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Matrix metalloproteinase expression and abnormal lung permeability are important determinants of outcome in IPF.

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Abstract (219)

Matrix metalloproteinases (MMP) degrade all the extracellular matrix components of the intersititium and may play a role in abnormal alveolar permeability which is a feature of idiopathic pulmonary fibrosis (IPF). This study aimed to evaluated the levels of MMP protein levels in patients with IPF and determine any relationship to treatment and markers of permeability.

Methods: 20 patients with IPF and 8 controls underwent bronchoalveolar lavage. MMP, TIMP, and VEGF levels were related to clinical outcome and protein permeability index

Results: MMP 3, 7, 8 and 9 were elevated in IPF lavage fluid and levels remain high despite treatment. Levels of MMP-3, 7, 8 and 9, VEGF and protein permeability index were higher in those who died early during follow-up. VEGF, MMP-8 and 9 were higher in those with a rapidly declining lung function over 1 year. Levels of MMP 3, 7, 8 and 9 correlated with an increased permeability index.

Interpretation: MMP levels are elevated in IPF patients and are not modulated by current standard treatment. MMP production through an interaction with the known vascular permogen, VEGF, is potentially associated abnormal capillary permeability and may potentiate the neoangiogenesis seen in IPF. These changes were greatest in those who died or progressed during follow-up suggesting that drugs targeting VEGF or MMP activity warrant assessment as novel therapy for IPF

Introduction (388)

Idiopathic pulmonary fibrosis (IPF) is a progressive lung disease of unknown aetiology[1]. Current theories speculate that IPF results from abnormal wound healing in response to multiple, microscopic sites of alveolar epithelial cell (AEC) injury and activation. Microscopic injury is associated with the formation of fibroblast/myofibroblast foci and epithelial cell dropout and the evolution of fibrosis. Disruption of the alveolar epithelium, altered AEC phenotypes, AEC loss and failure of epithelial repair are all distinctive features of IPF[2].

In addition to myofibroblast foci formation and epithelial cell injury, there is variable evidence of inflammation as evidenced by increased macrophage and neutrophil counts[3], intra-alveolar coagulapathy[4] and the formation of new blood vessels in the IPF lung. Abnormal angiogenesis has further been linked to the development of fibrotic disorders of the lung[5]. Together these changes result in an increase in the permeability of the alveolar capillary barrier which can be detected clinically by increased DTPA clearance[6].

Currently the molecular determinants of the abnormal angiogenesis and increased permeability are unclear as studies are somewhat conflicting. Some studies suggest that the pro-angiogenic ELR+ cytokines such as IL-8 or IP- promote neo-angiogenesis [7]. Other studies suggest that anti-angiogenic peptides such as pigment-epithelium derived factor (PEDF) are elevated whereas pro-angiogenic peptides such as vascular endothelial growth factor (VEGF) may be reduced[5]. To further complicate the situation it appears

that expression of these peptides in lung biopsies may be heterogenous – thus for example VEGF expression may be reduced within fibroblastic foci[5].

The formation of new blood vessels requires coordinated regulation of matrix proteolysis and endothelial cell migration. Matrix metalloproteinases (MMP) are essential for extracellular matrix (ECM) remodeling, wound healing, and angiogenesis and have been implicated in the pathogenesis of IPF[8]. Several studies have demonstrated elevated levels of MMP-1, MMP-8 and MMP-9 which occur in conjunction with alterations in the levels of their soluble inhibitors – TIMP-1 and TIMP-2 - in IPF. MMP-7 expression is elevated in both human IPF and murine models of fibrosis, whilst MMP-7 knockout mice have attenuated fibrotic reactions[9, 10].

Together the MMPs have the capability of degrading all the collagenous contents of the intersititium and may therefore play a role in abnormal alveolar permeability. The fact that VEGF, which is a known potent inducer of capillary permeability[11], acts in part via actions upon MMP production further supports a role for MMP expression in abnormal vascular permeability in IPF[12, 13].

The aims of this study were to systematically evaluate the levels of MMP and TIMP protein levels in patients with IPF and to determine the relationship of MMP levels to cellular inflammation, BALF protein permeability index and VEGF levels. In addition, we assessed whether MMP levels are influenced by standard treatment and relate to survival or decline in lung function in IPF patients.

Methods 491(500)

Subjects

20 patients with IPF diagnosed according to current American Thoracic Society (ATS) criteria were recruited from the specialist Interstitial Lung Disease clinic at University Hospital Birmingham, UK. Diagnosis was supported by open lung biopsy in five cases, histology post lung transplant in one case and high-resolution computed tomography (HRCT) evidence in all cases. Where open lung biopsy was performed, tissue was obtained from at least two sites, the upper and lower lobes of the same lung. Baseline bronchoscopy was performed during the investigation stage after referral, before the start of definitive treatment. 8 healthy individuals free from respiratory disease were recruited as controls. This study was approved by the local ethical committee and participants gave written informed consent.

Measurements

Patients underwent bronchoscopy and bronchoalveolar lavage (BAL) as described previously[14]. TIMP-1, TIMP-2 and VEGF were measured in BALF by ELISA kits (R&D). BALF MMP-1, 2, 3, 7, 8, 9, 12, and 13 were measured by Luminex array (R&D systems). Luminex array values for normal MMP levels have been included in a paper on ARDS which is currently under review by critical care medicine. IPF MMP-3 levels have been quoted in a paper upon endostatin cleavage which has been accepted for publication. Protein was measured using the Bio-Rad *DC* protein assay kit II. The protein permeability index (PPI) was calculated as the ratio of BALF to plasma protein as described previously(20).

Gelatin zymography

Gelatin zymography was carried out as previously described[15]. In brief BAL fluid was diluted 1/10 and 20µl aliquots mixed with 5µl loading buffer(0.25M Tris pH 6.8, 50% glycerol, 5% SDS, bromophenol blue) were run on 11% acrylamide gels impregnated with 0.1% gelatin at 180V for 3 ½ hours (buffer 25mM Tris, 190mM glycine, 0.1% SDS). After incubation in 2.5% Triton X for 1h with agitation and 2 brief washes in collagenase buffer (55mM Tris base, 200mM sodium chloride, 5mM calcium chloride, 0.02% Brij, pH 7.6), gels were incubated for 16 hours in fresh collagenase buffer at 37^{o}C. Gelatinolytic activity was detected by a single step stain/de-stain method using 0.02% Coomasie blue in 1:3:6 acetic acid: methanol: water. All experimental samples were run in parallel with 2ng recombinant MMP-9 (Oncogene) to standardize between gels.

MMP-9 activity assay. BAL fluid was diluted 1/25 or 1/50 and analysed using the MMP-9 fluorokine activity assay (R&D Systems, Europe) according to manufacturer's instructions. A standard curve of purified MMP-9 was run on the same plates (and pro-forms of MMP-9 standard were activated by 4-aminophenylmercuric acetate (APMA) for 2 hours before the addition of the fluorogenic substrate) to allow quantification. The minimal detectable concentration of MMP-9 using this assay is reported as 5pg/mlml (manufacturer's data). This assay takes into account the effect of TIMPs in the sample and gives a measure of net MMP-9 activity

Pulmonary function testing

Forced vital capacity (FVC) was measured using the Jaeger Compact system (Viasys Healthcare). Total lung diffusing capacity for carbon monoxide (TLCO) was measured by single-breath technique (Jaeger Compact system). Results were expressed as the percent of predicted values. Sequential lung function was obtained where possible 3 monthly as part of clinical follow-up. Rapid lung decline was defined as a consistent reduction (more than 10%) in FVC , TLC or TLco 1 year after presentation[1].

Statistics

IPF BALF protein data were non-parametric and are thus presented as median and interquartile range (IQR). Between group comparisons were performed using the Mann Whitney U test. A p value ≤ 0.05 for all data was considered significant. Correlations were made using Pearsons test after log transformation of non-parametric data. This study was considered hypothesis generating so a power calculation was not performed. Statistics were performed using Minitab 14.

Results

Demographics

20 patients with IPF and 8 healthy controls were recruited. A summary of patient characteristics is shown in *table 1*. 8 patients agreed to a sequential BAL 3-6 months after starting treatment with Prednisolone 0.5 mg/kg and azathioprine 1-1.5 mg/kg +/- N-acetylcysteine 600 mg tds daily. Patients had to be free from clinical suggestion of pulmonary infection in the 4 weeks prior to bronchoscopy.

Table 1: Demographics

	IPF	**Normal**
Patient numbers	20	8
Age (range)	70 (52-86)	45 (34-58)*
Sex: Male	16	4
Smoking status: current, ex, never	5,12,3	3,3,2
Pack years (SD)	30 (20)	21 (16)
FVC % predicted (SD)	73 (19.7)	N/A
TLco% predicted (SD)	49 (14.4)	N/A
TLC % predicted (SD)	75 (16.3)	N/A
Baseline Oxygen saturation % (SD)	94 (3)	97 (1)

FVC -forced vital capacity, TLco – Carbon monoxide diffusion factor (both expressed as percentage of normal), Values are given as the mean ± standard deviation (SD).*p=0.01

Of the 20 patients who were enrolled in this study 12 had died during the 3 year period of follow-up. The cause of death was deterioration of lung function decline causing respiratory failure (n=5), lung carcinoma (n=3), pneumonic infection (n=2), pulmonary embolus (n=1), myocardial infarction (n=1). One patient underwent lung transplantation during the follow-up period. Median time to death was 18 months from time of first bronchoscopy.

BALF Macrophage and Neutrophil counts are elevated in patients with IPF.

IPF BALF cell count 1260000/ml, (IQR 560000-3220000) was significantly increased compared to normal BALF cell count 412000/ml (253000-470000) p=0.0034. There was significantly elevated total alveolar macrophage (AM) count (IPF median 1030000 AMs/ml BALF (IQR 44600-274000), normal 39100 AMs /ml (IQR 245000-450000), P=0.0051). BALF % macrophage count was reduced compared to normal (median IPF %AM 88.3 (IQR 73.2-94.1), normal BAL % AM 96 % (IQR 95.5-97.7) p=0.031).

There was an increase in IPF BALF total and % neutrophil count compared to normal BALF counts. Median total IPF neutrophil count was 120000 cells/ml (IQR 56500-279000), normal total neutrophil count 400 /ml (IQR 52.5-956) cells/ml, p=0.0373. Median IPF BALF neutrophil% was 11.7% (IQR 5.9-26.8%), normal 2% (IQR 0.75-2.75%) p=0.016. There was an increase in % neutrophils (but not total neutrophils or macrophages) in patients who died during the follow-up phase (median died % neutrophil 25.0, IQR 13-62, survived % neutrophils 7.9, p=0.05)as has recently reported [3]. There

was no significant difference in BALF lymphocyte or eosinophil count between normal and IPF BALF in our cohort.

Matrix metalloproteinases are elevated in patients with IPF.

MMPs 7, 8 &9 represent the majority of MMP protein in IPF BALF (figure 1). MMPs 2,3,7,8 & 9 were significantly elevated from normal patients (table 2). In the 8 patients who underwent sequential bronchoscopy after treatment levels of MMP-2, MMP-3, MMP-7, MMP-8 and MMP-9 remained elevated (see table 2). BALF levels of MMP-12 and MMP-13 were largely undetectable in both normal and IPF BALF and are not demonstrated further. MMP levels did not correlate with age, sex, smoking status or pack year history (data not shown).

	Normal (n=8)	BAL 1 (n=20)	BAL2 (n=8)	P value BAL1 vs normal	P value BAL 2 vs normal	P value BAL 1 vs BAL 2
MMP-1	13.2 (13-13.45)	21.7 (13-53)	21.19 (13-43)	0.28	0.77	0.45
MMP-2	67 (67-177)	230 (74-1175)	1396 (74-2385)	0.0027	0.0066	0.17
MMP-3	9.7 (6.6-11.86)	24 (9.28-62.5)	22.7 (8.2-40.3)	0.011	0.056	0.3
MMP-7	102 (82-308)	4841 (1113-25026)	9126 (935-11024)	0.0012	0.0032	0.86
MMP-8	91.8 (65-472)	8547(1184-19767)	15381 (6602-27001)	0.0004	0.0022	0.79
MMP-9	321 (187-1795)	22281 (3416-66671)	36755 (10725-56954)	0.0005	0.0015	0.64

Table 2 BAL MMP levels in normals versus baseline BAL and follow-up bronchoscopy. Values are all in pg/ml and expressed as medians and interquartile range.

BALF MMP levels are elevated in those who have rapid progression of lung function 1 year after presentation.

BALF levels of MMP- 3, 7, 8 and 9 were elevated in those patients who had a rapid decline in lung function (n=8) compared to non- rapid progressing cases (n=12). However only the differences between MMP-8 and MMP-9 were significantly different see table 3. There were no important relationships between IPF BALF MMP 1, 2, 8, 12, or 13 with FVC, TLco or Kco. BALF MMP-7 correlated weakly with TLco (r=0.28, p=0.008) and MMP-3 correlated with FVC (r=0.20, p=0.048).

Table 3 MMP levels in patients whose lung function rapidly declined over 1 year compared to those where there was no significant decline. Rapid lung decline was defined as a consistent reduction (more than 10%) in FVC , TLC or TLco 1 year after presentation.

BAL MMP	Rapid lung function decline (n=8)	Non-rapid lung function decline (n=12)	P value
MMP-1	22 (13-86)	13 (13-18)	0.56
MMP-2	74 (74-1092)	297 (74-1323)	0.75
MMP-3	59.6 (23.4-92)	20.5 (4.3-27.8)	0.058
MMP-7	11095 (3505-32552)	4822 (260-17312)	0.29
MMP-8	26427 (11190-38160)	2533 (570-11431)	0.028
MMP-9	94747 (31730-181371)	9907 (1491-26947)	0.015

MMP levels are elevated in those who died during follow-up.

Our cohort of patients had mortality of 40% during the 3 years of follow-up. BALF levels of MMPs 3, 8 and 9 were elevated in those who subsequently died during the follow-up phase (see table 4). MMP levels in those who died did not predict the time to death.

Table 4: BALF MMPs and protein permeability are elevated in patients who die early during follow-up. Protein values are median (IQR).

	Died (n=8)	Survived (n=12)	P value (died vs survived)
MMP-1	13 (13-56)	16.6 (13-76.8)	0.79
MMP-2	74 (91-813)	74 (74-1092)	0.75
MMP-3	72 (28-91)	8 (3-28.5)	0.0081
MMP-7	10012 (4650-30399)	3187 (88-10105)	0.16
MMP-8	33705 (15276-41451)	1225 (373-6810)	0.0015
MMP-9	115834 (49055-167675)	3331 (877-15086)	0.0015
MMP-12	18	18	0.96
BAL protein	0.38	0.23	0.3415
PPI	0.01594 (0.008-0.044)	0.00434 (0.0033-0.0482)	0.04
Plasma protein	92	126	0.458
TIMP-1	15185 (7674-23593)	2245 (1070-7063)	0.01
TIMP-2	1448 (765-2102	488 (365-1693)	0.02

BALF TIMP-1 and TIMP-2 are elevated in patients with IPF.

TIMP-1 and TIMP-2 levels were elevated in first and sequential BALF compared to normal. Median normal BALF TIMP-1 was 505pg/ml (IQR 290-1062) versus IPF BAL TIMP-1 median 7343pg/ml (1830-18668) p=0.0044. IPF BALF TIMP-1 remained elevated in those who had treatment (n=8), IPF repeat BALF TIMP-1 9725 (IQR 2500-20230) p=0.0022 compared to normal (see figure 2).

Initial IPF BALF TIMP-2 levels (898 pg/ml (460-1836) p=0.0046) and IPF repeat BALF TIMP-2 levels (median – 1623 pg/ml (606-2296) p=0.0022) were elevated compared to normal 276 pg/ml (43-764) (see figure 2b). There were no relationships between TIMP-1 or TIMP-2 levels and static lung function at diagnosis. BALF TIMP-1 and TIMP-2 levels were elevated in those who died during follow-up as opposed to survivors (see table 4).

Molar ratios of MMP: TIMP are altered in IPF.

Calculation of the MMP: TIMP -1 molar ratio suggests that there is no difference between MMP-1 and 2 and normal. MMP-3 molar ratio is significantly reduced (IPF 0.00304 versus normal 0.02 p=0.0066). In contrast molar ratios were increased for MMP-7 (IPF 0.89 versus 0.25 normal, p=0.0374), MMP-8 (IPF 0.417 vs normal 0.092, p=0.0078) and MMP-9 (IPF 0.973, vs normal 0.171 p=0.018). **MMP: TIMP2 ratios were not elevated compared to normal for MMP-1,2,3 but were significantly elevated for MMP-7, 8 and 9 (data not shown).**

In order to confirm that protein levels reflected activity we went on to perform BALF MMP activity assays for MMP-9. We chose MMP-9 because it is the best validated assay for MMP activity.

MMP activity is elevated in patients with IPF.

MMP-9 was detected in 19/20 patients with IPF and 1 normal control patient by zymography. A representative gel is shown in figure 3. Fluorimetric MMP-9 activity was 23791 pg/ml, 5145-62787 pg/ml in IPF BALF compared to below detection limit in 7/8 samples (median allocated value 125 pg/ml, p=0.000). The addition of APMA, increased the activity of MMP-9 in IPF BALF from 23791 pg/ml to 52529 pg/ml, IQR 11728-134631, P=0.14) (see figure 4).

BALF VEGF levels in IPF

VEGF levels in IPF patients were not significantly different in patients with IPF compared to normal (median IPF 82 pg/ml IQR 25-222 versus normal BAL VEGF 140 pg/ml p=0.499) (see figure 5A). VEGF levels did not correlate with baseline lung function. Initial IPF BALF levels of VEGF correlated significantly with both protein permeability index (r=0.51, p=0.026) and with BALF MMP-3 (r=0.53, p=0.003), MMP-7 (r=0.36, p=0.02), MMP-8 (r=0.38, p=0.018), and MMP-9 (r=0.34, p=0.029) (data not shown).

Baseline BAL fluid VEGF levels were elevated in those patients who had a rapid decline in lung function over the first year of follow up (rapid progressors median 188 pg/ml (IQR 56-301.7) compared to IPF non-progressors median 38.4 pg/ml (IQR 16.8-152.1),

p=0.04) (see figure 5B). Baseline BAL fluid VEGF levels were also elevated in those patients who died (median 159pg/ml (62-272) compared to those surviving (median 34 pg/ml (13.9-70)) p=0.03 (figure 5C).

Protein Permeability index is elevated in patients with IPF.

IPF BALF protein permeability index was elevated 0.00439, (IQR 0.000853-0.00148) compared to normal 0.00196, IQR (0.000714-0.0026)p=0.044 (see figure 6a). Levels of PPI were greatest in those who died (0.01594) within the 3 years of follow-up (p=0.04) (table 4 and figure 6b). In contrast neither plasma protein levels nor BALF total protein had any relationship to outcome (table 4). Protein permeability index did not differ significantly in those who had a rapid decline in lung function (data not shown).

Discussion

MMPs capable of degrading various components of connective tissue matrices are believed to play a significant role in remodeling after parenchymal damage, resulting in tissue destruction or the induction of repair processes in pulmonary diseases[16]. In this study we have demonstrated that the predominantly elevated MMPs in IPF BALF were 7, 8 and 9. MMP-7 levels have previously been shown to be upregulated in microarray studies and in one lavage study of IPF patients[9, 17]. Another study has demonstrated elevated MMP-8 and neutrophil derived MMP-9 in BALF fluid [8]. Further studies by Suga et al confirm increased MMP-9 activity in IPF- particularly in those with rapid progression[18]. Similar to our findings a previous study did not show elevation of MMP-1 but did not confirm our finding of increased TIMP-1 levels [8].

In previous animal studies, it has been shown that MMP-12 has an important role in the development of lung fibrosis[19]. It is difficult to reconcile these findings with the low / undetectable levels of MMP-12 seen in our study. It is important to recognize that the tissue levels of MMP-12 do not necessarily clearly reflect BALF fluid levels due to the complex regulation of MMPs by local inhibitors, and clearly further studies are warranted in human disease to elucidate the role of MMP-12 in human lung fibrosis.

Matrix metalloproteinase activity is regulated in part by the activity of TIMPs. Thus simple BALF MMP immunoreactivity does not necessarily reflect bioactivity. To try and address this we analyzed the protein levels of TIMP-1 and TIMP-2 as well as the net BALF activity of MMP-9. The net MMP: TIMP molar ratios were elevated in IPF

compared to normal individuals for MMP 7, 8 and 9. The activity assay confirmed the increased activity of MMP-9 in the lavage fluid, a finding confirmed by zymography. This would suggest that elevated matrix metalloproteinase-9 is bioactive within the lungs of IPF patients.

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In this study we have tried to assess the relationship between MMP expression and the protein permeability index (PPI) (ratio of BAL: plasma protein). The PPI has been widely been used to assess the integrity of epithelial barrier function in patients with ARDS [20], and as the airway permeability index in patients with asthma[21]. To our knowledge no previous studies have assessed its utility in IPF patients. We have demonstrated an increased index compared to normal that relates to both VEGF, and MMP levels and is elevated in those who die early with IPF. The fact that neither plasma protein nor BALF protein had relationships to outcome supports the validity of the index in the setting of IPF.

In IPF, it has been recognized that the structural integrity of the alveolar wall depends on the basement membrane and that destruction of the subepithelial basement membrane may precede the development of alveolar fibrosis. A discontinuity of the basement membrane allows greater access for exudative factors and interstitial cells to the alveolar space – potentially promoting further tissue destruction and progressive fibrosis. In this study our finding of relationships between MMP levels and the protein permeability index supports a role for MMP-3, 7, 8 and 9 directly in the permeability of the alveolar barrier in IPF. The correlation between VEGF and both the PPI and MMPs supports a

role for VEGF in this abnormal permeability. VEGF is known to promote both neo-angiogenesis and increase vascular permeability as well as promoting epithelial proliferation and repair[11, 22, 23]. In addition, MMPs can activate matrix bound growth factors such as VEGF potentially increasing their bioavailability. Clearly the finding of elevated levels of PPI, MMP-3, 7 and 9 and VEGF in those patients who die or progress early suggests that these changes are of clinical significance.

Current treatment for IPF remains unsatisfactory and there is little evidence that the UIP pattern on lung biopsy ever regresses with treatment. A recent trial has, however, suggested that treatment with prednisolone, azathioprine and N-acetyl-cysteine slows progression[24]. Our data demonstrate clearly that, in the subgroup of patients who agreed to repeat bronchoalveolar lavage 3-6 months after such combination drug therapy, that this therapy does not have any suppressive activity upon BALF MMP immunoreactivity. This is despite the fact that a previous study suggests that steroid treatment does reduce MMP-9 production from IPF patients treated with steroids and azathioprine[25] but in that study patients were not individually studied consecutively pre and post treatment.

This study has several limitations. Firstly our patient population did not all have lung biopsies to prove UIP although they were well characterised according to international guidelines in a specialist clinic. Furthermore, the difference in age between the IPF patients and our healthy controls may be a confounding factor when comparing MMP levels. To address this we examined the relationship between age and MMP levels in IPF

and normal controls and found no correlation. Finally, it is important to understand that MMP levels in BALF do not necessarily reflect activity within the interstitium but our results do confirm findings from previous studies. As BAL only samples the alveolar component of the lung's response to disease, it is likely that the altered production of the other collagenases in sites not sampled by BAL can also contribute to the initiation of collagen remodelling in this disease.

In summary, this study has systematically assessed a range of MMPs known to be important in pulmonary disease in IPF patients both before and after treatment. MMP activity is raised and does not appear to be influenced by treatment. Levels of MMP correlated with an increased PPI and the known vascular permogen VEGF suggesting that MMP production is involved in abnormal capillary permeability and potentially the neoangiogenesis seen in IPF. The fact that these changes were greatest in those who died or progressed during the follow-up phase suggests significant clinical importance. In conclusion we suggest that drugs targeting MMP activity currently in development for COPD warrant assessment as novel therapy for IPF.

Acknowledgements:

DT and AR were funded by the Wellcome Trust. CO is a NIHR clinician scientist.

Figure Legends

Figure 1 Bronchoalveolar lavage fluid MMP levels expressed as a scatter plot – BAL data combined from baseline and follow-up bronchoscopy (n=28). This graph demonstrates that the majority of BALF MMP protein is MMP-7, 8 and 9.

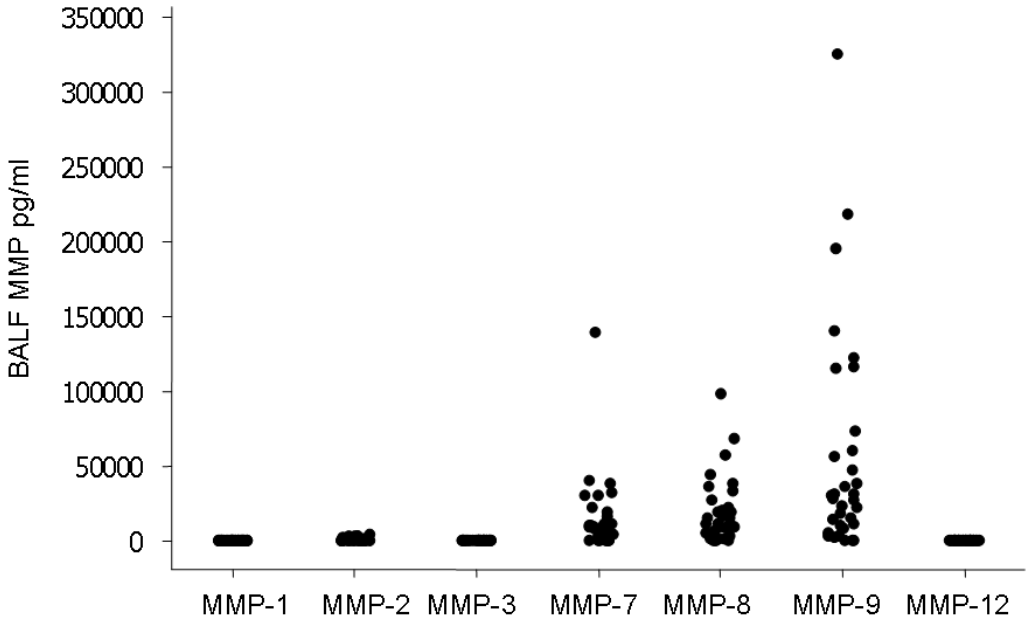


Figure 2: Figure 2 Boxplot of BALF levels of TIMP-1 and TIMP-2 are persistently elevated in patients with IPF. The *horizontal bar* represents the median and the *boxes* represent interquartile ranges. *Vertical lines* show minimum-maximum range.

Figure 2

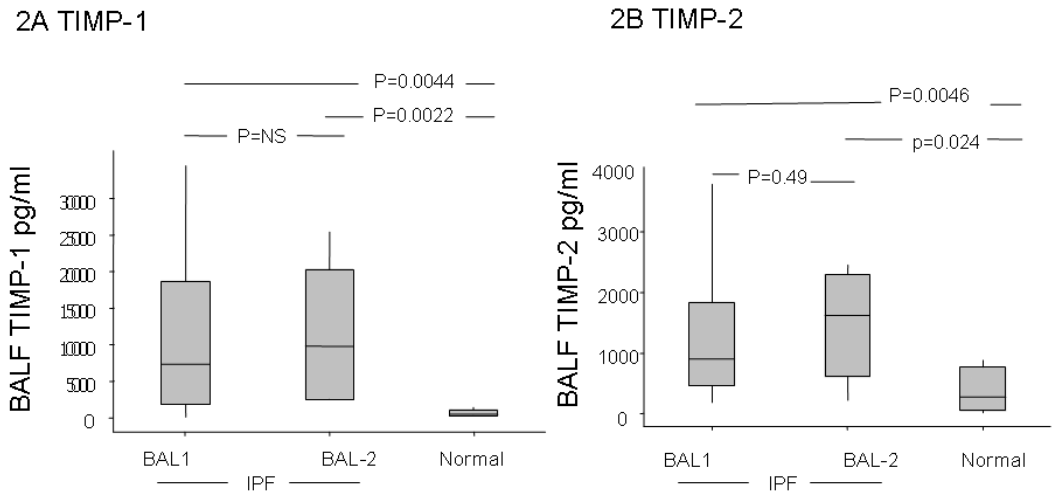


Figure 3 Representative zymogram demonstrating increased MMP activity in IPF BAL compared to normal.

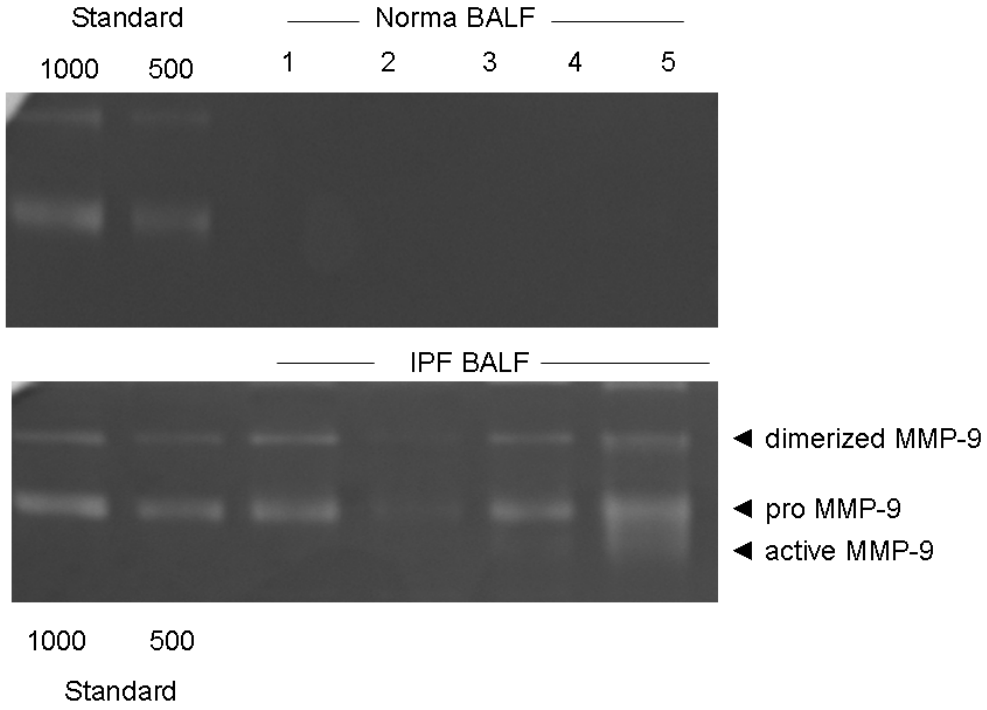


Figure 4: BALF MMP-9 activity assay – APMA maximally activates MMP-9. The *horizontal bar* represents the median and the *boxes* represent interquartile ranges. *Vertical lines* show minimum-maximum range.

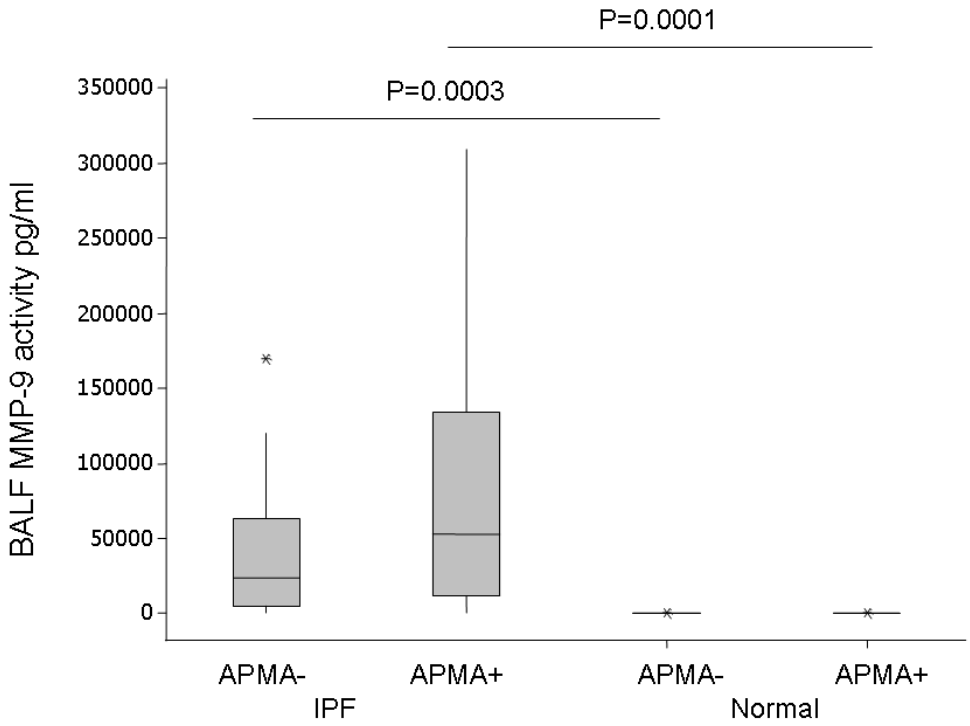


Figure 5a BALF VEGF is not different between normal and IPF patients (all 20 considered at baseline). The *horizontal bar* represents the median and the *boxes* represent interquartile ranges. *Vertical lines* show minimum-maximum range.

Figure 5b BALF VEGF is greater in those IPF patients who died during follow-up rather than survived.

Figure 5a

Figure 5b

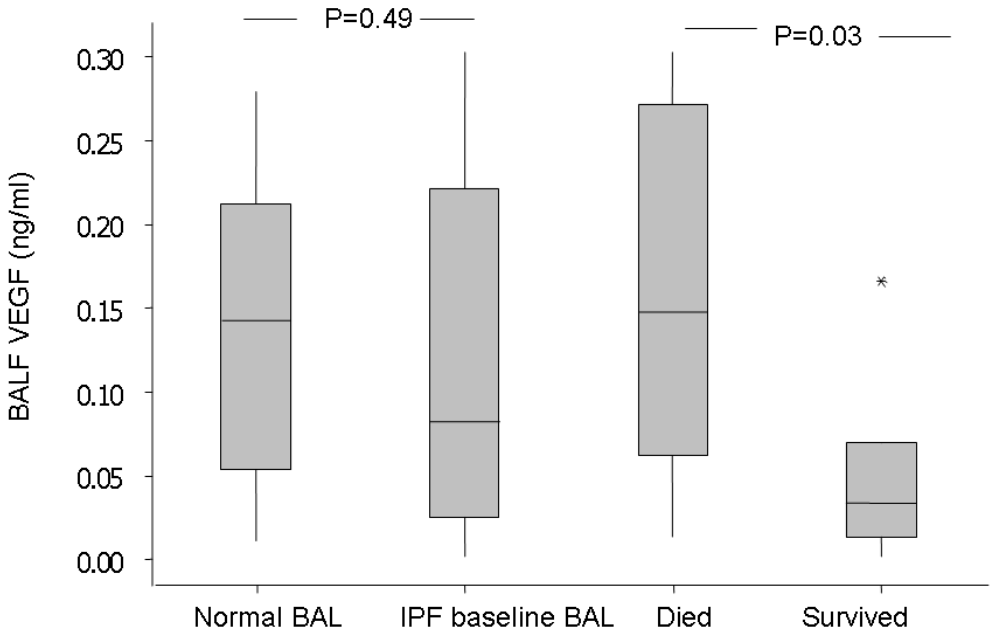
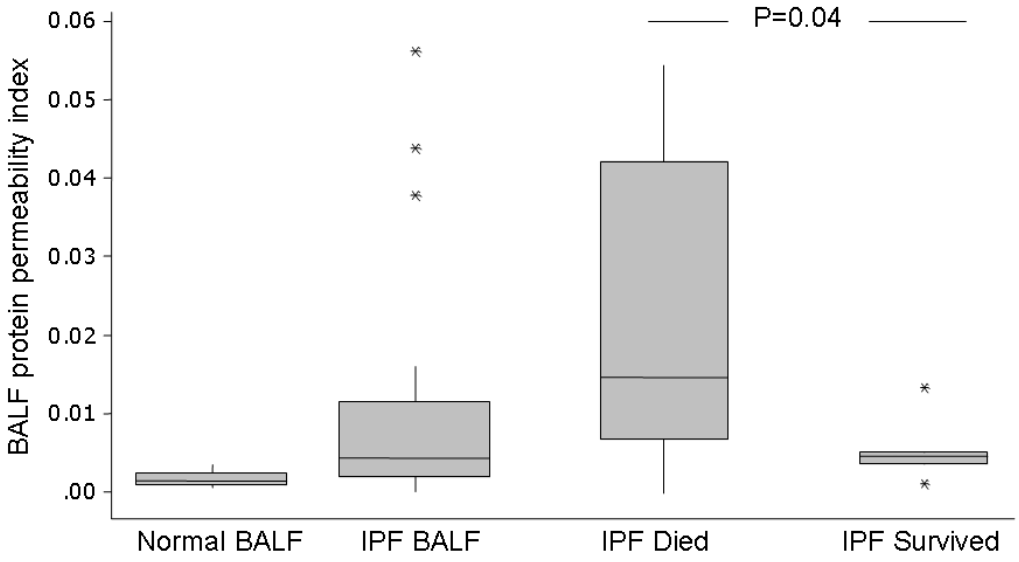


Figure 6 Protein Permeability index is increased in IPF patients compared to normal. IPF patients who died during followup have significantly elevated PPI compared to survivors. The *horizontal bar* represents the median and the *boxes* represent interquartile ranges. *Vertical lines* show minimum-maximum range.


P=0.04
BALF protein permeability index
0.06
0.05
0.04
0.03
0.02
0.01
.00
Normal BALF
IPF BALF
IPF Died
IPF Survived

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