Symptom clusters in palliative-stage cancer correlate with proinflammatory cytokine cluster

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Background: Patients with palliative-stage cancer often suffer from a variety of debilitating symptoms which have been shown to appear in clusters. It is suggested that cytokines cause many such symptoms, and elevated cytokine production has been shown to correlate with symptoms. However, symptom clusters have not been thoroughly analyzed in relation to cytokine clusters. The aim of the present study was to identify symptom clusters and cytokine clusters in Swedish cancer patients, and to investigate correlations between the identified symptom clusters and cytokine clusters.

Methods: The EORTC Quality of Life Questionnaire Core 15 Palliative Care questionnaire was completed by 110 cancer patients, with blood samples taken at two time points four weeks apart. Meso scale discovery (MSD) assays were used to analyze 23 cytokines. Statistical analysis was performed using principal component analysis (PCA) of symptoms and cytokines, followed by correlation analysis of the obtained clusters.

Results: Three symptom clusters were identified: (I) pain-sleep disorder, (II) gastro-intestinal-fatigue, (III) physical functioning. The cytokines were divided into three clusters that can be characterized as (I) pro-tumorigenic, (II) cell-mediated immune response and (III) proinflammatory. At the second time point, a fourth cytokine cluster was isolated (IV) immunostimulation. Correlations were found at both time points between the proinflammatory cytokine cluster and the physical functioning symptom cluster, and at the week four time point between the proinflammatory cytokine cluster and the gastro-intestinal-fatigue symptom cluster.

Conclusions: We show a correlation between symptom clusters and the proinflammatory cytokine cluster. Proinflammatory cytokines are known to cause symptoms that resemble palliative cancer symptoms. Increased knowledge of biochemical processes and their effect on patients' wellbeing may give clues for counteracting symptoms that affect quality of life (QOL) in palliative cancer care.

Keywords: Palliative-stage cancer; symptom cluster; cytokine cluster; palliative care; principal component analysis (PCA)

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Introduction

Patients with palliative-stage cancer are burdened by various manifestations both due to the cancer itself and other bodily processes related to the malignancy. As well as the local influence of the tumor or metastases, cancer patients report several general debilitating symptoms, due to the disease itself or from the treatments, with severe impact on quality of life (QOL). Associated symptoms common for all types of cancer are fatigue, pain, insomnia, gastrointestinal symptoms, anxiety and depressed mood (1). In advanced cancer, a patient may experience more than 13 symptoms simultaneously (2).

The symptoms reported by cancer patients share many features with the sickness behavior consisting of a coordinated set of adaptive behavioral changes that develop in people during an infection. This includes lethargy, depression, anxiety, malaise, loss of appetite, sleepiness, hyperalgesia, reduced hygiene and failure to concentrate. It is well established that this sickness behavior is a result of cytokines acting on the brain aiming to optimize the

recovery of the individual (3). In end stage cancer, however, these symptoms seem to have no benefit for the patient—they contribute instead to severe suffering.

Many of these cancer-associated symptoms result from the inflammatory reaction given by a tumor invading a host. Increasing knowledge about how inflammation affects the progress of cancer has led to inflammation being described as the disease's seventh hallmark (4). The inflammatory reaction is driven by cytokines and chemokines produced by the host, while the tumor itself also hijacks the system and produces cytokines to suppress the host's antitumor immunity and mediate migration, invasion and metastasis of malignant cells (5). Although different tumors have their own characteristics, several common features may be identified, and with increased knowledge of the connection between cytokines and symptoms, we may find new ways to increase the QOL in cancer patients. In fact, several available anti-cytokine treatments used in other contexts have been proven to reduce various symptoms. For instance, anti-tumor necrosis factor (TNF)-α and interleukin (IL)-6 inhibitors both improved depressive symptoms, and anti-TNF-α treatment reduced the fatigue experienced by cancer patients (6,7).

Generally speaking, patients experience several symptoms, and it would be beneficial to find strategies to treat them concurrently. Multiple concurrent symptoms are thought to function as symptom clusters. A cluster can contain two or more related symptoms which may or may not suggest a common etiology or underlying mechanism (8). There is presently no consensus on the definition of specific clusters (9). However, symptoms such as anxiety-fatigue-breathlessness, nausea-vomiting-appetite loss, and pain-fatigue-sleep disturbance are often reported as clusters in several studies (10-12). The result may vary depending on the method used, and more studies are needed to define clusters and find methods of treatment aimed at relieving patients' symptoms as much as possible.

Individual cytokines have been shown to correlate with specific symptoms (13). For example, TNF was shown to correlate with depression, and interleukin-1 receptor antagonist (IL-1RA) with fatigue. IL-6 is thought to predict worsening of symptoms but also more specifically affect appetite (14-17). Cluster analysis of cytokine levels

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Highlight box

Key findings
- A proinflammatory symptom cluster was found to correlate to a physical functioning and a gastro-intestinal-fatigue symptom cluster in patients with cancer in palliative stage. These findings suggest a connection between levels of pro-inflammatory cytokines in the blood of palliative cancer patients and symptom burden.

What is known and what is new?
- It is well established that cytokines cause different symptoms, it is also known that symptoms appear in clusters in several conditions. Different symptom clusters have been reported although a good definition still remains to be established. We identified three symptom clusters and four cytokine clusters. Correlations were found between the proinflammatory cytokine cluster and the physical functioning symptom cluster, and the gastro-intestinal-fatigue symptom cluster.
- We show for the first time that a cluster of cytokines correlate with clusters of symptoms in cancer.
- Knowing the causative role of cytokines opens new ways of treating adverse symptoms in various diseases, however verification in larger cohorts are needed.

Table 1 Overview of study enrollment

	Included	Excluded	%
Screened	493		
Not included according to criteria		33	
Remaining included	460		
Included			
Excluded according to criteria		219	48% of included
Remaining included	241		52% of included
PP			
Declined		90	37% of PP
Deceased before asked to participate		7	3% of PP
Accepted but did not participate		34	14% of PP
Participated in the study	110		46% of PP

PP, possible participants.

Table 2 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
At least 18 years old	Hematologic malignancy
Palliative-stage cancer	Pronounced leukopenia (LPK ≤1.0×10^9/L)
	Antibiotic treatment for ongoing infection
	Cognitive impairment*
	Patient too weak for the study*
	Patient's Swedish insufficient*

*, according to the patient's nurse or physician. LPK, leukocyte count.

in patients with chronic lymphocytic leukemia or breast cancer revealed clusters that were associated with prognosis and overall survival (18-20). To the best of our knowledge, no studies have reported correlation of cytokine clusters with symptom clusters. However, clusters of cytokine gene expression have been compared to symptoms. For instance, in breast cancer patients, the genetic variations in IL6, IL-13, and TNF-α were associated with the predefined symptom cluster of pain, fatigue, sleep disturbance, and depression (21).

This study aimed to identify symptom clusters and cytokine clusters in Swedish patients with palliative-stage cancer. Furthermore, we investigated correlations between the identified symptom clusters and cytokine clusters. Data and samples were collected at two time points in order to explore changes over time. PCA was carried out to identify symptom and cytokine clusters, followed by an exploration of associations between identified symptom and cytokine clusters. We present this article in accordance with the STROBE reporting checklist (available at https://apm.amegroups.com/article/view/10.21037/apm-22-974/rc).

Methods

Study population and procedures

Patients were recruited consecutively from three palliative home care units and one department of internal medicine located in southeast Sweden between April 2016 and April 2018: Department of Geriatrics and Palliative Medicine, The County Hospital in Kalmar; Department of Advanced Home Care, Linköping University Hospital; Department of Advanced Home Care and Department of Internal Medicine in Vrinnevi Hospital, Norrköping. An overview of study enrollment is presented in *Table 1*.

Data collection

A research nurse was recruited from each medical unit. The nurses screened inpatients consecutively according to inclusion and exclusion criteria—see *Table 2*. The study was

conducted in accordance with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from the patients prior to their entering the study. The study was approved by the Regional Ethical Review Board in Linköping (application No. 2015/383-31). Each patient was assigned an ID-number, and all analysis of data was performed blinded. Medical data (e.g., type of malignancy, metastasis, corticosteroid treatment, nonsteroidal anti-inflammatory drugs (NSAID) treatment, antibiotics, specific cancer treatment) were collected from the medical record by the nurse, and demographic data (e.g., home situation, educational background and smoking) were obtained from the patient. The nurse visited the patients in their homes on two occasions, with four weeks between the visits. On each visit, the patient answered the EORTC Quality of Life Questionnaire Core 15 Palliative Care (EORTC QLQ-C15-PAL), and body temperature was measured in the right ear. Blood samples were taken at the end of the visit. Analysis of C-reactive protein (CRP), lactate dehydrogenase (LD), albumin and procalcitonin was performed on serum using accredited routine methods at two clinical laboratories.

EORTC QLQ-C15-PAL questionnaire

The EORTC-QLQ-C15-PAL consists of 15 questions (Q1–15) that refer to 2 functional scales (physical functioning Q1–3 and emotional functioning Q13, 14), 2 symptom scales (pain Q5, 12 and fatigue Q7, 11), 5 single-symptom items (shortness of breath Q4, trouble sleeping Q6, loss of appetite Q8, nausea Q9, and constipation Q10), and the global QoL scale Q15. Fourteen items are rated from 1 (not at all) to 4 (very much), and QOL is rated from 1 (very poor) to 7 (excellent) (22). Question number 15 addressing QOL was excluded from the cluster analysis, since it has a different scaling.

Cytokine measurement

Blood samples for cytokine measurements were collected in ethylenediaminetetraacetic acid (EDTA) tubes. The tubes were stored on ice until processing in the laboratory. Within four hours of sampling, the blood samples were centrifuged (1,800 G, 10 minutes at 4 ℃) and frozen at –70 ℃. Until analysis, all samples were stored at the Linköping Biobank Facility.

Three patients were excluded from cytokine measurements due to hepatitis infection (two patients both at baseline and four weeks, one patient only baseline). Cytokines were analyzed using MSD technology (Mesoscale, Rockville, MD, USA). The following human kits were used: R-PLEX TNF-RII Antibody Set, U-PLEX Biomarker Group 1 Assays {interferon (IFN)-α2a IL-1RA, IL-3, IL-7, IL-9, IL-16, IL-17A, IL-17F, IFN-γ induced protein 10 (IP-10), V-PLEX Proinflammatory panel 1 (IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNF-α), and U-PLEX TGF-β Combo [transforming growth factor (TGF)-β1, β2, and β3]}. The cytokines were selected based on previous research on cancer related inflammation and symptoms (16,17,23). The cytokines IL-4, TGF-β1 and TGF-β2 were subsequently excluded from further analysis due to low readings, resulting in measurements from 20 cytokines used for analysis. Cytokine results from one patient were excluded due to outlying values.

Statistical analysis

Paired samples t-tests with Bonferroni-correction were performed for each question and the cytokine concentrations were analyzed using Wilcoxon signed-rank tests with Bonferroni-correction, investigating differences between baseline and the four weeks time point. Non-parametric tests were chosen for the cytokine measurements because the data was not normally distributed.

Cytokine values were normalized by calculating z-scores with the formula: $Z_x = (X_i - \overline{X})/S_x$, and PCA of the cytokine data was performed using the z-scores. SPSS software was used for all statistical analysis.

PCA

Baseline

Questionnaire data of question 1–14 EORTC QLQ-C15-PAL were subjected to factor analysis using PCA and orthogonal Varimax rotation. The Kaiser-Meyer-Olkin measure (KMO) was 0.754, indicating that the data were sufficient for factor analysis. 100% of KMO values for the individual items were above 0.5. The Bartlett's test of sphericity $\chi^2(91)=507.872$, P=0.000 showed that there were patterned relationships between the items. Iterating cut-off and numbers of factors resulted in three factors with at least three unique components that explain a cumulative variance of 53.6%. The scree plot confirmed the findings of retaining three factors.

PCA of cytokine values with orthogonal Varimax rotation gave a KMO of 0.696. Ninety percent of KMO values for the individual items were above 0.5 (IL-17F and

Table 3 Patient characteristics

Characteristics	Baseline	4 weeks
Total patients	110	90
Age (years)		
Mean ± SD	68±12.3	69±11.9
Median [range]	70 [23–89]	71 [23–88]
Gender		
Male	54 (49%)	45 (50%)
Female	56 (51%)	45 (50%)
Primary cancer site		
Lung	16	
Prostate	12	
Large intestine	12	
Breast	10	
Skin	7	
Ventricle/esophagus	7	
Peritoneum	5	
Pancreas	5	
Kidney	4	
Gallbladder/duct	4	
Liver	4	
Brain	4	
Bone	4	
Other	16	
Patients with metastases	94	75
Site of metastases		
Liver	34	26
Lung	23	19
Skeletal	38	32
Abdomen	14	11
Lymph node	20	17
Brain	12	9
Adrenal gland	5	3
Skin	4	3
Other/unknown	10	9
None	16	15

TNF-RII, which did not fit into the extracted components displayed values below 0.5). The Bartlett's test of sphericity $\chi^2(190)=1,488.010$, P=0.000 showed that there were patterned relationships between the items. Iterating cut-off and numbers of factors resulted in three factors with at least five unique components that explain a cumulative variance of 55.4%. The scree plot confirmed the findings of retaining three factors.

Four weeks

The same analysis was performed on data from the four-week time point. Analyzing the questionnaire, KMO was 0.785. Ninety-five percent of KMO values for the individual items were above 0.5. The Bartlett's test of sphericity: $\chi^2(91)=478.450$, P=0.000. Iterating cut-off and numbers of factors resulted in three factors with at least three unique components that explain a cumulative variance of 57.7%. The scree plot confirmed the findings of retaining three factors.

PCA on cytokine values with orthogonal Varimax rotation resulted in four factors with at least two unique components using a cut-off that explains a cumulative variance of 70.2%. KMO was 0.819, and 100% of KMO values for the individual items were above 0.5. The Bartlett's test of sphericity: $\chi^2(190)=1,753.392$, P=0.000. The scree plot confirmed the findings of retaining four factors.

Correlation of clusters

The data obtained from the PCA were used for correlation analysis of the different clusters. Linear correlation was performed calculating the Pearson correlation coefficient, r. Thus, clusters 1–3 from the symptom clusters at baseline were correlated with clusters 1–3 from the cytokine clusters at baseline, and consequently clusters 1–3 of symptoms and 1–4 of cytokines at four weeks were correlated.

Results

There were 110 patients that completed EORTC QLQ-C15-PAL at baseline. The average age was 68 years, with equal distribution between sexes (49% male, 51% female). Several different primary tumors were represented, and 85.5% of the patients presented with metastases. Of the 110

Table 4 Summary of EORTC C15-PAL scores at each visit

Question	Mean ± SD		Paired *t*-test, total (n)		
	Baseline	4 weeks	P	Baseline	4 weeks
Q1 short walk	2.15±1.15	2.47±1.30	0.007	109	90
Q2 in bed	2.55±0.93	2.72±0.91	0.050	109	90
Q3 need help	1.52±0.91	1.64±0.99	0.064	110	90
Q4 shortness of breath	1.86±0.94	2.14±1.10	0.005	110	90
Q5 pain	2.15±0.98	2.15±0.94	0.913	110	89
Q6 trouble sleeping	1.72±0.94	1.61±0.83	0.300	110	90
Q7 feebleness	2.52±0.87	2.57±0.94	0.581	108	90
Q8 lacking appetite	1.83±1.00	1.91±1.07	0.599	110	90
Q9 nausea	1.57±0.71	1.62±0.78	0.518	110	90
Q10 constipation	1.68±0.95	1.40±0.80	0.009	110	90
Q11 tiredness	2.69±0.77	2.85±0.97	0.193	109	90
Q12 pain interference	1.75±1.02	1.86±1.05	0.229	110	90
Q13 tenseness	1.57±0.77	1.80±0.99	0.013	110	90
Q14 depressed mood	1.84±0.86	1.92±0.93	0.572	110	90
Q15 total QOL	4.08±1.24	4.06±1.36	0.831	110	90

The P value for each pair is reported, significance level is P<0.003 according to Bonferroni correction analyzing 15 parameters. QOL, quality of life; SD, standard deviation.

patients, 90 patients (82%) participated four weeks later. The patient characteristics are summarized in *Table 3*. The drop in participation at the second time point was, in all cases, due to patients having deceased. EORTC QLQ-C15-PAL scores are summarized in *Table 4*. The symptoms with highest score at both time points were tiredness, fatigue and feebleness. The majority of scores, such as shortness of breath, trouble taking short walks, tenseness and fatigue, were higher (i.e., had worsened) after four weeks, whereas constipation was improved, although no statistical significance was seen using paired *t*-test with Bonferroni correction (see *Table 4*).

The data from the symptom questionnaire were subjected to factor reduction using PCA. Three clusters were identified at both timepoints (*Tables 5-7*). At baseline *Cluster 1* included symptoms of shortness of breath (Q4), pain (Q5), trouble sleeping (Q6), feebleness (Q7) and pain interfering with daily activities (Q12). *Cluster 2* was represented by lack of appetite (Q8), nausea (Q9), tiredness (Q11) and depressed mood (Q14), whereas *Cluster 3* described trouble taking a short walk (Q1), the patient needing to stay in bed during the day (Q2) and needing help

with basic daily activities (Q3). Questions 1–3 in *Cluster 3* are defined as "Physical functioning" according to EORTC C15-PAL (22).

At four weeks, *Cluster 1* included the two pain questions (Q5 and 12) as well as trouble sleeping (Q6) with the addition of tenseness (Q13) and depressed mood (Q14). *Cluster 2* contained Q8 lacked appetite and Q11 being tired as baseline, with the addition of Q4 shortness of breath and Q7 feebleness. *Cluster 3* at four weeks was the same as for baseline. The clusters are summarized in *Table 7*.

Blood samples for cytokine measurements were drawn on the same visit as the questionnaire was conducted. Cytokine data is summarized in *Table 8* and Figure S1. There were no significant changes between measurements at baseline and at 4 weeks. The cytokine data were subjected to factor reduction by PCA in a similar manner as the symptom data. Three cytokine clusters were identified at baseline and 4 cytokine clusters at 4 weeks, see *Tables 9,10* for details.

The clusters obtained from the symptom analysis were correlated to the clusters obtained from the cytokine data. At baseline, a significant correlation was obtained between

Table 5 Symptom cluster using principal component analysis, baseline

Cluster	Items	Component 1	Component 2	Component 3	Final communality
1	Q4 shortness of breath	0.525	−0.061	0.065	0.284
	Q5 pain	0.622	0.414	0.176	0.589
	Q6 trouble sleeping	0.660	−0.046	−0.213	0.483
	Q7 feebleness	0.631	0.309	0.413	0.664
	Q12 pain interference	0.676	0.426	0.155	0.663
2	Q8 lacking appetite	−0.162	0.755	0.161	0.622
	Q9 nausea	0.048	0.660	−0.390	0.590
	Q11 tiredness	0.430	0.588	0.184	0.564
	Q14 depressed mood	0.294	0.561	0.232	0.455
3	Q1 short walk	0.514	−0.081	0.611	0.643
	Q2 in bed	0.515	0.282	0.572	0.671
	Q3 need help	−0.005	0.142	0.823	0.697
	Q10 constipation	−0.119	0.382	0.348	0.282
	Q13 tenseness	0.297	0.443	0.083	0.291

Table 6 Symptom cluster using principal component analysis, 4 weeks

Cluster	Items	Component 1	Component 2	Component 3	Final communality
1	Q5 pain	0.712	−0.164	0.166	0.561
	Q6 trouble sleeping	0.549	0.051	0.006	0.304
	Q12 pain interference	0.693	0.372	0.139	0.638
	Q13 tenseness	0.743	−0.142	0.173	0.603
	Q14 depressed mood	0.709	0.142	0.284	0.604
2	Q4 shortness of breath	−0.086	0.785	−0.051	0.627
	Q7 feebleness	0.304	0.637	0.264	0.568
	Q8 lacking appetite	0.400	0.537	0.072	0.454
	Q11 tiredness	0.273	0.697	0.313	0.658
3	Q1 short walk	0.102	0.532	0.682	0.759
	Q2 in bed	0.246	0.432	0.704	0.743
	Q3 need help	0.021	0.109	0.806	0.663
–	Q9 nausea	0.530	0.307	−0.278	0.452
–	Q10 constipation	0.217	−0.356	0.517	0.441

Symptom Cluster 3 (Physical functioning) and *Cytokine Cluster 3* (r=0.258, P=0.008). At four weeks, the *Physical functioning symptom cluster* correlated with *Cytokine Cluster 2* (r=0.227, P=0.033). There was also a significant correlation between *Cytokine Cluster 2* and *Symptom Cluster 2* (r=0.223, P=0.036), summarized in *Figure 1*.

Table 7 Summary of symptom cluster analysis

Quest	Cluster		
	1st cluster	2nd cluster	3rd cluster
Baseline	Q4, Q5, Q6, Q7, Q12	Q8, Q9, Q11, Q14	Q1, Q2, Q3
4 weeks	Q5, Q6, Q12, Q13, Q14	Q4, Q7, Q8, Q11	Q1, Q2, Q3

Table 8 Summary of cytokine data

Cytokine	Mean ± SD (pg/mL)		Median (pg/mL)		Wilcoxon	Range (pg/mL)	
	Baseline	4 weeks	Baseline	4 weeks	P	Baseline	4 weeks
IFN-γ	8.56±7.35	10.88±16.65	5.70	6.11	0.79	0.04–37.36	1.67–114.38
IL-10	1.59±1.80	1.30±1.38	0.92	0.91	0.62	0.21–13.6	0.31–10.07
IL-12p70	0.73±0.81	0.74±0.88	0.48	0.43	0.39	0.03–4.95	0.12–5.18
IL-13	4.51±4.63	4.82±4.77	2.32	2.33	0.77	0.04–27.36	0.48–20.8
IL-1β	0.31±0.34	0.26±0.26	0.18	0.17	0.05	0.00–1.56	0.02–1.56
IL-2	0.75±1.25	0.68±1.10	0.34	0.32	0.93	0.03–7.37	0.02–6.93
IL-6	5.23±8.42	3.90±3.69	2.66	2.66	0.35	0.27–56.06	0.31–19.31
IL-8	10.78±22.87	11.09±23.05	4.29	4.15	0.02	0.76–173.68	0.72–179.23
TNF-α	3.02±1.80	3.00±1.59	2.52	2.68	0.36	0.00–12.11	0.70–9.11
TGF-β3	2.43±2.76	2.32±2.02	1.50	1.59	0.16	0.06–14.98	0.09–9.04
IFN-α2a	6.58±7.35	6.08±5.47	4.59	4.62	1.00	0.37–39.20	0.16–32.14
IL-16	228.27±93.31	227.39±100.42	214.67	211.96	0.42	68.78–559.63	62.82–517.08
IL-17A	49.00±32.53	45.01±27.86	42.46	39.55	0.57	9.69–184.85	7.76–150.22
IL-1RA	687.81±1,029.15	478.22±399.44	373.89	323.71	0.72	135.22–6,526.68	112.69–399.44
IL-3	85.09±99.09	77.04±74.92	47.94	46.93	0.96	5.76–510.88	0.72–45.41
IL-7	8.45±8.79	7.74±6.96	5.35	5.18	0.93	1.01–56.28	0.72–45.41
IL-9	1.48±1.46	1.25±1.22	1.03	0.92	0.25	0.04–7.85	0.12–6.44
IP-10	677.26±816.63	662.80±890.80	482.40	396.52	0.70	55.46–6,553.18	92.25–5,625.3
IL-17F	60.78±66.79	44.77±52.52	46.11	47.14	0.40	0.85–345.37	0.78–205.16
TNF-RII	19,875.4±14,596.2	19,216.0±14,196.2	17,245.5	16,754.1	0.89	26.38–77,340.6	157.36–62,232

Significance, P, referring to significance using Wilcoxon signed-rank test. Bonferroni corrected P value based on 20 cytokines gives significant difference if $P<0.0025$. SD, standard deviation; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; TGF, transforming growth factor; IP, induced protein.

Discussion

Three symptom clusters were extracted at both baseline and the four-week timepoint. The first cluster at both time points included symptoms of pain and sleep disturbance. The second cluster included gastro-intestinal and fatigue symptoms, and the third cluster at both time points represented physical functioning symptoms. Three cytokine clusters were identified at both time points, representing pro-tumorigenic, cell-mediated immune responses and proinflammatory cytokines. Additionally, at the four-week timepoint, a fourth cluster with immunostimulatory cytokines was identified. The proinflammatory cytokine

Table 9 Cytokine cluster using principal component analysis, baseline

Cluster	Items	Component 1	Component 2	Component 3	Final communality
1	IFN-α2a	0.959	0.070	0.107	0.935
1	IL-3	0.956	0.015	0.055	0.916
1	IL-7	0.915	−0.010	0.139	0.856
1	TGF-β3	0.808	−0.019	0.038	0.655
1	IL-17A	0.722	0.244	0.366	0.714
1	IL-9	0.701	−0.036	0.220	0.541
2	IL-12p70	−0.054	0.877	−0.070	0.777
2	IL-2	−0.047	0.821	−0.003	0.677
2	IL-13	−0.049	0.722	−0.068	0.528
2	IFN-γ	0.041	0.711	0.311	0.604
2,3	IL-1β	0.094	0.621	0.513	0.657
2	IL-10	0.045	0.476	0.250	0.292
3	TNF-α	−0.007	0.408	0.772	0.762
3	IL-16	0.194	0.062	0.717	0.555
3	IL-8	0.017	−0.083	0.647	0.426
3	IP-10	0.147	0.055	0.589	0.371
3	IL-1RA	0.136	−0.075	0.551	0.328
3	IL-6	0.025	0.240	0.548	0.358
–	TNF-RII	0.124	−0.074	0.258	0.087
–	IL-17F	0.064	0.180	−0.061	0.040

IFN, interferon; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor; IP, induced protein.

cluster correlated with the physical functioning and gastro-intestinal and fatigue symptom cluster.

Several studies have investigated symptom clusters in patients with cancer, but no overall accepted definition of the clusters has been presented. However, some clusters are reported in the literature more consistently than others, such as a gastro-intestinal symptom cluster with nausea, vomiting and occasional lack of appetite; a psychoneurological cluster including pain, fatigue and sleep disturbance sometimes associated with mood disturbances; and a respiratory symptom cluster with breathlessness, fatigue, anxiety and coughing (11). One problem with comparing different studies is that different methods have been used to evaluate the symptoms. This makes direct comparison difficult. In the present study the EORTC QLQ-C15-PAL—an internationally validated palliative care-specific health-related QOL instrument (22)—was used. This is a test with a small amount of questions (15),

which is important since the patients are often exhausted and have limited amounts of energy for answering questionnaires.

The present study identified three symptom clusters (I) pain-sleep disorder, (II) gastro-intestinal-fatigue, (III) physical functioning. Comparing the results of this study with one by Ganesh *et al.* which also used EORTC QLQ-C15-PAL at three different time-points in patients undergoing palliative radiotherapy reveals several interesting common features (12). As in the present study, the questionnaire data were analyzed by PCA, and several symptoms can be identified with similar distribution. The physical functioning symptom cluster isolated in the present study (questions 1–3) was also identified in the Ganesh study. In addition, questions 5, 6 and 12 addressing pain and trouble sleeping, as well as questions 4 and 7 representing shortness of breath and feebleness, gathered in the same clusters respectively in both studies. Gastrointestinal

Table 10 Cytokine cluster using principal component analysis, 4 weeks

Cluster	Items	Component 1	Component 2	Component 3	Component 4	Final communality
1	IL-3	0.960	0.045	0.045	0.074	0.932
1	IL-7	0.938	0.165	0.054	0.015	0.910
1	IFN-α2a	0.935	0.115	0.042	0.114	0.903
1	IL-9	0.850	0.171	0.049	0.227	0.806
1	TGF-β3	0.816	0.127	0.128	−0.102	0.709
1	IL-17A	0.687	0.466	0.008	0.242	0.747
2	IL-8	0.120	0.803	−0.070	−0.064	0.667
2	IL-6	0.111	0.734	0.140	0.035	0.572
2	IL-1RA	0.151	0.719	0.078	0.121	0.560
2	TNF-α	0.201	0.697	0.444	0.355	0.849
2	IL-10	0.081	0.625	0.339	0.058	0.515
2	TNF-RII	0.138	0.548	0.262	0.240	0.446
2	IL-16	0.421	0.488	0.253	0.308	0.574
3	IL-12p70	0.008	0.088	0.935	0.033	0.883
3	IL-2	−0.013	0.096	0.854	0.017	0.739
3	IL-13	0.021	0.138	0.797	0.171	0.684
3	IL-1β	0.130	0.472	0.695	−0.049	0.725
4	IFN-γ	0.088	0.008	0.238	0.864	0.811
4	IP-10	0.147	0.299	0.016	0.844	0.823
–	IL-17F	0.111	0.080	0.382	0.112	0.177

IL, interleukin; IFN, interferon; TGF, transforming growth factor; TNF, tumor necrosis factor; IP, induced protein.

symptoms (questions 8 and 9) were associated in the Ganesh study, whereas the present study only demonstrated this at the baseline timepoint (22).

The present study identified three cytokine clusters at baseline and four at four weeks. Cluster 1 at both timepoints contained the same cytokines: IFN-α2a, IL-3, IL-7, TGF-β 3, IL-17A and IL-9. Although the cytokine signaling processes are complex and often pleiotropic, there are studies suggesting that pro-tumorigenic activities may be represented in this cluster. TGF-β plays a key role in tumor-induced immunosuppression and stimulates production of factors that promote tumor growth—it is thought to be involved in tumor angiogenesis together with IL-3 (24). In addition, IL-17, recovered in the same cluster, induces VEGF which can induce TGF-β mediating angiogenesis (25,26). TGF-β levels in serum correlate with poor prognosis and increase the tumor's metastatic potential (27).

IL-12p70, IL-2 and IFN-γ cytokines found in cluster 2 at

baseline are cytokines with important roles in cell-mediated immune response against tumor cells (17). These cytokines have been shown to inhibit tumor growth and have been used experimentally as immunotherapy against cancer (28,29). In clinical tests, IL-2 administration resulted in reduced cognitive function and depressive symptoms (30,31).

The third cluster at baseline contains the classic pro-inflammatory cytokines IL-1β, IL-6 and TNF-α. IL-16 in the same cluster can stimulate secretion of these proinflammatory cytokines from monocytes and maturing macrophages (32). IL-8 is also considered to be a proinflammatory cytokine that increases in parallel with IL-6 and TNF-α as the cancer progresses (33,34). Pro-inflammatory cytokines are important for tumor growth, angiogenesis, and spreading of the tumor by metastases (35).

At the four-week time point, a fourth cluster was isolated containing IFN-γ and IP-10. IP-10, also known as CXCL10, is secreted in response to IFN-γ (36), known for

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Baseline		Correlation with symptom cluster			4 weeks		Correlation with symptom cluster		
Cytokine cluster		1	2	3	Cytokine cluster		1	2	3
		Pain-sleep	Gastro-intestinal-fatigue	Physical functioning			Pain-sleep	Gastro-intestinal-fatigue	Physical functioning
1	Pro-tumorigenic	r=0.088	r=-0.031	r=0.022	1	Pro-tumorigenic	r=-0.018	r=-0.004	r=-0.100
		P=0.372	P=0.753	P=0.824			P=0.869	P=0.971	P=0.352
	IFN-α2a					IFN-α2a			
	IL-3					IL-3			
	IL-7					IL-7			
	TGF-β3					TGF-β3			
	IL-17A					IL-17A			
	IL-9					IL-9			
2	Cell-mediated immune response	r=0.138	r=-0.043	r=-0.053	3	Cell-mediated immune response	r=0.010	r=0.134	r=-0.043
		P=0.157	P=0.661	P=0.592			P=0.926	P=0.212	P=0.688
	IL-12p70					IL-12p70			
	IL-2					IL-2			
	IL-13					IL-13			
	IL-1β					IL-1β			
	IFN-γ								
	IL-10								
3	Pro-inflammatory	r=0.094	r=-0.011	**r=0.258**	2	Pro-inflammatory	r=0.003	**r=0.223**	**r=0.227**
		P=0.338	P=0.912	**P=0.008**			P=0.980	**P=0.036**	**P=0.033**
	TNF-α					TNF-α			
	IL-16					IL-16			
	IL-8					IL-8			
	IL-6					IL-6			
	IL-1RA					IL-10			
	IP-10					TNF-RII			
	IL-1β								
					4	Immuno-stimulatory	r=0.083	r=-0.053	r=0.100
							P=0.440	P=0.620	P=0.349
						IFN-γ			
						IP-10			

Figure 1 Summary of cytokine clusters with correlation to symptom clusters. IFN, interferon; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor; IP, induced protein.

its immunostimulatory properties, may also have tumor-promoting properties helping in the selection of tumor cells with immuno-evasive phenotype (37).

A significant correlation was observed between the proinflammatory cytokine cluster (cytokine cluster 3) and the physical functioning symptom cluster as well as the second symptom cluster (including fatigue questions) at the four-week time point. These findings indicate a connection between proinflammatory activation and diminished everyday functioning. These findings are in line with the sickness-behavior initiated by the proinflammatory cytokines,

resulting in weakness, inability to concentrate, decreased interest in one's surroundings and anhedonia (38). Inhibiting the circulating cytokines may thus improve symptoms in patients with advanced cancer. Several anti-cytokine treatments are used for specific inflammatory conditions and the side-effects give clues as to how specific cytokines may impact symptom burden. For instance, fatigue has been linked to TNF-α. Patients with docetaxel treatment that received anti-TNF-α treatment using etanercept experienced less fatigue, while patients with rheumatoid arthritis—a disease where fatigue is a major burden for

the patients—reported significantly less fatigue after anti-TNF treatment (7,39,40). IL-6 can also cause fatigue, as demonstrated in patients with Castleman disease, a lymphoproliferative disorder characterized by increased IL-6 production, where IL-6 receptor administration reduced fatigue reported by the patients. Additionally, IL-6 has been shown to affect appetite and correlate with depression in cancer patients (39,41).

Few studies have investigated the association of symptom clusters with cytokine levels in the blood. In a Chinese study comparing symptom clusters with cytokine levels in 170 cancer patients, IL-6 was found to be associated with reported physical performance status, whereas no association was reported for TNF-α or IL-1β in this study (42). One study investigated the association between cytokine genes and symptom clusters, reporting an association between IL-6, TNF-α and IL-1β genes and the symptom cluster of pain, fatigue, sleep disturbance and, depression—however, the translation of the gene-expression to circulating plasma cytokines makes it difficult to compare data (21).

This study has shown correlations between cytokine clusters and symptom clusters, which may indicate that anti-cytokine treatment may have a place in palliative care of cancer patients.

It must, however, be remembered that other interventions may have good effect on the symptom burden. For instance, methylphenidate can improve fatigue and depression (43,44). Non-pharmacological treatments such as customized exercise, massage, psycho-educational and behavioral interventions have also proven effective (45-50). An optimal treatment should be individualized using a combination of the different options available.

The study population consisted of 110 cancer patients in a palliative disease stage. Although the heterogeneity, with many different cancer types, may have lowered the power to detect symptom and cytokine clusters related to specific kinds of cancer, we believe the results may be broadly generalizable for both symptom clusters and cytokine clusters, indicating common features in general palliative cancer care populations.

Conclusions

In conclusion, a pro-inflammatory symptom cluster was found to correlate with physical functioning symptoms as well as a fatigue symptom cluster. These findings suggest a connection between levels of pro-inflammatory cytokines in the blood of palliative cancer patients and symptom burden. One way to treat those symptoms may be to reduce circulating cytokine levels in the blood.

Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://apm.amegroups.com/article/view/10.21037/apm-22-974/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://apm.amegroups.com/article/view/10.21037/apm-22-974/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from the patients prior to their entering the study. The study was approved by the Regional Ethical Review Board in Linköping (application No. 2015/383-31).

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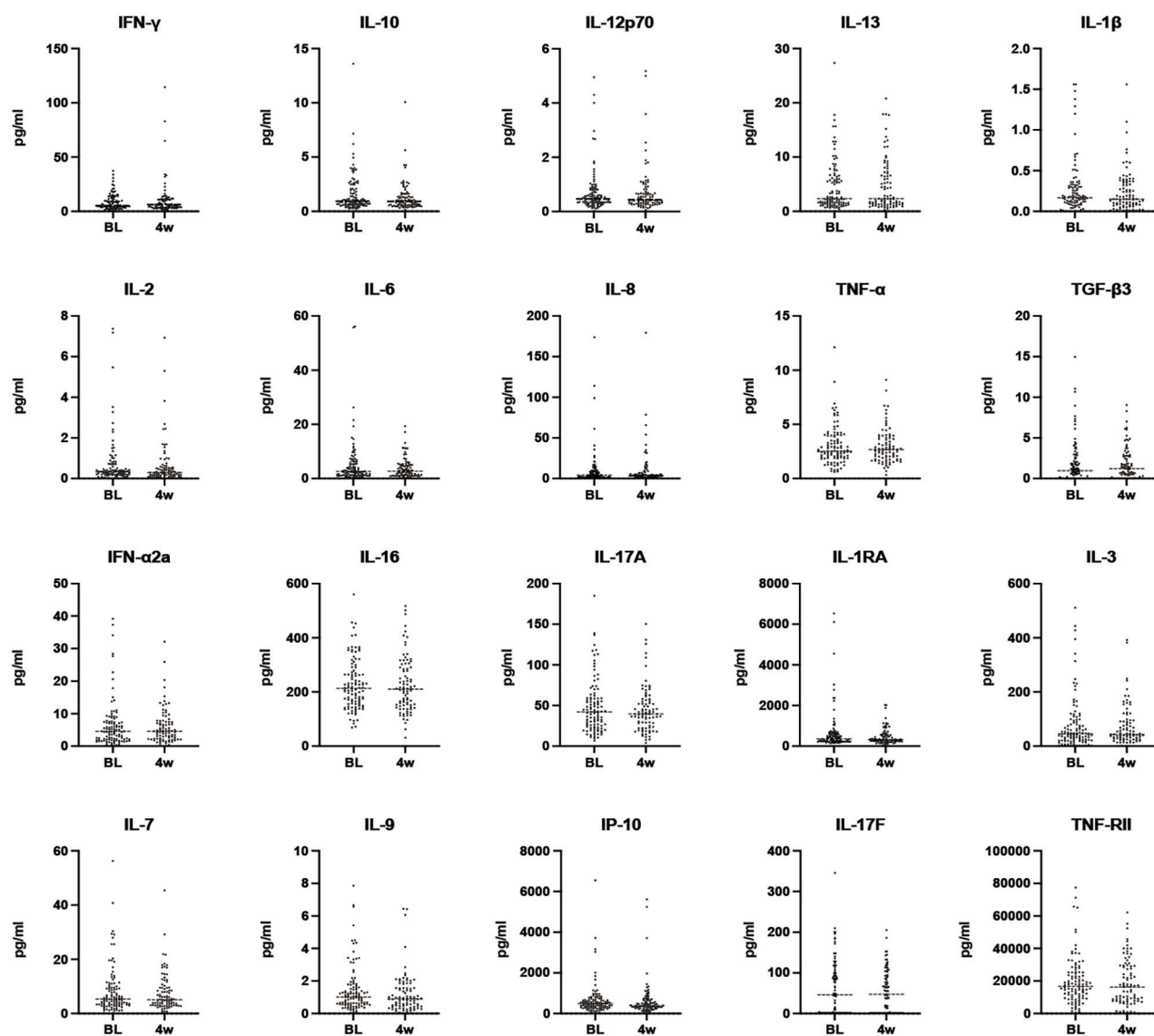
Figure S1 Cytokine levels at first visit (baseline, BL) and 4 weeks after the first visit (4w). All cytokines are reported in pg/mL. Dotted line represents mean.