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Rady Faculty of Health Sciences
Rady College of Medicine

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Student Name: Quinlan Richert **Date:** 08/05/16

Project Title: Systemic inflammation before and after antiretroviral therapy initiation as a predictor of immune response among HIV infected individuals in Manitoba

Primary Supervisor Name: Yoav Keynan

Department: Medical Microbiology, Internal Medicine, and Community Health Sciences

Co-Supervisor Name: Ken Kasper

Department: Medical Microbiology

Summary (250 words max single spaced):

Introduction: Despite the life-prolonging effects of Highly Active Antiretroviral Therapy (HAART), persons with HIV are still prone to higher rates of non-AIDS related morbidity (such as heart, kidney, and liver disease) than the general public. This is likely due to chronic immune activation and inflammation that persists in HIV-positive persons despite virological suppression.
What remains undetermined, however, is whether a link exists between chronic inflammation/immune activation and suboptimal immune recovery on HAART.
The hypothesis of the present study is that higher levels of systemic subclinical inflammation and immune activation are linked with suboptimal immune recovery on HAART.

Methods: Thirteen eligible patients from the Manitoba HIV program were enrolled and followed for up to two years; blood samples were drawn at 4 timepoints each, and concentrations of 21 proinflammatory markers were measured. Patients were grouped according to CD4:CD8 ratio recovery at viral suppression, and the inflammatory profiles of the two groups were compared.

Results and conclusions: APRIL and BAFF are higher in those with poor recovery at the point of suppression, but were also higher in this group at the onset of therapy and through the three additional follow-up visits. TNF-R1, CD163, and Osteopontin, were also in higher concentrations at the outset of therapy and beyond. These five molecules could thus see potential use in the future as biomarkers of likely poor immune recovery. Future work should focus on replicating these findings with larger cohorts.

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1. Introduction and Background

By and large, highly active antiretroviral therapy (HAART) has been a resounding success in the management of HIV. While in the past, an HIV diagnosis meant that eventual immunodeficiency was a virtual certainty, viral loads can now be effectively controlled down to zero in most cases (with strict drug compliance), and ten-year survival probabilities are now 78% (according to one 2016 meta-analysis) for patients on HAART.[1]

As HIV infection shifts from a fatal infection to a chronic, lifelong condition, so do the challenges surrounding its management. The present goal is to understand why people living with HIV, even with undetectable viral loads, still face higher rates of morbidity than those who are HIV-negative -- for example, those living with HIV experience higher rates of heart disease, neurocognitive decline, osteoperosis, as well as liver and kidney disease than the general public.[2]

The link between HIV pathogenesis and chronic immune activation has long been a subject of intense study; it has been shown in multiple studies that even when HIV-positive persons have achieved total viral suppression, some circulating markers of inflammation and immune activation remain elevated vis-à-vis HIV-negative persons.[3] A 2008 review by Alfano et al. speculated on the role for cytokines including IL-7, IL-15, IL-17, IL-18, IL-19, IL-20, IL-21, IL-23, and IL-27 in mitigating HIV replication and disease pathogenesis as well as their potential involvement in managing immune reconstitution in HIV-infected individuals.[4] More recently, a 2015 study by Wada et al. identified seven serologic biomarkers of inflammation that remained persistently high in HIV-suppressed men: CXCL10, sCD27, TNFR2, TNF-α, BAFF, CD14, and CRP; seven more were detected at levels significantly different from those in HIV-negative men (gp130, IL-8, CCL13, GM-CSF, and IL-12p70).[5] Regidor et al. showed that levels of IL-6 and CRP were unaffected by HAART initiation in HIV-positive men, while sCD27, sCD30, IgG, IgA, and CXCL13 decreased but did not normalize to HIV-negative levels.[6] Kamat et al. have posited that a constellation of CXCL9, CXCL10, sIL-2R, and sCD14 may have utility as a plasma biomarker for monitoring immune activation in patients with and without suppressed viremia on HAART.[7]

It is thought that immune activation and chronic subclinical inflammation is driven by persistent microbial translocation across the gut wall, which is initiated due to gut epithelium injury early in the disease course, and is able to continue due to incomplete repair even in patients started on HAART.[8] Furthermore, it has been suggested by several studies that this inflammation is linked to poor clinical outcomes.[9,10] What remains unknown, however, is whether this inflammation and immune activation is linked with suboptimal immune recovery (defined in our study as CD4:CD8 ratio <1), itself a surrogate lab marker for poor outcomes.[11,12]

The hypothesis of the present study is that higher levels of systemic subclinical inflammation and immune activation are linked with suboptimal immune recovery (represented by CD4:CD8 ratio) on HAART. The hope is that by identifying a specific constellation of inflammatory and immune activation markers that are linked with eventual suboptimal immune recovery, we can identify potential targets for adjunct therapies to HAART that can play a role in reducing the morbidity faced by people living with HIV, despite compliance with these essential and revolutionary antiretroviral drugs.

2. Materials and Methods

2.1 Population and setting

Thirteen eligible patients were selected from a cohort of 40 patients who were enrolled in the study and followed for between one to two years. We aimed to follow patients for one year with four total visits/sample collections spaced evenly apart (approximately three months) in order to monitor inflammatory response and immune activation over time; frequent missed appointments by patients caused us to extend the time between visits, with the greatest time between first and fourth (last) visit extending to two years.

Inclusion criteria dictated that a patient must have achieved an undetectable viral load (considered <50 copies per ml) by their final recorded visit with the clinical team in order to control for the effect of HAART non-compliance on inflammatory marker expression. Patients were enrolled after being linked to care following an HIV diagnosis and just prior to commencement of HAART under the Manitoba HIV program. All blood draws and clinical visits were conducted at the Health Sciences Centre (HSC) Internal Medicine Clinic.

Table 1 lists baseline characteristics for the 13 enrolled participants.

2.2 Ethics statement

This study was approved by the ethics committees of the University of Manitoba and HSC.

2.3 Cytokine/chemokine bead-based assays

Cell free supernatants were aliquoted, and frozen at −80°C. Two multiplex bead-based fluorescent assays were performed on 52 plasma samples (4 different timepoint samples per 13 enrolled participants) to quantify 21 markers of immune activation and inflammation. The LXSAHM 9 plex and 2 plex (R&D Systems, Minneapolis MN), were used to detect gp130, IL-10, IL-6Ra, MIP-1b/CCL4, TNF-R1, Chitinase 3-Like 1, IL-27, CD163, IL-34, TNF-R2, IFN-ɣ (samples were diluted 1:2), and CD14 (sample dilution required 1:50). Each kit was run separately by the same operator.

The Bio-Rad® Bio-Plex Pro™ Human Inflammation Panel 1, Single-plex were used to detect APRIL/TNFSF13, BAFF/TNFSF13B, INF-α2, IL-26, IL-20, IL-11, IL-32, Osteocalcin and Osteopontin (Bio-Rad, Mississauga, ON). Beads containing the antibodies were mixed all in one vial, 50 µl of the beads' suspension were used in each reaction, samples were diluted 1:4, and run simultaneously.

Prior to each assay, calibration and validation were performed. The assays were performed following manufacturers' instructions, respectively, using 50µl of samples and standards, in duplicates. The standards were reconstituted and serial diluted as per manufacturer's protocol to generate standard curves. Standards included all recombinant cytokines tested and were considered as positive controls for the procedure. Results were run on a Bioplex 200 instrument (Bio-Rad, Mississauga, ON), reported as mean fluorescence intensity and converted to pg/ml using the Bio-Plex® Manager version 6.0) (Bio-Rad, Mississauga, ON).

2.4 Statistical analysis

The overarching purpose of the statistical analysis was to determine whether there was an observable difference in concentration of multiple cytokines in patients with good compared to poor immune recovery both at the time where undetectable viral load (<50 copies per ml) was first achieved, and also throughout 4 visits representing the first one to two years of clinical management on HAART. Poor immune response was defined according to CD4:CD8 ratio because it is a more sensitive predictor of future adverse outcomes than the traditional CD4+ T cell count.[13] We considered patients who failed to achieve a CD4:CD8 ratio ≥ 1 by the time they first achieved virological suppression (< 50 copies/ml) to be poor immunologic responders.

Principal component analysis (PCA) was conducted using collected cytokine data from all patients at the point of earliest undetectable viral loads to determine which cytokines most contributed to the variability in the data set at that time. Component extraction was achieved using the principle axis method, and the rotation method employed was a Varimax rotation with Kaiser normalization. In interpreting the PCA, an inflammatory marker was considered to load on a given component if the loading factor was 0.6 or greater for that component and less than 0.6 for all other components. By using PCA, we could analyze all cytokines simultaneously, to determine whether a particular constellation of cytokines, when considered together (ie. those which loaded onto a common component at a given rotation) were disproportionately responsible for the total observed variation in the data set even if statistical significance was unfruitful when individual cytokines were compared in isolation.

Following PCA, paired boxplots were constructed (comparing concentrations for each inflammatory marker at TND for good vs poor recovery groups) and Mann-Whitney U tests ($\alpha = 0.05$) were conducted on the cytokines that loaded into each component to determine if they tended to preferentially segregate into the good immune recovery or suboptimal immune recovery group.

To observe, qualitatively, how concentrations of cytokines changed within and across the groups over time, average concentrations of each cytokine were compared for the good and poor immune recovery groups at each of the four timepoints. 95% confidence intervals were used for each.

Finally, Ingenuity Pathway Analysis (IPA) was used to determine whether the identified cytokines of interest, as determined by the above methods, shared involvement in any networks or canonical pathways, or implied the involvement of any well-described physiologic or

pathophysiologic processes. The background reference set used for p-value calculations was set to include only human genes listed in the Ingenuity Knowledge Base (IKB), and to exclude endogenous chemicals and genes described only in other species.

All statistical analysis, excluding IPA, was carried out using IBM SPSS® version 21.1.1.

3. Results

PCA, shown in Table 2, revealed two principal components or rotations of our 21-dimension data set accounting for an accumulated 43.135 percent of the total existing variation. Component 1 accounted for 21.825 percent of the total variation; component 2 accounted for 21.310 percent of the total variation. Of the 21 proinflammatory markers measured in this study, five loaded onto component 1 (TNF-R1, Chitinase 3-like-1, APRIL/TNFSF13, BAFF/TNFSF13B, and CD14) and four markers loaded onto component 2 (IL-6Ra, TNF-R2, IL-27, and gp130). The remaining 4 principal components accounted for progressively less variability in the data set and two or less proinflammatory markers loaded onto each. IL-34, IL-26, and IL-11 do not appear in the output because they all have the same variance across both poor and good immune responders (the same concentration was detected on each sample for these cytokines).

Mann-Whitney U tests were conducted for each individual proinflammatory marker, comparing cytokine concentrations between poor and optimal immune recovery groups at the point of initial virological suppression. Despite the PCA results, no proinflammatory marker in isolation achieved statistically significant difference in concentration between the good immune recovery and suboptimal immune recovery groups with an $\alpha = 0.05$.

However, when the complete distribution of the data is visually represented as seen in Figure 1, a trend toward increased concentrations in poor responders compared to good immune responders is observed in the following proinflammatory markers: IFN-ɣ, IL-10, CD163, IFN-α2, IL-32, Osteopontin, BAFF, and APRIL. The effect is most pronounced in BAFF, APRIL, IFN-ɣ, and IL-32, where the median concentration for poor responders is above the interquartile range (IQR) for good responders (and for APRIL and BAFF, aside from one outlier each, it is above the maximum value for good responders). Conversely, a trend toward increased concentrations in good responders is observed in the following markers: CD-14, TNF-R2, gp130, IL-6Ra, TNF-R1, Chitinase 3-like-1, IL-20, and Osteocalcin. The observed difference in median value is marginal, except in gp130, and IL-6Ra, where the median concentration for good responders exceeds the IQR for poor responders.

Data collection at 4 timepoints per patient allowed for the simultaneous observation of how proinflammatory marker concentrations changed over time within a group and how the concentrations of each marker compared between the two groups over time. Figure 2 depicts the average concentration of proinflammatory markers for both suboptimal and good recovery groups at all four visits. Five markers (TNF-R1, CD163, APRIL, BAFF, and Osteopontin (SPP1)) were observed in higher concentrations across all four timepoints in patients with poor CD4:CD8 recovery; of those five, three (APRIL, BAFF, and TNF-R1) appeared in the first two PCA components. Another five markers (IL-6Ra, Chitinase 3-like-1, IFN-a2, IL-20, and Osteocalcin)

were persistently elevated in patients with good CD4:CD8 recovery -- of those, only one, IL-Ra, appeared in the first two PCA rotations. Thus, of the nine cytokines in the first two PCA rotations, (that is, the two rotations accounting for an accumulated 43.135 percent of the variability in the data set), six were consistently (at all four visits) found to be on average higher or lower in either the good or suboptimal immune recovery groups. The cytokines in the first two components that did not consistently have higher averages in one immune recovery group over the other (ie. at all four visits) were CD14, TNF-R2, IL-27, and gp130. CD14 was observed at a higher mean concentration at every visit except for visit 2; TNF-R2 was observed in higher average concentrations in those with poor recovery at the first and last visits, but at the second and third visits, those with good recovery had higher average concentrations. IL-27 was consistently higher on average in those with good recovery except at visit 3, and gp130 was higher on average in those with good recovery at all visits except for at visit 2.

Ingenuity Pathway Analysis was used to determine whether important molecules in our dataset were overrepresented in previously described networks or canonical pathways. An IPA analysis was conducted to compare functions, relations, and canonical pathway overlap for molecules that were found to be elevated in the poor immune recovery group at all four visits (TNF-R1, CD163, APRIL, BAFF, Osteopontin/SPP1). Three of these molecules, APRIL, BAFF, and TNF-R1 were also present in the first principle component rotation. According to Ingenuity Knowledge Base (IKB) output, the shared functions/disease implications of these cytokines include rheumatic disease (all five are involved, p-value 2.22×10^{-11}) and all affect the "proliferation of cells," again this network involving all five markers (p value 1.31×10^{-10}). There is marginal overlap with canonical pathways -- "altered T and B cell signaling in rheumatoid arthritis" (p-value 7.21×10^{-7}) has 3.7 percent overlap with the input data set, while "communication between innate and adaptive immune cells" has 2.4 percent overlap (p-value 1.7×10^{-4}). A network demonstrating known direct and indirect interactions between the five molecules in the input data set is shown in Figure 3.

Proinflammatory markers elevated in all four visits in the good recovery group compared to the poor recovery group (IL-6Ra, Chitinase 3-like-1, IFN-a2, IL-20, and Osteocalcin) were also entered into IPA for analysis. A different set of networks and functions/canonical pathways was detected. Top involved network functions included "hematological system development and function and tissue morphology" (p-value 5.83×10^{-9}) and "cellular growth and proliferation" (p-value 2.46×10^{-8}) although none of the identified pathways incorporated all five of the molecules in the input dataset.

4. Discussion

4.1 Identification of important inflammatory molecules in poor immune responders

The results obtained in this small cohort study, while modest, demonstrated some consistency with prior established findings regarding inflammation and immune activation in the setting of HIV. However, they also suggest that, while total inflammation does not precipitously drop off in those with optimized immune recovery on HAART vis-à-vis those with suboptimal recovery, there is a distinct constellation of persistent immune activation and inflammation observed in those with poor immune recovery. We speculate that searching for and recognizing the individual

components of this constellation early on in disease management could have positive downstream effects.
PCA was used as a central modality in this experiment, because, with a low sample size and many variables simultaneously being measured and investigated (where each proinflammatory marker assayed is considered a separate variable or dimension), it was unlikely for a single marker in isolation to achieve a statistically significant difference in concentration between those with good and those with suboptimal immune recovery. As predicted, this was the case. However, the dimension reduction of PCA revealed that two "rotations" or principle components explained much of the variance in the data. The first principle component contained five proinflammatory markers: TNF-R1, Chitinase 3-like-1, APRIL/TNFSF-13, BAFF/TNFSF-13b, and CD14. The second principle component contained four proinflammatory markers: IL-6Ra, TNF-R2, IL-27, and gp130.

While Mann-Whitney U tests failed to show that any of the proinflammatory markers achieved statistically significant differences between groups on their own, some did appear, at viral suppression, to trend toward different median values and distributions between the two groups: the median concentration of BAFF is higher in the poor recovery group than value of the third quartile of the good recovery group, and the data covers a much broader, higher range. A similar pattern is evident for APRIL. On the other hand, TNF-R1 has a much broader data range in the poor recovery group, but the median concentration is marginally higher in good immunologic responders. The same is true of CD14. Thus, of the five cytokines in the first component, BAFF and APRIL exhibited the most promise in distinguishing poor from good immune responders.

In the second principal component, IL-6Ra has a higher median concentration in good responders, as does gp130. IL-27 has a broad data range in good responders, but a higher median concentration in poor immune recovery. TNF-R2 has a similar average across both good and suboptimal responders.

Other markers in the third through sixth principal components also demonstrated different median values between good and suboptimal responders (although, again, without achieving statistical significance at $\alpha = 0.05$): IFN-γ, IL-10, MIP-1b, CD163, and IL-32, but because of their inability to meet statistical significance and combined with their failure to sort into a high-ranking component, they were considered to be of less potential value than those that both ranked into a high component and demonstrated a tendency to higher concentrations in either the poor recovery group or the good recovery group.

The idea that some residual inflammation persists in HIV has been thoroughly investigated by others; our results are consistent with this, but suggest that the specific pattern of this inflammation is different depending on a patient's immune recovery status, specifically their CD4:CD8 ratio at the time of virological suppression. Thus far, our results predict that, of the most important markers of inflammation, that is, those ranking in the first two principal components, we expect a person with poor immune recovery to have an inflammation profile characterized by high BAFF and APRIL (median concentration is higher than the maximum observed value of good responders) and low gp130 and IL-6Ra (where the maximum observed concentration is less than the median value of the good responders).

Wada et al. demonstrated that in HIV-suppressed individuals on HAART, TNF-R2, BAFF, and CD14 were among the inflammatory biomarkers that remained elevated compared to in HIV-negative persons.[14] Our results go further to suggest that BAFF tends to be more elevated in those with poor immune recovery despite virological suppression compared to those with CD4:CD8 recovery ≥ 1 and viral suppression. This finding is of particular interest, because the potential utility of BAFF as a serum biomarker has been explored by others; Carbone et al. found that the cytokine was "independently associated with risk of AIDS after adjustment by clinical factors."[15] Thus, our research brings together Wada et al.'s findings that this cytokine remains elevated in HIV-suppressed individuals with evidence linking it to poor immune recovery (and the poor outcomes associated with it) and other independent research linking BAFF with adverse outcomes.

CD14 and TNF-R2, on the other hand, tended to segregate preferentially toward those with good immune recovery, although they still registered at observable levels in the poor immune recovery group. TNF-R2 is a member of the TNF-R super family, a related superfamily to the TNF superfamily, of which BAFF is a member; CD14 is a protein functioning in the translocation of microbiota across the gut wall, one mechanism thought to be driving persistent inflammation in virally suppressed patients.[16,17] Due to the marginal difference between the two groups, and the intuitive notion that TNF-R2 should be upregulated similarly to BAFF due to their closely associated superfamilies and functions, we must remain skeptical of these findings. The association of CD14 with adverse clinical outcomes in a follow-up paper by Wada et al. further emphasizes this point. These cytokines should be measured again in a bigger cohort in order to test the present finding.[18]

Using PCA, tests of statistical significance, and comparing median values at the point of viral suppression, offers a snapshot of the inflammatory profile of those with poor immune recovery and allows us to compare the two groups at a common point in treatment, since the time passed between visits for each patient were not always the same. We were also interested, however, in how the inflammatory profiles of patients with poor or good immune recovery evolved over time, from the beginning of treatment, through the achievement of viral suppression, and beyond. This information is of particular clinical importance, since it would in theory allow clinicians the ability to predict early in the treatment course those who would likely eventually reach optimal immune recovery and those who likely would not (within one to two years).

Using samples drawn from each patient at 4 separate visits, occurring over the span of between one and two years but always beginning at the initiation of treatment, we were able to discern that five cytokines were, on average, observed in higher concentrations in the poor immune recovery group. They were TNF-R1, APRIL, BAFF, CD163, and Osteopontin. Similarly, five cytokines were on average elevated across all four visits in the good recovery group: IL-6Ra, Chitinase 3-like-1, IFN-a2, IL-20, and Osteocalcin. Out of these 10 listed proinflammatory markers, half are represented in the first two principal components (four -- APRIL, BAFF, TNF-R1, and Chitinase 3-like-1 -- in the very first component), despite the fact that only BAFF and APRIL were elevated in the poor recovery group at viral suppression. The potential clinical implication is that, for example, for a patient found to have high BAFF, TNF-R1, and APRIL, and comparatively low IL-6Ra and Chitinase 3-like-1 early on in treatment, there would be a greater suspicion that the patient would have poor immune recovery at one to two years, compared to a patient with the opposite results. This observation and inference must, of course, be tempered by the fact that the differences in

average concentration were often quite marginal, and the 95% confidence intervals quite wide -- the finding should again be followed up in a larger study with a diverse, multisite cohort.

It is of note that two other markers from the first two PCA components were elevated preferentially in one group or the other at three out of four visits: CD14 was elevated in those with poor recovery at all visits except visit 2; gp130 was elevated in the good group at all visits except for visit 2. This is interesting because these are two molecules that Wada et al. found to be detected at abnormal levels in virally suppressed persons when compared to HIV-negative persons.[19] In regards to CD14, Serrano-Villar et al. observed a negative correlation between this biomarker and CD4:CD8.[20] Furthermore, a 2016 study associated unchanged concentrations of CD14 in virally suppressed HIV-infected people with serious non-AIDS events, including cardiovascular and renal disease, cirrhosis, non-AIDS defining malignancies, and death.[21]

4.2 Pathway elucidation

IPA showed that the cytokines that were elevated across all four visits in the poor immune recovery group were all involved in some common pathways involving the pathogenesis of rheumatoid arthritis and cellular proliferation, as well as demonstrating modest overlap with the canonical pathway responsible for B and T cell differentiation. Given this finding, it is important to confirm with future investigations whether rheumatologic morbidities are more prevalent in poor immune responders compared to those with optimized CD4:CD8 counts.

Pathway elucidation for the inflammatory markers that were increased in the good recovery group was less impressive, as not all input molecules sorted cleanly into a single network or function; furthermore, less overlap with canonical pathways was observed.

We can speculate that these IPA results imply one of two things: either that systemic inflammation persists in both poor and good immune responders, but with different networks and molecules preferentially upregulated, or that in poor immune reponders, inflammation is more “well-defined” and confined to well-known networks and pathways than the residual inflammation in good responders. This latter hypothesis could be tested by repeating a similar analysis as was conducted here, but also assaying for additional molecules identified in the networks related to poor and optimal responders.

4.3 Limitations, conclusions, future directions

We are aware of the limitations that exist in this study. We were limited in our ability to produce far-reaching and generalizable results by our small, single-centre cohort. Furthermore, no genetic-level data is reported here. Capitalizing on these areas for improvement in future studies could yield impressive results. In addition, the ability to follow patients in a longitudinal study lasting for many years could enable us (or others pursuing similar research) to directly measure inflammation against clinical, rather than laboratory, outcomes, or to confirm that suboptimal immune recovery persists in the identified patients for longer than one to two years. Given this, and our incidental and highly preliminary findings regarding potential rheumatologic inflammation

pathways involvement in poor immune responders, it would be interesting for the incidence of rheumatologic-type diseases to be followed as one of many possible adverse outcomes in a longitudinal analysis of that type.

Taking all of the above into account, we recognize the tentative nature of this study's conclusions. Nonetheless, we surmise that there are differing inflammatory patterns to be observed in patients with poor immune recovery, when poor immune recovery is defined as CD4:CD8 ratio < 1 at the time of viral suppression, compared to those with good immune recovery (CD4:CD8 ratio ≥ 1) at that time. APRIL and BAFF are higher in those with poor recovery at the point of suppression, but were also higher in this group at the onset of therapy and through three additional follow-up visits. In addition, TNF-R1, CD163, and Osteopontin, were also in higher concentrations at the outset of therapy and beyond. These five molecules could thus see potential use in the future as biomarkers of likely poor immune recovery -- TNF-R1, BAFF, and APRIL are likely to be the most important due to their high PCA ranking and consequently superior role in explaining variance in the data set. Simultaneously, we may expect to see IL-6Ra and Chitinase 3-like-1 register at lower concentrations than in other patients. Future work should focus on replicating these findings with larger cohorts, with particular focus on the molecules that ranked in high-priority principal components, and then subsequently determining whether this characteristic inflammation pattern has a role in *causing* poor immune recovery. If so, these molecules could serve as potential targets for adjunct therapies to HAART.

Finally, this study highlights the utility of using PCA to reveal results in a complex data set. The technique could be helpful in teasing out heretofore unrecognized aspects of the infection's pathogenesis.

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6. Tables and Figures

Table 1. Demographic characteristics of 13 enrolled HIV-positive patients

Parameter	**Value** n (%)
Male	7 (54)
Age	
18-40	6 (46)
40-60	7 (54)
Ethnicity	
African	1 (8)
Aboriginal First Nations	7 (54)
Caucasian	5 (38)
CD4+ T cell Nadir	
< 500 cells/μL	8 (62)
> 500 cells/ μL	5 (38)
Time to viral suppression	
< 1 year	10 (77)
>1 year	3 (23)
Time between first and last visit	
≤ 1 year	2 (15)
> 1 year	11 (85)
HCV coinfection	
Yes	1 (8)
No	12 (92)

Table 2. Principle Component Analysis using concentrations of 18 cytokines in HIV patients at the earliest detected point of viral suppression. IL-34, IL-26, and IL-11 are excluded from the analysis because they have equal values in every sample. Cytokines loading onto a given component are bolded and shaded.

Rotated Factor Pattern[a]						
	Components					
	1	2	3	4	5	6
TNF-R1	**.944**	-.115	.127	-.009	.078	-.062
Chitinase 3-like-1	**.872**	.126	-.184	.197	-.032	-.088
APRIL/TNFSF 13	**.837**	-.431	.140	-.075	.165	.233
BAFF/TNFSF 13B	**.678**	-.477	.096	-.473	-.171	.023
CD14	**.645**	-.209	.534	.255	-.046	-.392
IL-6Ra	-.122	**.909**	-.152	-.128	-.114	-.218
TNF-R2	.001	**.737**	.066	-.558	-.007	.169
IL-27	.024	**.735**	-.417	-.022	-.284	-.091
gp130	-.527	**.734**	.061	.175	-.182	.075
Osteopontin/SPP1	.283	-.675	.427	-.215	-.089	-.368
IFN-gamma	-.182	.532	-.446	-.035	-.495	.306
IL-20	-.114	-.177	**.835**	.354	.074	.204
Osteocalcin/BGLAP	.222	-.163	**.703**	-.344	-.261	-.369
IL-10	.185	-.028	.079	**.913**	-.166	-.021
IL-32	-.166	-.094	.163	**.576**	-.031	.517
IFN-a2	-.161	-.030	-.063	-.120	**.957**	.004
MIP-1b/CCL4	.380	-.307	.000	-.042	**.822**	-.111
CD163	.047	-.011	-.072	-.015	-.103	**.944**
Extraction method: Principal Component Analysis. Rotation method:Varimax with Kaiser normalization.						
a.The rotation converged in 11 iterations.						

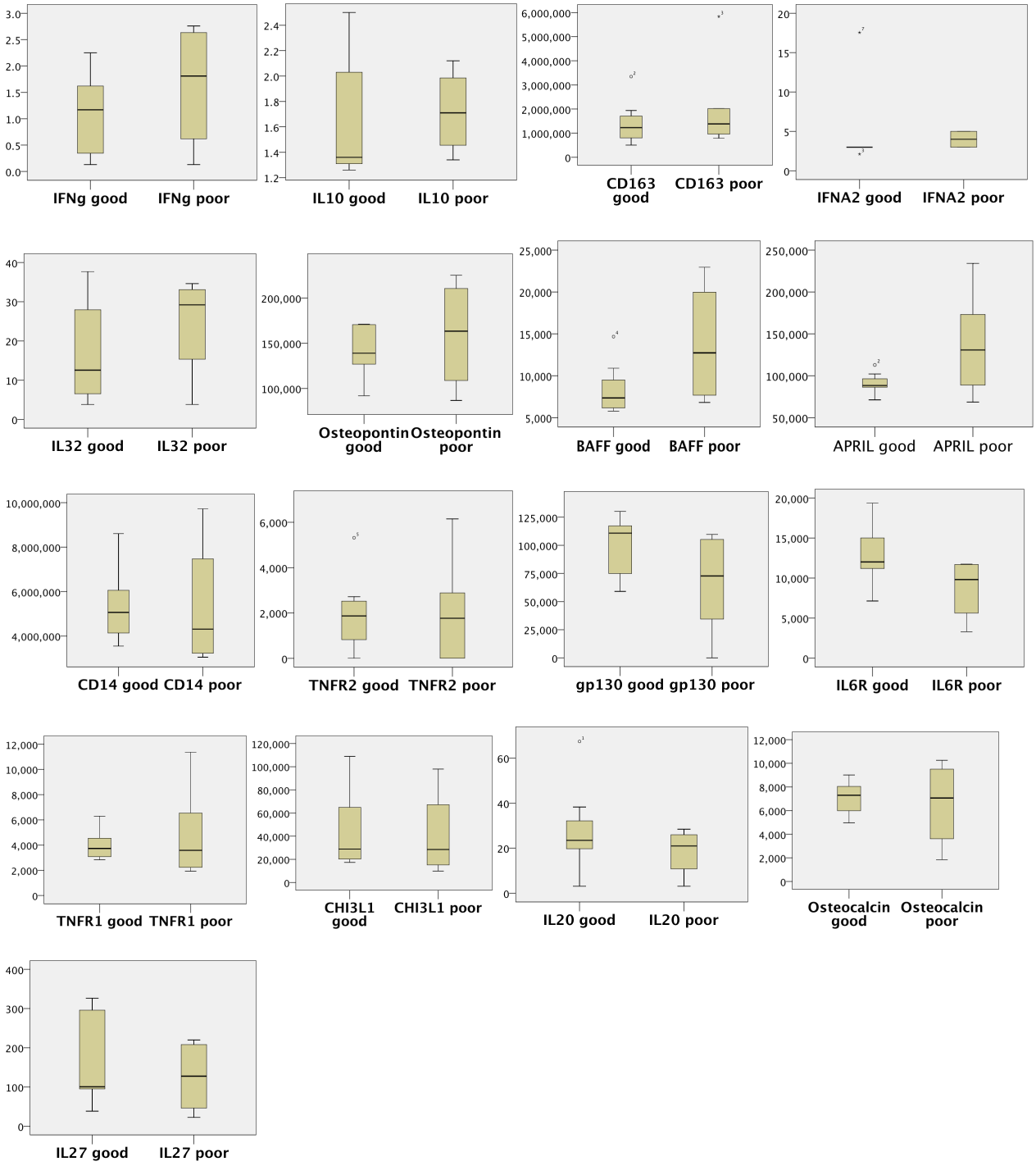

Figure 1. Boxplots comparing data range, including maximum, minimum, and IQR values, for cytokines where there was a visually discernible difference in median concentration between good and poor immune responders: IFN-γ, IL-10, CD163, IFN-α2, IL-32, Osteopontin, BAFF, APRIL, CD14, TNF-R2, gp130, IL-6Ra, TNF-R1, Chitinase 3-like-1, IL-20, Osteocalcin, and IL-27 are depicted. None achieved statistically significant differences. IL-11, IL-26, IL-34, and MIP-1b are excluded because the difference between the medians and/or data ranges was negligible. Y-axis values are reported in pg/ml.

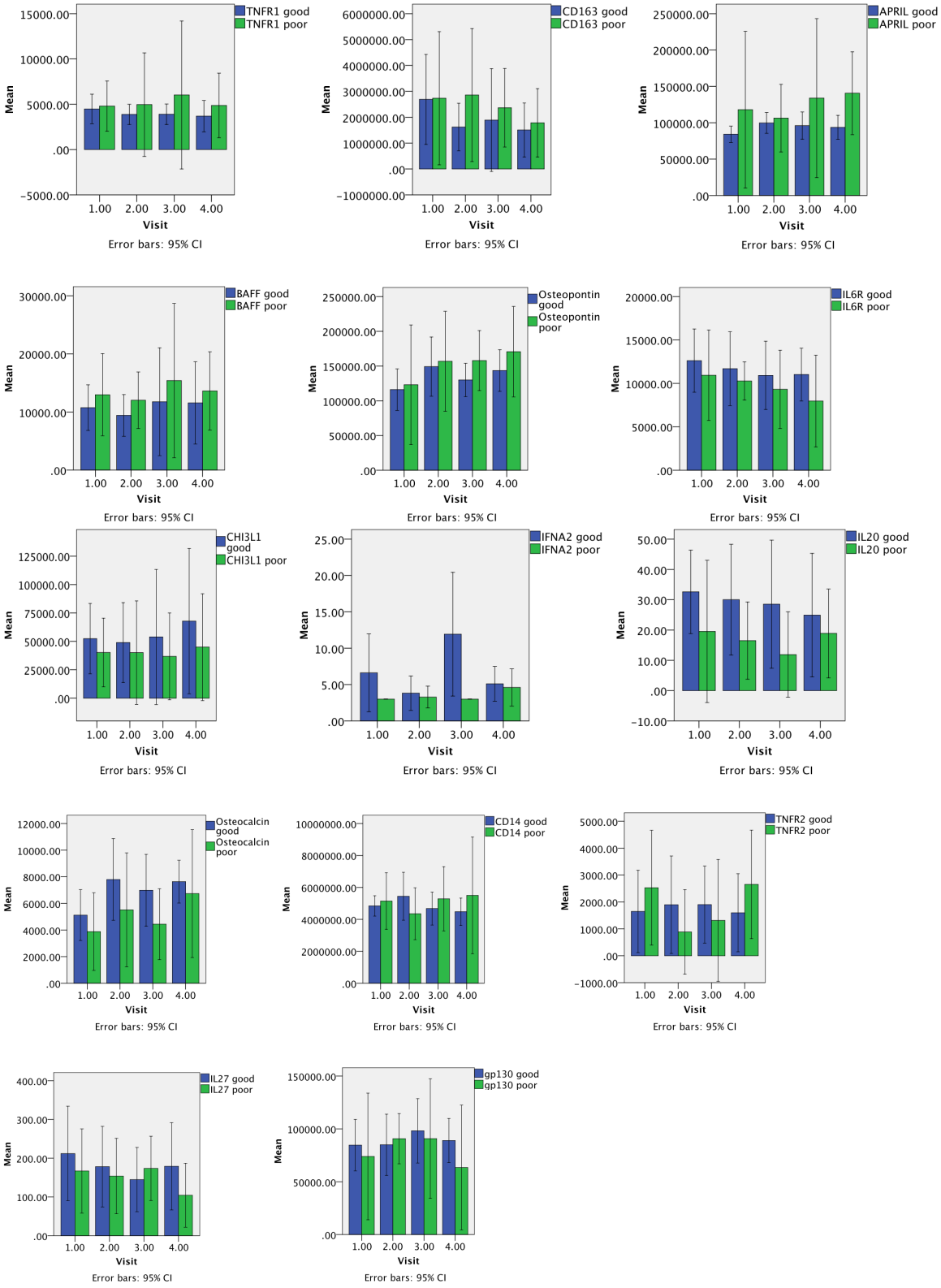

Figure 2. Mean values with 95% confidence intervals through all recorded visits for cytokines demonstrating a tendency to remain consistently elevated in good or poor immune responders. Y-axis units are in pg/ml.

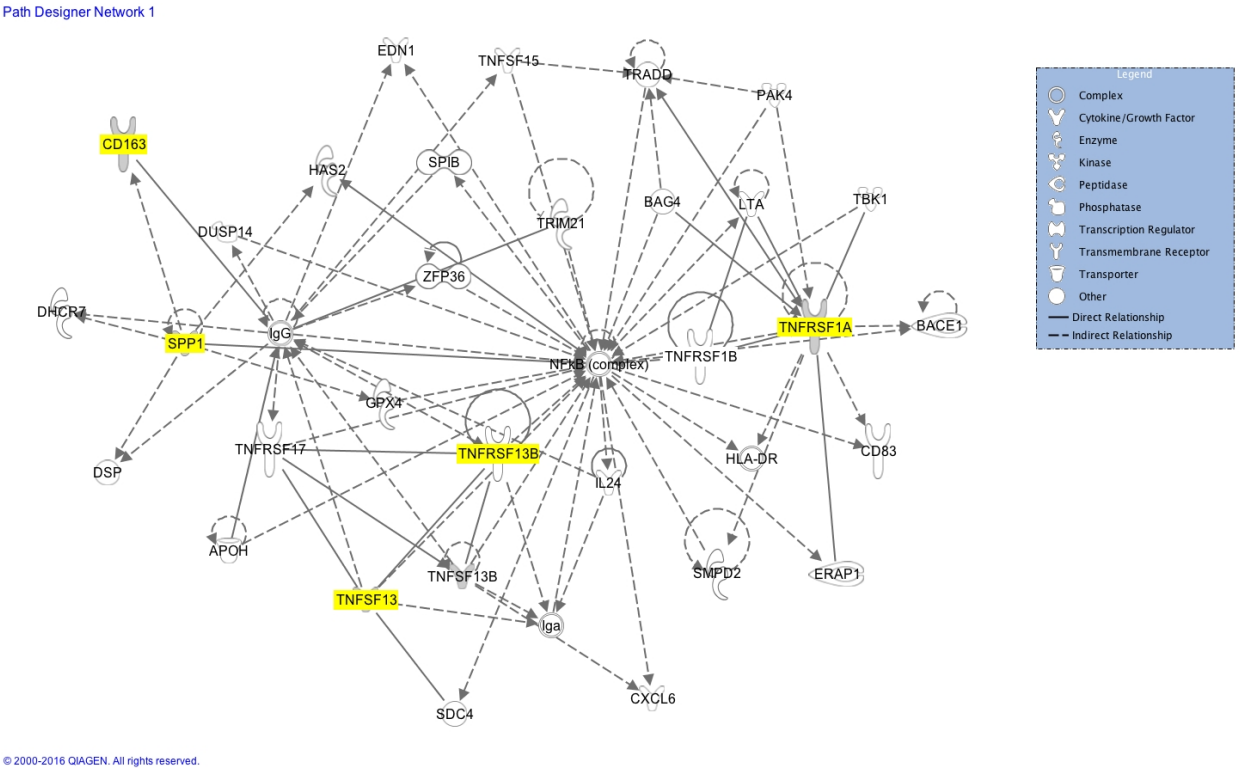

Figure 3. IPA-generated interaction pathway incorporating cytokines found to have higher means in the poor recovery group (compared to the good recovery group) across all 4 visits. TNF-R1 (TNFRSF1A), CD163, APRIL (TNFSF13), BAFF (TNFSF13B), and Osteopontin (SPP1) are represented on the network. Three of the above (APRIL, BAFF, and TNF-R1) were also sorted into the first principle component. Molecules from the input data set are highlighted in yellow.