Multi-analyte profiling and pathway analysis of plasma for proteins associated with cancer related fatigue syndrome in disease-free breast cancer patients after primary treatment

Minton O*
Coulton GR PhD St Georges University of London
Stone P MA MD FRCP St Georges University of London
*Corresponding author

Dr Ollie Minton PhD FRCP
Consultant and honorary senior lecturer in palliative medicine St George's University of London
Division of population health sciences and education
6th floor Hunter wing
St Georges University of London
Cranmer Terrace
London SW17 ORE
Phone: 02087252620
Email: ominton@sgul.ac.uk

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Abstract

Context A significant number of women treated for breast cancer develop long term fatigue afterwards. Previous research has suggested that fatigue may be due to a prolonged inflammatory response. However there are conflicting results and the exact nature of the disturbance remains unclear.

Objectives We wanted to identify any inflammatory markers associated with fatigue

Methods We recruited women from a breast cancer follow up clinic and categorised them on the basis of a diagnostic interview as to whether they met the criteria for cancer related fatigue syndrome (cases) or not (controls). We took plasma samples from each participant to analyse subsequently using a panel of 88 biological markers.

Results 90 samples were analysed in total (45 cases and 45 controls). A factorial Analysis of variance (using age as a fixed factor) demonstrated a number of differences in inflammatory cytokines. There were 28 significantly different analytes in total. Granulocyte colony stimulating factor (GCSF) was the most significantly different analyte ($p<0.001$). Many of the significant analytes were chemokine ligands found to be linked through an inflammatory pathway promoting T cell and granulocyte production and activation.

Conclusions Our results add further weight to the hypothesis that cancer related fatigue syndrome is associated with an increased pro-inflammatory immune response. Our findings indicate that these cytokine changes could underpin the subjective symptoms such as perceived muscle weakness and concentration difficulties experienced by women who feel fatigued after treatment.

Introduction:

The majority of women treated for breast cancer can expect to survive for the long term[1]. This means that many women are living with persistent side effects of treatment including fatigue[2]. The prevalence of post-treatment fatigue can be as high as 30% at up to two years following the completion of therapy[3]. This does, however, vary with how fatigue is measured[4]. One of the more robust methods to categorise significant fatigue is to use a case definition of cancer related fatigue syndrome . This is can be done by using a diagnostic interview[5] and based on similar criteria used for diagnosing chronic fatigue syndrome[6].

Cancer related fatigue syndrome is a clinical diagnosis which encompasses subjective aspects of fatigue. However the underlying biological pathogenesis is unclear and no objective testing of cancer related fatigue syndrome currently exists[7]. Most research has examined immunological disturbances and cytokine levels in relation to fatigue after completion of treatment. Fatigue levels with concurrent cytokine measurement have only been examined prospectively during radiotherapy for breast cancer [8 9]. Indirect evidence supporting an immunological mechanism for fatigue in chemotherapy comes from a small pilot trial of a tumour necrosis factor blocking drug –etanercept [10]. This demonstrated that etanercept reduced fatigue and thus allowed for higher doses of docetaxel chemotherapy to be used. The authors of a meta-analysis summarised the research investigating the relationship between fatigue and inflammatory markers[11] in relation to all tumour types both during after completion of systemic treatment. They found an association between fatigue and a number of pro-inflammatory cytokines with the best evidence being for a relationship between fatigue and interleukin 6, interleukin 1a and neopterin (secreted by activated macrophages). The authors of the review cited the wide range of outcome measures and experimental variation in different studies as factors which may explain the lack of consistent findings. All of these cytokines are associated with a prolonged inflammatory response and would fit with our current understanding of the patho-physiology of cancer related fatigue syndrome both occurring during and after treatment. However more

work is clearly needed before firm conclusions can be drawn about the relevance of any particular inflammatory marker. It is more than likely this inflammatory response peaks during treatment but persists in those patients that experience chronic fatigue [11 12].
There is evidence from studies conducted in disease-free post-treatment breast cancer survivors of a prolonged inflammatory response in a subset of fatigued women[13-17]. These studies have used a variety of techniques to examine laboratory markers of fatigue including examination of lymphocyte subsets and genetic polymorphisms as well as cytokine measurements. However the measures this group have used have altered over time.. The fatigue groups were also only identified using cut-off scores on quality of life scales, rather than a more robust application of cancer related fatigue syndrome criteria.
These limitations and *a priori* focus on a small number of pre-determined cytokines mean that the understanding of the pathogenesis of long term fatigue in breast cancer survivors is still limited. The aims of our study were to categorise breast cancer survivors into those meeting the criteria for cancer related fatigue syndrome and those who did not in order to examine differences in candidate biomarkers (including but not limited to the inflammatory cytokines previously identified). We also wanted to map differences onto known biological pathways in order to develop an explanatory model.

Materials and methods

Breast cancer survivors were recruited at St George's Healthcare NHS Trust. Recruitment was from January 2009 to May 2011. Approval was obtained from Wandsworth Ethics Committee prior to data collection (ref 08/H0803/182).
All patients who were clinically and radiologically disease-free between three months and two years after the end of their primary treatment (of any modality) were invited to participate.

Those patients with significant cognitive impairment, psychiatric history or medical co-morbidities on initial screening were excluded from the study (12 women in total). Participants underwent the following:

Diagnostic interview for cancer related fatigue

This interview determines whether the participant meets the four criteria for a diagnosis of cancer related fatigue syndrome [18]. Criterion A; The presence of two weeks of significant fatigue in the preceding month and the presence of at least five out of nine other fatigue-related symptoms. These include sleep and cognitive disturbance and functional impact of fatigue. Criterion B; The fatigue has a significant effect on work or self-care. Criterion C; The fatigue symptoms are a consequence of cancer or cancer therapy. Criterion D; The symptoms are not primarily a consequence of a co-morbid psychiatric disorder. The final criterion can be assessed clinically but the most robust method is to use a contemporaneous psychiatric interview. Participants with a significant current psychiatric history which was felt to be contributing to fatigue were excluded. This was usually a con-current clinically significant mood disorder.

Structured Clinical Interview for the Diagnostic and Statistical Manual (DSM) – IV (SCID)

The SCID provides a method for obtaining DSM-IV diagnoses. The procedure has been successfully used in previous studies examining CRF syndrome [19 20] and by our group [3]. All interviews were conducted by the same person (OM). This procedure allowed for the classification of women into cases of cancer related fatigue syndrome or controls (those who did not meet the criteria). The presence of a diagnostic mood disorder (such as major depression) meant we excluded those women from further analysis because of the potential for overlap in symptoms [21].

Group Analysis

The between group analysis was conducted on those meeting the criteria and an equal number of the control group (those patients with the lowest scores on the functional

assessment of cancer therapy fatigue subscale (FACT F[22]) in order to maximise the differences between groups). The FACT F is a thirteen item fatigue questionnaire widely used in the assessment of cancer fatigue. [23]..

Analysis of blood samples

A blood sample was obtained using Becton Dickinson (New Jersey USA)BD P100 proteomic vacutainer® kits. Blood was centrifuged at 2500g for fifteen minutes as per manufacturer's instructions. Blood was not taken at a specific time of the day but was taken contemporaneously with clinical data. Plasma was then aliquoted into 0.5ml micro-centrifuge containers and stored at -80 degree Celsius in a locked freezer until analysis at the end of the study. Single 100 microlitre aliquots were transported frozen on dry ice for analysis by Rules Based Medicine (RBM - Austin Texas USA). RBM (www.rulesbasedmedicine.com) is a commercial company which provides multiple immuno-assay testing through a designated panel. We used HumanMap® Antigen v1.6. This is a panel of 88 potential biomarkers which includes but is not restricted to interleukins, cancer antigens and other cytokines (see table 1). The panel has been generated by the company as is fixed in its makeup.

Table 1 List of 88 analytes from Rules Based Medicine

1. Adiponectin	2. Alpha-1-Antitrypsin
3. Alpha-2-Macroglobulin	4. Alpha-Fetoprotein
5. Apolipoprotein A-I	6. Apolipoprotein C-III
7. Apolipoprotein H	8. Apolipoprotein(a)
9. Beta-2-Microglobulin	10. Brain-Derived Neurotrophic Factor
11. Calcitonin	12. Cancer Antigen 125
13. Cancer Antigen 19-9	14. Carcinoembryonic Antigen
15. CD 40 antigen	16. CD40 Ligand
17. Complement C3	18. C-Reactive Protein
19. Creatine Kinase-MB	20. Endothelin-1
21. EN-RAGE	22. Eotaxin-1
23. Epidermal Growth Factor	24. Epithelial-Derived Neutrophil-Activating Protein 78
25. Erythropoietin	26. Factor VII
27. Fatty Acid-Binding Protein, heart	28. Ferritin
29. Fibrinogen	30. Fibroblast Growth Factor basic
31. Granulocyte Colony-Stimulating Factor	32. Granulocyte-Macrophage Colony-Stimulating Factor
33. Growth Hormone	34. Haptoglobin
35. Immunoglobulin A	36. Immunoglobulin E
37. Immunoglobulin M	38. Insulin
39. Insulin-like Growth Factor I	40. Intercellular Adhesion Molecule 1
41. Interferon gamma	42. Interleukin-1 alpha
43. Interleukin-1 beta	44. Interleukin-1 receptor antagonist
45. Interleukin-10	46. Interleukin-12 Subunit p40
47. Interleukin-12 Subunit p70	48. Interleukin-13
49. Interleukin-15	50. Interleukin-16
51. Interleukin-2	52. Interleukin-3
53. Interleukin-4	54. Interleukin-5
55. Interleukin-6	56. Interleukin-7
57. Interleukin-8	58. Leptin
59. Lymphotactin	60. Macrophage Inflammatory Protein-1 alpha
61. Macrophage Inflammatory Protein-1 beta	62. Macrophage-Derived Chemokine
63. Matrix Metalloproteinase-2	64. Matrix Metalloproteinase-3
65. Matrix Metalloproteinase-9	66. Monocyte Chemotactic Protein 1
67. Myeloperoxidase	68. Myoglobin
69. Plasminogen Activator Inhibitor 1	70. Pregnancy-Associated Plasma Protein A
71. Prostate-Specific Antigen, Free	72. Prostatic Acid Phosphatase
73. Serum Amyloid P-Component	74. Serum Glutamic Oxaloacetic Transaminase
75. Sex Hormone-Binding Globulin	76. Stem Cell Factor
77. T-Cell-Specific Protein RANTES	78. Thrombopoietin
79. Thyroid-Stimulating Hormone	80. Thyroxine-Binding Globulin
81. Tissue Factor	82. Tissue Inhibitor of Metalloproteinases 1
83. Tumor Necrosis Factor alpha	84. Tumor Necrosis Factor beta
85. Tumor necrosis factor receptor 2	86. Vascular Cell Adhesion Molecule-1
87. Vascular Endothelial Growth Factor	88. von Willebrand Factor

Pathway Mapping

The data obtained from this panel was then linked by existing biological relationships (such as binding or regulation) using Pathway Studio™ software (Ariadne Genomics, MarylandUSA). This is a software program that allows biological systems mapping and concurrent database searching to provide information on protein interactions. The software allowed interactions between the significant RBM anlaytes to be identified. This was done in two ways – 1) By using the software to find known links between identified proteins (from the RBM panel) and 2) Using a probability threshold ($P<0.05$) to find potential interactions between the RBM analytes and other proteins that enrich the pathway. This was based on a probability assessment made from electronic searching of the published literature extracted by Medscan software® part of the Pathway Studio™ software. This gave an indication of the highest probability linkages between the RBM analytes and other directly linked (but unmeasured) proteins. This was done in order to generate a system-level hypothesis for the mechanism of cancer related fatigue syndrome in this group and to place the statistical analysis within a biological network. The principle is to visually represent previously identified links between proteins. The more extensive the links, the more biologically active and potentially relevant to the pathogenesis of cancer fatigue it is. This biological linkage in analytes does not rely on the limitations of fatigue research discussed [11]

Statistical considerations

The RBM analyses have an upper and lower detectable limit for all analytes and any value below this level was reported as “missing”. Analytes for which more than 50% of data were missing were excluded from the analysis (12 analytes).

The measured analytes vary widely in their level of concentration even in normal plasma (from milligrams/ml to picograms/ml – a 10^9 fold difference between highest and lowest concentrations). Raw data from RBM was therefore initially transformed to normalise and

impute missing data. For certain analytes there was a small percentage of missing values (i.e. below the lowest detectable range). This was no more than twenty per cent of values in less than ten per cent of analytes. In order to be able to compare these analytes equally data were imputed. This was calculated by dividing the lowest measurable value overall for that analyte by two. This procedure was undertaken to ensure that there were no missing data and so all cases could be included in a multi-variate analysis.

Data was normalised to allow for direct comparison across all analytes. This was calculated by taking the original value minus the minimum value for each analyte divided by the range of analyte values. This transformation means that all analytes are scored between 0 and 1. Where 0 is the lowest possible value and 1 is the highest value for each analyte. This allows for direct comparison of the greatest differences between groups across all analytes. There were no major outliers and all samples were included in the analysis.

The role of age and other treatment and staging variables was assessed using an analysis of covariance (ANCOVA). This was undertaken to determine if any of these confounding variables contributed to the between group differences in cytokine levels. However this was an exploratory study and no correction was made for multiple analyses and the results have been interpreted in this context.

Results

The detailed clinical characteristics of this group have been reported in a separate paper [24]. In total 114 women were recruited the study. This gives a prevalence of 39% of Cancer related fatigue syndrome. This figure is in keeping with the wider literature [2]. There were no statistically significant differences between groups in mean age and time since completion of treatment. There is also no statistically significant difference in the frequency of lymph node

positivity (as a major staging variable) or treatment modalities employed (such as chemotherapy).Concurrent hormone usage was also not significantly different between the two groups.

Samples from 90 women were analysed (45 cases and 45 controls). There were no differences in routine laboratory measures (notably on full blood count or thyroid function) between groups. There was no difference on pathological staging or treatment modality between group effect on an analysis of co-variance. This demonstrated that these variables did not have a statistically significant impact on cytokine levels between groups.

However age was found to be a significant co-variate. Therefore a factorial ANOVA was conducted with the samples being divided into three different age groups (30-49;50-69; 70-89). The youngest participant was 30 years old and the oldest was 89 (there was no upper age limit on study entry). This age group allocation meant the age group distributions were matched between cancer related fatigue syndrome and control groups. This meant age became a fixed factor in the analysis and was controlled for.

We found twenty eight significantly different analytes between cancer related fatigue syndrome and controls. The greatest statistical difference was in Granulocyte Colony Stimulating Factor (GCSF) concentrations ($P<0.001$). However a number of chemokine ligands related to T cell and granulocyte proliferation were also found to be significantly different (CD40 antigen, macrophage inflammatory protein 1(MIP1 alpha) and chemokine ligand 5).

There were also a number of acute phase analytes (e.g. complement C3, apolipoprotein H and Aspartate transaminase) that were raised in the fatigued group. However these analytes did not contribute significantly to the computationally-derived networks.

The full list of significantly different analytes is shown in Table 2. All the analytes were raised in the fatigued group with the exception of Interleukin 1 receptor antagonist, Interleukin 13 and sex hormone binding globulin.

Table 2 Factorial ANOVA with three age groupings- Analytes tabulated in order of significance.

Analyte	Residual mean squares	F ratio	P value
Granulocyte colony stimulating factor (GCSF)- synonym CSF2	0.23	8.41	<0.001
Tissue inhibitor of metalloproteinases 1 (TIMP 1)	0.20	7.04	<0.001
Beta 2 microglobulin (B2M)	0.22	6.49	<0.001
Myoglobin	0.14	5.98	<0.001
Tumour necrosis factor beta (TNF-beta)	0.19	4.88	0.001
Aspartate transaminase (AST)	0.05	4.01	0.003
Brain derived neurotrophic factor (BDNF)	0.11	3.97	0.003
Thrombospondin 1	0.08	3.75	0.004
Apolipoprotein H	0.11	3.67	0.005
Vascular endothelial growth factor (VEGF)	0.13	3.65	0.005
Complement C3	0.11	3.52	0.006
CD 40 antigen	0.12	3.47	0.007
Interleukin 7 (IL7)	0.11	3.47	0.007
Pregnancy associated plasma protein A (PAPPA)	0.18	3.46	0.007
Fatty acid binding protein (heart)	0.06	3.36	0.008
T cell specific protein RANTES – synonym CCL5 (chemokine ligand 5)	0.07	3.32	0.009
Macrophage inflammatory protein 1 alpha (synonym CCL3)	0.06	3.26	0.01
Sex hormone binding globulin (SHBG)	0.13	3.22	0.01
Macrophage derived chemokine	0.10	3.13	0.01
Granulocyte-macrophage colony stimulating factor (GMCSF) synonym CSF3	0.07	2.90	0.02
Interleukin 1 receptor antagonist (IL1RN)	0.14	2.88	0.02

Fibrinogen	0.05	2.83	0.02
Alpha 2 macroglobulin(A2M)	0.09	2.77	0.02
Stem cell factor	0.07	2.69	0.03
Interleukin 18 (IL18)	0.08	2.55	0.03
Interleukin 15 (IL15)	0.11	2.58	0.03
Myeloperoxidase	0.06	2.57	0.03
Interleukin 8 (IL8)	0.08	2.54	0.03
Interleukin 13 (IL13)	0.07	2.33	0.04

Sixteen analytes linked significantly on Pathway Studio™ analysis. The remaining twelve significant analytes were added in to the proposed pathway but failed to demonstrate any linkage. The putative biological linkage and candidate relationship between these sixteen analytes is shown in figures 1. Figure 1 demonstrates the linkage between significantly different RBM analytes from table 2.

The addition of the previously identified cytokines from the literature (Tumour necrosis factor alpha, Interleukin 6 and neopterin) [11] did not meet the threshold probability needed to expand the pathway further. It is worth noting that individually these analytes (with the exception of neopterin which was not included) were not significantly different between cancer related fatigue syndrome and controls on the RBM panel.

Discussion

This is the first study to examine the biological pathogenesis of cancer related fatigue syndrome using a large immunoassay panel. We found significant differences between cases and controls. These differences were in a number chemokine ligands and pro inflammatory interleukins. Our data shows that a number of analytes including GCSF, MIP1 alpha, Interleukin 1 receptor antagonist, vascular endothelial growth factor (VEGF) and interleukin 18 (IL18) are significantly different between cancer related fatigue syndrome and controls. These analytes are discussed in detail as they are extensively biologically linked to each other and have also been previously identified as associated with cancer fatigue[11]. There is considerable overlap in function of a number of these chemokines (mainly secreted by activated granulocytes) so only this subset has been highlighted. Our more novel findings are discussed later.

Our results suggests that fatigue is linked to granulocyte activity with a T cell mediated response and may be due to reactivation of a latent viral infection or may reflect an auto-immune phenomenon. There was also a significant difference in myoglobin suggesting that a sub-clinical myositis may have a contributory role. Increased cytokine levels were linked to increased vascular endothelial growth factor (VEGF) which is intriguing as VEGF can alter the permeability of the blood brain barrier (BBB) and may be one of the mechanisms whereby cytokines can cross from the peripheral circulation to the central nervous system and thus cause the symptoms associated with cancer related fatigue syndrome.

There are a number of acute phase proteins that are raised in the fatigued group (AST, complement C3, fibrinogen and Apolipoprotein H). These analytes do not link into the proposed pathway but do support the hypothesis that there is an ongoing pro-inflammatory response linked to cancer related fatigue syndrome [25].

These findings of a mixed inflammatory response are in keeping with a number of previous reports. A study by Landmark-Høyvik and colleagues[26] examined gene expression in fatigued breast cancer survivors and found evidence of a predominately B cell mediated response in contrast to our study which suggests this is predominantly T cell mediated. Their findings suggested an altered B cell mediated immune response across several gene sets. While it is difficult to compare directly transcription rates with quantifiable immunoassay of proteins there does seem to be significant overlap. Both studies found evidence of a prolonged inflammatory response in the fatigued group.

Our findings are also in keeping with the studies by Bower and colleagues [13 14 16]. The authors of these papers have consistently found evidence of a pro-inflammatory response in breast cancer survivors with fatigue. However the exact nature has differed between studies because of the laboratory techniques used. The classification of the fatigued group in each of Bower's studies has been based on a quality of life cut-off score rather than a case based

approach. The consistent findings from these studies as a whole suggest a role for Interleukin 6, Interleukin 1b and TNF. It is likely there are multiple pathways for such a common symptom especially in those who have been treated for cancer. Although in our study these cytokines were not significantly different.

Our novel findings of the lower concentration cytokines such as IL 13 and 18 have not been previously associated with fatigue. These cytokines stimulate the production of IL 6 and TNF[27]. However they are also affected by other pathways including via suppression of cytokine signalling 1 (SOCS1) [27] and this may explain why there are no overall differences in TNF and IL6 in this study. As SOCS1 is an intracellular protein a further examination of SOCS1 gene transcription rates would be required to confirm or refute this hypothesis.

Our findings are at odds with work conducted by Orre et al[28] in which the authors found only a significant association with C reactive protein (CRP) in their analysis of fatigue in breast cancer survivors. However despite the large sample size in their study the fatigue measurement used was not contemporaneous to the blood analysis, as it is in our study. The authors also comment that CRP acts as an upstream marker for lower concentration cytokines such as IL6. Our group previously found a significant difference in CRP levels between fatigue and controls in breast cancer survivors when it was included in routine laboratory testing[3]. However, in another study we failed to find an association between CRP and severe fatigue in a large group of mixed advanced cancer patients[29]. It is likely that CRP is too non-specific to be a useful marker for fatigue as it is also an acute phase protein. CRP was included in our initial panel but was not significantly different between groups, most likely for this reason.

The link with myoglobin is intriguing as it suggests that fatigue may be associated directly with low grade myositis and is in keeping with the reported positive effects of exercise on fatigue[30]. Increased myoglobin levels have been correlated with cancer cachexia, measured muscle mass and composition[31]. However this was measured in advanced

cancer patients with established weight loss. In breast cancer survivors any effect on the muscle structure is likely to be more subtle[32]. The lack of difference in creatinine kinase between fatigue and controls supports this. The difference in IL 18 may be due to a low grade auto-immune myositis which has previously been linked with cancer patients[33]. Future exercise intervention studies should investigate its effect on serum concentrations of these inflammatory cytokines. However, the small observed effect of exercise interventions on fatigue [30] may suggest that it improves muscle function without significant impact on any associated inflammation.

Our findings may also explain the reported positive effects of psycho-stimulants on fatigue[34]. Inflammatory cytokines can act on the BBB by local direct diffusion from circulating plasma or activation of cranial nerve afferents – most notably the vagus nerve[35]. This may be why psychostimulants have been found to be effective in this group as they can directly affect the BBB[36].

An exploratory analysis to determine the power of the analytes to act as potential candidate markers demonstrated that all of the analytes with a P value of 0.01 or less (eighteen analytes) have 80% power to distinguish the fatigued group from controls with our current sample size. However this subset of significant analytes is not currently sufficient to be used in isolation as a predictive test for cancer related fatigue syndrome as many of the analytes included are of uncertain biological significance in this group.

This was an extensive immuno-assay analysis focusing on one of the most troubling symptoms patients experience both during and after treatment. The broad exploratory nature of the study has allowed us to develop hypotheses about the biological pathogenesis of cancer fatigue. This provides a basis to investigate further the relationship between a sustained inflammatory response and persistent fatigue and may provide a *rationale* for the development of more targeted therapies to prevent and treat this symptom.

While this is one of the largest studies of its kind, with a robust classification of fatigue cases, the absolute numbers are small. This study was exploratory in nature and the findings can therefore only be provisional. Our data was cross sectional and no pre-treatment baseline values were available. It is possible the observed differences were secondary to decreased activity rather than causative.

The cost of performing the analysis has limited the number of samples that we were able to evaluate. Dichotomising the group into cancer related fatigue syndrome and controls may mask small individual variations and may be why the proposed model cannot fully explain the full biology of cancer related fatigue syndrome. It is also likely that even if a pro-inflammatory response is the main driver of this set of symptoms there may be other processes at work which our panel has been unable to measure. However the similarity of our findings to previous work in this area strengthens these results. Although the observed differences may have potentially been due to type 1 errors, this is rendered less likely by the linkage to a common biological pathway associated with inflammation.

There are commercially available monoclonal antibodies that may be able to disrupt the pro-inflammatory process and minimise the prevalence of fatigue during and after treatment. This could include further trials of TNF alpha blockers or other commercially available monoclonal antibodies. . However the efficacy, toxicity and dosing regimen of these drugs would need to be evaluated in a clinical trial. It is possible that some of these variables may find a role as surrogate markers of early response to fatigue treatment or may identify sub-sets of patients who are particularly likely to respond to targeted therapy. This would not necessarily be limited to breast cancer as the mechanism is likely to overlap significantly across all cancer types.

Our future work aims to correlate, in prospective studies, changes in serum cytokine levels and other serum proteins with the diagnostic criteria for cancer related fatigue syndrome in

order to generate a model that may be used to understand the pathology and develop novel avenues for treatment of this debilitating condition.

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Legend for Figure 1

Computationally generated (Pathway Studio™) network of putative relationships between significantly different RBM analytes (derived from Table 2). This map shows previously published molecular relationships.

Dotted arrows indicate regulation relationships (arrow heads indicating the direction and a plus sign indicating up-regulation). Solid lines indicate expression relationships (direction of the arrow indicating the regulator and regulated molecule, plus signs indicate increased expression). A flat headed line indicates reduced expression of that protein. Dark single lines indicate binding relationships only.

KEY
B2M- beta 2 microglobulin
BDNF- brain derived neurotrophic factor
CCL3- synonym for Macrophage inflammatory protein 1 alpha
CCL5- synonym for T cell specific protein RANTES
CSF2 – synonym for granulocyte colony stimulating factor
CSF3 – synonym for granulocyte-macrophage colony stimulating factor
CD40 – CD40 antigen
Interleukin 7/13/15/18 respectively
Il1RN- Interleukin 1 receptor antagonist
MB – Myoglobin
SOCS1 – suppressor of cytokine signalling 1
TIMP1- Tissue inhibitor of metalloproteinases 1
XCl1 synonym- Tumour necrosis factor beta

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