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Inflammatory Cell Differentiation and Chemotaxis and Extracellular Tissue Repair Markers Are Correlated with Pulmonary Dysfunction in HIV Infected Individuals Presenting with Community-Acquired Pneumonia

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Prior studies have shown that HIV patients develop permanent pulmonary dysfunction following an episode of community-acquired pneumonia (CAP). However, the mechanism causing pulmonary dysfunction remains an enigma. HIV patients experience chronic inflammation. We hypothesized that CAP exacerbates inflammation in HIV patients resulting in an accelerated decline in lung function. A prospective cohort pilot study enrolled HIV patients hospitalized in Medellin, Colombia, with a diagnosis of CAP. Sixteen patients were eligible for the study; they were split into 2 groups: HIV and HIV+CAP. Plasma, sputum, and pulmonary function test (PFT) measurements were retrieved within 48 h of hospital admission and at 1 month follow-up. The concentrations of 13 molecules and PFT values were compared between the 2 cohorts. The HIV+CAP group had lower lung function compared to the HIV group; forced vital capacity (FVC)% predicted and forced expiratory volume in 1 s (FEV_1)% predicted decreased, while FEV_1/FVC remained constant. APRIL, BAFF, CCL3, and TIMP-1 correlated negatively with FVC% predicted and FEV_1% predicted; the relationships however were moderate in strength. Furthermore, the concentrations of BAFF, CCL3, and TIMP-1 were statistically significant between the 2 groups ($P \leq 0.05$). Our results indicate that HIV patients with CAP have a different inflammatory pattern and lower lung function compared to HIV patients without CAP. BAFF, CCL3, and TIMP-1 were abnormally elevated in HIV patients with CAP. Future studies with larger cohorts are required to verify these results. In addition, further investigation is required to determine if BAFF, CCL3, and TIMP-1 play a role in the process causing pulmonary dysfunction.

Keywords: inflammation, cytokine, lung function, pulmonary dysfunction, HIV, CAP

Introduction

After its discovery, HIV has become one of the fastest growing pandemics in recent history. Its unique pathogenicity and global dissemination has made it one of the most devastating infectious diseases. In 2005, HIV related deaths peaked at 2.3 million (Maartens and others 2014). The situation has since changed, in part, due to the development and use of combination antiretroviral therapy (cART). The success of cART has been well documented, HIV related mortality has been drastically reduced, and HIV-infected individuals now have a more promising prognosis (Kohli and others 2006). However, studies have shown that even after viral load suppression and restoration of systemic immune function following cART, the risk for lower respiratory tract infections such as community-acquired pneumonia (CAP) remains higher in HIV patients vis-à-vis HIV negative individuals (Cillóniz and others 2017). The increased susceptibility is multifactorial and, in part, related to alterations in host defense in the respiratory tract; this includes abnormalities to the mucociliary clearance apparatus and impairment of the innate and adaptive immune responses (Chinnapaiyan and others 2017). The combination of mucus impaction, bacterial entrapment within the lower respiratory tract, and

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inadequate clearance by the immune system facilitates the infectious process that leads to recurrent pulmonary infection in HIV infected individuals (Chinnapaiyan and others 2017). It has been proposed that these changes are attributed to the direct effects of the HIV on bronchial epithelial cells, persistent immune activation, and inflammation within the alveolar space (Shellito 2004; Brune and others 2016; Chinnapaiyan and others 2017). However, despite the high incidence of CAP in HIV-infected individuals, little is known about the effects of respiratory infection on lung function in this population.

Several studies have investigated various inflammatory patterns in HIV-infected individuals with pneumonia. Perenboom and others in their study characterized the pulmonary inflammatory response in HIV infected individuals with *Pneumocystis jiroveci* pneumonia (PJP). Interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6, and TNF soluble receptors were quantified, and IL-1β was significantly higher in individuals with HIV and PJP coinfection compared to HIV negative individuals (Perenboom and others 1997). Likewise, in a study by Isrëal-Biet and others (2004), IL-10, RANTES, CCL3, and CCL4 were markedly decreased in HIV infected patients with PJP. Further analysis identified a negative correlation between IL-10, RANTES, and CCL3 with pulmonary viral load, which was elevated in these patients (Isreal-Biet and others 2004). In 2015, Keynan and others (2015) characterized similar cytokines in a cohort of HIV patients infected with CAP. Moreover, they identified unique inflammatory patterns specific for certain infecting agents. For example, *Mycobacterium tuberculosis* infection was associated with elevated levels of IL-10, IL-12, IL-13, IL-17, Eotaxin, GCSF, CCL3, and CCL4. Patients with fungal infections were associated with low levels of IL-1RA, IL-8, TNF-α, and VEGF (Keynan and others 2015).

Pulmonary dysfunction as a complication in the HIV population is increasingly appreciated and is found to occur at accelerated rate in older individuals, males, and those with higher baseline chronic obstructive pulmonary disease severity rates (Li and others 2018). Shaw and others (1988) showed that the transfer factor for carbon monoxide and forced expiratory volume in 1 s (FEV_1) were reduced in HIV-infected individuals with PJP. Morris and others (2000) also identified a decline in lung function in patients with HIV, and they further noted that PJP in HIV-infected individuals resulted in an accelerated decline, most notably airflow obstruction. The deterioration in lung function persisted over time (Morris and others 2000).

Current studies have either focused on the inflammatory pattern or lung function, but what remains unknown is the relationship between inflammatory response and loss of lung function in HIV patients after an episode of CAP; therefore, further exploration is required.

It has been well documented that HIV patients experience chronic inflammation and persistent immune activation despite cART (Deeks and others 2013). Lung epithelium responds to the HIV virus by releasing pro-inflammatory mediators, which damage the epithelial barrier. Disruption of the epithelial barrier promotes bacterial translocation, which results in ongoing immune activation and inflammation (Brune and others 2016). We hypothesized that CAP infection worsens inflammation in HIV patients in turn leading to a decline in lung function. The aim of this prospective cohort pilot study was to identify potential biomarkers that can be used to predict pulmonary dysfunction and also identify potential targets for therapeutic intervention to mitigate the deterioration in lung function. To our knowledge this is the first study that directly investigates inflammation and its relationship with lung injury specifically in HIV patients following CAP infection.

Materials and Methods

Population and setting

A prospective cohort pilot study was performed using the data collected from patients hospitalized with CAP at Hospital Universitario San Vicente Fundación and Clínica SOMA in Medellín, Colombia. Patients were enrolled in the study following their visit to one of these institutions. Patients were eligible for the study if they were 18 years or older. The remaining inclusion criteria mandate that plasma and induced sputum samples, as well as pulmonary function test (PFT) values, were taken at the time of admission and at 1 month follow-up. The main exclusion criteria were chronic lung disease and immunosuppression that were not associated with HIV. The control group for this study was composed of HIV patients without CAP. The inclusion criteria for the control group dictated that patients must have a diagnosis of HIV and were 18 years or older, who provided written informed consent and did not plan to move within the 1 year of follow-up. A total of 67 patients were recruited for the study; 30 patients were excluded from the study, died, or were lost to follow-up. The remaining 37 patients were enrolled in the full study. As this is an ongoing study, we conducted a pilot study using a subset of enrolled patients ($n = 16$) for whom we had plasma, induced sputum, and PFT values at admission and 1 month follow-up. Data collection for the remaining patients is still in process. Study participants were separated into 2 groups: HIV+CAP and HIV. A diagnosis of CAP was confirmed by identifying new pulmonary infiltrates on a chest computed tomography or radiograph in the presence of respiratory symptoms. Individuals with HIV+CAP underwent bronchoalveolar lavage to study the infecting pathogen. The HIV group without CAP infection served as the control group in this study. Plasma and sputum samples and PFT measurements were retrieved within 48 h of hospital admission and at 1 month follow-up.

Baseline characteristics of the 16 participants are shown in Table 1.

Ethics statement

This study was reviewed/approved by the University of Manitoba Health Research Board, the Universidad Pontificia Bolivariana and Universidad de Antioquia Faculty of Medicine's Ethics Committees, and the Ethics Committee of Hospital Universitario San Vicente and Clinica SOMA. All patients enrolled in the study provided written consent for their participation. Additional consent was obtained from patients regarding the storage and analysis of the collected samples.

Data collection

Plasma and sputum samples were collected at Hospital Universitario San Vicente Fundación and Clínica SOMA, stored at −80°C at Universidad Pontificia Bolivariana's

Table 1. Baseline Characteristics of the 16 Participants in the Study, HIV+CAP Group (n=9), Control HIV Group (n=7)

Characteristics	HIV+CAP group (n=9)	HIV group (n=7)
	Median (IQR)	
Age, years	35 (25–40)	33 (26–43)
CD4 count, cells/μL	193 (63–297)	97 (32–300)
Viral load, copies/mL	43,699 (6,308–257,036)	196,155 (1,766–729,152)
	n (%)	
Male	7 (78)	7 (100)
Female	2 (22)	0 (0)
Pneumocystis jirovecii	2 (22)	
Mycobacterium tuberculosis	6 (66)	
Streptococcus pneumoniae	1 (11)	
CURB65 score ≥3	0 (0)	
ART use	3 (33)	3 (33)
Smokers	3 (33)	2 (29)

CURB65 is a clinical score used to predict severity and mortality risk in patients that have CAP; a score of ≥3 is considered high severity. In the HIV+CAP group 3 patients had a CURB65 score of 1, and 6 patients had a score of 0, which are considered low severity and mortality risk.

ART, antiretroviral therapy; CAP, community-acquired pneumonia; IQR, interquartile range.

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laboratory, and sent to the University of Manitoba, Winnipeg for analysis. PFT values were obtained from the patients using a portable spirometer. The procedure was performed following American Thoracic Society (ATS) guidelines. A portable spirometer was used to measure forced vital capacity (FVC), FEV_1, and FEV_1/FVC ratio. FEV_1 is the maximum volume of air that can be forcefully exhaled in 1 s. FVC is the total volume of air that can be forcefully exhaled. The FEV_1/FVC ratio is the portion of a person's vital capacity that is exhaled in first second of expiration (Morris 1976). Each subject was required to perform 3 satisfactory attempts, but not more than 8 consecutive maneuvers. FEV_1% predicted and FVC% predicted were calculated using the actual measured FEV_1 and FVC values and their estimated predicted FEV_1 and predicted FVC values. Predicted FEV_1 and predicted FVC are estimated values from the healthy general population based on gender, age, and height. FEV_1% predicted and FVC% predicted is a method to standardize measured FEV_1 and FVC by comparing it to the estimated values from the general population (Morris 1976). In this study lung dysfunction was defined as a decline in lung volumes beyond reference values following ATS criteria, which was measured in terms of FVC% predicted, FEV_1% predicted, and FEV_1/FVC. Normal value for FEV_1% predicted and FVC% predicted is defined as >0.8, and the normal value for FEV_1/FVC is >0.7. Different lung diseases will have different patterns of FEV_1% predicted, FVC% predicted, and FEV_1/FVC values.

Selecting the panel of biomarkers

Before the experiment, we conducted a systematic review summarizing the current literature regarding inflammation and pulmonary dysfunction in HIV patients. The panel of inflammatory markers selected for this study was guided by the results from the systematic review. The molecules include inflammatory cytokines, regulators of extracellular matrix (ECM) remodeling, and markers of microbial translocation.

Immunoassay

Induced sputum supernatants were separated from cell pellets using centrifugation, and the cell-free supernatants were aliquoted and stored at −80°C until required. In total, 64 samples were used for this study (ie, plasma and induced sputum samples from 2 time points for each of the 16 patients).

Commercially available multiplex and singleplex bead-based fluorescent assays and Enzyme-Linked Immunosorbent Assay (ELISA) Kits were used to quantify the inflammatory proteins of interest. LXSAHM 6 plex and singleplex (R&D Systems, Minneapolis, MN) were used to detect APRIL, BAFF, CCL3, CCL4, CCL7, CD163, MMP-9, TGF-β1, TGF-β2, and sCD14. ELISA was used to detect CICP (Quidel, San Diego, CA) and FABP-2 (R&D Systems). The assays were performed according to manufacturer's instructions, using 50 μL per sample. Standards were reconstituted and serially diluted following the manufacturer's instructions to generate standard curves. Standards included the reconstituted molecules being tested for and were considered as positive controls for the experiment. The assays for plasma samples were run in duplicates. Results were run on a Bio-Plex 200 instrument (Bio-Rad, Mississauga, ON) and reported as mean fluorescence intensity and converted to pg/mL using the Bio-Plex® Manager version 6.0 (Bio-Rad).

Statistical analysis

The data collected were analyzed using IBM SPSS© version 24 and Prism. Statistical analysis was performed on the data collected at the time of admission and 1 month follow-up. The main purpose was to determine whether or not the concentration of the molecules differed between the 2 groups and if they correlated with the clinical outcome, which was pulmonary dysfunction.

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Principal component analysis (PCA) was conducted using the data from the plasma samples of all patients collected at the time of admission to identify which molecules accounted for the greatest variation between the 2 groups. The principal component factor was used to extract each principal component, which was followed by varimax rotation. A molecule was considered to load onto a given component if the loading factor was 0.6 or greater for that given component and less than 0.6 for the other components. We selected for principal components that accounted for at least 10% of the total variance.

Dot plots were generated for the molecules that loaded onto the PCA components that accounted for at least 10% of the total variance. This allowed for a qualitative comparison of the molecules between the 2 groups. The molecules that loaded onto these principal components were included in subsequent analysis. Wilcoxon test ($\alpha = 0.05$) was used to quantitatively evaluate the differences in the concentrations of the molecules between the 2 groups at each time point. This was done to determine if any molecules were statistically different between the 2 groups in this study. TIMP-1 is a natural inhibitor of MMP-9 and binds in a 1:1 manner; therefore, their ratio was also analyzed using Wilcoxon test.

Correlations between the molecules that accounted for the majority of the variation between the 2 groups and PFT measurements were explored using Spearman's rank correlation, to assess the relationship between inflammation and pulmonary dysfunction. The correlation was considered statistically significant if $P \leq 0.05$.

Ingenuity pathway analysis (IPA) was performed to determine if the important molecules in our study were implicated in any canonical pathways or any pathological and physiological processes. Only human genes listed in the Ingenuity knowledge base were included for core analysis.

Results

Sixteen participants were included in this study; 9 participants were in the HIV+CAP group, and 7 were in the HIV group. Table 2 shows the molecule concentrations in plasma for both groups at admission. The majority of the molecules were undetectable in the sputum samples with the exception of MMP-9. In the HIV+CAP group, the median (interquartile range [IQR]) concentration for MMP-9 in induced sputum was 918.3 pg/mL (623.03–1,270.67) at admission and 215.85 pg/mL (133.60–1,169.94) at 1 month follow-up. In contrast, in the HIV group, the median (IQR) concentration for MMP-9 was 16,540.57 pg/mL (6,364.34–21,257.72) at admission and 4,723.77 pg/mL (2,486.75–15,840.27) at 1 month follow-up. The sputum concentration of MMP-9 was significantly higher in the HIV group at admission compared to the HIV+CAP group [95% confidence interval (CI), 1,915.71–11,429.08, $P = 0.016$].

PCA for plasma samples revealed 4 principal components for our data set. Principal component 1 accounted for 31.51% of the total variation, principal component 2 accounted for 23.10%, and principal component 3 accounted for 10.42%. Together, the 3 principal components account for 65.03% of the total variation between the 2 groups, as shown in Table 3. The fourth principal component was not selected because it accounted for less variability in the data.

The distribution of the data for the molecules that loaded onto the first 3 principal components is shown in Fig. 1. At the time of admission CCL3 and TIMP-1 demonstrated a trend toward higher concentrations in the HIV+CAP group compared to the HIV group. The lower quartiles in the HIV+CAP group are greater than the upper quartiles of the HIV group. Conversely, at the 1 month follow-up BAFF demonstrated a trend toward higher concentrations in the HIV+CAP group compared to the HIV group. In the HIV+CAP group the lower

Table 2. Concentration of the Molecules Measured in the Plasma Samples from the HIV+CAP Group ($N = 9$) and HIV Group ($N = 7$) at Hospital Admission

	HIV+CAP group	*HIV group*
	Admission median (IQR)	
APRIL	1,323.93 (972.12–1,677.97)	977.01 (878.82–1,241.89)
CCL3[a]	127.79 (116.95–151.54)	90.90 (90.90–94.70)
CCL7	39.02 (28.66–39.02)	35.30 (34.63–39.60)
BAFF	1,114.86 (475.08–1,931.09)	669.36 (436.29–866.97)
CCL4	133.00 (100.00–178.04)	100.00 (100.00–100.00)
CD163	2.10×10^6 (6.40×10^5–4.83×10^6)	7.21×10^5 (5.44×10^5–1.85×10^6)
sCD14	4.59×10^6 (2.92×10^6–4.84×10^6)	3.55×10^6 (2.26×10^6–4.82×10^6)
MMP-9	8.10×10^4 (3.36×10^4–1.39×10^5)	7.10×10^4 (6.10×10^4–1.94×10^5)
FABP-2	1,830.00 (1,345.00–1,935.00)	2,305.00 (1,480.00–2,447.50)
TGF-β1	3.14×10^4 (2.36×10^4–3.27×10^4)	3.49×10^4 (2.49×10^4–4.95×10^4)
TGF-β2	17.00 (17.00–126.325)	17.00 (17.00–92.05)
CICP	9.41×10^7 (8.04×10^7–1.79×10^8)	1.12×10^8 (1.06×10^8–1.72×10^8)
TIMP-1[a]	1.84×10^5 (1.42×10^5–2.66×10^5)	8.27×10^4 (5.22×10^4–1.08×10^5)
MMP-9/TIMP-1	0.44 (0.11–1.02)	1.59 (0.79–2.99)

Molecule concentration: pg/mL.

The plasma concentration of CCL4 in the HIV group was similar in value between the individual participants. All of the molecules in Table 2 with the exception of MMP-9/TIMP-1 ratio were included in the PCA; the data from all 16 patients were included in the analysis. Subsequently the molecules that loaded onto the first 3 principal components were included in Wilcoxon test and Spearman's correlation; again the data from all 16 participants were included in those analyses.

[a]TIMP-1 and CCL3 were detected at significantly higher plasma concentrations in the HIV+CAP group at the time of admission compared to the HIV group without CAP ($P \leq 0.05$).

PCA, principal component analysis.

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Table 3. Principle Component Analysis Using the Plasma Concentrations of the 13 Molecules Measured in HIV+CAP Patients and HIV Patients at the Time of Hospital Admission

Rotated component matrix

	Component			
Molecules	*1*	*2*	*3*	*4*
CCL4	0.87[a]	–0.14	0.28	0.17
BAFF	0.86	0.03	–0.13	0.23
TIMP-1	0.85	0.34	0.13	–0.14
APRIL	0.82	0.25	0.03	–0.15
CCL3	0.67	–0.43	–0.18	–0.23
FABP-2	0.08	0.82	–0.02	0.24
TGF-β1	0.13	0.67	0.41	0.20
sCD14	0.48	0.62	–0.23	–0.21
TGF-β2	–0.03	0.61	0.18	–0.12
MMP-9	0.10	0.09	0.94	0.00
CICP	–0.15	0.60	0.68	0.06
CCL7	0.14	0.11	0.00	0.87
CD163	0.48	0.03	–0.08	–0.59

Extraction method: PCA.
Rotation method: varimax with Kaiser normalization.
Gray shading indicates the molecules within each component.
A molecule loads onto a principal component if its correlation with the component is >0.6 and <0.6 in every other component.
Principal component 1 consists of CCL4, BAFF, TIMP-1, APRIL, and CCL3 and accounts for 31.5% of the total variation seen in this data set. Principal component 2 consists of FABP-2, TGF-β1, sCD14, and TGF-β2 and accounts for 23.1% of the total variation seen in this data set. Principal component 3 consists of MMP-9 and CICP and accounts for 10.42% of the total variation seen in this data set. Together the 3 components comprise 65.03% of the total variation seen in this data set.
[a]Rotation converged in 6 iterations.

quartile for BAFF was greater than the upper quartile of the HIV group. Subsequently, Wilcoxon test was performed on the molecules shown in Fig. 1 at admission and 1 month follow-up. At admission, the HIV+CAP group had significantly higher concentrations of CCL3 (95% CI, 92.00–131.75, $P = 0.028$) and TIMP-1 (95% CI, 102,010.14–218,914.86, $P = 0.014$) compared to the HIV group. Conversely, at 1 month follow-up the concentration of BAFF (95% CI, 508.39–1,077.56, $P = 0.003$) was found to be significantly higher in the HIV+CAP group compared to the HIV group. This is consistent with observed differences seen in Fig. 1. In addition, the ratio of MMP-9/TIMP-1 was significantly lower in the HIV+CA group compared to the HIV group at 1 month follow-up (95% CI, 0.54–1.44, $P = 0.023$). The remaining molecules did not differ significantly between the 2 groups.

Table 4 shows the PFT values at the time of hospital admission and at 1 month of follow-up. We did not perform statistical analysis comparing PFT indices (FEV_1% predicted, FVC% predicted, and FEV_1/FVC) between the HIV+CAP group and HIV group because we defined abnormal lung function based on ATS criteria; FEV_1% predicted <0.8, FVC% predicted <0.8, and FEV_1/FVC <0.7 are considered abnormal lung function clinically. At admission, the HIV+CAP group demonstrated lower lung function compared to the HIV group. In the HIV+CAP group, FVC% predicted and FEV1% predicted were less than 0.80 indicating clinically abnormal lung function, while FEV_1/FVC ratio was within the reference range for normal. FVC% predicted, FEV1% predicted, and FEV_1/FVC ratio were all normal in the HIV group. The impairment in lung function did not resolve after 1 month of follow-up in the HIV+CAP group. Lung function remained normal in the HIV group after 1 month of follow-up.

Table 5 shows the correlation between the molecules measured in plasma at admission and FEV_1% predicted and FVC% predicted values at 1 month follow-up. As shown in Table 5 the concentrations of APRIL, BAFF, CCL3, and TIMP-1 at admission were found to correlate negatively with PFT values measured at 1 month of follow-up (FEV_1% predicted and FVC% predicted); the correlations were statistically significant. TIMP-1 had the strongest negative correlation with FVC% predicted (rs = –0.689, 95% CI, –0.89 to –0.28, $P = 0.004$). The correlation between BAFF and FVC% was predicted (rs = –0.639, 95% CI, –0.86 to –0.19, $P = 0.009$). ARPIL had the strongest negative correlation with FEV_1% predicted (rs = –0.561, 95% CI, –0.83 to –0.07, $P = 0.026$). The correlation between CCL3 and FEV_1% was predicted (rs = –0.55, 95% CI, –0.83 to –0.06, $P = 0.028$). The correlation between BAFF and FEV_1% was predicted (rs = –0.505, 95% CI, –0.81 to –0.003, $P = 0.048$). The remaining biomarkers demonstrated weak correlations with FEV_1% predicted and FVC% predicted and were not statistically significant.

IPA shows that 7 molecules in our data set are involved in inflammation response (APRIL, BAFF, CCL3, CCL4, MMP-9, TIMP-1, and TGF-β1). Furthermore, 3 of these 7 molecules (BAFF, CCL3, and TIMP-1) were found to be statistically significant between the 2 groups. A network depicting the direct and indirect relationships between the 7 molecules and other molecules involved in those processes is shown in Fig. 2. Based on the Ingenuity Knowledge Base (IKB) output, the shared disease and function component of IPA showed that 6 of our 7 molecules (CCL4, ARPIL, BAFF, MMP-9, TIMP-1, and TGF-β1) are involved in inflammation of various organs ($P < 2.34 \times 10^{-7}$). Five of the 7 molecules are implicated in chemotaxis of lymphocytes, phagocyte transmigration, and migration (CCL3, CCL4, TGF-β2, MMP-9, TIMP-1, and TGF-β1) ($P < 3.32 \times 10^{-6}$–$P < 2.34 \times 10^{-10}$).

Discussion

None of the patients in the HIV+CAP group had a CURB65 score ≥3, which is a clinical indication for high severity and mortality in CAP infection. Despite not having clinically severe CAP infections, the results of this study show that the HIV+CAP group had lower lung function compared to the HIV group. The impairment in lung function following CAP is consistent with prior findings; however, the pattern of pulmonary dysfunction is not in keeping with the obstructive pattern observed by Morris and others (2000). To reduce confounding variables for pulmonary dysfunction, we excluded patients with preexisting lung diseases. Interpretation of the PFT results based on ATS criteria suggests that the patients in the HIV+CAP group could be experiencing a restrictive ventilation defect where FEV_1% predicted and FVC% predicted are less than 0.8, while FEV_1/FVC is >0.7. A study by Aaron and others (1999) demonstrated that combining FVC and FEV_1/FVC measurements can improve the predictive ability of spirometry in identifying restrictive

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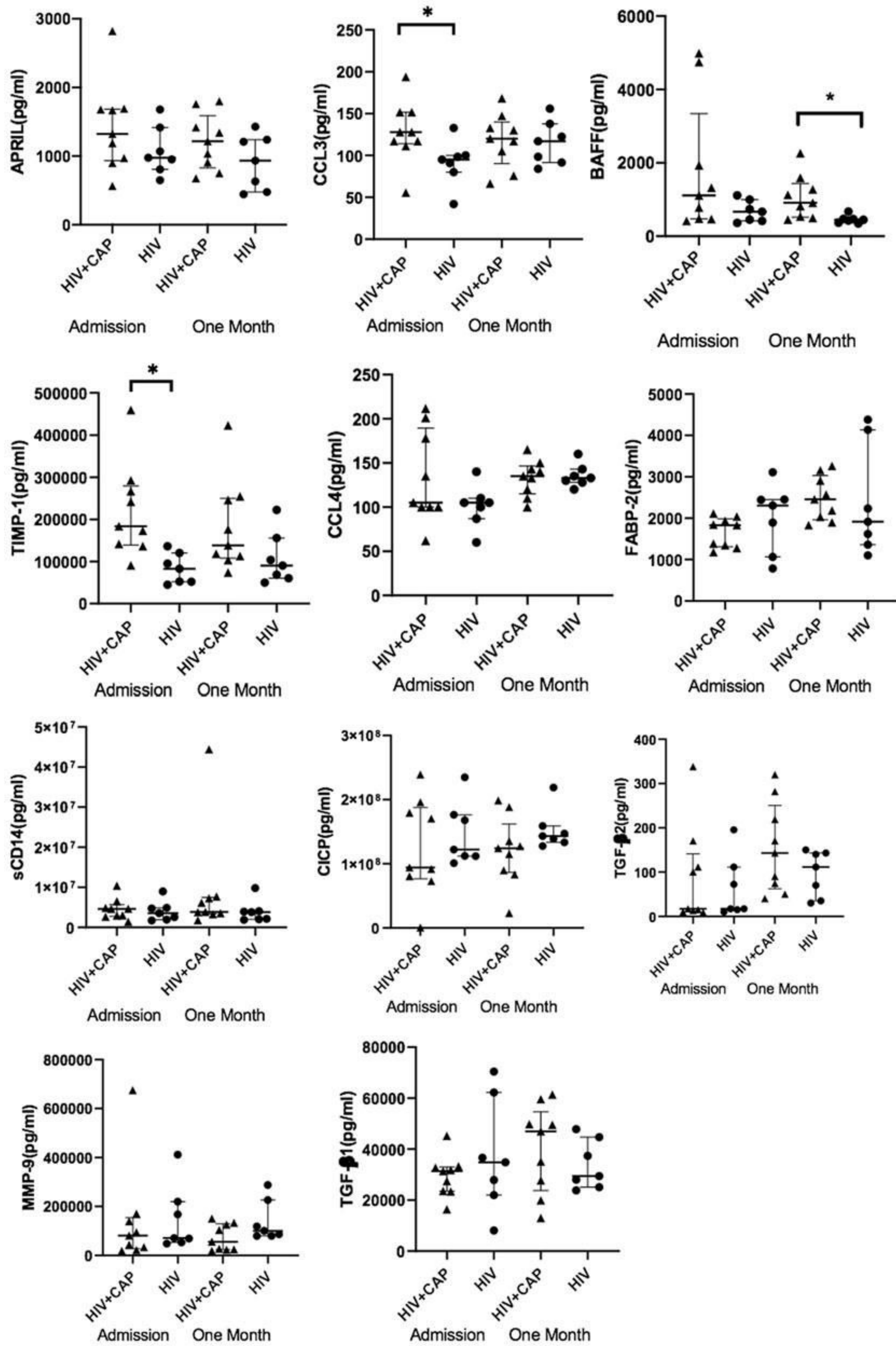

FIG. 1. Plasma concentrations of APRIL, BAFF, CCL3, CCL4, TIMP-1, sCD14, TGF-β1, TGF-β2, FABP-2, CICP, and MMP-9 measured in HIV+CAP patients compared to HIV patients. Immunoassays were performed on plasma samples from all 16 participants included in the study at admission and after 1 month follow-up. The *horizontal axis* indicates the time points when the molecules were measured, and the *vertical axis* indicates the measured plasma concentration of the molecule. Each *symbol* represents a data point from a single individual. ▲ Indicate individual data points from the HIV+CAP group. • Indicate individual data points from the HIV group. The *large horizontal bar* is the median, and the 2 *smaller horizontal bars* represent the first and third quartiles. At the time of admission, the concentrations of CCL3 and TIMP-1 were measured at significantly higher concentrations in the HIV+CAP group compared to the HIV group. At 1 month of follow-up the concentration of BAFF was measured to be significantly elevated compared to HIV group. The concentrations of the remaining molecules were not statistically different between the 2 groups at either time point. *Indicates $P \leq 0.05$. CAP, community-acquired pneumonia.

Table 4. Indices for Pulmonary Function Test Measured Using Portable Spirometry for HIV+CAP Group and HIV Group at the Time of Hospital Admission and at 1 Month Follow-Up

	Groups		*Groups*	
	HIV+CAP group	*HIV group*	*HIV+CAP group*	*HIV group*
	Admission		*1 Month*	
	Median (IQR)			
Measured FEV_1, L	2.09 (1.80–3.37)	3.51 (3.11–4.12)	2.72 (1.74–3.08)	3.47 (3.17–4.08)
Predicted FEV_1, L	3.74 (3.18–4.27)	3.91 (3.11–4.11)	3.77 (3.24–4.27)	3.89 (3.38–4.08)
FEV_1% predicted	0.65 (0.51–0.81)	0.95 (0.91–1.00)	0.66 (0.55–0.85)	0.91 (0.89–1.07)
Measured FVC, L	2.85 (1.83–4.27)	4.21 (4.19–4.98)	2.99 (2.17–3.66)	4.26 (3.73–4.61)
Predicted FVC, L	4.42 (3.81–5.18)	4.59 (4.03–4.83)	4.51 (3.76–5.18)	4.57 (4.03–4.83)
FVC% predicted	0.74 (0.49–0.78)	0.96 (0.91–0.98)	0.64 (0.53–0.84)	0.93 (0.87–1.04)
Measured FEV_1/FVC	0.87 (0.79–0.89)	0.85 (0.83–0.87)	0.85 (0.84–0.91)	0.86 (0.85–0.88)
Predicted FEV_1/FVC	0.83 (0.82–0.840)	0.85 (0.84–0.86)	0.83 (0.82–0.85)	0.85 (0.84–0.85)

HIV+CAP group was found to have abnormal FEV_1% predicted and FVC% predicted compared to the HIV group at the time of admission and 1 month follow-up. Six out of 9 patients in the HIV+CAP group had persistent abnormal FEV_1% predicted and FVC% predicted values. All of the HIV patients without CAP had normal FEV_1% predicted and FVC% predicted values. FEV_1% predicted and FVC% predicted <0.8 are considered abnormal based on ATS criteria. The measured FEV_1/FVC ratio was normal for both groups; FEV_1/FVC ratio >0.7 is considered normal based on ATS criteria.

Data from all 16 patients were included for each pulmonary function test parameter.

ATS, American Thoracic Society; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity.

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ventilation defects if FEV_1/FVC ratio is greater than 0.80. The median (IQR) FEV_1/FVC in the HIV+CAP group was 0.87 (0.79–0.89). However, total lung capacity (TLC) and diffusing capacity of the lung for carbon monoxide (DLCO) will need to be measured to confirm a restrictive ventilation defect.

Table 5. Correlations Between the Concentration of the Molecules Measured at Admission with Pulmonary Function Test Indices (FEV_1% Predicted and FVC% Predicted) at 1 Month Follow-up, Using Data From all Participants

Molecules measured in plasma	*FEV_1% predicted* Correlation coefficient (rs_1)	P value	*FVC% predicted* Correlation coefficient (rs_2)	P value
CCL3	–0.554[a]	0.028	–0.475	0.065
APRIL	–0.561[a]	0.026	–0.495	0.053
BAFF	–0.505[a]	0.048	–0.639[a]	0.009
CCL4	–0.476	0.063	–0.411	0.065
CD14	–0.138	0.609	–0.062	0.822
MMP-9	0.262	0.326	0.394	0.132
FABP-2	0.188	0.484	0.327	0.217
TGF-β1	0.063	0.818	0.232	0.385
CICP	–0.024	0.935	0.135	0.617
TIMP-1	–0.499	0.051	–0.689[a]	0.004
TGF-β2	0.063	0.818	0.00	>0.999

The molecules that loaded onto the first 3 principal components were included in the analysis.

rs_1: The correlation coefficient between the concentration of the molecules measured at the time of admission in both groups and their FEV_1% predicted values at 1 month follow-up.

rs_2: The correlation coefficient between the concentration of the molecules measured at the time of admission in both groups and their FVC% predicted values at 1 month follow-up.

CCL3, APRIL, and BAFF had significant negative correlations with FEV_1% predicted at 1 month follow-up ($P \leq 0.05$), while TIMP-1 and BAFF had significant negative correlations with FVC% predicted at 1 month follow-up ($P \leq 0.05$).

[a]Correlation is significant at the $P < 0.05$ level (2-tailed).

$CD4^+$ T cell count is a potential contributing factor for the different patterns of pulmonary dysfunction, observed in the above studies. The HIV patients infected with CAP in the study by Morris and others (2000) had a mean (range) $CD4^+$ T cell count of 337 (8–1,046). Similarly, in our study the mean (range) $CD4^+$ T cell count was 185(9–378) in the HIV+CAP group. Furthermore, in HIV-infected individuals, lower $CD4^+$ T cell count has been found to be associated with worse immune dysfunction. A study by Drummond and others (2015) demonstrated that higher viral loads and lower $CD4^+$ count levels are associated with greater degrees of lung function decline over 1 year in HIV patients.

It is also possible that infection by different respiratory pathogens is a contributing factor for the different patterns of lung injury observed. In the study by Morris and others (2000), the patients were infected with *Pneumocystis* and bacterial pneumonia. For our study the majority of the patients in the HIV+CAP group were infected with *Mycobacterium*. Keynan and others (2015) demonstrated that distinct inflammatory patterns were associated with the different respiratory pathogens causing pneumonia in HIV infected individuals. It is possible that different patterns of inflammation lead to different types of lung injury.

The majority of the molecules we tested failed to show a correlation with PFT values at 1 month follow-up with the exception of ARPIL, BAFF, CCL3, and TIMP-1. This constellation of molecules demonstrated a negative correlation with FEV_1% predicted and FVC% predicted, which was shown to be significant; however, the relationships were moderate in strength. Due to the limited sample size in our study, further testing with larger cohorts should be done to evaluate the ability of these molecules to serve as biomarkers for pulmonary dysfunction in HIV patients with CAP.

Along with lung function we were also interested in the inflammatory response of our population. Prior studies have shown that HIV patients have chronic inflammation despite

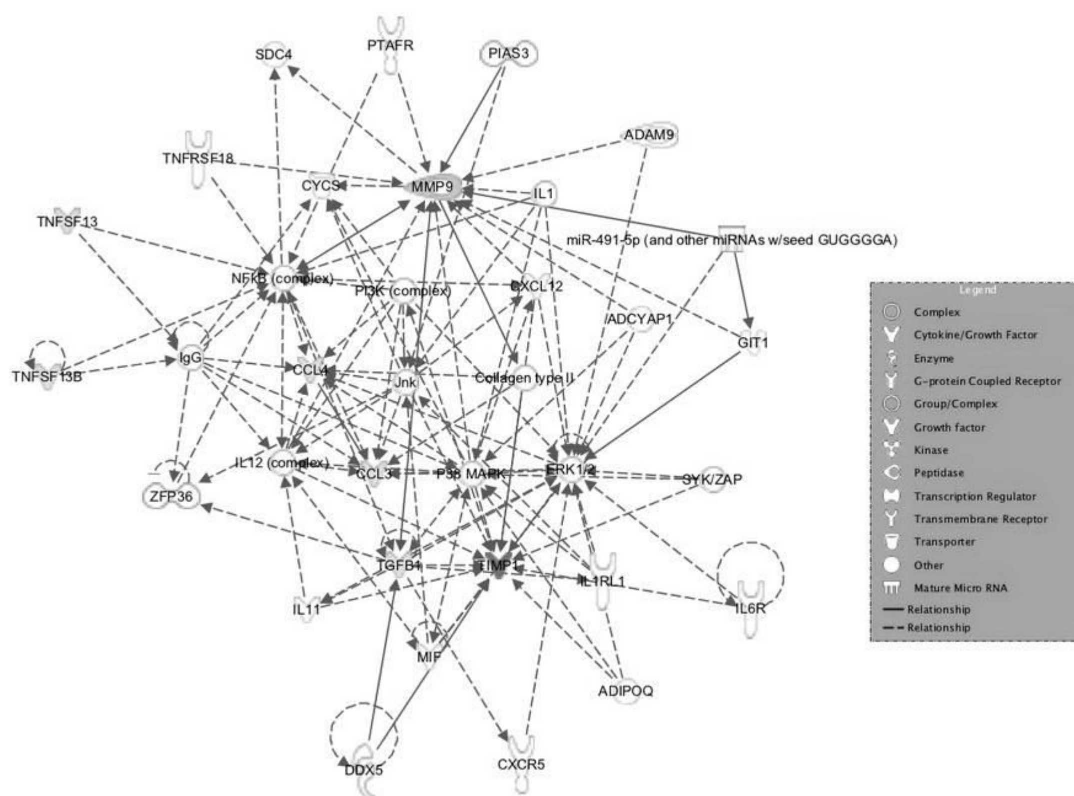

FIG. 2. Ingenuity pathway analysis generated network incorporating the molecules that loaded onto the first 3 principal components of the principal component analysis. Molecules with color were investigated in this study. The figure depicts the interaction between APRIL, BAFF, CCL3, CCL4, MMP-9, TIMP-1, and TGF-β1 in inflammation response in humans.

the use of cART, suggesting an inability to control the inflammatory response in these patients (Wada and others 2015; Richert and others 2017). Studies to date have shown that BAFF and TIMP-1 are elevated in HIV patients (Richert and others 2017; Xing and others 2017). This study goes further to suggest that BAFF, TIMP-1, and CCL3 are elevated even more so in HIV patients infected with CAP. We speculate that these 3 molecules may have downstream effects on lung function in HIV patients with CAP.

IPA showed that 7 of the molecules (APRIL, BAFF, CCL3, CCL4, MMP-9, TIMP-1, and TGF-β1) are involved in a common network for inflammation response in humans. These 7 molecules include inflammatory cytokines and regulators of ECM remodeling. They are involved in specific cellular processes important to inflammation response, such as chemotaxis of lymphocytes and phagocytes, as well as extravasation of phagocytes. This finding is of particular interest; as mentioned earlier our study shows that BAFF, CCL3, and TIMP-1 are significantly elevated in the HIV+CAP group compared to the HIV group. It is possible that abnormal expression of these 3 molecules dysregulated the inflammation response seen in HIV patients infected with CAP by disrupting these cellular processes, which in turn activates a pathway that leads to pulmonary dysfunction.

Several studies have tried to evaluate the involvement of BAFF, CCL3, and TIMP-1 in pathophysiology of various lung diseases. It has been well documented that unbalanced expression of TIMP-1 can lead to excessive collagen deposition over time (Arpino and others 2015). CCL3 is a member of the CC chemokine family (Baba and Mukaida 2014). Yang and others (2011) investigated the role of CCL3 pulmonary fibrosis following lung injury. It was shown that $Ccr1^{-/-}$ mice and $CCL3^{-/-}$ mice had less fibrosis and lower mortality compared to wild-type mice following lung injury. A similar study was conducted by François and others (2015) to evaluate the role of BAFF in pulmonary fibrosis. BAFF is a member of the tumor necrosis factor superfamily 13 (Mackay and Browning 2002). François and others (2015) showed that after lung injury $BAFF^{-/-}$ mice had less tissue damage and fibrosis compared to the wild-type mice. It would be interesting to see if these 3 molecules contribute to a similar profibrotic process in HIV patients following CAP infection.

There are several limitations to our study. The sample size may have been underpowered to detect a significant difference between the 2 groups for some of the molecules we tested. The small sample size also limits the generalizability of our results to larger populations. Other limitations include the duration of follow-up and the lack of pre and post bronchodilator testing because salbutamol was not used during spirometry. A 1 month follow-up period may not be sufficient to determine if the decrements in lung function are permanent. In addition, despite showing significant increases in

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BAFF, CCL3, and TIMP-1 in HIV patients following CAP infection, we did not measure its effect on immune cell function; therefore, subsequent testing is required to determine if BAFF, CCL3, and TIMP-1 are involved in the pathophysiologic process causing pulmonary dysfunction in HIV patients infected with CAP.

However, this is an ongoing study; plasma, sputum, and PFT measurements will also be collected at 4 months, 6 months, and 1 year follow-up. The next step involves quantifying the molecules from the new patient samples at each time point and correlating this information with PFT values from new time points. This will allow us to determine if we can replicate the results of this study with a larger cohort. The longer follow-up period would allow us to observe the evolvement of the host's inflammatory pattern and lung function. It is also necessary to perform full PFT measurements TLC and DLCO to confirm the restrictive ventilation defect in the HIV+CAP group.

Conclusion

In summary, we found that HIV patients infected with CAP have a different inflammatory pattern compared to HIV patients without CAP coinfection and have lower lung function as demonstrated by FEV_1% predicted and FVC% predicted values that were below the cutoff for normal after 1 month follow-up. We believe that the inflammatory process in HIV patients infected with CAP plays a key role in facilitating pulmonary dysfunction in this population. BAFF, CCL3, and TIMP-1 were significantly elevated in HIV patients infected with CAP; further investigation is required to evaluate their role in pulmonary dysfunction.

Author Disclosure Statement

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