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Xenotropic Murine Leukemia Virus-related Virus-associated Chronic Fatigue Syndrome Reveals a Distinct Inflammatory Signature

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Abstract. *Background: The recent identification of xenotropic murine leukemia virus-related virus (XMRV) in the blood of patients with chronic fatigue syndrome (CFS) establishes that a retrovirus may play a role in the pathology in this disease. Knowledge of the immune response might lead to a better understanding of the role XMRV plays in this syndrome. Our objective was to investigate the cytokine and chemokine response in XMRV-associated CFS. Materials and Methods: Using Luminex multi-analyte profiling technology, we measured cytokine and chemokine values in the plasma of XMRV-infected CFS patients and compared these data to those of healthy controls. Analysis was performed using the Gene Expression Pattern Analysis Suite and the Random Forest tree classification algorithm. Results: This study identifies a signature of 10 cytokines and chemokines which correctly identifies XMRV/CFS patients with 93% specificity and 96% sensitivity. Conclusion: These data show, for the first time, an immunological pattern associated with XMRV/CFS.*

Chronic fatigue syndrome (CFS) is a poorly understood disease of unknown etiology, which is commonly characterized by innate immune defects, chronic immune activation and dysregulation, often leading to neurological maladies [reviewed in (1)]. It can also involve other biological systems such as the musculoskeletal, gastrointestinal and endocrinological systems (2-4). Although several common symptoms are primarily reported and predominate, they may differ among individuals, are often intermittent and can persist for years, frequently resulting in substantial disability (5). Some of the most commonly reported physical symptoms include muscle weakness and pain, tender or swollen lymph nodes and chronic flu-like symptoms (6). Memory and concentration impairment, blurred vision, dizziness and sleep abnormalities represent some of the cognitive symptoms typically observed while immunological symptoms often manifest themselves through viral reactivation, RNase L dysregulation, decreased natural killer (NK) cell function and susceptibility to opportunistic infections (7-12). NK cell dysregulation may be associated with viral reactivation or viral persistence and may also lead to malignancy (13, 14). Indeed, clinical observations corroborate pathological manifestations in CFS as viral reactivations, particularly herpes virus such as cytomegalovirus (CMV), Epstein-Barr virus (EBV) and human herpes virus-6 (HHV-6), are common occurrences (15-17). Moreover, epidemiological studies have reported increased incidences of lymphoma associated with CFS outbreaks (18). These clinical observations suggest that a compromised innate immune system may play a role in CFS pathology.

The completion of the human genome project enabled positional cloning studies to identify the *RNASEL* gene as the hereditary prostate cancer allele-1 (HPC1) (19). This discovery prompted Robert Silverman and his colleagues to search for a viral component to hereditary prostate cancer. Using a viral micro-array and tissue biopsies from

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individuals with hereditary prostate cancer they identified and sequenced the complete genome of a novel human gammaretrovirus, very similar in sequence to xenotropic murine leukemia virus and therefore termed the new virus xenotropic murine leukemia virus-related virus (XMRV) (20). Subsequent studies performed in our laboratory identified and isolated infectious XMRV in the blood of 67% of CFS patients (21). This work was performed using multiple techniques including PCR, electron microscopy showing budding viral particles, Western blot analysis of viral proteins and serology confirming that infected patients express antibodies to XMRV envelope proteins. In addition, gene sequencing and phylogenetic analysis confirmed these patients were indeed infected with XMRV that was >99% identical to previously published sequences but was obviously distinct from the only existing XMRV molecular clone, VP62 (20). Taken together, this work clearly rules out any possibility of gross contamination and additionally, represents the first identification and isolation of naturally occurring infectious XMRV. The connection between CFS and XMRV was further supported by the studies of Lo *et al*., who identified murine leukemia virus (MLV)-related sequences in the blood of 86% of CFS patients, further establishing a retroviral association with CFS (22).

Presently, three families of retroviruses are known to infect humans; the human immunodeficiency viruses (HIV), the human T-cell leukemia viruses (HTLV) and now the human murine leukemia-related viruses. Both HIV and HTLV are known to dysregulate the innate immune system and promote the production of inflammatory cytokines and chemokines (23, 24). In light of the association between XMRV and CFS, it is not surprising that some of the most salient observations in CFS are the differences in cytokines and chemokines when compared to healthy controls (8). Previous reports, however, addressing the role of these molecules in CFS have produced conflicting results. Much of this emerges from such hindrances as small sample size, a limited number of cytokines surveyed at one time, insufficient patient population stratification, and insufficient negative control subjects. This has resulted in inconsistent reports in the literature for a number of cytokines including interleukins (IL) 6, 10 and 12. In spite of these conflicting results, a number of cytokines and chemokines have consistently been show to be associated with different subgroups of CFS. For instance, Natelson *et al*. showed elevated levels of IL-8 and IL-10 in the cerebral spinal fluid of patients with sudden, influenza-like onset CFS when compared to healthy controls (25). Additionally, Chao *et al*. have show neopterin and IL-6 to be up-regulated in subsets of CFS patients, indicative of a pro-inflammatory immune condition (26). However, these studies did not analyze the complex relationships between multiple cytokines and clinical disease.

By applying conventional statistical analysis and 'machine logic' algorithms to the multiplex data, it is possible to identify cytokines and chemokines that are differentially expressed between two groups. To support this premise, we have used the xMap® multi-analyte profiling technology that allows simultaneous measurements of multiple biomarkers in serum or plasma. In this study, a panel of 26 cytokines, chemokines and growth and angiogenic factors were analyzed in blood plasma of CFS patients and healthy control subjects. This study revealed a signature of 10 cytokines and chemokines, which showed a specificity of 93% and sensitivity of 96% in diagnosing XMRV-associated CFS in this patient cohort.

Materials and Methods

Patients and controls. One hundred and eighteen specimens from patients who tested positive for XMRV and with a confirmed diagnosis of CFS at the time of collection were obtained from the Whittemore-Peterson Institutes' sample repository. All specimens used in this study were heparinized plasma and represented a female to male ratio of approximately 2 to 1, consistent with previously reported CFS distributions. Patients scheduled their physician appointment approximately two months in advance of the time their blood was drawn and therefore, the observed results are unlikely to correlate with any short-term condition or fluctuation that prompted the physician visit. All patients described in this study represent well-defined cohort of CFS patients who are known to have a 'viral or flu-like' onset and have tested positive for XMRV by PCR and/or serology. One hundred and thirty-eight control plasma samples were provided by a local medical practice, under University of Nevada, Reno IRB approval, also with a female to male ratio of approximately 2 to 1. Control subjects donated blood during a routine health check-up and were determined to be healthy at the time of their visit.

Collection and storage of blood plasma. Ten milliliters of peripheral blood was drawn from subjects by venipuncture using standardized phlebotomy procedures. Handling and processing was similar for all groups. Blood samples were drawn using green-capped vacutainer® collection tubes with the anti-coagulant sodium heparin (Becton Dickinson, Franklin Lakes, NJ, USA) and processed within 6 hours of draw. Plasma samples were separated by centrifugation at room temperature, aliquoted and stored at –80°C until analysis. Samples were not subjected to more than 1 freeze-thaw cycle.

Multiplex analysis. The xMap® technology (Luminex® Corporation, Austin, TX, USA) is a suspension assay that combines standard sandwich immunoassay principles with flow cytometry. Whereas a conventional sandwich immunoassay uses a microtiter plate as the solid support, xMap® technology uses styrene microspheres, each having a unique fluorescent signature specific to a particular cytokine of interest and conjugated to a capture antibody specific to that cytokine. This technology allows the multiplex analysis of up to 100 individual cytokines in a single microtiter plate well (27). The xMap® plasma assays were done in 96-well microplate format according to the manufacturer's protocol (Invitrogen Camarillo, CA, USA). The human 25-Plex cytokine panel (Invitrogen catalog # LHC0009) was used to analyze plasma samples according to the

manufacturer's instructions on Luminex® 100 or 200 analyzers with MasterPlex® CT control software and MasterPlex® QT analysis software (MiraiBio division of Hitachi Software San Francisco, CA, USA). The Invitrogen Human Cytokine 25-Plex panel consists of the following cytokines and chemokines: eotaxin, GM-CSF, IFN-α, IFN-γ, IL-1β, IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40/p70), IL-13, IL-15, IL-17, IP-10, MCP-1, MIG, MIP-1α, MIP-1β, RANTES, and TNF-α. To generate a standard curve, 3-fold serial dilutions of multiplex standard, provided in the 25 cytokine panel assay kit, were prepared in assay diluent and pipetted in duplicate. In addition to the Invitrogen control standards, a set of 12 clinically validated cytokines were also used as an external control (Tri-level Cytokine Controls Catalog # CY5006; Randox, Crumlin, County Antrim, UK). Patient plasma and the external controls were diluted 1:1 in assay diluent and pipetted in duplicate. A select number of samples (on the average of 10) were measured in 4 separate assays in order to determine intra-assay variation. Each assay was validated against an ELISA, demonstrating 92-99% correlations (information provided by Invitrogen). Analysis of experimental data was carried out using five-parameter curve fitting. Interferon beta (IFN-β), which is not available on the Luminex® platform, was analyzed by conventional ELISA (PLB InterferonSource, Piscataway, NJ, USA).

Gamma-delta (γ-δ) T-cell clonality assay. As part of their routine clinical work-up, a large proportion of patients were commercially screened (Laboratory Corporation of America) for γ-δ T-cell clonality, using the InVivoScribe T-Cell Receptor Gamma Gene Rearrangement Clonality Assay for the ABI capillary-based analyzer (Catalog # 1-207-0021; InVivoScribe Technologies, San Diego, CA, USA). T-cell receptor gamma chain gene rearrangement assays are often used to identify clonal populations of γ-δ T-cells, which are highly suggestive of T-cell and some immature B-cell malignancies. Positive results are given as a monoclonal population twice the polyclonal population background (28-30). These results were used to stratify patients into two groups in order to evaluate differences in cytokine and chemokine expression.

Statistical methods. In order to determine differences in cytokine and chemokine values and distributions between patient and control groups we initially performed Kolmogorov-Smirnov tests for normality, which revealed that the data were not normally distributed. Calculating the log of cytokine values produced nearly normally distributed values. Data values were log transformed and Student's *t*-test was used to analyze differences between patients and controls. Although log-transformed laboratory values were used for all statistical analyses, mean and standard error of untransformed values are presented for clarity. Multiple regression analysis was used to examine the impact of age and gender on the observed results for each group. In order to establish differences between the three groups of patients, correlation analysis was made between the five most significant cytokines/chemokines using the Pearson Correlations method. Three subgroups were analyzed with this method: the control group, the CFS patients with γ-δ T-cell clonality and the CFS patients without γ-δ T-cell clonality. Correlations were then used to evaluate if differences existed between subgroups. Classification analysis was carried out using the Random Forest (RF) classification algorithm (31). The RF algorithm uses an ensemble of unpruned classification or regression trees produced through bootstrap sampling of the training data set and random feature selection in tree generation. Prediction is made by a majority vote of the predictions of the ensemble. The strength of the analysis was evaluated by an 'out of bag' sampling without replacement of the original data. The RF is an attractive method since it handles both discrete and continuous data, it accommodates and compensates for missing data, and it is invariant to monotonic transformations of the input variables. The RF algorithm is uniquely suited for cytokine and chemokine analysis in that it can handle highly skewed values well and weighs the contribution of each cytokine or chemokine according to its relatedness with others. Two dimensional cluster analyses were made with the Gene Expression Pattern Analysis Suite (GEPAS) version 4.0 using the SOTA method and Pearson correlation coefficient for distance of the log transformed data. In addition to clustering of cytokines and chemokines, conditions of clustering were also analyzed.

Results

Analysis of plasma concentrations of cytokines and chemokines in XMRV-infected CFS patients. In order to develop a cytokine/chemokine signature, it was first necessary to determine the extent of differential expression between a large patient population and a verified healthy, age- and gender-matched control population. To investigate any potential difference between these two populations, the concentrations of 26 different plasma markers, belonging to the various immunologically functional classes, were evaluated by Luminex® or ELISA, as described above. Analyses were performed using the plasma samples of healthy control subjects and from patients with a confirmed diagnosis of CFS, representing an approximate female to male ratio of 2:1 respectively (Table I). Multiple regression analysis was used to examine the impact of age and gender in addition to the case or control group. These variables generally only explained 20% of the variation in six of the cytokine levels (IL-6, IL-12, TNF-α, IP-10, eotaxin and MCP-1). This difference did not affect the overall significance of the respective cytokine. Of the 26 cytokines and chemokines assayed, 19 were differentially expressed as determined by log-transformed Student's *t*-test at the 99% confidence level: 11 were up-regulated, 8 were down-regulated and 7 did not reach statistical significance when comparing the CFS group to the control group (Table II). The majority of up-regulated factors were members of the CXC or CC chemokine family. The greatest statistical difference was seen in chemotactic factor IL-8, a major mediator of the inflammatory response, with a mean value of 1045±245 pg/mL in patients compared to a mean of only 13.1±1.6 pg/mL as seen for the healthy controls. The other up-regulated chemokines, also from the CXC or CC family, were MIP-1β, MIP-1α, IP-10, eotaxin, MCP-1 and RANTES; two members of the cytokine family, IL-2 and TNF-α, were also up-regulated.

Of the cytokines and chemokines that were statistically down-regulated, the most significant was the T-cell inflammatory inhibitor IL-13. Although statistically

Table I. *Demographics of XMRV-CFS Patients and Controls by age and gender. Female to Male ratio is approximately 2 to 1, consistent with reported Ratios found in CFS populations.*

Gender	Age (years)		
	Mean	Median	Range
Female CFS			
N=69	53.9	54.0	26-74
Male CFS			
N=49	54.0	56.5	20-82
Female Control			
N=97	48.6	49.0	22-82
Male Control			
N=41	46.7	48.0	25-61

Table II. *Plasma cytokine and chemokine values of patients and controls given in pg/mL. Significance is at the 99% C.I. by the Student's t-test of the log-transformed data.*

Protein	Patients N=118 Mean (pg/mL)	(S.E.)	Controls N=138 Mean (pg/mL)	(S.E.)	Significance *P*-value
Up-regulated					
IL-8	1045	(254)	13	(1.6)	<0.0001
MIP-1β	1985	(556)	164	(41	<0.0001
MIP-1α	763	(216)	91	(19)	<0.0001
TNF-α	148	(53)	13	(4.3)	<0.0001
IL-6	336	(87)	29	(11)	<0.0001
IL-2	113	(56)	28	(10)	<0.0001
IP-10	98	(16)	32.8	(3.0)	<0.0001
Eotaxin	271	(19)	95.8	(6.5)	<0.0001
IL-12	289	(20)	211	(31)	0.0001
MCP-1	468	(42)	421	(41)	0.0003
Rantes	27107	(3400)	9564	(1012)	0.0018
Down-regulated					
IL-13	28.2	(3.6)	85.5	(6.5)	<0.0001
IL-5	7.35	(0.66)	21.1	(4.9)	<0.0001
IL-7	33	(11)	78	(6.9)	<0.0001
MIG	48.2	(9.0)	80	(12)	<0.0001
IFN-α	35	(5.9)	60	(4.3)	<0.0001
IL-1RA	1010	(363)	1277	(429)	<0.0001
GM-CSF	108	(23)	166	(28)	<0.0001
IL-4	39.6	(3.9)	55	(9.3)	0.0003
No significance					
IL-1β	118	(21)	88	(21)	0.62
IL-2R	289	(45)	476	(143)	0.09
IL-10	70	(21)	49	(12)	0.56
IL-17	52	(5.6)	56	(5.6)	0.15
IFN-γ	16.3	(1.4)	13.7	(0.70)	0.072
IL-15	78	(26)	117	(57)	0.93
IFN-β	284	(80)	196	(70)	0.28

All mean values are significant at the 99% C.I. by the Student's *t*-test of log-transformed data.

significant but down-regulated to a lesser extent were IL-5, IL-7, MIG, IFN-α, IL-1RA, GM-CSF and IL-4. Those factors that did not reach significance were IL-1β, IL-2R, IL-10, IL-17, IL-15, IFN- γ and IFN-β (Table II).

Comparison of γ-δ T-cell clonal and non-clonal XMRV-infected CFS patients and healthy controls. To investigate the possibility that patients with clonal populations of γ-δ T-cells represent a discrete subgroup of XMRV-infected CFS, we analyzed the same cytokines and chemokines using two approaches: the first approach was by cluster and correlation analysis with the Gene Expression Pattern Analysis Suite (GEPAS) version 4.0 using the SOTA method and Pearson correlation coefficient for distance of the log transformed data. The second approach was by looking at inter-subgroup correlations of five of the most significant cytokines/chemokines, again using the Pearson correlation method. Twenty subjects from each group (patients with γ-δ T-cell clonality, patients without γ-δ T-cell clonality, and controls) were chosen at random for cluster analysis. Prior to analysis, the raw cytokine/chemokine values were monotonically transformed by taking the log of each value and were additionally normalized against the entire study population median cytokine value (Figure 1). Cluster analysis segregated study subjects into two primary groups; the first group predominantly contained the control subjects (20 out of 41) and the XMRV/CFS subjects without γ-δ T-cell clonaity (15 out of 41), and this cluster was further sub-classified into two minor clusters of predominantly controls (19 out of 24) and XMRV/CFS patients (13 out of 17). The second cluster mostly contained the clonal patients (14 out of 19). These data clearly support the concept of unique XMRV/CFS subgroups based upon γ-δ T-cell clonality.

To further demonstrate that the three groups (patients with γ-δ T-cell clonality, patients without, and controls) represent discrete populations, we performed correlation analysis on five of the most significant cytokines and chemokines (IL-8, IL-6, MIP-1β, MIP-1α, and TNF-α). Correlation between IL-6 and IL-8 was significantly different between the three groups, as was the correlation between IL-8 and MIP-1β and MIP-1α. In addition, almost perfect correlation was observed between TNF-α, MIP-1β and MIP-1α in the control group, however, the correlation was less strong in the patient population with γ-δ T-cell clonality and even less in the non-clonal patients (Table III).

Random forest signature. To develop a cytokine and chemokine signature that could ultimately lead to a diagnostic tool, we utilized the Random Forest data mining software package using patient status as the target variable and the cytokine and chemokine values as the predictive variables. The model was made using tree type classification mode, building 500 decision trees and 'out of bag' testing

without replacement, using three predictors at each decision node. All 26 cytokines and chemokines were used in the initial model and the final model was refined using only the 10 most significant cytokines/chemokines identified in the initial model (Figure 2). The final model accurately identified 128 out of the 138 controls (93% specificity) and accurately identified 113 out of 118 patients (96% sensitivity) (Table IV).

Discussion

The purpose of this study was to evaluate differences in cytokine and chemokine profiles between XMRV-infected CFS patients and healthy control subjects. This study clearly demonstrates XMRV-infected CFS patients display an inflammatory cytokine and chemokine signature that distinguishes them from healthy control subjects. Although the correlation between XMRV and CFS is considerable, previous studies by our laboratory and those reported by Lo *et al*. have shown a small but significant proportion of the control population is also infected with XMRV (21) or potentially other human murine leukemia related viruses (22). The clinical significance of this is unknown at this time; however, the availability of complete viral genome sequences from many symptomatic and asymptomatic individuals may lead to a greater understanding as to why some individuals manifest symptoms of neuroimmune disease while others do not. If future studies establish that disease status is not determined by XMRV variation, it is possible that other conditions, such as a compromised immune system, predisposing genetics, or co-infections with other pathogens, may be necessary to manifest disease. Although these relationships are complicated, multiplex suspension arrays afford the opportunity to analyze the complex relationships between cytokines and clinical disease and to determine if clinical subgroups of disease also exist. Indeed, such analysis has proven effective in delineating other diseases with subtle differences, such as distinguishing noninflammatory from idiopathic ocular uveitis (32). Although the determination of XMRV infection is presently not a definitive stand-alone diagnostic tool for neuroimmune diseases or malignancies, a combination of XMRV and cytokine/chemokine analysis may prove to be a reliable diagnostic strategy and may assist in monitoring the success of treatment. Such analysis may also give insight into the pathology responsible for the progression from asymptomatic to symptomatic XMRV infections. For instance, the viral reservoir of XMRV is not known, however, if the cellular infiltrate involved in the production of IL-8 is identified, the viral reservoir may be revealed. Additionally, the subtle but significant decrease in IFN-α, as compared to the levels of healthy controls, may be involved in the persistent viral reactivation seen in CFS patients. Hata *et al*. have reported that low constitutive levels of type I IFN are required for the proper induction of acute type I IFN production as well as the proper priming of other cytokine responses (33). A basal level of IFN below a normal threshold results in the inability of the host to mount the proper innate immune response, including viral clearance (34). Certainly, the observed decrease in IFN-α and the refractory production of IFN-γ and IFN-β is consistent with clinical observations in CFS regarding their inability to control viral infections properly. These observations may also provide clues to disease pathology and suggest treatment options.

In addition to investigating differences in cytokine and chemokine values between patients and controls, we also used retrospective clinical results for γ-δ T-cell clonality as a second level of stratification. This was done for the following reasons: Previous exploratory data analysis of our patient cohort revealed that at least 10 patients, who originally were diagnosed with CFS, subsequently developed lymphoma (particularly mantle-cell and CLL) later in life and all were positive for XMRV as well as γ-δ T-cell clonality.

Table III. *Correlation analysis of the five most significant up-regulated cytokines and chemokines, stratified by patients who do not display a clonal population of γ-δ T-cells (γ-δ Neg), patients with clonal populations of γ-δ T-cells (γ-δ Pos), and healthy controls (Controls).*

	IL-6	IL-8	TNF-α	MIP-1α
γ-δ Neg				
IL-8	0.335	-	-	-
TNF-α	0.735	–0.004	-	-
MIP-1α	0.981	0.228	0.0781	-
MIP-1β	0.778	0.108	0.628	0.987
γ-δ Pos				
IL-8	0.771	-	-	-
TNF-α	0.571	0.096	-	-
MIP-1α	0.945	0.0633	0.0779	-
MIP-1β	0.864	0.506	0.0840	0.996
Control				
IL-8	–0.048	-	-	-
TNF-α	0.626	–0.024	-	-
MIP-1α	0.760	–0.067	0.939	-
MIP-1β	0.742	–0.024	0.922	0.981

By Pearson correlation analysis.

Table IV. *Predictive success of Random Forest Model of 10 cytokines and chemokines. The final model accurately identified 128 out of the 138 controls (93% specificity) and accurately identified 113 out of 118 patients (96% sensitivity).*

Actual class	Total cases	Accuracy (%)	Control N=138	Positive N=118
Control	138	92.75	128	10
Positive	118	95.76	5	113

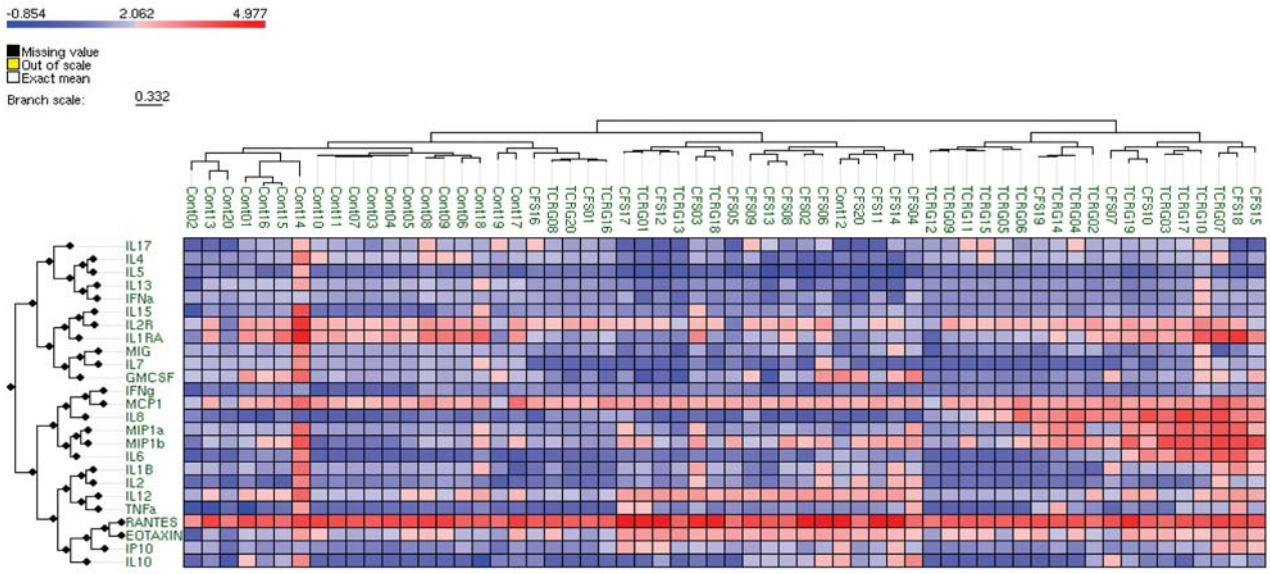

Figure 1. *Two-dimensional SOTA method cluster analysis with Pearson correlation distance of 20 controls (Cont#), 20 XMRV/CFS patients (CFS#) and 20 XMRV/CFS patients with clonal populations of γ-δ T-cells (TCRG#), all chosen at random. Red indicates cytokines/chemokines that are increasingly up-regulated whereas blue indicates cytokines/chemokines that are increasingly down-regulated. Branching represents relatedness between cytokines/chemokines or relatedness between subjects.*

Additionally, when available, samples that were collected prior to the development of lymphoma and subsequently tested were all found to be positive for XMRV. Interestingly, a large percentage of CFS patients who were positive for XMRV were also found to display clonal populations of γ-δ T-cells, but have never developed lymphoma. Furthermore, a proportion of this cohort has repeatedly tested positive for active herpes virus infections, including HHV-6A, CMV and EBV. A previous report by Lusso *et al*. has shown γ-δ T-cells to be susceptible to HHV-6A infection, inducing the expression of CD4 *de novo* (35), and Vrsalovic *et al*. have shown an association between HHV-6 infection and γ-δ T-cell clonality (36). To investigate this phenomenon further, we elected to stratify our data by γ-δ T-cell clonality status to determine if these patients represented a unique subgroup of XMRV-infected CFS patients, perhaps predisposed to lymphoma.

γ-δ-T-Cells play an active role in the regulation and resolution of pathogen-induced immune responses; they accumulate at sites of inflammation associated with viral, bacterial and parasitic infections and in autoimmune diseases (37-40). Consistent with a γ-δ T-cell involvement, our analysis shows the up-regulation of MIP-1β, MIP-1α, TNF-α and IL-10, all of which are produced by γ-δ T-cells. Recently, Gu *et al*. investigated the correlation between circulating cytokines and chemokines and the risk of developing B-cell non-Hodgkin lymphoma and reported that an increased level of IL-13 has a protective effect regarding the development of B-cell lymphoma, whereas patients with

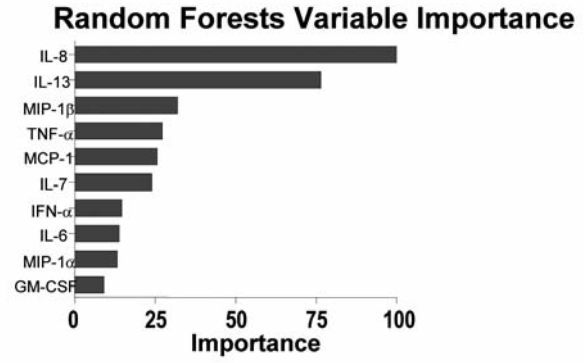

Figure 2. *Random Forest prediction. Horizontal bars represent the relative importance that each cytokine or chemokine contributes to the predictive nature of the signature.*

increased inflammatory cytokines and chemokines are at greater risk (41). This study supports the hypothesis that CFS patients are at greater risk for developing lymphoma as a consequence of their inflammatory condition.

The results of this study suggest that multiplex cytokine and chemokine analysis in conjunction with XMRV testing may serve as a useful diagnostic for CFS. In addition, these results further support the description of XMRV-related CFS as an inflammatory disease and may explain, in part, an increased risk of lymphoma associated with XMRV-infected CFS patients.

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