Title

Neutrophil extracellular trap formation linked to idiopathic pulmonary fibrosis severity and survival

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Abstract

Rationale: Neutrophil counts in bronchoalveolar lavage fluid in idiopathic pulmonary fibrosis are associated with worse outcomes; however, the underlying mechanisms are unknown. Neutrophil extracellular trap formation is associated with worse outcomes in several chronic lung diseases however, there is an unknown role in idiopathic pulmonary fibrosis.

Objectives: To determine the relationship between neutrophil extracellular trap formation in the lungs of idiopathic pulmonary fibrosis patients and clinical outcomes.

Methods: In a discovery cohort of 156 patients with idiopathic pulmonary fibrosis, we measured neutrophil extracellular trap markers in bronchoalveolar lavage fluid, including extracellular DNA, DNA-myeloperoxidase complexes, calprotectin, and neutrophil elastase. We assessed the correlation of these markers with baseline pulmonary function and survival. In a subset of 50 patients, label-free quantitative proteomics was performed to identify the source of extracellular DNA. A validation cohort of 52 patients was similarly assessed.

Measurements and Main Results: Neutrophil extracellular trap markers in the discovery cohort were significantly correlated with worse pulmonary function ($p<0.03$). Higher levels of these markers predicted worse survival after adjusting for gender, age, and baseline physiologic severity (hazard ratio range: 1.79–2.19). Proteomics revealed a significant correlation between extracellular DNA levels and proteins related to neutrophil extracellular trap formation. The validation cohort showed that extracellular DNA levels were associated with reduced pulmonary function ($p=0.04$) and trend towards worse survival (hazard ratio=2.16).

Conclusions: Neutrophil extracellular trap formation markers were associated with disease severity and worse survival in idiopathic pulmonary fibrosis. These findings suggest neutrophil extracellular trap formation contributes to lung injury and decreased survival and may represent a potential therapeutic target.

[Abstract Word Count: 250 (minus headings)]

Background

Idiopathic pulmonary fibrosis (IPF) is a devastating and universally progressive condition with survival of 3 to 5 years from the time of diagnosis [1]. Current treatment options with antifibrotic therapy have been shown to slow lung function decline compared to placebo but fail to improve survival [2, 3]. Given the limitations of currently available therapies and the grim prognosis of IPF, the discovery of novel mechanisms of lung injury and targetable endotypes of IPF could offer new therapeutic targets.

Previous data have highlighted the role of bronchoalveolar lavage fluid (BALF) neutrophilia and serum neutrophil-to-lymphocyte ratio in prognostication for IPF patients [4, 5]. However, the mechanism by which the presence of neutrophils results in worse outcomes in IPF remains unclear. One possible link between increased neutrophils and worse lung injury in IPF patients is neutrophil extracellular trap formation (NETosis) [6]. NETosis occurs when neutrophils expel their nuclear DNA in complex with granule-derived proteins, which can contribute to the damage of surrounding tissues (Figure 1). These NET scaffolds play a direct role in innate immune functions to clear pathogenic debris but are also implicated in lung injury where exaggerated immunologic responses to chronic and acute inflammation is associated with worse prognosis, such as in asthma, bronchiectasis, and acute lung injury [6-8]. NETs also induce key pathways of pulmonary fibrosis [9-12], and NET inhibition in animal models can resolve lung fibrosis [9, 13]. Given the known association of BAL neutrophil counts and worse IPF survival and the role of NETosis in acute and chronic lung injuries, we hypothesize that increased levels of NET remnants in the lung of patients with IPF are associated with worse clinical outcomes.

Methods

Study participants - Discovery cohort. We utilized BAL fluid samples from the interstitial lung disease (ILD) biorepository at National Jewish Health (NJH) that included patients with ILD who were prospectively enrolled into a Specialized Center of Research Study at NJH. A subset of these patients have been previously reported on [4]. We identified 156 patients with adequate BALF volume available who met the current IPF diagnostic criteria [14], which was confirmed by contemporary chart review (JJS, MKD). All BALF samples were collected between 1986 to 2009. Clinical and demographic information was extracted from the medical record to include demographics, medications, and pulmonary function testing (PFT) results at the time of BALF collection (+/- 90 days). Date of death was confirmed with a query of the United States Center for Disease Control's National Death Index (CDC NDI).

Study participants - Validation cohort. The validation cohort consisted of previously obtained BALF samples from two independent cohorts of IPF patients: the University of California San Francisco (UCSF) ILD biorepository and the Weighing Risks and Benefits of Laparoscopic Anti-Reflux Surgery in Patients with IPF (WRAP-IPF) cohort [15]. We identified 27 IPF patients from the UCSF cohort and 25 patients from the WRAP-IPF cohort who had adequate BALF volume available. UCSF samples were collected between 2013-2018 and WRAP-IPF samples were collected between 2014-2016. Clinical and PFT data were collected as part of the research study. Date of death up to five years after enrollment for the UCSF cohort was confirmed using the CDC NDI. Survival data was only available for the 52-weeks following BALF collection in the WRAP-IPF cohort based on this study's design. In the validation cohort, 27/52 (52%) had assessment of survival or death at 5 years. Mean length of follow-up for survival assessment of these 27 individuals was 3.5 years.

BALF collection. BALF was collected using saline lavage. Cell count and differential were determined using an unspun BALF aliquot in the discovery cohort. BALF cell-free supernatant was obtained following centrifugation and stored at -80^{0}C until analysis.

PFTs. Pulmonary function testing was performed under normal conditions in routine clinical care, for prospective research purposes, or in the case of WRAP-IPF subjects, prior to surgical procedure. Percent predicted forced vital capacity (FVC%) and percent predicted diffusing capacity for carbon monoxide (DLCO%) at the time of BALF collection (+/- 3 months) were used to determine baseline disease severity.

NET remnant testing. All BALF cell-free supernatant from the discovery cohort was tested for levels of NET remnants using multiple complementary but distinct assays, including an immunofluorescence assay for extracellular DNA (exDNA; Invitrogen Quanti-iT^{TM} PicoGreen® dsDNA assay), ELISA for calprotectin (Werfen), ELISA for NE (Abcam), and research-based sandwich ELISA for MPO-DNA. Given limitations of BALF volume, only exDNA was tested for BALF samples in the validation cohort. For the commercially available assays, exDNA, calprotectin and NE, manufacturers protocols for testing were followed. For MPO-DNA, previously published protocols were used [16]. Briefly, a high-binding EIA/RIA 96-well plate (Costar) was coated overnight at 4ºC with anti-human MPO antibody (Bio-Rad0400-0002) in coating buffer from Cell Death Detection ELISA kit (Roche). The plate was then washed three times with 0.05% Tween 20 in PBS and blocked with 1% BSA in PBS for one hour at room temperature (RT). Following three more washes, BALF samples were diluted 1:2 in the blocking buffer and incubated for one hour at RT. The plate was then washed five times, followed by incubation with anti-DNA antibody for one hour at RT (HRP-conjugated, Cell Death kit, diluted 1:100 in blocking buffer). After 5 more washes, the plate was developed with 3,3',5,5'-TMB substrate (Invitrogen) followed by a 2N sulfuric acid stop solution. Absorbance was measured at

a wavelength of 450 nm. A background control well was run for each sample that included the BALF sample without including the primary anti-MPO antibody and background absorbance level was subtracted from each sample to account for any non-specific background binding of the sample.

BALF label-free quantitative proteomics.

Fifty patients from the discovery cohort who had adequate BALF were selected for proteomics testing (Supplemental Table 1). BALF proteins were then isolated using acetonitrile precipitation as described in the Supplemental Methods section. In brief, BALF samples were thawed and centrifuged to remove cellular remnants and particulates. A 100uL aliquot of supernatant was reduced, alkylated, and proteins then precipitated with 3x volume ice cold acetonitrile (ACN) overnight at -20 ^{o}C. The protein was pelleted and digested overnight with trypsin and analyzed by nanoLC-MS/MS using a Vanquish Neo nano-UPLC interfaced directly to the Orbitrap Ascend Tribrid mass spectrometer (Thermo Fisher) equipped with a FAIMS source. Spectra were searched with Proteome Discoverer 3.0 against the human database downloaded from Uniprot on May 5, 2023 and a database of 155 common contaminants.

Statistical Analysis. Correlations between log transformed levels of NET remnants (exDNA, DNA-MPO, calprotectin, and NE), BALF% neutrophils, FVC% predicted, and DLCO% predicted were calculated using Pearson's correlation coefficient. Linear regression models were used to compare FVC% and DLCO% with log transformed BALF exDNA, MPO-DNA, calprotectin, and NE levels adjusted for age and sex. Cox proportional-hazards (PH) models were used to assess the association between survival time (up to 5-years) and log transformed BALF exDNA, MPO-DNA, calprotectin and NE levels. For the discovery cohort, multivariate Cox PH models were used to determine associations with death adjusting for GAP score [17] and use of

immunosuppressing medications (i.e. corticosteroids, mycophenolate mofetil, azathioprine, cyclophosphamide). Notably, no patients in the discovery cohort were taking anti-fibrotic medications as all samples were collected prior to 2014 when antifibrotic medications were FDA-approved. Given the small sample size, multivariate Cox PH models were not applied to the validation cohort. Using the maximal log rank statistic for a continuous variable (surv_cutpoint() function in R) to determine the optimal point for discriminating between two groups, we dichotomized each cohort into high and low BALF NET remnant groups. In this cut point analysis, we determined optimal levels for discriminating survival associated with each BALF NET remnant. Any patient with a BALF NET remnant level greater than the optimal cut point was categorized into the high group, and those with a level less than the cut point were categorized into the low group. Kaplan-Meier curves were used to visualize the survival function for high and low NET remnant groups. In all models, a p-value <0.05 was considered statistically significant.

For the subset of subjects who underwent proteomics analysis, protein identification and abundance were interpreted in Proteome Discoverer 3.0 [18]. Source exDNA pathways were categorized as apoptosis, necrosis, active secretion via exosomal regulation, or NETosis [19]. Gene sets associated with these pathways were searched on GSEA-MSigDB (Supplemental table 2) and compared with proteins detected in our samples. Proteins detected in less than 30% of samples were excluded from analysis. Expression heatmaps of identified proteins were created based on Euclidian distance. Pearson correlations between the normalized abundance of each protein and levels of exDNA (natural log transformed) were calculated in addition to p-value and false-discovery rate.

All analysis were performed in R version 4.1.3.

Results

Patient characteristics

In the discovery cohort (n=156), the mean age was 66.1 years, and the majority of patients were male (64.1%) ever smokers (66.7%) (Table 1). The mean (SD) baseline % predicted FVC was 66.3 (17.5) and % predicted DLCO was 50.8 (16.0). Within the 5 years following BALF collection, 69.9% of participants had died. Twenty-nine percent of the cohort was taking immunosuppressing medications included prednisone, azathioprine, or mycophenolate mofetil at the time of BALF collection.

IPF patients in the validation cohort (n=52) were older (mean age 68.7 years) and more often male (82.7%) compared to the discovery cohort (Table 1). Similar to the discovery cohort, the majority of patients were ever smokers (61.5%). Mean baseline FVC% was 73.2 (16.6)] with mean baseline DLCO% predicted of 42.8 (11.9). With a mean follow-up of 2.4 years for assessment of survival, (13/52) 25% of patients in the validation cohort had died. At the time of BALF collection, 42% of the validation cohort were taking anti-fibrotic medications, and 0% were taking immunosuppressing medications.

Correlation between BALF NET remnant levels and BALF neutrophils

There was a strong positive correlation among the four different measures of BALF NET remnants (Figure 2). The percentage of BALF neutrophils significantly correlated with the levels of each BALF NET remnant (exDNA: $r=0.49$, $p<0.01$; MPO-DNA: $r=0.34$, $p<0.01$; calprotectin: $r=0.51$, $p<0.01$; NE: $r=0.46$, $p<0.01$) (Figure 2).

BALF NET remnant levels correlate with lung disease severity

In the discovery cohort, there was a significant negative correlation between lower FVC% predicted and higher BALF NET remnant levels [exDNA: r=(-)0.34, p<0.01; MPO-DNA: r=(-)0.19, p=0.03; calprotectin: r=(-)0.30, p<0.01; NE: r=(-)0.22, p<0.01] (Figure 2, Supplemental Figure 1). Similarly, there was a negative correlation between lower DLCO% predicted and higher BALF NET remnant levels (exDNA: r=(-)0.26, p<0.01; MPO-DNA: r=(-)0.23, p<0.01; calprotectin: r=(-)0.29, p<0.01; NE: r=(-)0.22, p<0.01) (Figure 2). Using linear regression models, this inverse relationship was significant for each NET remnant upon adjustment for age and sex for FVC% predicted (exDNA: p<0.01; MPO-DNA: p=0.02; calprotectin: p<0.01; NE: p<0.01) and DLCO% predicted (exDNA: p<0.01; MPO-DNA: p=0.01; calprotectin: p<0.01; NE: p=0.01).

Given that the strength of the correlation between exDNA and disease severity was the strongest amongst NET remnants, BALF exDNA was quantified in the validation cohort and correlated with disease severity. As in the discovery cohort, there was a significant relationship between lower FVC% predicted and lower DLCO% predicted and higher BALF exDNA [r=(-)0.29, p=0.04 and r=(-)0.30), p=0.04, respectively]. In linear regression models adjusted for age and sex, this inverse relationship was maintained, although no longer statistically significant (FVC%: p=0.07, DLCO%: p=0.08).

BALF NET remnants are associated with worse survival

In unadjusted models, higher levels of each of the four BALF NET remnants was associated with worse 5-year survival in the discovery cohort (exDNA: HR=1.32, 95% CI 1.15–1.53, p<0.01; DNA-MPO: HR=1.19, 95% CI 1.05–1.36, p<0.01; calprotectin: HR=1.27, 95% CI

1.07–1.51, p<0.01; NE: HR=1.17, 95% CI 1.04–1.31, p=0.01). In models adjusted for GAP score and use of immunosuppression, exDNA, DNA-MPO and calprotectin remained significantly associated with worse survival (exDNA: HR=1.30, 95% CI 1.09–1.55, p<0.01; DNA-MPO: HR=1.18, 95% CI 1.02–1.38, p<0.05; calprotectin: HR=1.23, 95% CI 1.00–1.51, p<0.05;).

For each NET remnant test, IPF patients were stratified into groups based on high vs. low BALF NET levels. In the discovery cohort, for all four measures of NET remnants, the high BALF NET group had significantly worse survival compared to IPF patients with low BALF NET remnant levels [exDNA: HR=2.57, 95% CI 1.76–3.76, p<0.01; DNA-MPO: HR=2.15, 95% CI 1.43–3.24, p<0.01; calprotectin: HR=2.00, 95% CI 1.31–3.05, p<0.01; NE: HR=2.06, 95% CI 1.41–3.01, p<0.01] (Figure 3). Each of these associations remained significant after adjusting for GAP score and use of immunosuppression [exDNA: HR=2.19, 95% CI 1.33-3.60, p< 0.01; DNA-MPO: HR=1.98, 95% CI 1.20–3.26, p<0.01; calprotectin: HR=1.79, 95% CI 1.05–3.03, p=0.03; NE: HR=1.82, 95% CI 1.12-2.94, p=0.01]. Despite the limitations of sample size and length of follow up for determination of death, when IPF patients in the validation cohort were stratified into groups by high and low BALF exDNA levels, there was a trend towards worse survival in the high BALF exDNA group (HR=2.16, 95% CI 0.7–6.67, p=0.17) (Supplemental figure 2).

Untargeted proteomics analysis to evaluate for source of exDNA in BALF

To investigate the source of exDNA in BALF, we performed label-free, quantitative proteomics in a subset of the discovery cohort (n=50, Supplemental Table 3.) and queried the resultant protein library for proteins in pathways associated with the generation of exDNA. Pathways included necrosis, apoptosis, exosome regulation, and NETosis. We identified 9 proteins associated with these pathways at adequate abundance across the 50 samples from

our BALF proteomics library (Table 2). The expression levels of these 9 proteins in each IPF patient in relation to BALF exDNA were displayed in the heatmap in Figure 4. When evaluating the correlation between BALF exDNA levels and these 9 BALF protein abundances, only the three NETosis associated proteins significantly correlated with BALF exDNA levels (Table 2). There was no significant correlation between BALF exDNA levels and the proteins associated with non-NET sources of exDNA.

Discussion

In this study we found evidence of NET remnants in the lung of patients with IPF. Importantly, we found that higher BALF NET remnant levels were associated with more severe lung disease as measured by baseline FVC% predicted, DLCO% predicted and worse 5-year survival. Additionally, we confirmed the presence of NET remnants in the lungs of patients from a second IPF cohort and found a similar association between NET remnants and disease severity further supporting the strength and rigor of our findings. While NETosis has been shown to have important associations with lung injury, disease severity and survival in other pulmonary conditions [6-8], this is the first evidence that NETs may be a contributor to IPF survival and disease severity.

There are several interesting links between NETosis and known risk factors for IPF. Tobacco smoke and *in vitro* neutrophil stimulation by purified MUC5B induces neutrophils to undergo NETosis, and smoking and genetic variants associated with increased MUC5B production are known risk factors for IPF [20-24]. Additionally, in mouse models, inhibition of NETosis reduces bleomycin-induced lung injury [25].

Of the four NET remnant measures included in this study, exDNA had the strongest associations with our clinical outcomes of lung disease severity and survival. While NETs are

only one possible source of exDNA in the lung, our agnostic proteomics analysis was able to distinguish the source of BALF exDNA as being from NETosis rather than other non-NETosis sources of exDNA, such as apoptosis, exosome regulation, or necrosis. Studies to date have demonstrated that some NET associated proteins can induce lung fibroblast proliferation and myofibroblast differentiation[9, 10]. Several NET proteins have also been found to be cytotoxic to alveolar epithelial cells[12]. Future studies should explore the direct effects of different NET protein cargo, including NET-derived exDNA, on lung injury in IPF.

The data from our study also offer a possible explanation for the previous immunologic phenomena observed in IPF patients. For instance, previous associations between elevated BALF neutrophil count and poor prognosis in IPF may well be mediated by those neutrophils undergoing NET formation. There are also increased circulating autoantibodies demonstrated in patients with IPF [26-29]. NETosis is associated with autoantigen exposure and the development of autoantibodies associated with diseases including rheumatoid arthritis, scleroderma, anti-neutrophil cytoplasmic antibody vasculitis, and inflammatory myositis syndromes [16, 30-34]. Given the role of NETs in autoantibody development, these observations suggest that NETosis may be an important entity to better understand the complex relationship between IPF and fibrotic forms of autoimmune ILDs [35-39].

There are important considerations to interpreting these data that require future prospective study. For instance, our discovery cohort was large and had robust outcome data, however these samples were collected in a previous era of diagnosis and treatment and therefore the role of immunosuppression should be considered in survival and NETosis observations. Additionally, because BALF is a rarely obtained fluid for clinical or research purposes in IPF, there were few sources of validation globally and our validation cohort was limited as a result. For example, the validation cohort was small, which limited our power to adjust for multiple confounders in regression models. Also, follow-up data for survival in our validation cohort was too short to robustly determine associations with survival.

It is important to highlight that these data find an association between BALF levels of NET remnants and clinical outcomes; however, it remains unclear if NETosis and associated exDNA is directly pathogenic in IPF or if it represents a marker of upstream lung injury. Also, the nature of BALF collection methods and variance in era of collection limits conclusions about specific thresholds for clinical cut-offs in these data. Prospective study with uniform collection methods will be required to understand a clinically important threshold that can be validated to develop a NETosis 'biomarker' that could be used for assessment of prognosis and potentially inform treatment decisions in patients with IPF. Future study should also explore peripheral, circulating markers of NETosis given the lack of widespread bronchoscopy in IPF clinical practice. In addition, it will be important to identify the etiology for the increased lung levels of NET remnants in some patients with IPF. For example, are their neutrophils more prone to NETosis or did they encounter more factors that induce NET formation?

In conclusion, NETosis is a relatively under-explored possible mechanism of lung injury in IPF, and our data find associations between lung NETosis markers and worse clinical outcomes. Future work is needed to understand if this represents a novel target for IPF drug discovery or a pathogenic mechanism of lung injury for some IPF patients.

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Figure 1.

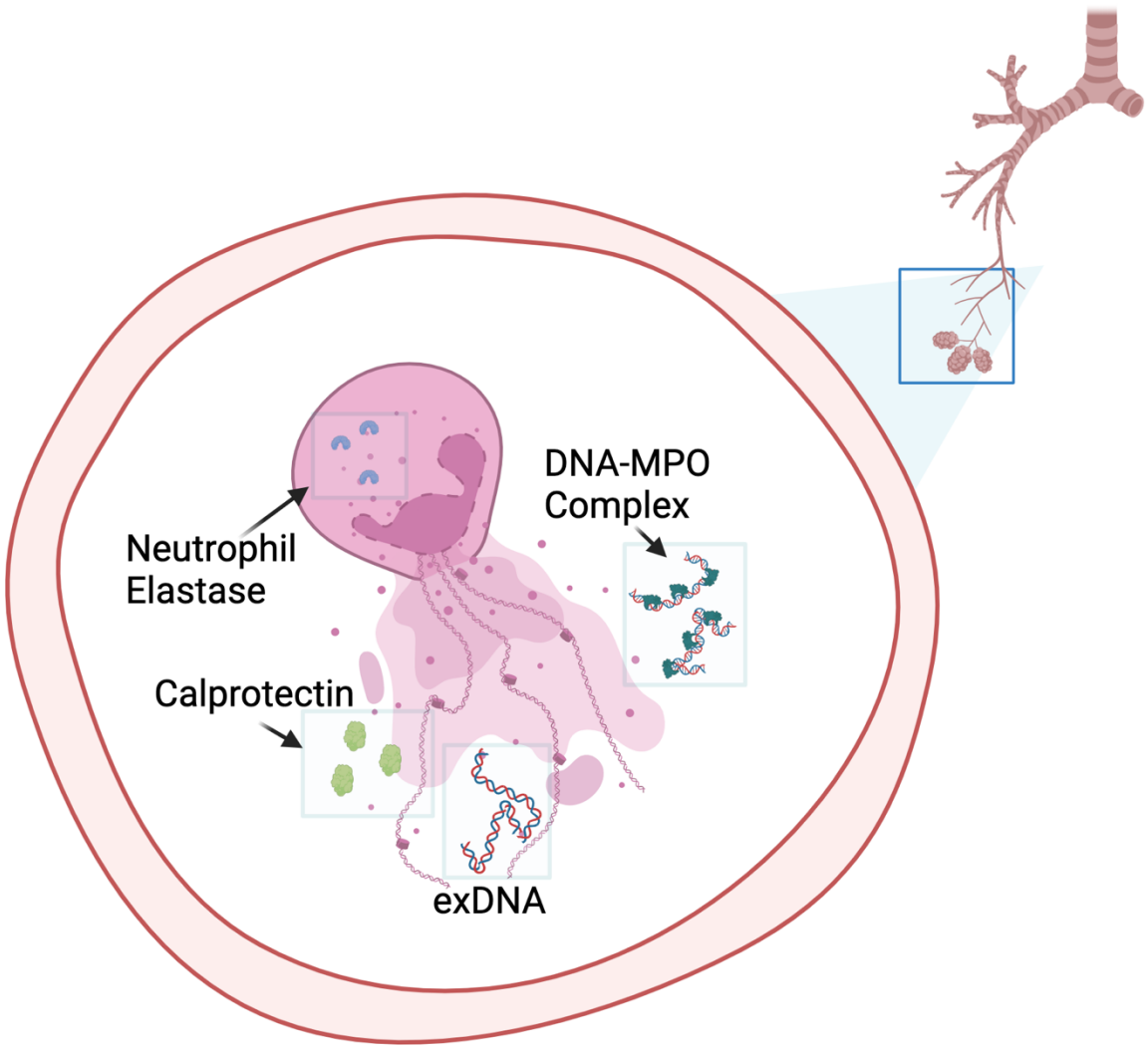

Figure 1. . Neutrophils in the lungs after undergoing stimulation to release their intracellular protein cargo via neutrophil extracellular trap formation (NETosis). This material includes extracellular DNA (exDNA), calprotectin and DNA-myeloperoxidase (DNA-MPO) complexes. NETosis is mediated by the activity of neutrophil elastase, which is also detectable in the extracellular environment. The presence of exDNA has been associated with immunologic and inflammatory reactions that likely result in direct lung and airway injury.

Table 1.

	Discovery cohort (n=156)	***Validation cohort (n=52)***	***P-value***
Age, mean (SD)	66.1 (7.6)	68.7 (5.7)	0.01
Sex, n (% male)	100 (64.1%)	43 (82.7%)	0.02
Smoker, n (% ever)	104 (66.7%)	32 (61.5%)	0.9
FVC% predicted, mean (SD)	66.3 (17.5)	73.2 (16.6)	0.01
DLCO% predicted, mean (SD)	50.8 (15.9)	42.8 (11.9)	<0.01
On immunosuppression, n (%)	45 (28.9%)	0	-
On antifibrotic, n (%)	0	22 (42.3%)	-
Survived up to 5-years, %	30.1%	*17.3%	0.10
Time to death in days, mean (SD)	1326.9 (1403.6)	1269.0 (994.8)	0.8
Time to death in years, mean (SD)	3.6 (3.8)	3.5 (2.7)	0.8
BAL % Neutrophil, mean (SD)	8.8 (9.6)	unknown	-
GAP score, mean (SD)	3.1 (1.1)	4.4 (1.2)	<0.01

Table 1. SD: standard deviation, FVC: forced vital capacity, DLCO: diffusion capacity for carbon monoxide, BAL: bronchoalveolar lavage, GAP: gender, age and physiology score. * Survival data only available for the 52-week period of observation for those patients in the validation dataset whose samples were derived from WRAP-IPF.

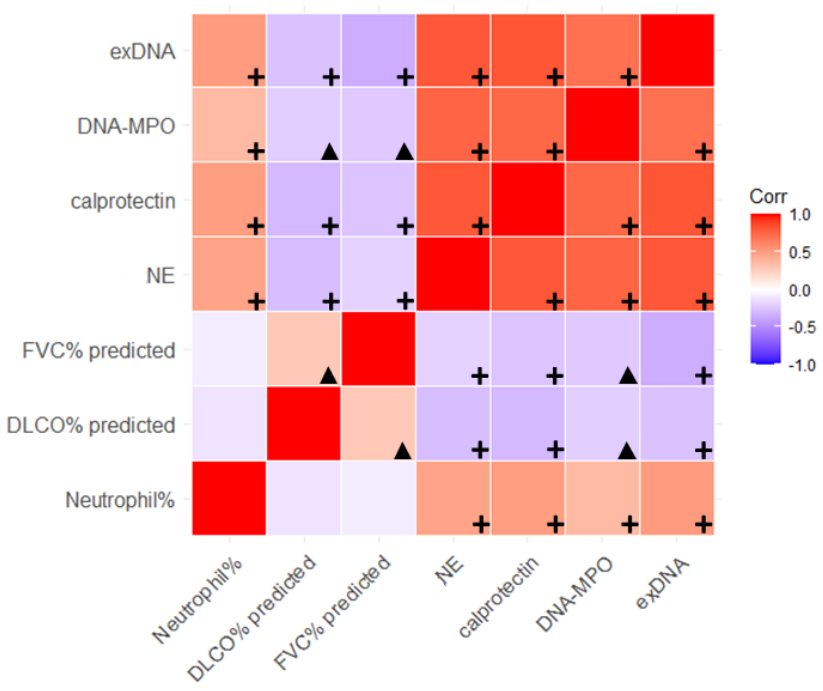

Figure 2. Correlogram highlighting degree of correlation across the four NET remnants, neutrophil percentage in BALF samples, and the negative correlation with FVC and DLCO (% predicted) across the discovery cohort. A red box color indicates a positive correlation with darker reds indicating greater strength of correlation and purple boxes indicate negative correlation with darker colors indicating stronger correlation, based on Pearson's correlation coefficient. Black plus signs indicate correlations with p-value <0.01 and black triangles indicate significant correlations with p-values between 0.01 and 0.05

Figure 3.

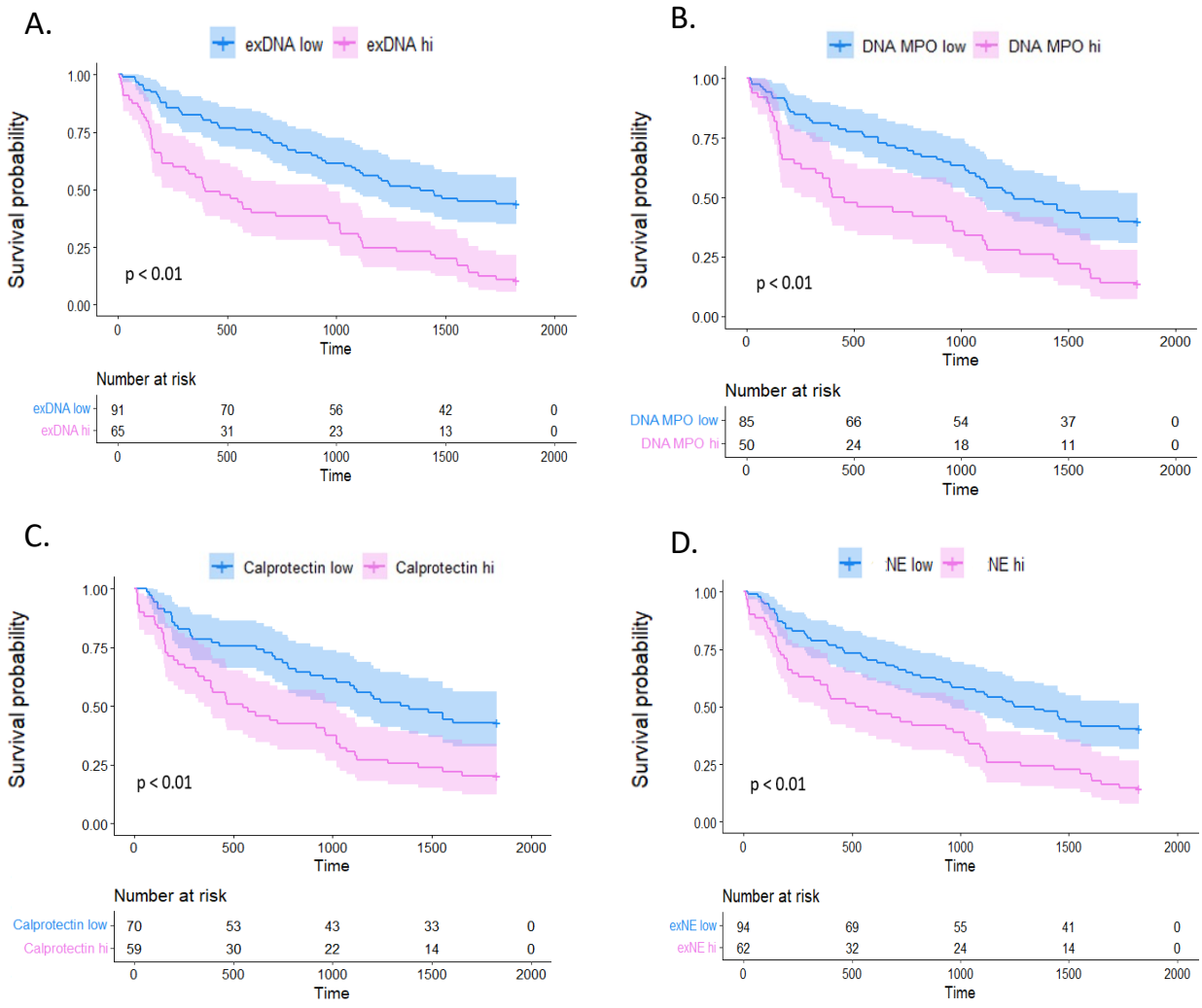

Figure 3: Kaplan-Meier survival curves in discovery cohort. Each pane represents one of the NETosis markers tested within the discovery cohort which was divided into high and low NETosis marker levels by optimal cut-off threshold. Patients with NETosis marker levels higher than the optimal cutoff threshold are represented in pink and those with NETosis marker levels below the threshold are in blue. Survival probability is represented by the y-axis and the time to death in days is plotted on the x-axis. Panel A shows results from exDNA: HR=2.57, 95% CI 1.76–3.76, p<0.01; Panel B shows results from DNA-MPO: HR=2.15, 95% CI 1.43–3.24, p<0.01; Panel C shows results from calprotectin: HR=2.00, 95% CI 1.31–3.05, p<0.01; Panel D shows results from NE: HR=2.06, 95% CI 1.41–3.01, p<0.01.

Figure 4.

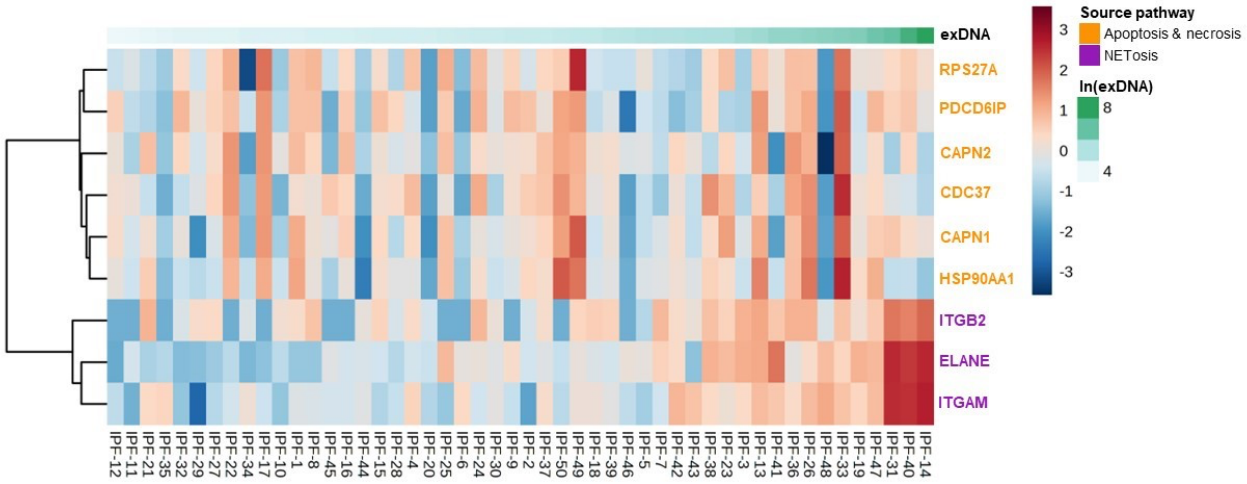

Figure 4. Heatmap of BALF protein expression levels. In this heatmap, each column represents one IPF subject and each row represents one of the nine exDNA associated proteins. Proteins associated with apoptosis and/or necrosis are in orange and proteins associated with NETosis are in purple. IPF subjects are arranged based on their BALF exDNA levels, which are noted on the top of the figure in green, with subjects on the left side of the heatmap having the lowest BALF exDNA levels and those on the right having the highest BALF exDNA levels. Each box represents the expression level of each protein for each IPF subject where a darker red box indicates higher expression of that protein in that subject and a darker blue box indicates lower protein expression in that subject. There is a visual relationship where the NETosis proteins (in purple) have increased expression from left to right, which corresponds to increasing BALF exDNA levels. This relationship is not seen for the proteins associated with non-NETosis sources of exDNA. Proteins include: RPS27A: Ribosomal protein S27A; PDCD6IP: Programmed cell death 6 interacting protein; CAPN2: Calpain 2; CDC37: Cell-division cycle co-protein 37; CAPN1: Calpain 1; HSP90AA1: Heat shock protein alpha family class A, member 1; ITGB2: Integrin beta 2; ELANE: Elastase, neutrophil-expressed; ITGAM: Integrin alpha, subunit alpha M.

Table 2.

Pathway	Protein	Correlation coefficient	p-value	FDR
NETosis	ELANE [19]	0.86	<0.01	<0.01
NETosis	ITGAM [20]	0.78	<0.01	<0.01
NETosis	ITGB2 [20]	0.60	<0.01	<0.01
Apoptosis	CAPN1 [21]	0.18	0.22	0.49
Necrosis	RPS27A [22-23]	0.15	0.30	0.53
Necrosis/Exosome Regulation	PDCD6IP [24-25]	0.10	0.48	0.73
Apoptosis	CAPN2 [21]	-0.08	0.60	0.74
Necrosis	HSP90AA1 [26]	0.06	0.66	0.74
Necrosis	CDC37 [27]	0.04	0.77	0.77

FDR: False-discovery rate.

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