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Pleural fluid cytokine levels at baseline and over time are associated with time to IPC removal – an exploratory study

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Abstract

BACKGROUND: The behavior of pleural fluid cytokine levels (PFCs) and their association with pleurodesis after indwelling pleural catheter (IPC) placement is unknown.

Objective: We conducted a prospective exploratory study to obtain preliminary data on PFC levels after IPC placement.

Methods: The PFC panel consisted of four cytokines ((interleukin -8 (IL-8), vascular endothelial growth factor (VEGF), total (but not activated) transforming growth factor betas (TGF-βs), and fibroblast growth factor (bFGF)), measured across five time points (T0: insertion; T1: 24–48 hours; T2: 72–96 hours; T3: 1 week; and T4: 2 weeks). Profile plots were used to identify patterns of change of PFC levels. Correlation matrices for each PFC over time were computed, and area under the curve (AUC) categories were used to compare the cumulative incidence of IPC removal.

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Author contributions: IIW and HBG were principal investigator and was responsible for study design, analysis, management, writing, and editing. WL and XT were responsible for pleural fluid processing., RC, JDM, CR, LY, DS, MS, JJ, CR, AAR, DFK, HL, SS, FF, FM, BX, LN, RM, LM, TS, MVR, GAE were responsible for data collection, study oversight, analysis and writing. SM, RC, JDM, CR, LY, DS, MS, JJ DO, MRV, MH NG, LN, AH, LB, SF, DB, RC, GE, VS, AS performed the procedures and helped with data collection, data entry, study oversight, analysis and writing. All authors participated in reviewing and editing the manuscript.

Conflict of interest: The authors have no conflict of interest to disclose.

Auto pleurodesis was defined as elective catheter removal due to decreased drainage within 90 day of insertion.

RESULTS: A total of 22 patients provided complete data. Except for IL-8, the majority of PFCs demonstrated strong positive correlations across measurement time points. Patients with high AUCs for IL-8, bFGF, and VEGF had a higher cumulative incidence of IPC removal by 90 days than did patients with low AUCs.

CONCLUSION: This is the first study to evaluate longitudinal changes of pleural cytokine levels with respect to likelihood of IPC removal, and provide early evidence that the cytokine profile may be associated with the outcome of pleurodesis induced by IPCs. However this is an exploratory study and further studies are needed to assess if these findings can be validated in further studies.

Keywords

pleural effusion; cytokines; pleurodesis

Introduction

Malignant pleural effusion (MPE) is associated with poor prognosis and a median survival of 3–6 months; in addition, it can cause significant dyspnea, resulting in poor quality of life.[1, 2] Treatment of MPE centers around symptom palliation that can be achieved with use of several treatments, such as intermittent thoracentesis, chest tube drainage followed by pleurodesis, thoracoscopic drainage followed by pleurodesis, or pleurodesis using a long-term indwelling pleural catheter (IPC).

IPCs are an attractive option for management of MPE, and in the past two decades, placement of IPCs has become more common because they are cost-effective, do not require hospitalization for placement, and provide both symptoms palliation and spontaneous pleurodesis.[3, 4] Almost 50% of patients in whom IPCs are placed can achieve spontaneous pleurodesis and catheter is removed.[5]

The mechanisms underlying pleural inflammation and spontaneous pleurodesis after IPC placement are poorly understood. Several studies have evaluated the inflammatory pathways and pleural fluid cytokines (PFCs) involved in chemical pleurodesis, but to date, there are no accurate biochemical criteria to help select suitable candidates for pleurodesis. Along with several pleural vasoactive mediators such as vascular endothelial growth factor (VEGF) and host-elaborated transforming growth factor-β (TGF-β), involved in pleural fluid formation, there are pleural mediators, angiopoietins, and chemokines that have been associated with chemical pleurodesis, including interleukin-8 (IL-8) and basic fibroblast growth factor (bFGF).[6–9]

The behavior of PFC after IPC placement is unknown. Further elucidation of changes in PFCs may help define factors that enhance spontaneous pleurodesis, thus helping to select the optimal modality for different patients, and further our understanding of the process behind “autopleurodesis”. In addition, data on changes in the inflammatory milieu may predict the probability of successful pleurodesis.

The main objective of this prospective exploratory study was to assess whether changes in PFCs (IL-8, VEGF, TGF-β, and bFGF) over time was associated with the rate of IPC removal over a 90-day monitoring period.

Methods

This prospective study was conducted to acquire preliminary data regarding PFCs after placement of an IPC. The PFC panel consisted of four PFCs (IL-8, VEGF, total TGF-βs, and bFGF) previously associated with pleurodesis in MPE, measured across five specified time points (T0: insertion of IPC; T1: 24–48 hours; T2: 72–96 hours; T3: 1 week; and T4: 2 weeks). Eligibility included patients aged 18 years or older with MPE who were undergoing IPC placement. Patients who were excluded were those 1) who had undergone previous pleural intervention other than thoracentesis for MPE on the ipsilateral side, including IPC or chest tube placement and/or thoracotomy; 2) with a history of chemical or mechanical pleurodesis and a history of thoracotomy within a year; 3) who were unable or unwilling to give informed consent; 4) who were unable or unwilling to return for additional fluid collection at each predefined time point; 5) with an Eastern Cooperative Oncology Group (ECOG) of >2) who received intrapleural chemotherapy including instillation of cisplatin or cytarabine; 6) who had chylous effusions, defined as pleural fluid analysis for triglyceride content of greater than 110 mg/dL or pleural fluid chylomicrons;7) patients with pleural fluid infection or other comorbidities that could influence pleural fluid productions (i.e decompensated heart failure).

Informed consent was obtained from all participants. Approval from the Institutional Review Board was obtained, and protocol number PA2016–0126 was assigned.

Definitions

Malignant pleural effusion was defined as pleural effusion and the presence of metastatic disease in the pleural fluid proven by either cytology or biopsy.[20]

Successful auto pleurodesis was defined as elective catheter removal due to decreased drainage within 90 day of insertion with no evidence of pleural fluid on imaging. The period of 90 days was chosen based on a review of evidence showing that elective catheter removal due to spontaneous pleurodesis occurred at a median of 56 to 90 days in 50% of patients.[5, 10]

Time to pleurodesis or time to IPC removal was defined as the number of days between IPC insertion and elective IPC removal at 90 days.

Pleural fluid processing

Within 30 minutes after the pleural fluid was removed, the fluid was placed in a citrated tube, and the sample was then iced to be transported to the laboratory; at the laboratory, the sample was spun at 1500 rpm, and the pellet and supernatant were separated before the sample was stored at −80° centigrade. Samples were processed in triplicate by Meso Scale Discovery, a division of Meso Scale Diagnostics LLC. We used V-PLEX Custom Human Biomarkers for human IL-8, human VEGF, human bFGF, and 96-well 4-Spot Prototype (MSD) TGF-B Triplex N45ZA-1 5.0 ANALYTES: TGF-β1, TGF-β2, and TGF-β3. We

measured the total (i.e. combined latent and activated) TGF-beta levels, but not that of the activated forms.

MesoScale Discovery (MSD) technology uses electro-chemo-luminescence detection technology using SULFO-TAG labels, which emit light upon electrochemical stimulation initiated at the electrode surface of multi-array spots microplates. Thus, we measured TGF-βs using multiplexed antibodies TGF-b 1, 2 and 3 MSD chemo-luminescent system for simultaneous measurement of all three analytes from a single biological sample[11]

Study Procedures and Fluid Processing

After informed consent was obtained, the study enrolled patients being treated for MPE as part of their standard medical care. After enrollment, pleural fluid was collected, and cytokines were measured at five distinct time points, which included T0: insertion of IPC; T1: 24–48 hours; T2: 72–96 hours; T3: 1 week; and T4: 2 weeks. Details of fluid processing are found in the online supplement. All patients followed the same standardized pleural fluid drainage algorithm shown in supplemental e-Figure 1.

Statistical Analysis

Descriptive statistics were used to summarize PFC at each specified time. Measurements were summarized by using the mean, median, standard deviation, and range. A scatter plot matrix was used to visually assess the correlation between cytokines for each time point of interest. Two patients were missing information on all six PFCs. One patient was missing cytokine information at T2, whereas another patient was missing cytokine information at T3. The last observation carried forward was applied in these circumstances, thus providing complete data on cytokine profiles for 22 patients.

Profile plots were used to characterize changes in PFCs from baseline, to identify patterns in the trajectory of PFC measurements over time, to visually assess variability, and to identify any potential outliers. Differences between baseline levels and subsequent levels at other evaluation times were computed to quantify the change in PFCs over the evaluation time points. After characterizing the profiles for each of the six PFCs and for exploratory purposes, patients were categorized into two cohorts: patients who had elective catheter removal versus those who did not.

A linear mixed model was introduced to assess the IPC removal cohort by time interaction while adjusting for baseline levels. Due to the limited sample size, *P* value adjustment was not considered. Correlation matrices for each PFC over time were computed, and the interclass correlation (ICC) was estimated to describe the portion of residual variance attributed between patients.

Information from individual profile plots suggested that the area under the curve (AUC) was a reliable measure quantifying the PFC experience for each patient. Therefore, the AUC for each PFC was computed by using the trapezoidal rule to summarize measures from T0 to T4 for each patient. With AUCs, low versus high pleural cytokine categories were developed by using portioning at the median.

AUC categories were used to compare the cumulative incidence of IPC removal, which was derived by using one minus the Kaplan-Meier (1-KM) estimate. Patients who had died before 90 days, with indwelling catheters in place, were censored at time of death. Cumulative incidence estimates were provided at days 30, 60, and 90 by AUC low versus high category, and the log-rank test was used to compare distributions. *P* values of <0.05 were considered as statistically significant and warranting further investigation. Analyses were conducted with use of Stata software (Release 15. College Station, TX; StataCorp LLC).

Results

A total of 32 patients underwent IPC treatment for MPE; however, analyzable data were not available for 10 patients due to lost to follow up or early death prior to 2 weeks collection period. Complete data were available for 22 patients, who were included in our analysis, which consisted of 10 men (45%) and 12 women (55%). The median patient age was 60 years (range: 22 to 76 years), and the median amount of fluid drained was 1000 ml (Table 1). Of the 22 patients, 11 had their IPC removed within 90 days of achieving auto pleurodesis; 6 did not achieve pleurodesis within 90 days; and 5 died with a catheter in place and were censored at their time of death.

The trend among patients who achieved auto pleurodesis and had IPC removed was a median level of bFGF that was significantly higher at baseline and at 2 weeks; the median level of IL-8 was significantly higher at 2 weeks; and the median level of VEGF was higher at all time points in those patients with IPC electively removed. Median levels of total TGF-βs did not show significant change over time (Table 2). Due to the high ICC estimates, strong outliers, and the small dataset, we focused on AUC levels (low vs high) for each PFC rather than on slopes of PFC response profiles, since AUCs provided an alternative, signaling that PFC trajectories had elevated levels within the assessment domain.

Aside from several outlying values identified for PFCs, individual profile plots showed that mean response trajectories remained relatively stable across time for the majority of PFCs, with IL-8 as the only exception. In addition compression between pleurodesis/IPC removal and no pleurodesis/No IPC removal is shown in Figure 1. Moreover, correlation analyses were conducted for each PFC. Although there was some degradation in the correlations from baseline to day 14, the majority of PFCs demonstrated strong and positive correlations across measured time points. Therefore, patients with a high PFC at baseline were expected to have high PFCs at subsequent time points. Supplemental e-Table 1 summarizes correlations, illustrating that repeated responses occurring in the same patient were highly correlated at adjacent time points. IL-8 was the exception. Temporal variability of PFC measurements over a 2-week period is shown in supplemental e-Figure 2

Assessments of the ICC suggested a higher between-subject relative to within-subject residual variability (supplemental e-Table 2). From the ICC, we noticed that 70% of the residual variance was between subjects for total TGF-b1, and more than 90% of the residual variance was between subjects for total TGF-b2, TGF-b3, and VEGF. Furthermore, findings from the linear mixed model found no significant pleurodesis cohort by time interaction

after baseline adjustment except for IL-8. For IL-8, the pleurodesis by time interaction was statistically significant ($P = .007$), evidence of the association between patients with steeper IL-8 trajectories and IPC removal within 90 days. Except for IL-8, the covariates describing baseline levels for all PFCs were statistical significant ($P < .001$).

The cumulative incidence was used to compare the rate of IPC removal between patients with low versus high PFC AUCs. Patients with a high AUC for IL-8, bFGF, and VEGF had a higher cumulative incidence of IPC removal at 90 days (Table 3). The cumulative incidence (CumInc) of IPC removal for patients with high bFGF AUCs was 38%, 53%, and 84% at 30, 60, and 90 days, respectively. For patients with low bFGF AUCs, the CumInc was 0%, 0%, 44% at the same time assessments ($P = .005$). (Figure 2) For IL-8, the CumInc for patients with high AUCs was 38%, 48%, and 90% at 30, 60, and 90 days, respectively. Patients with low IL-8 AUCs had a CumInc of 0%, 0%, 28% at the same time assessments ($P = .001$). Similarly for VEGF, the CumInc for patients with high AUCs was 38%, 50%, and 81% at 30, 60, and 90 days, respectively. Patients with low VEGF AUCs had a CumInc of 0%, 0%, and 28% at the same time assessments ($P = .006$). For patients with high AUCs for bFGF, IL-8, and VEGF, the median time to IPC removal was approximately 60 days. Patients with low AUCs for these PFCs never reached a median time to IPC removal.

Discussion

This study is the first to assess PFCs in patients with IPCs longitudinally at scheduled intervals and to correlate PFC levels with outcome of achieving auto pleurodesis and elective IPC removal. In this small exploratory study, it appears that IL-8, VEGF, and bFGF are associated with auto pleurodesis in patients who are treated with an IPC. In addition, except for IL-8, all PFCs demonstrated strong and positive correlation across all measurement time points such that the first measurement correlates well with the last measurement.

The finding of higher IL-8 in those patients who achieved auto pleurodesis builds on the findings by Goodman et al., who tested the hypothesis that mesothelial cells play a role in regulating inflammatory responses within pleural space and provide a mechanism whereby mesothelial cells can respond to inflammatory stimuli in the pleural cavity with increased levels of IL-8.[6] This finding also builds on those of van den Heuvel et al., who evaluated the inflammatory process that causes pleurodesis, after the administration of talc, and concluded that talc-induced inflammation is characterized by an influx of polymorphonuclear cells, followed by an accumulation of macrophages.[12, 13] IL-8 has also been detected in inflammatory and infectious pleural effusions,[14, 15] in synovial fluid from patients with inflammatory rheumatic diseases,[16] and in the gastrointestinal mucosa in patients with active ulcerative colitis.[17] The concentration of IL-8 in our study increased over time, suggesting that either the underlying disease or inflammatory response to the IPC may influence the inflammatory process. However, we did not demonstrated a strong correlation across all measurement time points. Even though our finding could be causally related with pleurodesis, it could also be coincidental and we are interpreting this finding cautiously.

The finding of higher VEGF in those patients who achieved auto pleurodesis is similar to findings of pleurodesis in rabbit models, which describe an acute inflammatory reaction due

to pleurodesis that increases pleural fluid VEGF.[18] VEGF, which is present in areas of inflammation, has been found to be increased in exudative effusions and has been implicated in the genesis and maintenance of pleural effusion.[19]

The finding of higher bFGF in patients who achieved auto pleurodesis builds on findings by Antony et al., who demonstrated that patients who underwent successful pleurodesis after intrapleural talc insufflation had significantly higher levels of bFGF in their pleural fluid than did those who did not respond to pleurodesis.[8] *In vitro*, pleural mesothelial cells stimulated with talc released higher levels of biologically active bFGF, compared with controls, and it is believed that fibrin network formation and adhesions between visceral and parietal pleura are responsible for pleurodesis. In addition, patients who achieved successful chemical pleurodesis showed markedly increased levels of bFGF in the pleural fluid.[20, 21]

Our findings are different than those presented in another study of six patients by Shojaee et al, who demonstrated that pleural fluid TGF-β1 concentration in MPE increased significantly 2 weeks after IPC insertion, compared with the level at the time of IPC insertion. Plasminogen activator inhibitor-1 and VEGF levels did not significantly increase in that study.[22] TGF-β stimulates the synthesis of collagen and fibronectin by fibroblasts, increasing the extracellular matrix.[6–9] TGF-β has been suggested to be the main profibrotic agent in the setting of chemical pleurodesis.[14] Furthermore, recombinant TGF-β2, an isoform of TGF-β, has been used in experimental animal models to produce pleurodesis and has been shown to be at least as effective.[23–26] These differences were unexpected since TGF-β is a potent profibrotic cytokine and may be related to different methodology being used to assess PFC. We used MSD technology that simultaneously measures total TGF- β s (both active and latent TGF- β).[11] In addition the study by Shojaee et all used median measurements over time in a very small number of patients and this change could've been observed by chance. Nevertheless it is very helpful to know both active and total TGF-β levels simultaneously to better delineate how TGF-β is involved in pleurodesis however in this study we only measured total TGF-β. Total TGF-βs level were noted to be elevated in multiple pathological conditions including breast cancer[27], non-small cell lung cancer[28], hepatocellular carcinoma[29], and dilated cardiomyopathy[30, 31]. In addition we did use MSD to measure TGF-βs and in a study that compared a bioassay and enzyme-linked immunosorbent assay (ELISA), the bioassay demonstrated that both active and total tissue TGF-β levels were significantly higher in post-myocardial infarction than in sham myocardium. ELISA was markedly less sensitive in detecting both active and total TGF-β levels than our bioassay and failed to show any statistically significant difference in TGF-β levels between myocardial infarction and sham myocardium.[31] We believe that MSD technology is a more sensitive technology however since we only measured the total TGF-β the data should be interpreted with caution. Data on longitudinal changes in pleural PFCs is very scarce and is limited to just a few cytokines. In a study by Thomas et al., the authors performed longitudinal PFC testing from 35 patients for MCP-1, VEGF, IL-6, and IL-8 and concluded that pleural fluid MCP-1 increased significantly over time.[32] The study did not evaluate correlation to the outcome of pleurodesis, and unlike in our cohort, in which patients drained their catheter daily, their patients drained the effusion via the IPC when they were symptomatic, and the frequency of drainage varied from daily to every 2 weeks.

Our study had the limitation of being a small exploratory study and best for hypothesis generating. Also, change in PFCs, may be secondary to tumor response to treatment, tumor progression, and multitude of underlying patient and disease factors that are not accounted for. In addition, we had to exclude 10 patients due to incomplete data points, resulting from many patients finding frequent visits to the clinic for fluid removal to be cumbersome; also, 5 patients died, and we do not know if they would have achieved pleurodesis, and this uncertainty could have introduced bias. Finally, due to the feature of multiplex assay, the VEGF signal in our study was much higher than other analytes designed on the same plate with reasonable dilution of specimens. Specifically, at the T4 time point, the VEGF signal either tended or was close to being saturated in the Meso Scale Discovery platform, among which some readings were in the above-fit-curve range, and the results should be interpreted with some caution.

This study to show that PFCs are likely involved in pleurodesis induced by IPCs, with higher levels of IL-8, VEGF, and bFGF in those patients who achieved auto pleurodesis. Except for IL-8, the majority of PFCs demonstrated strong and positive correlations across all measurement time points, and this evidence is useful for further studies directed toward developing biochemical criteria to help in the selection of suitable candidates for pleurodesis and in developing agents that can mediate inflammatory pathways. Further studies are needed to assess if these findings can be validated in further studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations:

MPE	malignant pleural effusion
IPC	indwelling pleural catheter
PFT	Pleural fluid cytokines

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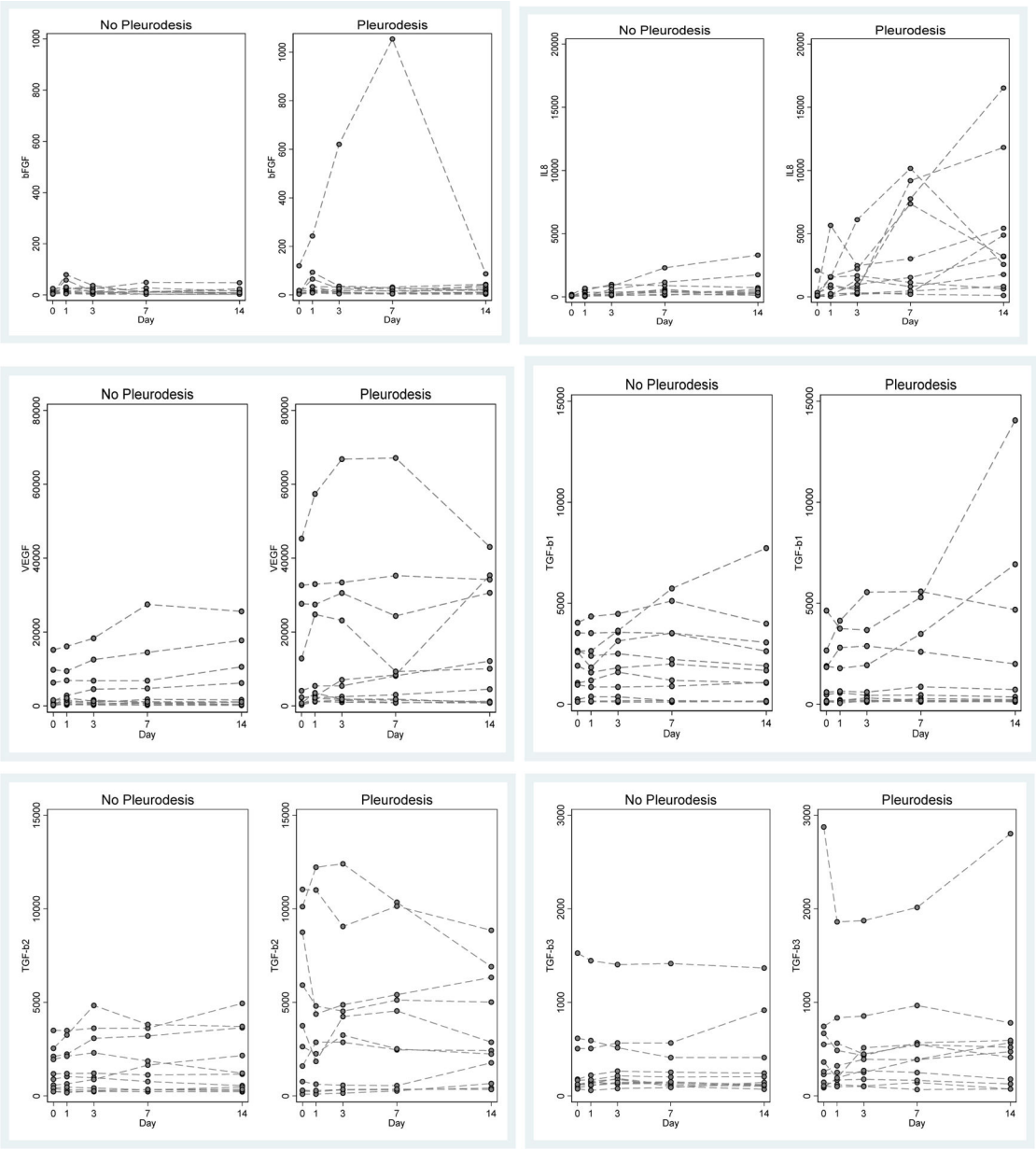

Figure 1:
Individual profile plots by pleural fluid cytokines and by pleurodesis (bFGF, IL-8, total TGF-b's, and VEGF)

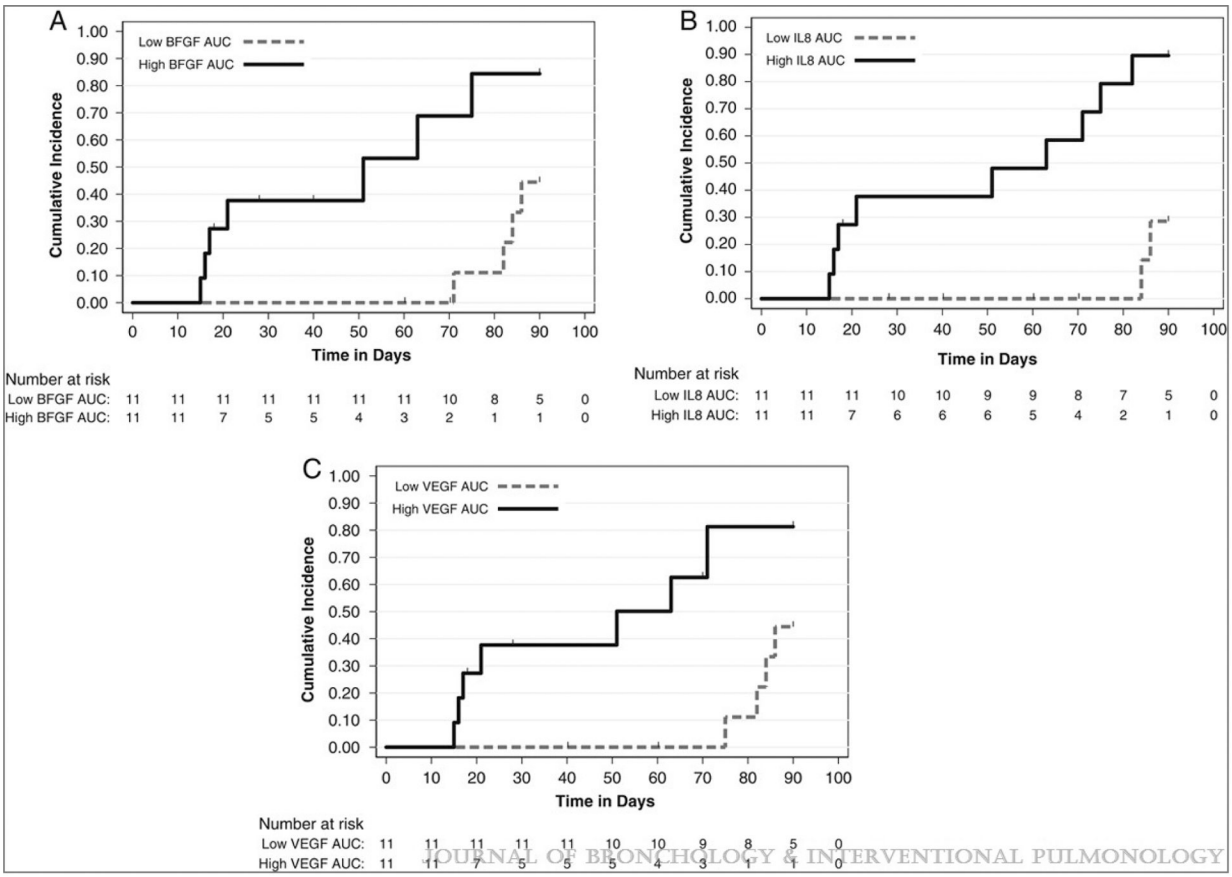

Figure 2:

Cumulative incidence plot (low vs high area under the curve): A: bFGF, B: IL-8, and C: VEGF

Table 1

Patient Characteristics

	N = 22 (%)
Age, years	
Mean ± SD	56.2 ± 15.5
Median (min to max)	60 (22 to 76)
Gender	
Female	12 (54.6)
Male	10 (45.4)
Race	
White	13 (59.09)
Black	3 (13.64)
Hispanic	5 (22.73)
Asian	1 (4.55)
Cancer diagnosis	
Breast	7 (31.82)
Lung	6 (27.27)
Other *	9 (4.55)
Amount of fluid drained (ml)	
Mean ± SD	1045 ± 473
Median (min to max)	1000 (300 to 2200)
Side of indwelling pleural catheter placement	
Left	9 (40.91)
Right	13 (59.09)

* "Other" includes: colon, gastric, mesothelioma, ovarian, pancreatic, prostate, rectal, renal cell, and sarcoma

Table 2.

Summary of Cytokines that Reached Pleurodesis Within 90 Days

	No (N = 6)	Yes (N = 11)	P-value	Total (N = 17)
Summary of BFGF				
Time 0				
Mean ± SD	4.63 ± 1.94	20.51 ± 33.56	0.0208	14.9 ± 27.68
Median (min to max)	4.09 (2.85 to 7.79)	9.55 (3.28 to 120.30)		6.9499998 (2.85 to 120.30)
Time 24–48 hours				
Mean ± SD	28.35 ± 27.24	50.88 ± 68.78	0.3657	42.92 ± 57.55
Median (min to max)	23.08 (4.91 to 79.28)	19.15 (8.67 to 242.80)		19.15 (4.91 to 242.80)
Time 72–96 hours				
Mean ± SD	12.1 ± 12.84	72.5 ± 182.14	0.1594	51.18 ± 147.21
Median (min to max)	7.66 (3.06 to 37.15)	16.44 (4.59 to 620.71)		11.31 (3.06 to 620.71)
Time 1 week				
Mean ± SD	9.03 ± 4.69	109.33 ± 313.61	0.1914	73.93 ± 252.82
Median (min to max)	11.15 (3.06 to 13.48)	15.62 (4.02 to 1054.39)		12.05 (3.06 to 1054.39)
Time 2 weeks				
Mean ± SD	9.48 ± 7.44	27.55 ± 23.84	0.0444	21.17 ± 21.26
Median (min to max)	8.71 (2.68 to 23.61)	19.97 (3.10 to 87.14)		12.1 (2.68 to 87.14)
AUC BFGF				
Mean ± SD	163.98 ± 102.47	1001.82 ± 2454.63	0.1078	706.11 ± 1984.78
Median (min to max)	158.95 (50.65 to 319.87)	258.38 (76.02 to 8390.62)		229.22501 (50.65 to 8390.62)
Summary of IL-8				
Time 0				
Mean ± SD	75.39 ± 37.28	343.05 ± 590.55	0.1914	248.58 ± 485.58
Median (min to max)	77.4 (24.28 to 114.84)	124.43 (39.76 to 2081.83)		96.230003 (24.28 to 2081.83)
Time 24–48 hours				
Mean ± SD	186.17 ± 200.74	1167.75 ± 1612.57	0.0704	821.31 ± 1368.07
Median (min to max)	98.88 (13.96 to 514.43)	724.39 (36.53 to 5660.13)		266.66 (13.96 to 5660.13)
Time 72–96 hours				
Mean ± SD	414.56 ± 335.14	1539.24 ± 1704.6	0.0562	1142.29 ± 1469.04
Median (min to max)	300.79 (114.52 to 991.32)	975.45 (215.25 to 6108.06)		612.09003 (114.52 to 6108.06)
Time 1 week				
Mean ± SD	598.46 ± 371.61	3816.43 ± 3947.67	0.0875	2680.67 ± 3506.55
Median (min to max)	493.11 (162.00 to 1163.65)	1539.89 (202.08 to 10166.08)		897.04999 (162.00 to 10166.08)
Time 2 weeks				
Mean ± SD	604.82 ± 600.05	4636.94 ± 5112.12	0.012	3213.84 ± 4515.65
Median (min to max)	397 (104.91 to 1750.07)	3173.5 (112.50 to 16531.11)		1750.0699 (104.91 to 16531.11)
AUC IL8				
Mean ± SD	6969 ± 4782.11	43760.51 ± 37519.05	0.009	30775.27 ± 34862.54
Median (min to max)	4903.91 (2308.99 to 14456.05)	23146.94 (2834.78 to 103954.00)		14975.12 (2308.99 to 103954.00)

	No (N = 6)	Yes (N = 11)	P-value	Total (N = 17)
Summary of total TGF-B1				
Time 0				
Mean ± SD	1398.47 ± 1378.87	1162.72 ± 1461.54	0.8407	1245.92 ± 1393.81
Median (min to max)	1012.34 (123.62 to 3531.14)	483.51 (82.47 to 4635.28)		600.28003 (82.47 to 4635.28)
Time 24–48 hours				
Mean ± SD	1278.32 ± 1272.68	1315.74 ± 1550.26	1.00	1302.53 ± 1417.24
Median (min to max)	1018.69 (133.52 to 3522.43)	571.08 (63.74 to 4136.32)		647.04999 (63.74 to 4136.32)
Time 72–96 hours				
Mean ± SD	1571.8 ± 1482.75	1457.22 ± 1837.35	0.9199	1497.66 ± 1673.36
Median (min to max)	1221.48 (114.46 to 3562.62)	441.1 (116.35 to 5550.09)		604.28003 (114.46 to 5550.09)
Time 1 week				
Mean ± SD	1568.07 ± 1563.76	1748.48 ± 2135.76	0.9199	1684.81 ± 1903.41
Median (min to max)	1040.95 (114.46 to 3522.80)	456.67 (126.30 to 5581.78)		867.01001 (114.46 to 5581.78)
Time 2 weeks				
Mean ± SD	1356.78 ± 1230.5	2689.96 ± 4389.06	0.8407	2219.42 ± 3597.82
Median (min to max)	1071.63 (144.72 to 3060.38)	365.6 (125.26 to 14053.40)		725.17999 (125.26 to 14053.40)
AUC TGF-B1				
Mean ± SD	20705.2 ± 19685.23	25958.12 ± 33658.76	0.9199	24104.15 ± 28911.28
Median (min to max)	15174.56 (1812.25 to 46234.86)	6212.96 (2036.35 to 97266.12)		10390.24 (1812.25 to 97266.12)
Summary of total TGF-B2				
Time 0				
Mean ± SD	1329.49 ± 1363.71	4111.98 ± 4172.77	0.2689	3129.93 ± 3652.71
Median (min to max)	658.77 (230.02 to 3501.36)	2634.4 (99.00 to 11038.46)		1596.38 (99.00 to 11038.46)
Time 24–48 hours				
Mean ± SD	1459.64 ± 1513.17	3692.98 ± 4243.26	0.4214	2904.74 ± 3630.3
Median (min to max)	751.09 (175.36 to 3493.36)	2255.11 (98.93 to 12222.38)		1845.08 (98.93 to 12222.38)
Time 72–96 hours				
Mean ± SD	1736.02 ± 1984.5	3873.75 ± 3903.92	0.3149	3119.26 ± 3444.56
Median (min to max)	698.38 (264.24 to 4835.21)	3251.09 (151.67 to 12404.91)		2869.52 (151.67 to 12404.91)
Time 1 week				
Mean ± SD	1533.93 ± 1701.62	3823.37 ± 3719.29	0.2278	3015.34 ± 3289.74
Median (min to max)	558.37 (322.34 to 3816.11)	2517.12 (266.47 to 10349.66)		2464.8601 (266.47 to 10349.66)
Time 2 weeks				
Mean ± SD	1718.48 ± 2061.88	3440.72 ± 2901.04	0.1317	2832.87 ± 2703.39
Median (min to max)	514.49 (278.36 to 4945.72)	2423.71 (346.44 to 8852.58)		2240.1699 (278.36 to 8852.58)
AUC TGF-B2				
Mean ± SD	22513.56 ± 25179.88	52287.8 ± 49868.3	0.1914	41779.25 ± 44356.74
Median (min to max)	8353.97 (4287.96 to 55075.76)	36137.89 (4446.90 to 141713.42)		35750.746 (4287.96 to 141713.42)
Summary of total TGF-B3				
Time 0				

	No (N = 6)	Yes (N = 11)	P-value	Total (N = 17)
Mean ± SD	419.73 ± 564.55	557.59 ± 803.18	0.5465	508.93 ± 712.32
Median (min to max)	151.21 (75.30 to 1526.56)	260.25 (87.95 to 2874.90)		230.12 (75.30 to 2874.90)
Time 24–48 hours				
Mean ± SD	418.03 ± 527.33	463.36 ± 514.46	0.4817	447.36 ± 502.81
Median (min to max)	189.43 (59.92 to 1445.91)	251.22 (100.39 to 1860.71)		221.8 (59.92 to 1860.71)
Time 72–96 hours				
Mean ± SD	439.58 ± 502.38	492.94 ± 506.36	0.5465	474.1 ± 489.71
Median (min to max)	221.71 (79.28 to1403.95)	391.29 (101.10 to 1874.01)		271.48999 (79.28 to 1874.01)
Time 1 week				
Mean ± SD	429.68 ± 513.93	550.1 ± 548.28	0.4817	507.6 ± 523.4
Median (min to max)	201.79 (90.56 to 1414.93)	389.99 (68.33 to 2014.62)		387.37 (68.33 to 2014.62)
Time 2 weeks				
Mean ± SD	476.75 ± 537.46	600.83 ± 768.13	0.6875	557.04 ± 680.27
Median (min to max)	194.45 (71.16 to 1367.14)	472.59 (74.35 to 2803.65)		412.07999 (71.16 to 2803.65)
AUC TGF-B3				
Mean ± SD	6187.52 ± 7220.26	7581.11 ± 8329.83	0.6153	7089.25 ± 7754.28
Median (min to max)	2685.16 (1371.54 to 19711.10)	5571.5 (1141.01 to 30743.73)		5387.1396 (1141.01 to 30743.73)
Summary of VEGF				
Time 0				
Mean ± SD	1376.93 ± 2414.69	11762.51 ± 15975.89	0.035	8097.01 ± 13693.51
Median (min to max)	478.36 (134.75 to 6295.39)	2311.1 (296.12 to 45284.12)		745.02002 (134.75to45284.12)
Time 24–48 hours				
Mean ± SD	1842.58 ± 2550.83	14605.92 ± 18598.93	0.021	10101.21 ± 16054.95
Median (min to max)	634.74 (296.57 to 6874.28)	3489.2 (1143.42 to 57380.80)		2195.95 (296.57 to 57380.80)
Time 72–96 hours				
Mean ± SD	1800.04 ± 2512.52	15899.53 ± 20890.9	0.016	10923.24 ± 17971.6
Median (min to max)	916.98 (266.70 to 6813.00)	5407.15 (1004.13 to 66815.98)		1785.59 (266.70 to 66815.98)
Time 1 week				
Mean ± SD	1674.19 ± 2542.72	14595.26 ± 20582.05	0.012	10034.88 ± 17529.82
Median (min to max)	832.9 (225.24 to 6813.00)	8128.32 (825.23 to 67123.98)		1782.71 (225.24 to 67123.98)
Time 2 weeks				
Mean ± SD	2239 ± 4096.85	15820.22 ± 16513.53	0.021	11026.85 ± 14847.11
Median (min to max)	711.07 (164.76 to 10563.39)	10061.64 (811.32 to 43031.78)		1173.42 (164.76 to 43031.78)
AUC VEGF				
Mean ± SD	25896.97 ± 40755.28	211133.43 ± 259132.97	0.012	145755.86 ± 225418.68
Median (min to max)	11474.5 (3426.43 to 108341.48)	113531.49 (13519.15 to 828954.31)		24193.99 (3426.43 to 828954.31)

*AUC: Area under the curve

Table 3.

Cumulative Incidence of IPC Removal by Cytokine Levels

	No. of Patients	No. of^ Events	Cumulative Incidence (95% CI)			P-value
			30 Days	60 Days	90 Days	
Overall Demographics	22	11	0.18 (0.07, 0.42)	0.24 (0.11, 0.48)	0.62 (0.40, 0.84)	
Age, years						
<60	10	5	0.20 (0.05, 0.59)	0.20 (0.05, 0.59)	0.67 (0.34, 0.95)	0.703
≥60	12	6	0.17 (0.04, 0.52)	0.26 (0.09, 0.61)	0.58 (0.31, 0.86)	
Gender						
Female	12	4	0.17 (0.04, 0.52)	0.27 (0.09, 0.63)	0.42 (0.17, 0.79)	0.204
Male	10	7	0.20 (0.05, 0.59)	0.20 (0.05, 0.59)	0.77 (0.48, 0.96)	
Cytokine levels						
BFGF						
Low AUC	11	4	0.00 (--)	0.00 (--)	0.44 (0.20, 0.80)	0.005
High AUC	11	7	0.38 (0.16, 0.72)	0.53 (0.25, 0.86)	0.84 (0.51, 0.99)	
IL-8						
Low AUC	11	2	0.00 (--)	0.00 (--)	0.28 (0.08, 0.74)	0.001
High AUC	11	9	0.38 (0.16, 0.72)	0.48 (0.23, 0.80)	0.90 (0.63, 0.99)	
Total TGF-β1						
Low AUC	11	7	0.27 (0.10, 0.63)	0.36 (0.15, 0.70)	0.68 (0.40, 0.92)	0.473
High AUC	11	4	0.09 (0.01, 0.49)	0.09 (0.01, 0.49)	0.53 (0.23, 0.89)	
Total TGF-β2						
Low AUC	11	4	0.09 (0.01, 0.49)	0.09 (0.01, 0.49)	0.48 (0.21, 0.84)	0.288
High AUC	11	7	0.27 (0.10, 0.63)	0.36 (0.15, 0.70)	0.74 (0.44, 0.96)	
Total TGF-β3						
Low AUC	11	4	0.09 (0.01, 0.49)	0.09 (0.01, 0.49)	0.53 (0.23, 0.89)	0.473
High AUC	11	7	0.27 (0.10, 0.63)	0.36 (0.15, 0.70)	0.68 (0.40, 0.92)	
VEGF						
Low AUC	11	4	0.00 (--)	0.00 (--)	0.44 (0.20, 0.80)	0.006
High AUC	11	7	0.38 (0.16, 0.72)	0.50 (0.24, 0.83)	0.81 (0.47, 0.99)	

*AUC (area under the curve)

^Number of events = number of elective catheter removal by 90 days