. For the differential methylation analyses (A), of the 60,725 genes in the genome, we first narrowed our evaluation to a candidate list of 90 genes within the nuclear factor kappa B signaling pathway as defined by the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Then, using the Illumina EPIC and 450K microarrays, we only evaluated for

trans expression-associated CpGs (eCpGs) on the initial 90 genes. Differential methylation analyses were done for the resulting 3,785 eCpGs on 56 genes for the EPIC sample. Results of the differential methylation tests for the EPIC sample were then evaluated in an independent sample (B).

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Chapter 8

Conclusions, Implications for Clinical Practice, and Directions for Future Research

CONCLUSIONS

The purposes of this dissertation research were to: 1) review the conceptual basis for using variable-centered versus patient-centered analytic approaches in symptom cluster research; 2) systematically review studies published since 2016 that evaluated for symptom clusters in patients receiving primary or adjuvant chemotherapy; 3) evaluate the stability and consistency of symptom clusters across time and across three symptom dimensions (i.e., occurrence, severity, and distress); 4) identify common and distinct symptom clusters across various types of cancer; and 5) evaluate for associations between psychological and gastrointestinal symptom clusters and epigenetic regulation of inflammatory genes in a heterogeneous sample of oncology patients.

In Chapter One, conceptual and methodological issues within symptom cluster research were identified and served as areas of inquiry for this dissertation research. One issue was whether the dimension of the symptom experience that is used to create a symptom cluster affects the number of types of clusters that are identified. A second issue was whether common and distinct clusters could be identified across various types of cancer and/or treatment(s). In addition, while psychological and gastrointestinal symptom clusters are the most common clusters among patients receiving chemotherapy,[1] we noted that the symptoms within these clusters vary across studies. Given that this variability may be due to differences in the statistical methods and/or instruments used to identify the symptom cluster across studies, we suggested that an evaluation of common symptom clusters within a single study was warranted. In addition, the hypothesis that symptoms cluster together due to shared, underlying biological mechanism(s) was discussed and the paucity of research in this area of scientific inquiry was highlighted. We hypothesized that dysregulation of inflammatory processes through epigenetic regulation (i.e., DNA methylation) may underlie these symptom clusters.

In Chapter Two, we compared and contrasted the conceptual basis for using variable-centered versus patient-centered analytic approaches in symptom cluster research; reviewed their strengths and weaknesses; and compared their applications in symptom cluster research. We reported that EFA was the most common statistical approach for studies that used a variable-centered approach. Among studies that used a patient-centered approach, latent variable modeling was the most common method used. For both approaches, relatively few studies evaluated the underlying mechanisms for various symptom clusters.

In addition, we identified a need to develop clear criteria to determine the stability and consistency of symptom clusters. The establishment of these criteria will allow researchers to determine within and across studies whether symptom clusters change over time and/or across dimensions of the symptom experience. Furthermore, these criteria can be used to evaluate stability and consistency of symptom clusters across studies of patients with different cancer types and/or chronic conditions.

Chapter Three reported the results from a review of 23 studies that evaluated for symptom clusters in patients receiving primary or adjuvant chemotherapy from 2017 through 2021. Across these studies, the MSAS was the most common instrument and EFA was the most common statistical method used to identify symptom clusters. While psychological, gastrointestinal, and nutritional clusters were the most commonly identified clusters across studies, only the psychological cluster remained relatively stable over time. One major conclusion from this review was that clear criteria are needed to evaluate the stability of symptom clusters across time and dimensions. We suggested that the term stability should be used to describe whether or not the same clusters are identified across study samples, dimensions, and/or over time. While consistency should be used to describe whether the symptoms within a cluster remain the same across these conditions.

In Chapter Four, a report on the symptom dimensions of occurrence, severity, and distress of 38 symptoms and an evaluation of the stability and consistency of symptom clusters

across the three dimensions was done. In addition, we identified common and distinct symptom clusters across four types of cancer (i.e., breast, gastrointestinal, gynecological, lung). The psychological, gastrointestinal, weight gain, respiratory, and hormonal clusters were stable across all three symptom dimensions. Our findings suggest that psychological, gastrointestinal, and weight gain clusters are common across various types of cancer while respiratory and hormonal clusters are cancer-specific.

Building on our findings from the first four chapters, in Chapter Five, we reported on new methods to assess consistency of symptom clusters over time and symptom dimensions. Using these new criteria, we evaluated the stability and consistency of symptom clusters across a cycle of chemotherapy, three symptom dimensions, and four types of cancer (i.e., breast, gastrointestinal, gynecological, lung). Psychological, weight gain, gastrointestinal, and respiratory clusters were stable over time and dimensions. Only the psychological, weight gain, and respiratory clusters were consistent across time and dimensions.

In Chapters Six and Seven, we reported on findings from an evaluation of the associations between the occurrence of psychological and gastrointestinal symptom clusters and levels of DNA methylation in putative regulatory regions of inflammatory genes. As reported in Chapter Six, our findings suggest that increased *CD40* expression through hypomethylation of six promoter eCpG loci is involved in the occurrence of a psychological symptom cluster in patients receiving chemotherapy. In Chapter Seven, our findings suggest that increased *LTB* expression through hypermethylation of a trans eCpG locus is involved in the occurrence of a gastrointestinal symptom cluster in patients receiving chemotherapy.

IMPLICATIONS FOR CLINICAL PRACTICE

Findings from this dissertation research highlight the significant symptom burden experienced by oncology outpatients receiving chemotherapy. Prior to the start of their second or third cycle of chemotherapy, patients reported on average 13.9 symptoms. Across a cycle of chemotherapy, this symptom burden remained relatively consistent, with patients reporting on

average 14 symptoms one week and 12.2 symptoms two weeks after receipt of their chemotherapy. These findings underscore that ongoing symptom assessment and management need to be central foci during outpatient oncology care.

In addition, while lack of energy was the most common and severe symptom reported by patients, “I don’t look like myself” was the most distressing. These findings suggest that the most common symptom is not always the most distressing. While symptom occurrence and severity are assessed most often, clinicians need to evaluate multiple dimensions of the symptom experience to address the complex needs of patients with cancer.

Given this complexity, across all patients with cancer, we suggest that symptoms within stable clusters (i.e., psychological, gastrointestinal, weight gain) need to be assessed on a routine basis. For example, in our study that identified a psychological symptom cluster in patients prior to the start of their second or third cycle of chemotherapy,[2] symptoms within this cluster were some of the most common and severe (i.e., worrying, difficulty concentrating, feeling sad) and distressing (i.e., “I don’t look like myself”) symptoms experienced by these patients. Given the stability and consistency of this cluster over time,[3] it is imperative to routinely assess for these symptoms and initiate interventions and/or referrals to psychological support services.

While stable across cancer types, the structure and composition of a gastrointestinal symptom cluster varies across time, symptom dimensions, and cancer types.[3] This variability has a number of plausible explanations, including: differential effects of specific chemotherapy regimens on the gastrointestinal mucosa; differential effects of the cancer itself (e.g., colon cancer versus breast cancer) on the gastrointestinal tract; and/or variations in the relationships among various symptoms that are associated with specific types of cancer (e.g., feeling bloated in gastrointestinal cancers). Given that gastrointestinal symptoms are extremely common and distressing (e.g., nausea, change in the way food tastes, constipation)[2] and persist over time,[3]

routine assessment for these symptoms and initiation of referrals to nutrition services are warranted throughout cancer treatment.

In terms of specific cancer diagnoses, symptom assessments need to be tailored to the unique symptoms and symptom clusters experienced in these patients. Specifically, specific symptoms within the hormonal cluster need to be assessed in patients with breast and gastrointestinal cancers and symptoms within the respiratory cluster need to be assessed in patients with gynecological or lung cancer.

RECOMMENDATIONS FOR FUTURE RESEARCH

While symptom cluster research is growing at a rapid pace, multiple opportunities for conceptual and methodological growth remain. As outlined in Table 2.1, many of these opportunities relate to how a symptom cluster is defined and conceptualized, and most notably, the stability of a symptom cluster. In Chapters Two and Three, we identified a lack of clarity and consensus on the definition of and methods used to evaluate the stability of symptom clusters across time, symptom dimensions, and studies. To address this issue, we proposed clear definitions for the terms “stability” and “consistency” and approaches for their evaluation. Given that our cross-sectional[2] and longitudinal studies[3] are the first to use these new definitions and methods to evaluate the stability and consistency of symptom clusters across time, symptom dimensions, and cancer types, future studies need to determine their validity and clinical utility.

Consistent with findings from a previous review[1] and our systematic review[4] that identified that psychological and gastrointestinal clusters are the most common clusters in patients receiving chemotherapy; we identified that psychological and gastrointestinal clusters were common across four distinct cancer types (i.e., breast, gastrointestinal, gynecological, lung). While stable and consistent after the commencement of chemotherapy, it is unclear if the psychological cluster occurs as a result of the chemotherapy, the cancer itself, or stress associated with a cancer diagnosis. In studies that evaluated for symptom clusters prior to and throughout the administration of chemotherapy, findings are mixed. For example, among

patients with high grade brain[5] and breast cancer,[6] a psychological cluster was identified prior to the start of cancer treatment. However, for patients newly diagnosed with acute myelogenous leukemia, this cluster was not identified until after chemotherapy induction.[7] Future research is needed to evaluate the factors that contribute to the time to onset and development of the psychological cluster.

In contrast, the stability and consistency of the gastrointestinal cluster were found to vary across time, dimensions, and cancer types. The dynamic nature of this cluster is consistent with previous reports. For example, in three studies[8-10] that evaluated for symptom clusters across two or more cycles of chemotherapy, while stable, the symptoms within the gastrointestinal cluster were not consistent. Additional research is warranted to examine how the gastrointestinal cluster evolves during chemotherapy and how it differs by cancer type. Findings from this research will provide direction for mechanistic studies.

In addition, we identified symptom clusters distinct to specific cancer types (i.e., hormonal for women with breast or gynecological, and respiratory for patients with gynecological or lung cancers). Of note, the specific symptoms within these clusters were comprised of symptoms that were added to the MSAS; namely: hot flashes in the hormonal cluster and chest tightness and difficulty breathing in the respiratory cluster. Additional research is warranted to identify the optimal symptom inventories to identify common and disease-specific symptom clusters.[11]

As identified in our conceptual and systematic reviews, research that evaluates the mechanisms that underlie symptom clusters are limited. Given that our studies are the first to evaluate for associations between the occurrence of the psychological and gastrointestinal symptom clusters and epigenetic regulation of inflammatory genes, future studies are warranted to confirm our findings. Additional research is warranted to determine the best methods to evaluate the relationship between symptom cluster scores and levels of methylation.

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