PLEURAL FLUID CALPROTECTIN LEVELS IN BENIGN AND MALIGNANT PLEURAL EFFUSIONS AND ITS SIGNIFICANCE IN PREDICTING SUCCESS OF PLEURODESIS IN MALIGNANT PLEURAL EFFUSIONS

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In partial fulfillment of the requirement for the degree

of Doctorate of Medicine (DM)

Pulmonary, Critical Care and Sleep Medicine

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AIIMS Jodhpur

DECLARATION

I hereby declare that the thesis titled **"PLEURAL FLUID CALPROTECTIN LEVELS IN BENIGN AND MALIGNANT PLEURAL EFFUSIONS AND ITS SIGNIFICANCE IN PREDICTING SUCCESS OF PLEURODESIS IN MALIGNANT PLEURAL EFFUSIONS"** embodies the original work carried out by the undersigned in All India Institute of Medical Sciences, Jodhpur.

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CERTIFICATE

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Dr Amartya Chakraborti

LIST OF ABBREVIATIONS

PE	**Pleural effusion**
MPE	Malignant pleural effusion
AUC	Area Under Curve
BAL	Broncho Alveolar Lavage
BMI	Body Mass Index
CAD	Coronary Artery Disease
CBC	Complete Blood Count
CI	Confidence Interval
CKD	Chronic Kidney Disease
CT	Computed Tomography
CRP	C- Reactive Protein
CXR	Chest X-ray
ECG	Electrocardiogram
ICU	Intensive care unit
KFT	Kidney Function Tests
LFT	Liver Function Tests
ROC	Receiver operating curve
SD	Standard deviation
TLC	Total Leucocyte counts
PT/INR	Prothrombin time/International normalized ratio
CLD	Chronic liver disease
CKD	Chronic Kidney disease
CAD	Coronary Artery Disease

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SUMMARY

Introduction- Differentiation of malignant from benign effusions is often difficult due to the lower yield of cytopathological examination of the pleural fluid. This exposes a patient, clinically suspected to have malignant effusion to a battery of invasive tests like thoracoscopy, bronchoscopy and imaging-guided biopsies. There is a dearth of biomarkers that can reliably differentiate between these two aetiologies . Even after diagnosis of malignant pleural effusion , pleurodesis failure remains a constant risk and no biomarker has been found which can reliably predict pleurodesis success or failure. Keeping this in mind, this study was done to see the efficacy of a novel biomarker in pleural fluid -Calprotectin in diagnosing malignant pleural effusions and its capacity in predicting pleurodesis success or failure.

Materials and Methods- This was a prospective observational ,time bound study, carried 1were enrolled over a period of 18 months . All patients over the age of 18 with pleural effusion were enrolled , the exclusion criteria being no consent , pregnant females and pus on thoracocentesis. After clinical history and examination , routine blood investigations, pleural fluid was sent for cytopathology , biochemical examinations like protein , sugar , ADA , CRP, ferritin , LDH , IL-6 and microbiological examinations for aerobic , fungal organisms and tuberculosis. Malignant effusions were diagnosed on the basis of either cytopathology or histopathology on tissues obtained by biopsy. Benign effusion was diagnosed on basis of clinical history , biochemical and microbiological examinations. Pleural fluid Calprotectin level was measured by a quantitative ELISA test (Krishgen -Human Calprotectin Elisa 96 test). Pleurodesis was carried out in patients with malignant pleural effusion if clinically indicated. Lignocaine was used as an anesthetic and Inj Doxycycline was used as a sclerosing agent.

Statistical analysis was done using MedCalc Version 20.115. The accuracy of calprotectin levels for discriminating MPE from BPE were evaluated using receiver operating characteristic (ROC) curves. Sensitivity, specificity, positive or negative Likelihood ratios, and their corresponding confidence intervals (CIs) were calculated. The degree of significance in this study was taken to be below 0.05 ($P<0.05$ was considered significant).

Results- Out of the 63 patients who underwent the study , 31(49.2%) had malignant effusion while the rest 32 (50.8%) patients had benign effusion. Parapneuomonic effusion was seen in 17(26.9%) patients , tubercular effusion in 12(19.05%) patients while 3(4.8%) patients had transudative effusion. On multiple logistic regression , pleural fluid Calprotectin level was found to independently differentiate malignant from benign , parapneumonic and tubercular pleural effusions. Area under curve(AUC) of Calprotectin as a differentiator of malignant and benign effusion was 0.845. In 15 patients with malignant pleural effusion who underwent pleurodesis , five patients had pleurodesis failure. Calprotectin levels were also significantly different between patients who had pleurodesis success or failure.

Conclusion- Pleural fluid Calprotectin has the potential to become a veritable marker of malignancy in pleural fluid. It also can be a reliable marker of pleurodesis success or failure. Further studies with a larger sample size and varied population are required to establish its role in the management of suspected malignant pleural effusion.

INTRODUCTION

Pleural effusion (PE) can occur as a consequence of more than 50 recognized etiologies and there exists diagnostic challenges in differentiating benign or infective effusions from malignant effusions. Thoracentesis is the first and simplest procedure for the diagnosis of PE. Pleural fluid biochemical examinations help the clinician to broadly separate the effusions into transudative and exudative effusions with the help of the Lights criteria [1] . But to make the final aetiological diagnosis the clinician has to rely on cytological and microbiological methods. Although the specificity of pleural fluid cytology for establishing malignant pleural effusion(MPE) is 100%, the sensitivity varies from 46% (95% CI 42-58%)[2].When the cytology results are negative, more invasive methods such as a pleural biopsy , fibreoptic bronchoscopy thoracoscopy and CT/USG guided biopsies are required which are associated with their own set of morbidity and complications. New biomarkers are being studied to improve the diagnosis of PE like survivin, CEA ,VEGF, fibulina-3, CA-19-9 and their combinations [3,4,5].

Calprotectin is a heterocomplex of the two S100 calcium binding proteins, S100A8 (calgranulin A) and S100A9 (calgranulin B). Both these proteins are expressed strongly in myeloid cell lines [6]. They are released after the interaction of innate immunity cells with any microorganism and help in the adhesion of leucocytes to the endothelium and their migration to the sites of inflammation[6]. It also plays a critical anti-apoptotic role in tumor biology , whereby their low values lead to tumour progression [6]. Hence it has been postulated that levels of calprotectin will be low in malignant lesions that have metastasized and led to development of malignant pleural effusion whereas calprotectin levels will be raised in patients with effusions due to benign disease processes like inflammation and infection. There are few studies that have looked into the diagnostic role of Calprotectin in pleural effusions but they have an important shortcoming[7,8,9] . They all have clubbed tubercular , parapneumonic, transudative and other causes of non malignant effusion into one heading of benign effusion

and have not studied Calprotectin levels in these individual scenarios. In our country , infective pleural effusions are extremely common and tuberculosis being a master masquerader of malignancy often poses a diagnostic dilemma to the clinician. We aimed to correct this lacuna in our prospective cross sectional study. Secondly, we also studied the role of Calprotectin during the management of malignant pleural effusions by predicting success of pleurodesis .

OBJECTIVES

Primary:

To see the association of pleural fluid calprotectin levels with malignant and benign pleural effusion.

Secondary:

To find if any relationship exists between pleural fluid calprotectin levels and the success rate of pleurodesis in patients with malignant pleural effusion.

REVIEW OF LITERATURE

Botana-Rial et al (2020) carried out a multicentre study in to validate calprotectin as a diagnostic biomarker of PE in clinical settings. A total of 425 patients were recruited, and the pleural fluid samples collected had BPE in 223 cases (53.7%) or MPE in 137 patients (33%). Calprotectin levels ranged from 772.48 to 3,163.8 ng/mL (median: 1,939 ng/mL) in MPE, and 3,216–24,000 ng/mL in BPE (median: 9,209 ng/mL; $p < 0.01$), with an area under the curve of 0.848 [95% CI: 0.810–0.886]. For a cut-off value of ≤ 6,233.2 ng/mL, they found 96% sensitivity and 60% specificity, with a negative and positive predictive value, and negative and positive likelihood ratios of 96%, 57%, 0.06, and 2.4, respectively. Multivariate analysis showed that low calprotectin levels was a better discriminator of PE than any other variable [OR 28.76 ($p < 0.0001$)][7].

Otero et al (2018) carried out a study in which Calprotectin concentration was measured in 156 individuals diagnosed with exudative PE (67 malignant and 89 benign). Calprotectin accuracy for discriminating between malignant and benign PE was evaluated using receiver operating characteristic (ROC)curves. Univariate and multivariate logistic regression were performed to test the association between calprotectin levels and malignant PE. It was found that Calprotectin levels were significantly lower in malignant pleural fluid (257.2 ng/ ml, range: 90.7–736.4) than benign effusions (2627.1 ng/ ml, range: 21–9530.1). The area under the curve was 0.963. A cutoff point of p736.4 ng /ml rendered a sensitivity of100%, with a specificity of 83.15%. Logistic regression demonstrated a strong association between calprotectin and malignancy (adjusted OR 663.14)[8].

Mohammed et al (2018) did a similar study in patients with pleural effusions and found that s Pleural calprotectin level in MPEs (229.2±168.6 ng/ ml) was significantly lower than its level of infectious pleural effusions (3202.2±1304.8 ng/ml; P<0.001). The cutoff value of

calprotectin level for the diagnosis of MPE was less than or equal to 730.5 ng/ml, with 95% confidence interval and the area under the curve was 0.999, the corresponding sensitivity was 96.7 and the specificity was 100% ($P<0.001$)[9].

In a study by Luo et al (2015), the role of combined levels of calprotectin and CXCL12 were looked at in diagnosis of malignant pleural effusions. It was found that Calprotectin and CXCL12 levels of patients with MPE were significantly lower than that of BPE and tuberculous PE ($P < 0.05$). The area under the curve (AUC) of calprotectin and CXCL12 was 0.683 and 0.641 in MPE and BPE, and a combination of calprotectin 500.19 ng/ mL and CXCL12 6.11 ng/mL rendered a sensitivity and specificity of 48.72% and 78.57%, respectively. While in MPE and tuberculous PE, the AUC of calprotectin and CXCL12 was 0.696 and 0.690, and a combination of calprotectin 421.73 ng/mL and CXCL12 3.71 ng/mL presented a sensitivity and specificity of 25.64% and 95.45%, respectively. Multivariate logistic regression demonstrated that both calprotectin and CXCL12 were independent predictors of MPE[10].

MATERIALS AND METHODS

Study setting - Department of Pulmonary Medicine at All India Institute of Medical Sciences, Jodhpur

Study design – Prospective observational study

Study participant - All patients with undiagnosed pleural effusions presenting to pulmonary medicine through OPD or IPD of AIIMS Jodhpur for a period of 18 months from date of ethical clearance.

Study duration - 24 months

Inclusion criteria- All patients aged more than 18 years with undiagnosed pleural effusions and giving consent to participate in the study.

Exclusion criteria- 1)Patients showing frank pus on the diagnostic thoracentesis.

2)Pregnant females

Calculation of sample size:

This was a time bound study. All patients satisfying the inclusion criteria during the enrolment period of 18 months were included in the study.

Methodology -

Institute ethics committee approval was taken prior to the commencement of the study. All patients satisfying the inclusion criteria during the study period were enrolled under the study after acquiring the informed written consent. Routine clinical examination were done along with a proper clinical history. Blood investigations like CBC, LFT, KFT, Serum electrolytes, Sputum for AFB were done along with coagulation parameters like PT/PTT/INR and virological markers for HIV, Hepatitis-B and Hepatitis-C. All patients underwent a chest

radiography, ultrasound chest and if necessary, a CECT thorax. Thoracentesis was done under ultrasound guidanceand 50 ml of pleural fluid sent for –

(a)Physical examination, including color, aspect, turbidity, and specific gravity.

(b) Chemical examination, including total proteins, glucose, and lactate dehydrogenase (LDH), where effusions was classified into exudates or transudates according to Light's criteria. If the effusion had any of the following three properties, the effusion was classified into exudates:(i) A ratio of the concentration of total proteins in pleural fluid to serum total proteins of more than 0.5.(ii) An absolute value of LDH of more than 200 IU.(iii) A ratio of pleural fluid LDH to serum LDH of more than 0.6.

(c) Bacteriological examination: including culture and sensitivity, pleural fluid examination for Gram stain and ZN stain and CBNAAT.

(d)Cytological examination followed by differential cell count.

(e)Pleural fluid ferritin and CRP

(f) Pleural fluid Calprotectin level which was measured by a quantitative ELISA test.

USG guided closed pleural biopsies or thoracoscopy was done in patients in whom diagnosis could not be made even after the above mentioned tests. If clinically indicated , other invasive tests like Fibre optic bronchoscopy /CT/USG guided biopsies were done from other sites as per the discretion of the treating physician. The diagnosis of tuberculous effusion was based on the presence of positive stain /culture/CBNAAT for Mycobacterium tuberculosis in the pleural fluid, sputum, or pleural biopsy or the presence of typical caseating granulomas in the pleural biopsy. A clinical diagnosis of tuberculous pleural effusion was made in absence of positive biopsy features in patients with exudative pleural effusion with ADA>40U/L and positive clinical history of fever,weight loss and suggestive radiological picture.

In patients with malignant pleural effusion, when clinically indicated, tube thoracostomy was done. Pleurodesis was attempted after full lung re-expansion as confirmed by a chest radiograph and ultrasound chest. After taking informed written consent, drug sensitivity test was done for lignocaine and doxycycline. In case of hypersensitivity to doxycycline , other sclerosing agent like povidone iodine was used. Premedication with Injlignocaine(3 mg/kg; maximum 250 mg) was done followed by administration of inj. Doxycycline(500mg) through the intercostal tube(ICT)[11]. The intercostal tube was clamped for 1 h after Inj doxycycline administration. After 1 hour the clamp was removed and subsequent fluid drainage noted daily. NSAIDS or steroid administration was withheld for a period of 48 hours and in case of post procedure pain, Tramadol was used as an analgesic. Pleurodesis failure was considered when there is continued drainage of pleural fluid(>250 ml/day) even after 48 hours or if there is incomplete symphysis between visceral and parietal pleura as evidenced by chest radiography or ultrasound chest[11].Association between levels of calprotectin in the pleural fluid and success of pleurodesis was studied.

Statistical analysis:

Statistical analysis was done using MedCalc Version 20.115. Quantitative data was represented as means and standard deviation if normally distributed or as medians and interquartile range if non-normally distributed. Categorical data was presented as number and percentages. The accuracy of calprotectin levels for discriminating MPE from BPE were evaluated using receiver operating characteristic (ROC) curves. Sensitivity, specificity, positive or negative Likelihood ratios, and their corresponding confidence intervals (CIs) was calculated. The degree of significance in this study was taken to be below 0.05 ($P<0.05$ was considered significant).

Ethical Consideration

Once approved by the Research committee, the protocol was submitted to the Institute Ethics Committee for ethical clearance. A written informed consent was taken from all eligible participants. Participants were fully informed about the study and its utility.

RESULTS

Demographic data

In our study, 63 patients were enrolled out of which 43 (68.25%) were male while the rest 20(31.75%) were female. The mean ± standard deviation of the age of these patients was 54.22 ± 15.26 years. This data is represented in the form of a histogram in Figure 1. Hypertension was the commonest comorbidity seen in 25(31%) patients, followed by Diabetes seen in 22(27%) patients and CAD seen in 5(6%) patients. The distribution of comorbidities among patients is represented in the form of a pie chart in Figure 2. Addiction history was present in 39(53.97%) patients, out of which the commonest was smoking, opium, and alcohol , found in 41.27%, 14.29%, and 4.76%, respectively. Among smokers, the mean ± standard deviation of the Smoking Index(SI) was 933.33 ± 362.1. Past or recently diagnosed history of malignancy was also taken during our evaluation and positive history was found in 6(9.52%) patients. This demographic data is represented in Table 1.

Table 1: Demographic data of study population

S no.	Parameters	
1	Age(yrs) mean ± sd	54.22 ± 15.26
2	Gender(n,%)	
	Male	43 (68.25%)
	Female	20 (31.75%)
3	Comorbidities (n,%)	
	Diabetes	22 (34.92%)
	Hypertension	25 (39.68%)
	COPD	2 (3.2%)
	CAD	5 (7.93%)
	CLD	2 (3.2%)
	CKD	2 (3.2%)
	None	24(29%)
4	Addiction (n,%)	
	Smoking	26 (41.27%)
	Alcohol	3 (4.76%)

	Opium	9 (14.29%)
	Tobacco chewing	4 (6.35%)
	None	29 (46.03%)
5	H/O of previous malignancy (n,%)	6(9.52%)

Figure 1 – Histogram of the age of study participants

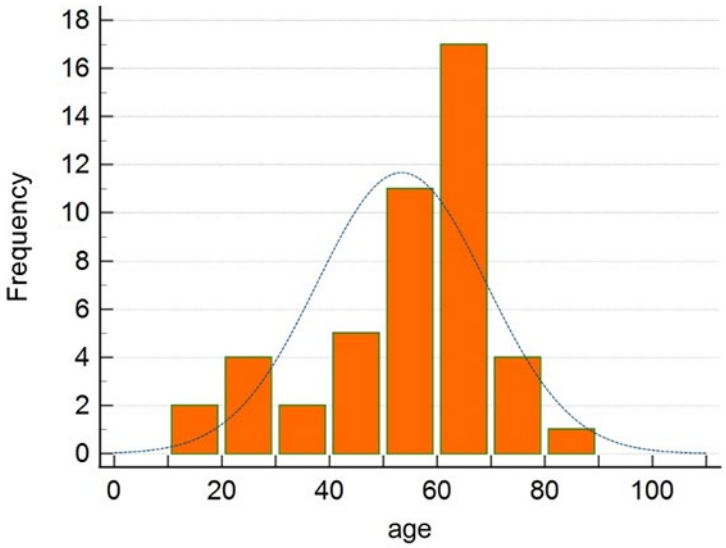
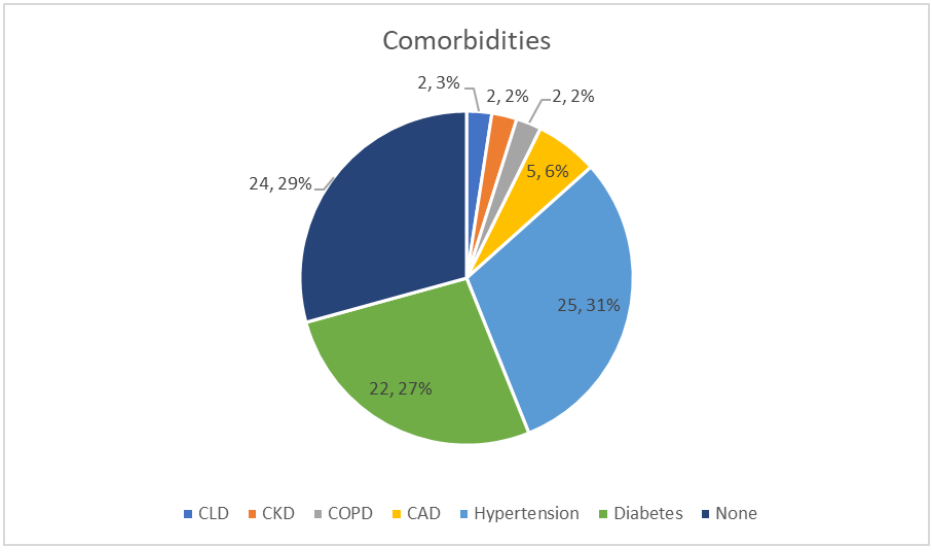


Figure 2 – Pie chart of comorbidities of patients



Symptomology

The commonest symptoms seen in the study population were Cough and Chest pain, both seen in 62(98.41%) patients, followed by Shortness of breath seen in 51(80.95%) patients and loss of weight in 43(68.25%) patients. Haemoptysis was seen in 4.76% of patients.

Table 2: Symptomology of the study population

S No.	Symptomology	(n,%)
1.	Cough	62 (98.41%)
2.	Chest Pain	62 (98.41%)
3.	Loss of weight	43 (68.25%)
4.	Shortness of breath	51 (80.95%)
5.	Haemoptysis	3 (4.76%)

Diagnostic Interventions

Thoracocentesis was the commonest intervention undertaken in 36(57.14%) patients, followed by CT/USG guided biopsy and Fibreoptic bronchoscopy, which both were undertaken in 8 (12.7%) patients. Thoracoscopy was carried out in 6(9.52%) patients. This data is represented in Table 3.

Table 3: Diagnostic interventions used in the study population

S No.	Diagnostic interventions	(n,%)
1	Thoracocentesis	36 (57.14%)
2	Thoracoscopy	6 (9.52%)
3	CT/USG guided biopsy	8 (12.7%)
4	EBUS/EUS	3 (4.76%)
5	Fibreoptic bronchoscopy	8 (12.7%)
6	FNAC	2 (3.17%)

Pleural fluid cytopathological and microbiological yield

Atypical cells were found in 15(23.81%) patients in the cytological examination of pleural fluid while inflammatory cells were found in 47(74.6%) patients. Out of the 31 patients with malignant pleural effusion, almost 50% had positive atypical cells. AFB was seen in 1 sample of pleural fluid. The mean $\pm$ sd of Neutrophil lymphocyte ratio (NLR) was 2 ± 4.46.

Pleural fluid for CBNAAT was positive in 7(11.11%) patients. Out of the 17 patients diagnosed with parapneumonic effusion, pleural fluid gram staining was positive in 7(41.12%) patients. All these 7 samples had a growth on aerobic culture and yielded E.coli, Klebsiella pneumonia, and Staphylococcus aureus in 3(42.86%), 3(42.86%), and 1(14.29%) patients respectively. Pleural fluid samples were all negative for KOH mount and fungal cultures were summarily negative.

The yield of Pleural fluid Biochemistry

The yield of the various biochemical parameters on pleural fluid examination is enumerated in Table 4 below.

Table 4: **Pleural fluid biochemical parameters**

S No.	Laboratory paarmeters	
1	Pl. fluid ADA (mean±sd)	25.18 ± 21.72 U/L
2	Pl.fluid Protein (mean±sd)	4.3 ± 1.2 mg/dl
3	Pl.fluid sugar (mean±sd)	89.48 ± 52.59
4	Pl.fluid LDH (mean±sd)	440.59 ± 225.72 U/L
5	Pl fluid Ferritin(mean±sd)	2259.01 ± 2349.0625 ug/ml
6	Pl.fluid IL 6(mean±sd)	15563.48 ± 24590.39 pg/ml
7	Pl.fluid CRP (mean±sd)	39.95 ± 62.15 mg/dl
8	Pl.fluid Calprotectin (mean±sd)	706.45 ± 356.04 ng/ml

Correlation between pleural fluid biochemical markers and Calprotectin levels

Correlation analyses were done between Calprotectin levels and pleural fluid biochemical parameters. Scatter diagrams done and Pearson correlation coefficient were calculated(r).The correlation coefficients of ADA ,LDH,CRP ,IL-6 and Ferritin with respect to Calprotectin

were 0.08,0.07,0.10,0.03 and 0.23 respectively . Ferritin values were did not achieve statistically significant correlation(p=0.07).

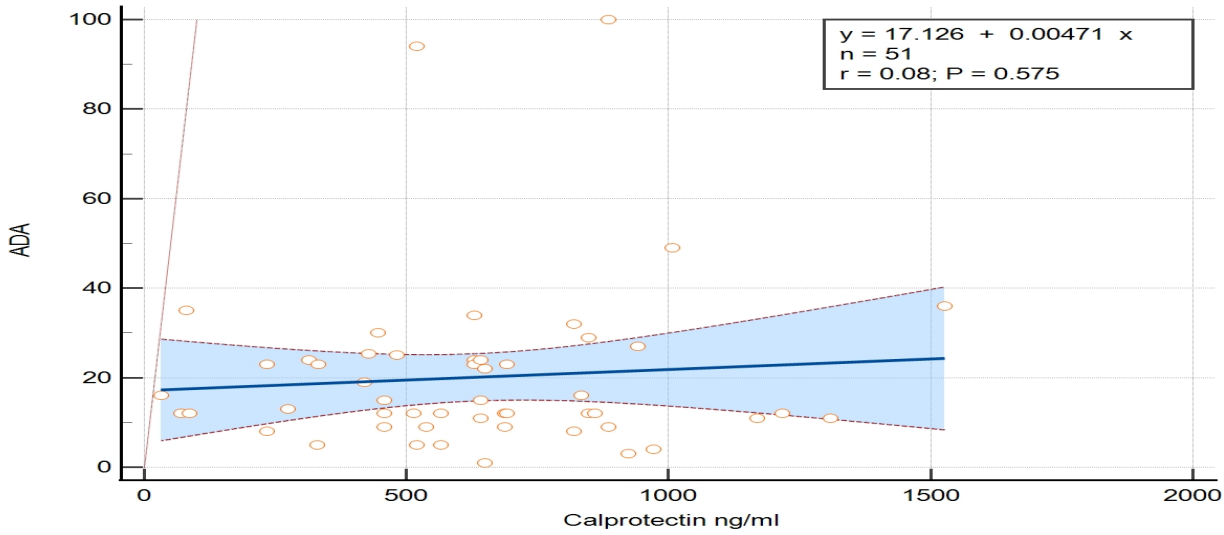


Figure 3: Scatter diagram of ADA and Calprotectin ng/ml

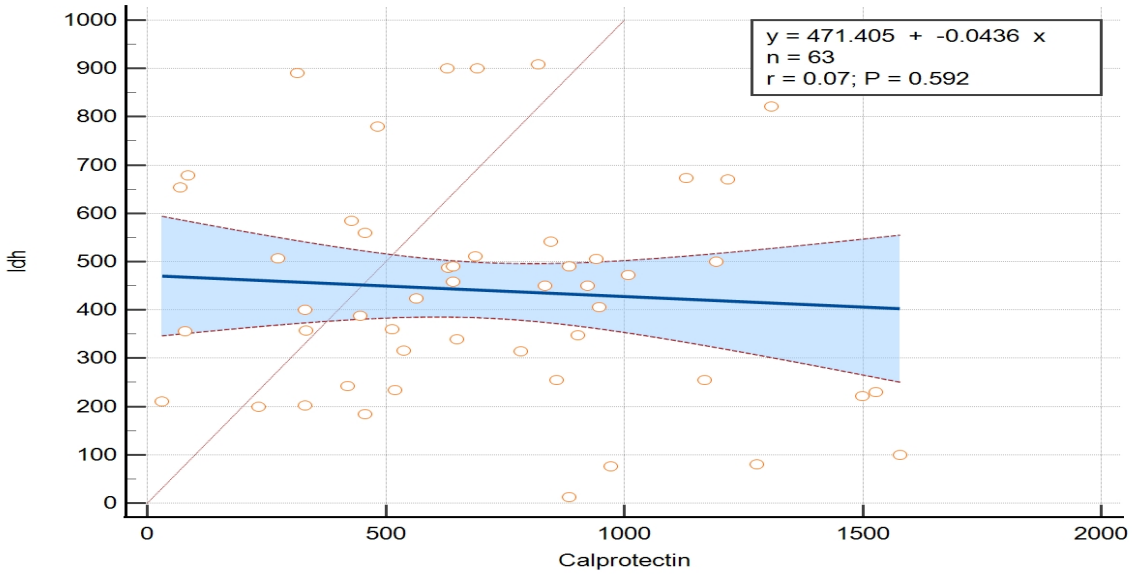


Figure 4: Scatter diagram between LDH levels and Calprotectin

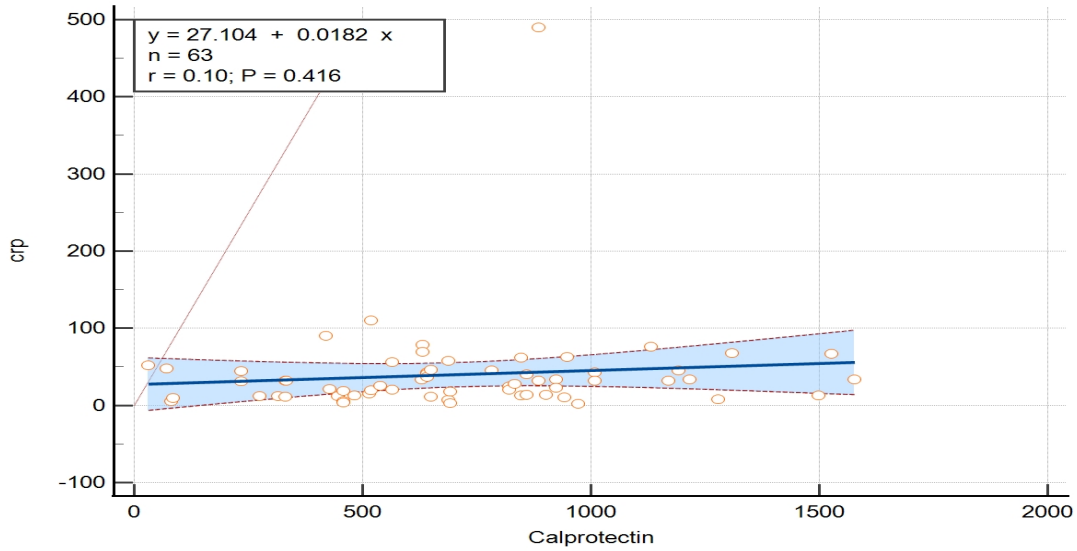


Figure 5: Scatter plot of CRP levels with Calprotectin in pleural fluid

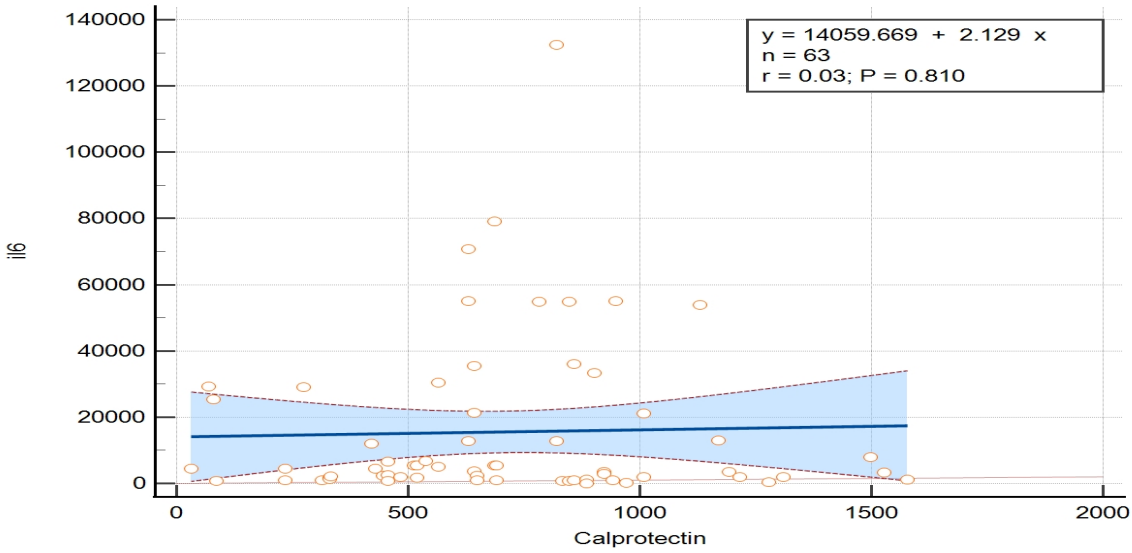


Figure 6 : Scatter diagram between IL-6 and Calprotectin levels

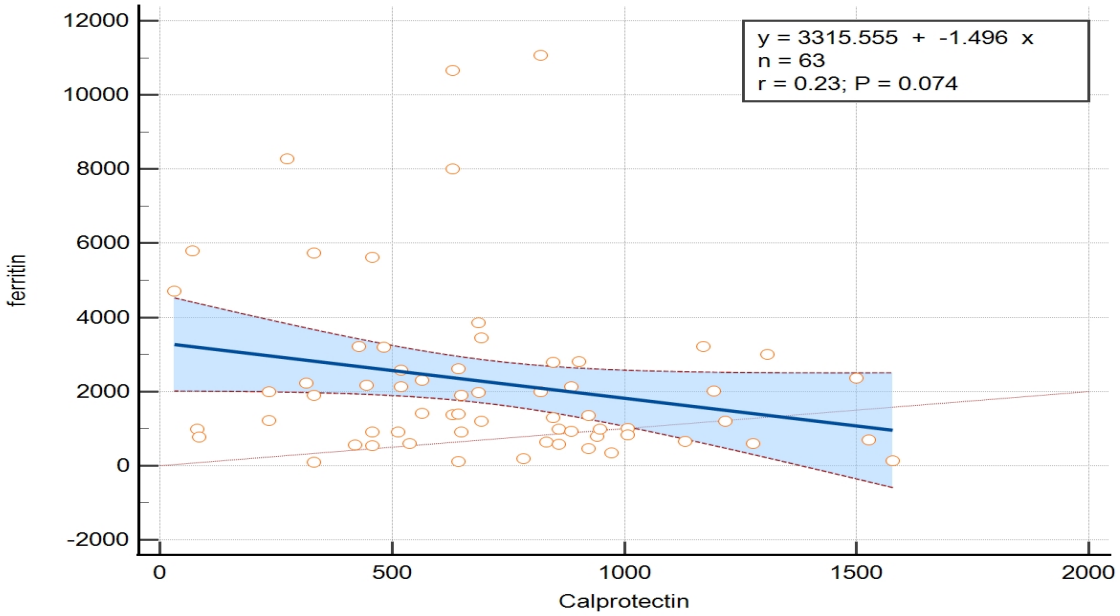


Figure 7: Scatter diagram with Ferritin and Calprotectin levels

Aetiology of pleural effusion

Exudative effusion was diagnosed in 60(95.24%) patients while transudative effusion was diagnosed in 3 (4.76%) patients. Out of the patients with exudative effusion(n=60), malignancy was diagnosed in 31(49.21%), tubercular in 12(19.05%), and parapneumonic effusion in 17(26.98%) patients.

Histological diagnosis of malignant pleural effusion

Adenocarcinoma was the most prevalent cause of malignant pleural effusion, seen in 22 (70.96%) patients. Squamous cell and small cell carcinoma were seen in 3(9.68%) and 2(6.45%) patients with malignant pleural effusion respectively. Mesothelioma and rare spindle cell carcinoma were seen in 1 patient each. Two cases of malignant effusion could not be typed into specific histology.

Relationship of Calprotectin levels between various clinical factors

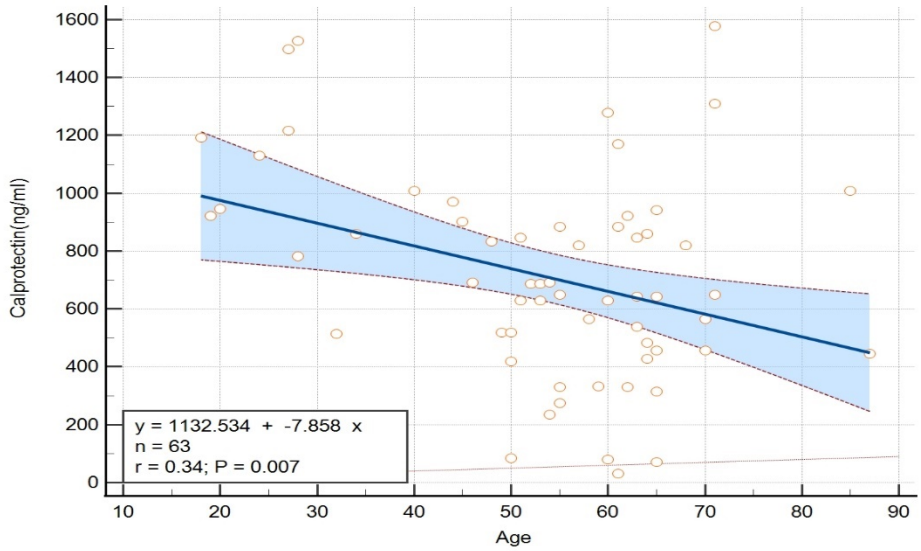


Figure 8: Scatter diagram of Calprotectin levels versus age

Pleural fluid Calprotectin levels seemed to have an inverse relationship with age as we see in Figure 8. Increase in age was associated with a decrease in Calprotectin levels with a Pearson coefficient(r) of 0.34 and p-value <0.007.

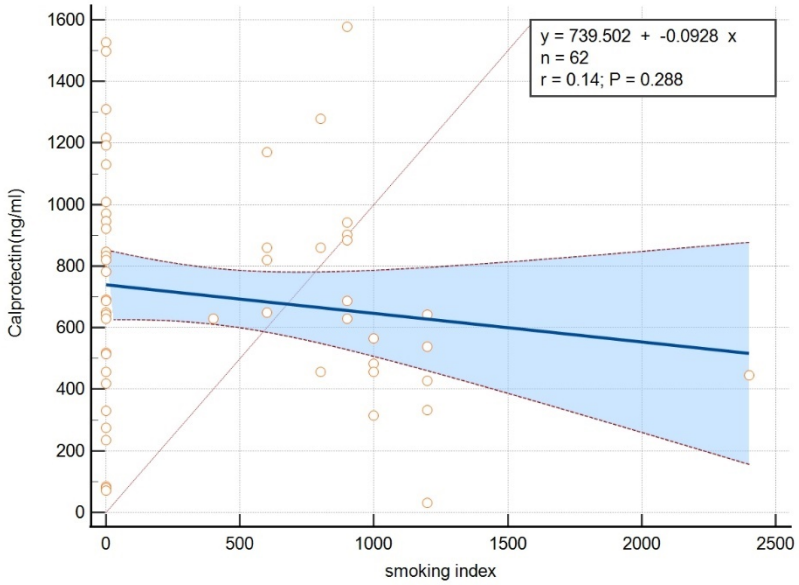


Figure 9: Scatter diagram of Calprotectin levels and Smoking index

Among smokers, no significant correlation was seen between Calprotectin levels and smoking index with an r =0.14 and p value =0.288. We also found that 14 out of 26 smokers (53.8%) had malignant effusion while the rest 12(46.2%) had benign effusions. Smoking status also had no relationship with Calprotectin levels (p=0.74).

Table 5: Relationship between calprotectin levels and histological variants of malignant effusion

S No.	Histological diagnosis of malignant effusion	Calprotectin ng/ml (mean ± sd)	p value (one way ANOVA)
1	Adenocarcinoma	497.59+ 255.68	0.503
2	Squamous cell carcinoma	573.37± 64.64	
3	Small cell carcinoma	431.55 ± 279.63	
4	Others	410.54 ± 270.72	

Calprotectin levels in pleural fluid did not have a statistically significant relationship with different histological variants of malignancy with a p value of 0.503.

The mean(sd) of Pleural fluid Calprotectin levels in patients with prior h/o of cancer (475.59 ± 289.92 ng/ml) was lower than that in patients with no such history (632.04 ± 358.31 ng/ml) but however it did not reach statistical significance (p=0.09 , Students t test for means)

Comparison of biochemical parameters between transudative and exudative effusions

S.No	Parameters	Transudative effusion(n=3)	Exudative effusion(n=60)	p value	Statistical test used
1	Age(mean±sd)	51.67± 11.6 yrs	54.24 ± 15.59 yrs	0.84	Students t test
2	Calprotectin(mean±sd)	706.39±257.53ng/ml	706.45 ± 361.89 ng/ml	0.9	Students t test
3	ADA (mean±sd)	8.33±8.04 U/L	54.35±15.48U/L	**<0.0001**	Students t test
4	IL-6 (mean±sd)	27.47±2655.12 pg/ml	16219.49 ±25018.77pg/ml	**0.0006**	Students t test
5	CRP (mean±sd)	8.53 ±8.08 mg/dl	33.50 ±23.39 mg/dl	**0.01**	Students t test
6	Ferritin(mean±sd)	1565.3±1661.3 ug/ml	2293 ± 2383.14 ug/ml	0.55	Students t test

Table 6: Difference in pleural fluid biochemical parameters between transudative and exudative effusions

As we see in Table 6, ADA, IL-6, and CRP levels were statistically higher in exudative effusion concerning transudative effusion. However, no statistically significant difference was seen in Calprotectin and ferritin levels between these two groups of effusion.

Comparison in biochemical parameters between tubercular and malignant effusions

On univariate analysis, age , SI, Pleural fluid Calprotectin, ADA, and protein levels were found to be significantly different between tubercular and malignant effusions. On multiple logistic regression, Calprotectin achieved statistical significance(p=0.022) while the rest factors were grossly insignificant. Data is enumerated in form of a table below. ROC analysis revealed that Calprotectin differentiated malignant from tubercular effusion with an AUC of 0.91(95% CI 0.79- 0.98) and p-value <0.001. The optimal cutoff point to predict malignancy

was ≤ 691.094 ng/ml with a Youden index =0.8 and Sensitivity ,Specificity,positive, and negative likelihood ratio(LR) of 83.87, 91.67, 10.06, and 0.18 respectively.

Table 7: Relationship between parameters in tubercular and malignant pleural effusion

S.No	Parameters	Tubercular effusion (n=12)	Malignant effusion (n=31)	p value	Univariate Statistical test used	Multiple logistic regression p-value
1	Age (yrs) (mean±sd)	37.33 ± 18.38	59.73 ± 9.78	**<0.0001**	Students t test	0.25
2	SI (mean±sd)	500 ± 635.71	266.67 ± 400.76	0.16	Students t test	
3	Calprotectin(mean±sd)	1035.68± 334.61 ng/ml	499.1±247.34ng/ml	**0.0001**	Students t test	**0.022**
4	ADA (mean±sd)	46.83±20.48 U/L	17.53± 8.11 U/L	**<0.0001**	Students t test	0.41
5	IL-6 (mean±sd)	22671 ± 21870.61pg/ml	19122.18 ± 29830.82 pg/ml	0.35	Students t test	
6	CRP (mean±sd)	34.21± 20.5mg/dl	27.5± 27.72mg/dl	0.19	Students t test	
7	Ferritin (median ,IQR)	2122(822.5-4486.75)ug/ml	904.3(593.2-1954.5)ug/ml	0.07	Mann Whitney test	
8	LDH (mean±sd)	351.5 ± 170.48 U/L	487.41 ± 217.80 U/L	0.06	Students t test	
9	Protein (mean±sd)	4.43 ± 0.88 mg/dl	5.06± 0.54 mg/dl	**0.008**	Students t test	0.33
10	NLR (mean±sd)	3.33 ± 7.09	0.91 ±2	0.27	Mann Whitney test	

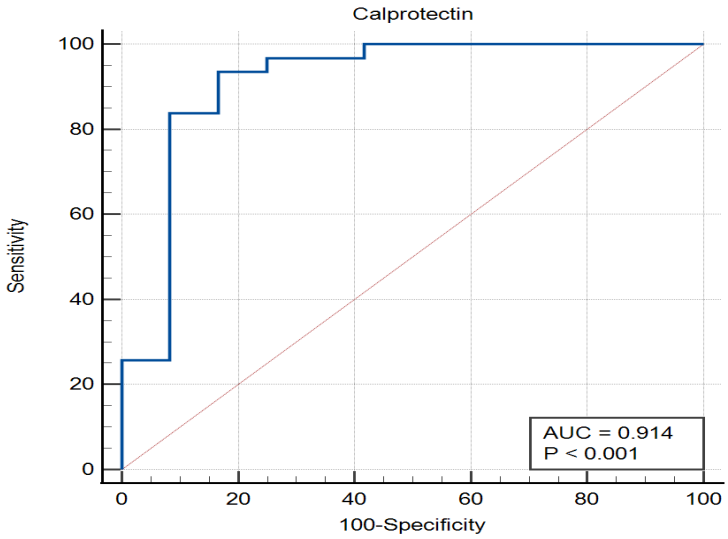


Figure 10: ROC of Calprotectin in the prediction of tubercular/malignant effusion

Comparison in parameters between malignant and benign effusions

On univariate analysis, there was a statistically significant difference in Calprotectin and ADA levels between malignant and benign pleural effusion. Calprotectin emerged as the sole independent predictor of malignant pleural effusion after multiple logistic regressions. ROC analysis revealed that Calprotectin was a reliable differentiator of malignant from benign effusion with an AUC of 0.85(95%CI -0.73- 0.92) and p value<0.001. The optimal cut-off value for malignant effusion came out to be ≤686.807 ng /ml with a Sensitivity, Specificity, positive and negative LR of 80.65, 71.87, 2.87, and 0.27respectively.

Table 8: Relationship between biochemical parameters in malignant and benign pleural effusion

S.No	Parameters	Malignant effusion (n=31)	Benign effusion (n=32)	p value	Univariate analysis	Multiple logistic regression p-value
1	Age (yrs) (mean±sd)	59.77 ± 9.62	48.84 ± 17.76	**0.004**	Students t test	0.39
2	SI (mean±sd)	500 ± 635.72	281.25 ± 384.74	0.11	Students t test	0.29
3	Calprotectin(mean±sd)	499.11±247.34 ng/ml	907.30±331.19 ng/ml	**<0.0001**	Students t test	**0.0007**
4	ADA (mean±sd)	17.53±8.24 U/L	29.84 ±24.94 U/L	**0.012**	Students t test	0.36
5	IL-6 (mean±sd)	19122 ± 30323.92 pg/ml	12144.47 ± 17142.87 pg/ml	0.27	Students t test	
6	CRP (mean±sd)	27.5 ± 22.07 mg/dl	36.98 ± 24.20	0.11	Students t test	
7	Ferritin (median ,IQR)	2122(822.5-4486.75) ug/ml	1322.5(763.5-2065)ug/ml	0.12	Mann Whitney test	
8	LDH (mean±sd)	487.42 ± 217.8 U/L	410.15 ± 216.78 U/L	0.16	Students t test	
9	Protein (mean±sd)	4.43 ± 0.88 mg/dl	4.1 ± 1.36 mg/dl	0.25	Students t test	
10	NLR (median,IQR)	0.5(0.28-0.76)	0.5(0.14-1)	0.95	Mann Whitney test	

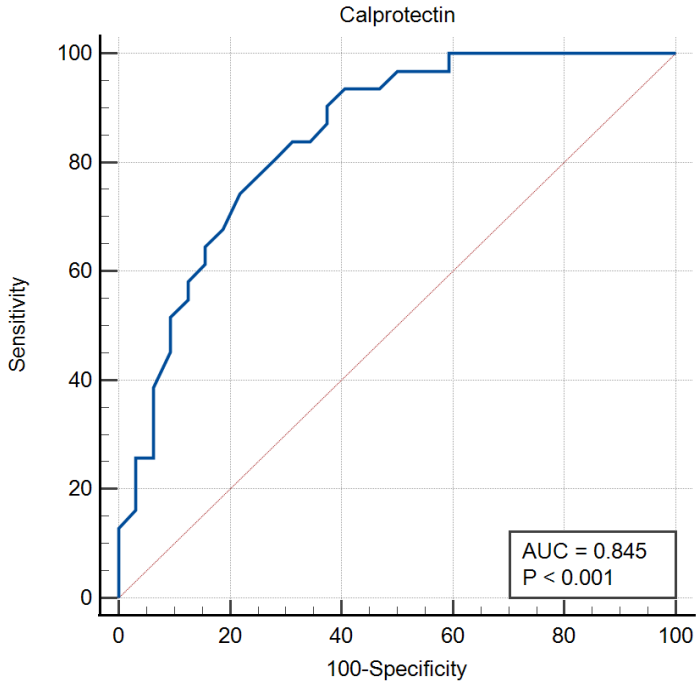


Figure 11: ROC of Calprotectin in malignant /benign pleural effusion

Comparison in parameters between malignant and parapneumonic effusions

Univariate analysis showed that only Calprotectin and CRP levels in the pleural fluid were significantly different between malignant and parapneumonic effusions. On multiple logistic regression, both Calprotectin and CRP emerged as independent predictors of malignant /parapneumonic effusion. ROC analysis showed that Calprotectin achieved an AUC of 0.81 (95% CI 0.67- 0.91) with $p<0.001$. The optimal cut-off value for malignant effusion came out to be ≤564.82 ng /ml with a Sensitivity, Specificity , positive and negative LR of 63.52, 82.35, 3.59, and 0.45 respectively. The required data is represented in the form of a table and figure below.

Table 9: Relationship between biochemical parameters in malignant and parapneumonic pleural effusion

S.No	Parameters	Malignant effusion (n=31)	Parapneumonic effusion (n=17)	p value	Statistical test used	Multiple logistic regression p value
1	Age (yrs) (mean±sd)	59.78 ± 9.62	56.47 ±14.12	0.4	Students t test	
2	SI (mean±sd)	577.42 ± 607.02	341.18 ± 396.96	0.11	Students t test	
3	Calprotectin(mean±sd)	499.10 ± 247.34 ng/ml	852.14 ± 322.63ng/ml	**0.0005**	Students t test	**0.0043**
2	ADA (mean±sd)	17.53 ± 8.25U/L	21.65 ± 22.68U/L	0.47	Students t test	
3	IL-6(median, IQR)	800(1170-28239.25)pg/ml	800(1702.75-7345)pg/ml	0.5	Mann - Whitney test	
4	CRP (mean±sd)	27.49± 22.08mg/dl	43.96±24.55 mg/dl	**0.03**	Students t test	**0.0464**
5	Ferritin(mean±sd)	2122(822.5 - 4486.75)ug/ml	1401 (1159 - 2165.75)ug/ml	0.48	Mann Whitney test	
6	LDH (mean±sd)	487.42 ± 217.80U/L	455.71 ± 202.75U/L	0.61	Students t test	
7	Protein (mean±sd)	4.44 ± 0.88 mg/dl	3.92± 1.05mg/dl	0.07	Students t test	

8	NLR (median, IQR)	0.5(0.28 -0.77)	0.5(0.30-3.17)	0.46	Mann - Whitney test	

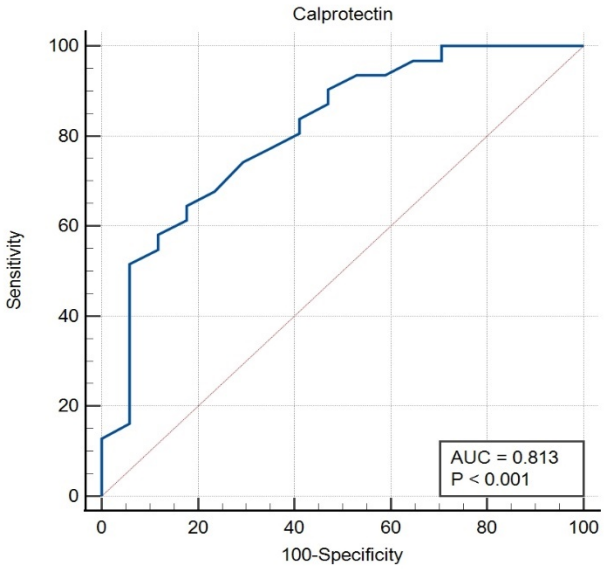


Figure 12: ROC of Calprotectin in differentiation of malignant and parapneumonic effusion

Comparison in parameters between tubercular and parapneumonic effusions

On univariate analysis , pleural fluid Calprotectin levels were not significantly different between tubercular and parapneumonic effusion . However , parameters like Age , IL-6 ,ADA. Pleural fluid Protein had statistically significant difference between tubercular and parapneumonic effusion. However on multiple logistic regression , this statistical significance disappeared other than pleural fluid protein which almost achieved significance(p=0.07).The respective data is given in Table 10.

Table 10: Relationship between parameters in patients with tubercular and parapneumonic effusion

S.No	Parameters	Tubercular effusion	Parapneumonic effusion	p value	Univariate Statistical test used	Multiple logistic regression p-value
1	Age (yrs) (mean±sd)	37.33 ± 18.38	59.73 ± 9.78	**0.0037**	Students t test	0.99
2	Calprotectin(mean±sd)	1035.68± 334.61 ng/ml	852.14± 322.63 ng/ml	0.15	Students t test	
3	ADA (mean±sd)	46.83±20.48 U/L	21.65±22.68 U/L	**0.006**	Students t test	0.38
4	IL-6 (mean±sd)	22671 ± 21870.61pg/ml	6372.06 ± 8642.37 pg/ml	**0.035**	Students t test	0.14
5	CRP (mean±sd)	34.21± 20.5mg/dl	43.96± 24.55mg/dl	0.28	Students t test	
6	Ferritin (mean±sd)	1200.15±877.36ug/ml	1691.71 ± 783.59 ug/ml	0.13	Students t test	
7	LDH (mean±sd)	351.5 ± 170.48 U/L	455.71 ± 202.75 U/L	0.16	Students t test	
8	Protein (mean±sd)	4.43 ± 0.88 mg/dl	3.92± 1.05mg/dl	**0.002**	Students t test	0.07
9	NLR (mean±sd)	3.34 ± 7.09	3.30 ±5.52	0.99	Students t test	

Comparison of factors between patients with pleurodesis success and failure

Univariate analysis showed that only Calprotectin and Ferritin levels in the pleural fluid were significantly different between patients with pleurodesis success and those with failure. ROC analysis showed that Calprotectin achieved an AUC of 0.95 (95% CI 0.74- 1) with p<0.001. The optimal cut off value for malignant effusion came out to be >332.3 ng /ml with a Sensitivity , Specificity ,positive and negative LR of 100, 80, 5.0 and 0 respectively. This data is represented in the form of Table 11 and Figure 13. 8 out of 10 patients(80%) with successful pleurodesis had Karnofsky score more than 70 while 1 out of 5(20%) patients with pleurodesis failure had Karnofsky score more than 70.This difference was statistically significant (p=0.02). Radiologically massive malignant effusion engulfing the entire hemithorax was seen in 2 out of 10(20%) patients with pleurodesis success and 4 out of 5(80%) patients with pleurodesis failure .This difference also came out to be statistically significant(p=0.025).

Figure 13: ROC of Calprotectin and pleurodesis success

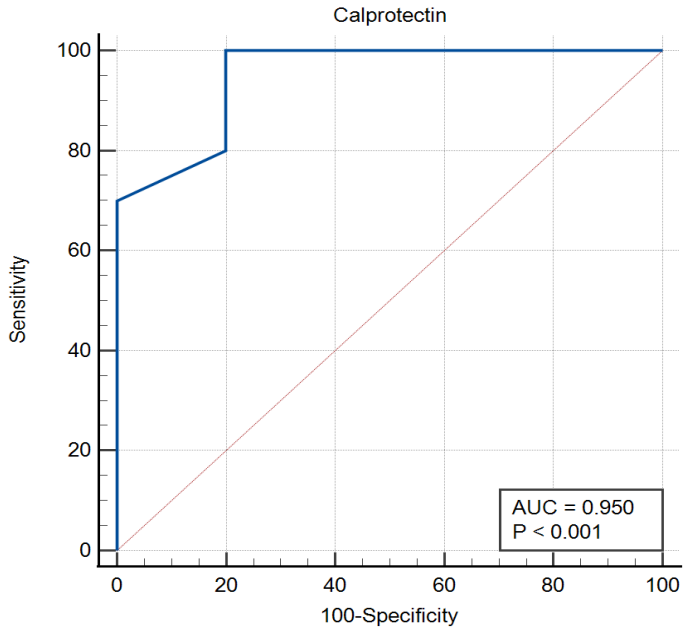


Table 11: Relationship between parameters in patients with pleurodesis success and failure

S.No	Parameters	Pleurodesis success	Pleurodesis failure	p value	Statistical test used
1	Age (yrs) (mean±sd)	65.4± 8.55	62± 5.74	0.44	Students t test
2	SI (mean±sd)	966.67 ± 717.63	680± 626.09	0.46	Students t test
3	Calprotectin(mean±sd)	635.7±196.5 ng/ml	232.9±179.6 ng/ml	**0.0033**	Students t test
4	ADA (mean±sd)	18.65±8.08 U/L	15.8±4.32U/L	0.39	Students t test
5	S.Albumin(mean±sd)	3.38 ± 0.21 g/dl	3.72 ± 0.97 g/dl	0.18	Students t test
6	IL-6 (mean±sd)	7786.6 ± 16638.3 pg/ml	14298.2 ±13671.4 pg/ml	0.43	Students t test
7	CRP (mean±sd)	20.11 ±18.71 mg/dl	32.72 ±17.31	0.23	Students t test
8	Ferritin(mean±sd)	1801.5±1104.24 ug/ml	6021.6±1334.02 ug/ml	**0.0005**	Students t test
9	LDH (mean±sd)	552.4 ± 173.73U/L	382.6 ± 199.36 U/L	0.15	Students t test
10	Protein (mean±sd)	4.66 ± 0.97 mg/dl	4.87 ±0.74 mg/dl	0.65	Students t test
11	NLR (mean±sd)	0.45 ± 0.49	0.55 ± 0.20	0.59	Students t test
12	Pl.Sugar(mean±sd)	107 ± 29.22 g/dl	77.8 ± 17.52 g/dl	**0.025**	Students t test

Discussion

This prospective cross sectional study was undertaken to study the relationship between Calprotectin levels in patients with pleural effusion and their respective aetiologies. A total of 63 patients were enrolled after proper informed consent . The mean(standard) deviation of age in our study population was 54.22 $\pm$ 15.26 years which is comparable to the studies of Sanchez-Otero N et al and Luo et al[8,10]. Males comprised 68.25% of the patients while females comprised the rest 31.75%. This gender distribution is also similar to the study by Luo et al and Sanchez-Otero N et al with male participation amounting to 69% and 66%[8,10]. Hypertension was the commonest comorbidity seen in 39.68% of patients, followed by Diabetes seen in 34.92% of patients, while 29% of patients had no known comorbidities.

Smoking as an addiction was found in 41.27% of patients while alcohol consumption was seen in 4.76% of patients. Similar smoking addiction rates of 42.82% and 41% were also found in studies by Botana-Rial M et al and Luo et al[7,10].

In our study exudative effusion was diagnosed in 95.24% of patients while transudative effusion was diagnosed in 4.76% of patients. This is contrary to the study by Botana-Rial M et al in which 86.82% of patients had exudative effusions while 13.2% had transudative effusions[7]. Malignancy was found however in 31/63(49.2%) of our patients while 51.8% of patients had benign effusions. Similar to our study , Luo et al also found in their study that 41.05% of patients had malignant effusion while the rest 58.95% had benign effsuion[10]. However , in the study by Botana-Rial M et al , lower rates malignant pleural effusion was diagnosed in 33 % patients while the rest 67% patients had benign effusion[7]. The rates of tubercular effusion in our study was 19.04%(12/63) which is comparable to the studies by Botana-Rial M et al and Sanchez-Otero N et al , in which 12% and 19.04% of patients were found to have tubercular effusion respectively [7,8]. However , in the study by Luo et al , tubercular effusion comprised about 46.31 % of all the effusion. This high percentage may be

due to the fact that the study was conducted in a Respiratory Unit in which patients with primarily extrapulmonary disorders were less likely to be admitted .

In our study , the age distribution among the various aetiologies was found to be heterogenous. Age in the population with malignant effusion was significantly higher than that with benign effusion($p<0.0001$). Similar difference was found to exist when comparing tubercular effusion and malignant effusion. However this difference ceased when age was compared between patients with malignant and parapneumonic effusion($p=0.4$). Age difference between malignant and benign effusion were seen in studies by Botana-Rial M et al and Sanchez-Otero N et al [7,8]. In the study by Mohammed et al , a statistically significant difference in age was seen in patients with malignant and tubercular effusion[9].

In our study pleural fluid Calprotectin level was found to be an inverse linear relationship with Age with a Pearson coefficient of $r= -0.34$ and a p value of 0.007. No studies have looked at the correlation of Calprotectin levels in pleural fluid and age of patients. There are few studies that have looked at relationship between age and faecal Calprotectin levels. One of those studies by Park et al , faecal Calprotectin levels were positively correlated with age of patients (Pearson $r=0.3779$, $p=0.0096$)[12]. In another study by Joshi et al , faecal Calprotectin levels in healthy volunteers were significantly higher with more than 60 years than those with age less than 60[13]. We believe that since in our study the patients in malignant effusion group have significantly lower Calprotectin levels and higher age than those in benign effusion, this correlation has crept up in the results . This mostly is a confounding factor , as we found that in Multiple logistic regression age no longer remained an independent parameter.

Smoking was not found to be related to Calprotectin levels in our study . In the study by Sanchez-Otero N et al , smoking status was associated with malignant pleural effusion and

lower Calprotectin levels[8]. However we found in our study that smoking status was not different between malignant and benign pleural effusion(53.8% and 46.2% , p =0.58). Hence we believe no significant relationship was achieved between either Smoking Index or smoking status with Calprotectin levels.

In our study the mean(sd) levels of Calprotectin levels in transudative , exudative , tubercular , malignant and parapneumonic effusions came out to be 706.39+257.53 , 706.45 + 361.89 , 1035.68± 334.61 , 499.1±247.34 and 852.14 ± 322.63 ng .ml respectively . On comparing transudative and exudative effusion , Calprotectin levels were not significantly different . This may be due to the fact only 3 patients in our study had transudative effusion. In the study by Botana-Rial et al, the calprotectin levels ranged from 400.0 to 548.5 ng/mL in transudates [7].

On comparing malignant and benign pleural effusion , the mean Calprotectin levels in malignant and benign effusion were 499.1±247.34 and 907.30±331.19 ng/ml respectively($p<0.05$). On logistic regression , Calprotectin emerged as an independent parameter in predicting malignancy with an AUC of 0.85 and cut off value ≤686.807 ng /ml . In the study by Sanchez-Otero N et al , a cut off of less than 736.4 ng /ml was the required Calprotectin level with an AUC of 0.96[8] . In the study by Luo et al and Mohammed et al , the cut off and AUC of Calprotectin were ≤500.19 ng/ml and ≤ 730.5 ng/ml , 0.6 and 0.9 respectively[10,9].

Pleural fluid Calprotectin levels in malignant and tubercular effusion were also significantly different on univariate analysis . On logistic regression, it was an independent predictor of malignancy with a cut off of ≤691.09 ng/ml and AUC of 0.91 . In the study by Sanchez Otero N et al , the cut off to differentiate malignant from tubercular effusion was less than 612 ng/ml with sensitivity and specificity (95% CI) of 98.51 (92–100) and 96.67 (82.8–

99.9) respectively [8] . In the study by Luo et al , the cut off value and AUC of Calprotectin were ≤421.73 ng/ml and 0.69 respectively in predicting malignant effusion from tubercular effusion . In our study ADA levels although was found to be significantly different on univariate analysis , lost its significance on logistic regression in separating malignant from tubercular effusion . This is an important matter because in an endemic country like India , many times it is difficult to separate malignant from tubercular effusion . Both are predominantly lymphocytic , exudative and other than fever both have similar presenting complaints[14] . So in these situations, a physician depends on a biomarker like ADA to diagnose the condition. But our study indicates that Calprotectin can emerge as a better predictor than ADA in differentiating a malignant effusion from tubercular.

Differentiation of parapneumonic effusion from malignant effusion is sometimes easier owing to the more acute history and neutrophilic cytology of pleural fluid . But malignant effusion can have neutrophilic cytology also as seen in 20% of patients and to make things more difficult both malignant and parapneumonic effusion have ADA less than the tubercular range of >40U/L[15]. In our study also NLR was not significantly different between these two groups indicating prevalence of neutrophilic malignant effusion. CRP levels were higher in the parapneumonic effusion group indicating a higher inflammation but however the ADA levels were similar. In this scenario , pleural fluid Calprotectin levels can help to differentiate between these two categories with similar laboratory parameters. The cut off and AUC of Calprotectin levels in our study was ≤564.82 ng /ml and 0.81 respectively . In comparison , Sanchez Otero et al , found that the cut off of Calprotectin in differentiating malignant and parapneumonic effusion was ≤532.5 ng /ml with a sensitivity and specificity (95% CI) of 95.52 (87.5–99.1) and 96.55 (82.2–99.9) respectively[8].

Calprotectin is a made up of two calcium binding proteins S100A8 (calgranulin A) and S100A9 (calgranulin B). This hetero complex is found in cytoplasm of inactivated

phagocytes. On the elevation of intracellular Calcium levels, one of two things can happen . Either they translocate to the plasma membrane and are presented as heterodimers attached cell membranes of monocytes or are secreted into the extracellular matrix , each having its own functions[16]. In areas of acute inflammation, monocytes with expressed Calprotectin dimers are the predominant cell type and are exuberant producers of TNF-α and IL-1β. These monocytes are involved in production of Reactive oxygen species , phagocytosis and clearance of debris . They have been also shown to influence migration of leucocytes by modulation of cytoskeletal tubulin and thus affects propagation of the inflammatory pathway[17].Extracellular action of Calprotectin is primarily by chelating ions other than Calcium like $Mn^{2+}$and Zn^{2+} after they have been released into the extracellular matrix by neutrophils. These ions are essential for proper functioning of micro organisms and by depleting their active stores , this dimer helps in containing infection.

In tumour cells , however , Calprotectin acts as an apoptotic factor. It depletes intracellular Zinc levels which leads to cleaving of Procaspase-3 to Caspase-3 which finally leads to cell death .Another pathway is by mitochondrial-mediated intrinsic pathway of apoptosis[18]. Thus , lower levels of Calprotectin should be associated with anti-apoptotic events leading to tumour progression , metastasis especially to pleural cavity leading to pleural effusion as is in the case with our study. In a study by Ghavami et al , it was seen that at serum levels less than 25 ug/ml Calprotectin is even associated with tumour cell growth while the higher serum values are associated with tumour suppression[19]. This tumorogenic effect of Calprotectin was found to be achieved by RAGE (Receptor of advanced glycation end products) ligation and Mitogen-activated protein kinases (MAPK) activation[19] .

It follows that Calprotectin plays a dual rule of apoptotic and inflammatory at high levels and anti apoptotic at lower levels. Hence it is a very good in detecting a malignant effusion not only because there is a lack of severe inflammation , but also due to the fact that a metastatic

malignant pleural effusion will also have lower Calprotectin levels due to the fact that it indicates tumour growth and spread . This may also be the reason why Calprotectin levels were not found to have any significant correlation with the other studied biomarkers like CRP ,LDH, IL-6 and Ferritin which are inflammatory markers and while Calprotectin has a dichotomous role , both as an inflammatory and tumour growth marker.

Continuing with the above discussion we can see why Calprotectin is a good discriminator between malignant and benign inflammatory /infective effusion. But when it came to tubercular and parapneumonic effusion , pleural fluid Calprotectin levels were not significantly different between these two groups($p=0.15$). Pleural fluid levels of IL-6 ,Protein and ADA levels were found to be significantly higher in patients with tubercular effusion than parapneumonic effusion on univariate analysis. In the study by Mohammed et al , statistically significant difference was also not seen in Calprotectin levels between tubercular and parapneumonic effusion($p=0.92$)[9]. It thus emerges that Calprotectin is not a good discriminator between tuberculosis and parapneumonic effusion as both are inflammatory /infective clinical conditions.

Diagnosis of malignant pleural effusion is followed by its management which includes chemoradiotherapy , molecular targeted therapies and in cases of recurrent effusion causing symptoms , intercostal tube insertion followed by pleurodesis. Pleurodesis failure is common in malignant effusion leading to increase in hospital stay , risk of empyema and pain due to prolonged ICD placement. In the study by Saleh et al , 22% of patients with malignant effusion had pleurodesis failure [20]. In our study , 5 out of 15 (33%) patients undergoing pleurodesis had failure while 67% had successful pleurodesis . In the study by Martinez et al in which 120 malignant patients undergoing chemical pleurodesis were studied , risk factors for pleurodesis failure were Pleural fluid glucose (<60 mg/dl), Karnofsky performance status (<70), size of the effusion in chest radiographs (massive effusion) and pleural LDH

(>600U/l)[21]. In the meta analysis by Hassan et al , following factors were found to be related with pleurodesis success - smaller volume of effusion pre-pleurodesis , full lung re-expansion post effusion drainage , shorter duration of tube drainage, higher pleural fluid glucose, lower LDH and lower pleural tumour burden[22] . The tumour burden score was a visual one , done during thoracoscopy to estimate the extent of involvement of parietal pleura and a score was given from 0-9 (where higher scores correspond to the more extensive involvement)[21]. In our study , we believe that Calprotectin can be used as a surrogate for tumour burden due to its established relationship with tumour biology . We saw that patients with successful pleurodesis had significantly higher Calprotectin levels which can be associated with lower tumour burden while those with pleurodesis failure , had lower Calprotectin levels , which can be associated with higher tumour burden . This finding is of import because we also see that pleural fluid Ferritin which is an inflammatory marker was found to be significantly lower in the pleurodesis success group. Keeping in mind that the objective of a sclerosing agent is primarily to augment the pleural inflammatory process leading to a successful pleurodesis , we believe that our results show that in the backdrop of malignant effusion that is similar to both groups , it's the tumour burden that determines the pleurodesis success , not its inflammatory state before pleurodesis.

The levels of the Calprotectin in pleural fluid in our study was carried out by the Krishgen Human Calprotectin GENLISA^{TM} ELISA kit , in which two monoclonal antibodies are used instead of one monoclonal antibody which is usually used in other ELISA kits . Presence of double antibodies and sandwich technique leads to better sensitivity and specificity. Variation exists in estimation of Calprotectin levels when different types of ELISA kits are used . Casado-Rey et al in their study looked at the diagnostic efficacy of two different ELISA kits , namely , ELISA(fCAL) Buhlmann Laboratories and Quantum Blue(QB sCAL)quantitative Lateral Flow Assay. It was found that the results of the QB sCAL method were an average

2,100 units higher than those obtained by ELISA fCAL method. In malignant effusion cases ELISA fCAL gave a cut off of less than 6323.2 ng/ml while QB sCAL gave a cut off of less than 9750 ng/ml[23]. In the study by Botana-Rial M et al , it was found that the optimal cut off of less than 6233.2.ng/ml was associated with malignant effusion which is quite different from the cut offs calculated in our study . ELISA fCAL kit was used in the above mentioned study also which use a single monoclonal antibody[7]. Our study cut off s corroborated well with studies done by Luo et al and Sanchez Otero et al in which SEK504Hu ELISA kit (USCN Life Science Inc. Wu Han) and sandwich ELISA kit (Hycult Biotechnology Uden, The Netherlands) were used[10,8]. We believe that use of different ELISA kits and different antibodies used can lead to different Calprotectin levels and cut offs. This thought is echoed in the studies by Sanchez Otero et al and Kristinsson et al[8,24]. Calprotectin has the potential to be a veritable biomarker of malignancy and inflammation in pleural cavity but however further standardisation in ELISA kits and sample processing is necessary to achieve a single accepted cut off value .

LIMITATIONS

1. All patients with exudative benign effusions were infective, either tubercular or parapneumonic. The data set did not include other causes of exudative effusion like Connective tissue disorders .

2.The study sample was small and was a single centre study.

CONCLUSIONS- Pleural fluid Calprotectin has the potential to become a veritable marker of malignancy in pleural fluid. It also can be a reliable marker of pleurodesis success or failure. Further studies with a larger sample size and varied population are required to establish its role in the diagnosis and management of suspected malignant pleural effusion.

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ANNEXURE -1 Informed Consent Form(English)

All India Institute of Medical Sciences

Jodhpur, Rajasthan

Thesis / Thesis Title of dissertation: Pleural fluid calprotectin level in benign and malignant pleural effusion and its significance in predicting success of pleurodesis in malignant pleural effusion..

DM Student Name: Dr Amartya Chakraborti Tel. No.9599700325

Patient / Patient Volunteer Identification Number:

_____

_____

I, _____ Son of or Daughter of _____

Residentof_____

_____

I confirm that I have had the opportunity to ask questions.

I understand that my participation is voluntary and I am aware of my right to pull out of the study at any time without any reason.

I understand that the information collected about me and any of my medical records can be viewed by the person responsible for _____

I allow these individuals to access their records.

Date: _____

Location: _____

Signature / Signature Left Thumb Impression

To certify that the above consent has been received in my presence.

ANNEXURE 2 : Informed Consent form(Hindi)

अखिल भारतीय आयुर्विज्ञान संस्थान

जोधपुर, राजस्थान

थीसिस / थीसिस शोध प्रबंध का शीर्षक: सौम्य और घातक फुफ्फुस बहाव में फुफ्फुस द्रव कैलप्रोटेक्टिन स्तर और घातक फुफ्फुस बहाव में प्लुरोडेसिस की सफलता की भविष्यवाणी करने में इसका महत्व।

डीएम छात्र का नाम: डॉ अमर्त्य चक्रवर्ती दूरभाष। संख्या 9599700325

रोगी / रोगी स्वयंसेवी पहचान संख्या:

_____

_____

मैं, _____ _____ का पुत्र या पुत्री

_____

_____

_____ के निवासी मैं पुष्टि करता हूं कि मुझे प्रश्न पूछने का अवसर मिला है।

मैं समझता हूं कि मेरी भागीदारी स्वैच्छिक है और मैं बिना किसी कारण के किसी भी समय अध्ययन से बाहर निकलने के अपने अधिकार से अवगत हूं।

मैं समझता हूं कि मेरे और मेरे किसी भी मेडिकल रिकॉर्ड के बारे में एकत्र की गई जानकारी को _____ के लिए जिम्मेदार व्यक्ति द्वारा देखा जा सकता है **I**

मैं इन व्यक्तियों को उनके रिकॉर्ड तक पहुंचने की अनुमति देता हूं।

तारीख: _____

जगह: _____

हस्ताक्षर / हस्ताक्षर बाएं अंगूठे का निशान

प्रमाणित करने के लिए कि उपरोक्त सहमति मेरी उपस्थिति में प्राप्त हुई है।

ANNEXURE -3 : PATIENT INFORMATION SHEET

Name of the patient:

Patient ID.:

Aim of the study: Pleural fluid calprotectin level in benign and malignant pleural effusion and its significance in predicting success of pleurodesis in malignant pleural effusion.

Study site: Department of Pulmonary Medicine All India Institute of

Medical Sciences, Jodhpur, Rajasthan.

Study procedure: After routine clinical examination and history taking ,Chest x ray and CECT (if indicated)scan of the patient will be done.Some amount of fluid will be taken by piercing a needle thorugh the chest of the patient and sent for some tests.Skin will be numbed with medication before needle is inserted.If the diagnosis is not made by the tests done on the fluid ,patient will be further taken up for thoracoscopy orUSG/CT/FOB guided biopsy.

Likely benefit: Study will help the patient to get a faster and more confident diagnosis regarding their pleural effusion

Confidentiality: All the data collected from each study participant will be kept confidential.

Risk: Enrollment in above study poses no substantial risk to any of the study participant and if

any point of time participant want to withdraw himself/ herself, he/ she can do so voluntarily at

any point of time during the study.

For further information / questions, the following personnel can be contacted:

Dr. Amartya Chakraborti, Senior Resident,

Department of Pulmonary Medicine,

All India Institute of Medical Sciences, Jodhpur, Rajasthan.

Mob-9599700325

ANNEXURE -4: Patient Information Sheet(Hindi)

रोगी सूचना पत्र

रोगी का नाम:

रोगी आईडी:

अध्ययन का उद्देश्य: सौम्य और घातक फुफ्फुस बहाव में फुफ्फुस द्रव कैल्प्रोक्टिन स्तर और घातक फुफ्फुस बहाव में फुफ्फुसावरण की सफलता की भविष्यवाणी में इसका महत्व।
अध्ययन स्थल: पल्मोनरी मेडिसिन विभाग अखिल भारतीय संस्थान
चिकित्सा विज्ञान, जोधपुर, राजस्थान।
अध्ययन प्रक्रिया: नियमित नैदानिक परीक्षण और इतिहास लेने के बाद, चेस्ट एक्स रे और CECT (यदि संकेत दिया गया है) तो रोगी का स्कैन किया जाएगा। कुछ मात्रा में तरल पदार्थ को सुई की थ्रूग छांटकर मरीज के सीने में डाला जाएगा और कुछ परीक्षणों के लिए भेजा जाएगा। । सुई डालने से पहले दवा के साथ सुन्न कर दिया जाएगा। यदि निदान तरल पदार्थ पर किए गए परीक्षणों द्वारा नहीं किया जाता है, तो रोगी को थोरैकोस्कोपी orUSG / CT / एफओबी निर्देशित बायोप्सी के लिए आगे ले जाया जाएगा।
संभावित लाभ: अध्ययन से रोगी को अपने फुफ्फुस बहाव के बारे में अधिक तेजी से और अधिक आश्वस्त निदान प्राप्त करने में मदद मिलेगी
गोपनीयता: प्रत्येक अध्ययन प्रतिभागी से एकत्र किए गए सभी डेटा को गोपनीय रखा जाएगा।
जोखिम: उपरोक्त अध्ययन में नामांकन से अध्ययन के किसी भी प्रतिभागी को और यदि हो तो कोई जोखिम नहीं है
किसी भी समय प्रतिभागी स्वयं / खुद को वापस लेना चाहता है, वह स्वेच्छा से ऐसा कर सकता है
अध्ययन के दौरान किसी भी समय।
अधिक जानकारी / प्रश्नों के लिए, निम्नलिखित कर्मियों से संपर्क किया जा सकता है:
डॉ। अमर्त्य चक्रवर्ती, वरिष्ठ निवासी,
पल्मोनरी मेडिसिन विभाग,
अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर, राजस्थान।
मोब -9599700325

ANNEXURE -5: **PROFORMA FOR DATA COLLECTION**

Name- …… Age-…… Gender…….

Contact no- ……Occupation……

Marital status……..Educational qualification …….

Symptoms and duration-

1.cough-

2.chest pain-

3.hemoptysis-

4.shortness of breath-

5.loss of weight/loss of appetite-

History of addiction(smoking/tobacco/alcohol/opium/others)-

Previous history of medical illness(Asthma/Diabetes/hypertension/others):-

History of previous malignancy-

Blood investigations

CBC- Hb(g/dl),TLC,DLC

LFT- S.Bil/SGOT/SGPT/proteins/albumin/ALP

KFT- urea/creatinine

PT/PTT/INR-

Pleural fluid investigations-

Appearance-

Specific gravity-

Presence of coagulum-

Cytology-

Cell differentials-

Pl.protein(g/dl)	Pl.sugar(g/dl)	Pl.ADA	Pl.LDH	Pl.calprotectin	Pl.ferritin	Pl.CRP

Pleural fluid gram stain/ ZN stain-

Pleural fluid pyogenic culture-

Pleural fluid CBNAAT-

Pleural fluid cytology

Histopathological reports

IHC report(if required)

Final Diagnosis-

Pleurodesis flowchart

Checklist: 1.Complete reexpansion of lung.

2.Symphysis between visceral and parietal pleura.

3.ICD in situ and properly working.

4.Drug sensitivity done for lignocaine/doxycycline?

Time :Premedication: Inj Lignocaine(3mg/kg, max 250mg) dissolved in 0.9% NS , made upto 50

ml and administered through ICD.

Time:Sclerosing agent : Inj Doxycycline(500mg) administered through ICD.

InjTramadol(50mg) i.m stat in case of pain

Time: ICD released after being clamped for 1 hour.

Pleural fluid drainage day 1 after pleurodesis-

Pleural fluid drainage day 2 after drainage-

CXR 48 hours after pleurodesis- fluid reaccumulation present?

Visceral and parietal pleura apposed?

Pleurodesis success/failure-

ANNEXURE 6 – IEC Certificate

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2021/3482 **Date: 12/03/2021**

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2021/3317**

Project title: **"Pleural fluid calprotectin levels in benign and malignant pleural effusions and its significance in predicting success of pleurodesis in malignant pleural effusions"**

Nature of Project:	**Research Project Submitted for Expedited Review**
Submitted as:	**D.M. Dissertation**
Student Name:	**Dr. Amartya Chakraborti**
Guide:	**Dr. Nishant Kumar Chauhan**
Co-Guide:	**Dr. M.K.Garg, Dr. Naveen Dutt, Dr. Ramniwas, Dr. Mithu Banerjee, Dr. Ravisekhar Gadepalli & Dr. Deepak Vedant**

Institutional Ethics Committee after thorough consideration accorded its approval on above project.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

Please Note that this approval will be rectified whenever it is possible to hold a meeting in person of the Institutional Ethics Committee. It is possible that the PI may be asked to give more clarifications or the Institutional Ethics Committee may withhold the project. The Institutional Ethics Committee is adopting this procedure due to COVID-19 (Corona Virus) situation.

If the Institutional Ethics Committee does not get back to you, this means your project has been cleared by the IEC.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma
Member Secretary

Member secretary
Institutional Ethics Committee
AIIMS,Jodhpur

Basni Phase-2, Jodhpur, Rajasthan-342005; **Website:** www.aiimsjodhpur.edu.in; **Phone:** 0291-2740741 Extn. 3109
E-mail : ethicscommittee@aiimsjodhpur.edu.in; ethicscommitteeaiimsjdh@gmail.com