

ANA positivity and complement level in pleural fluid are potential diagnostic markers in discriminating lupus pleuritis from pleural effusion of other aetiologies

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ABSTRACT

Objective Lupus pleuritis is the most common pulmonary manifestation of systemic lupus erythematosus (SLE). We aimed to compare various biomarkers in discriminating between pleural effusions due to lupus pleuritis and other aetiologies.

Methods We determined in 59 patients (16 patients with SLE and 43 patients without SLE) pleural fluid levels of high-mobility group box 1, soluble receptor for advanced glycation end products (sRAGE), adenosine deaminase (ADA), interleukin (IL) 17A, tumour necrosis factor- α , antinuclear antibodies (ANA), and complements C3 and C4.

Results We found significant differences in the pleural fluid level of sRAGE, ADA, IL-17A, C3 and C4, and in the proportion of ANA positivity, among lupus pleuritis and other groups with pleural effusion. Specifically, ANA positivity (titre $\geq 1:80$) achieved a high sensitivity of 91%, specificity of 83% and negative predictive value (NPV) of 97% in discriminating lupus pleuritis from non-lupus pleural effusion. A parallel combination of the level of C3 (<24 mg/dL) and C4 (<3 mg/dL) achieved a sensitivity of 82%, specificity of 89% and NPV of 93% in discriminating lupus pleuritis from non-lupus exudative pleural effusion.

Conclusions In conclusion, ANA, C3 and C4 in pleural fluid are useful in discriminating lupus pleuritis from pleural effusion due to other aetiologies with high NPV.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease that likely leads to serious complications.¹ Lupus pleuritis is the most common pulmonary manifestation of SLE, with a prevalence of 45%–60%.² It is sometimes even the initial presentation in patients with SLE.³ However, there are other causes of pleurisy, such as infections, congestive heart failure and malignancy.⁴ The differential diagnosis of lupus pleuritis is challenging but crucial for early optimal treatment. Analyses of pleural fluid in patients with lupus pleuritis revealed mostly exudative

KEY MESSAGES

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT?

⇒ There lacks a direct comparison between various biomarkers in the pleural fluid for lupus pleuritis.

WHAT DOES THIS STUDY ADD?

- ⇒ ANA positivity achieved high negative predictive value in discriminating lupus pleuritis from non-lupus pleural effusion.
- ⇒ Combination of C3 and C4 achieved a high negative predictive value in discriminating lupus pleuritis from non-lupus exudative pleural effusion.

HOW MIGHT THIS IMPACT ON CLINICAL PRACTICE OR FUTURE DEVELOPMENTS?

⇒ These biomarkers are useful in the differentiation between lupus pleuritis and pleural effusion due to other aetiologies.

changes, dominated with either neutrophils or lymphocytes, and with decreased levels of complements C3 as well as C4, and the presence of ANA.⁵ Nevertheless, the diagnostic values of various potential biomarkers for lupus pleuritis have not been directly compared in a single study. Furthermore, the distribution of these biomarkers in non-lupus pleural effusion may vary among the different aetiologies, which has not been fully explored in previous studies.

High-mobility group box 1 (HMGB1), a DNA-binding nuclear protein, is an endogenous damage-associated molecular pattern.⁶ HMGB1 promotes an inflammatory response through the receptor for advanced glycation end products (RAGE).⁷ Soluble RAGE (sRAGE) is a truncated form of RAGE and primarily acts as a decoy receptor to capture proinflammatory ligands like HMGB1.⁸ Several studies have demonstrated increased

circulating levels of HMGB1 and decreased levels of sRAGE in patients with autoimmune diseases such as rheumatoid arthritis (RA)^{9,10} and SLE.^{11–13} Therefore, perturbations in the levels of HMGB1 and sRAGE are postulated to be present in the pleural fluid of these patients.

Herein, we determined the pleural fluid levels of biomarkers potentially useful in discriminating lupus pleuritis from pleural effusion of different aetiologies.

METHODS

Patients

We prospectively enrolled 16 consecutive patients with SLE diagnosed according to the 1997 American College of Rheumatology criteria¹⁴ presenting with pleural effusion between March 2015 and December 2020. Their median disease duration was 5 years and half of them had lupus nephritis. The disease activity for SLE was evaluated by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI).¹⁵ Of the patients, 11 had lupus pleuritis, 4 had fluid overload (3 with nephrotic syndrome and 1 with heart failure) and 1 had malignant pleural effusion (cancer of unknown primary). We also enrolled 43 patients without SLE: 11 with infection-related pleural effusion (5 parapneumonic pleural effusion and 6 empyema), 18 with malignant pleural effusion based on pathological findings (8 lung cancer, 4 gynaecological cancer, 2 breast cancer, 2 gastrointestinal cancer, 1 hepatoma and 1 transitional cell carcinoma of the urinary bladder) and 14 with fluid overload (9 with heart failure and 5 with hepatic hydrothorax). The above diagnoses were made retrospectively at the discretion of the treating physician. In particular, lupus pleuritis was diagnosed based on associated symptoms, exclusion of other possible causes and treatment response. We determined whether a pleural effusion is exudative or transudative based on Light's criteria.¹⁶ Written consent from each participant was obtained.

Determination of HMGB1 and sRAGE

The level of HMGB1 in the pleural effusion was determined using an ELISA kit (Chondrex, Redmond, Washington, USA) according to the manufacturer's instructions. In brief, 100 µL of the capture antibody were added in each well at 4°C overnight. Then we added 50 µL pleural fluid sample or protein standards. Then 100 µL of the detection antibody were added and incubated at 37°C. After rinsing, 100 µL of streptavidin peroxidase were added and incubated at room temperature for 30 min. Detection was performed with tetramethylbenzidine solution. Optical density was measured at 450 nm using a DAR800 microplate reader (Cortez Diagnostics, California, USA). One sample from the lupus pleuritis group did not undergo determination of HMGB1. The level of sRAGE in the pleural effusion was determined using an ELISA kit (Quantikine, R&D Systems, Abingdon, UK) in accordance with the manufacturer's instructions. Briefly,

we added 100 µL of diluent to each well. We then added 50 µL of the patient's pleural fluid per well and incubated at room temperature for 2 hours. After rinsing, we added 200 µL of detection antibody conjugated to horseradish peroxidase and incubated at room temperature for 2 hours. Detection was performed with tetramethylbenzidine solution. Optical density was measured at 450 nm using Thermo Multiskan EX Microplate Photometer (Thermo Fisher Scientific). For each sample, duplicate measurements were made to obtain an average value.

Measurement of IL-17A and TNF-α levels

The pleural fluid level of two proinflammatory cytokines, interleukin 17-A (IL-17A) and tumour necrosis factor-α (TNF-α), was determined with ELISA (Quantikine) and chemiluminescent ELISA (QuantiGlo, R&D Systems), respectively. Optical densities were measured at 450 nm for IL-17A using Thermo Multiskan EX Microplate Photometer (Thermo Fisher Scientific) and the luminescence of TNF-α was determined using Beckman Coulter DTX 880 Multimode Detector (Beckman Coulter).

Determination of pleural fluid levels of ANA, C3, C4 and ADA

The pleural fluid level of ANA was determined with indirect immunofluorescence using a Hep-2 cell line (Medical & Biological Laboratories, Nagoya, Japan). The level of C3 and C4 was measured with immunoturbidimetry (Siemens Healthcare Diagnostics, Tarrytown, New York, USA). Adenosine deaminase (ADA) activity was evaluated before June 2016 using the endpoint method (Denka Seiken, Japan),¹⁷ and after June 2016 using the enzymatic method (InnoChem, Pyeongtaek, South Korea).¹⁸ Values obtained with these two methods were harmonised using Passing-Bablok regression.^{19,20} One patient without SLE and with malignant pleural effusion did not undergo C3 level determination. One patient without SLE and with malignant pleural effusion, and one without SLE and with fluid overload did not undergo C4 level examination. One patient without SLE and with infection-related pleural effusion did not undergo ADA activity examination.

Statistical analyses

Statistical analyses were performed using Stata V.15.0 software. Quantitative data were presented as median and IQR unless specified otherwise. Kruskal-Wallis test and χ^2 test were performed to assess differences between patients with lupus pleuritis and patients without SLE and with pleural effusion due to other aetiologies. For between-group comparisons of numerical variables (lupus pleuritis vs infection-related pleural effusion, malignant pleural effusion and fluid overload), non-parametric Mann-Whitney U test and χ^2 test were used. Bonferroni's correction was undertaken for multiple comparisons. The diagnostic performance for lupus pleuritis of each biomarker and their combinations was determined. The area under the receiver operating characteristic curve (AUC) was calculated using MedCalc statistical software

Table 1 Baseline characteristics

| | Lupus pleuritis (n=11) | Infection-related pleural effusion (n=11) | Malignant pleural effusion (n=18) | Fluid overload (n=14) |
|-------------------------|------------------------|---|-----------------------------------|-----------------------|
| Demographics | | | | |
| Age (years)* | 28 (23–44) | 71 (54–75)† | 69 (53–80)† | 69.5 (58–81)† |
| Female sex (%)* | 9 (82) | 2 (18)† | 10 (56) | 3 (21)† |
| Pleural effusion | | | | |
| pH | 7.1 (6.8–7.4) | 7.1 (6.5–7.4) | 7.4 (7.1–7.5) | 7.1 (7.0–7.4) |
| White cell count (/μL)* | 380 (28–874) | 9350 (1782–33 398)† | 485 (300–1116) | 435.5 (210–1047) |
| Neutrophils (%)* | 8 (3–27) | 81 (35–94)† | 6 (0–17) | 5 (2–11) |
| Lymphocytes (%)* | 24 (10–52) | 9 (5–27) | 38.5 (21–74) | 49 (38–62) |
| Protein (mg/dL)* | 3.7 (2.2–4.5) | 4.1 (2.9–4.9) | 3.7 (3.3–4.2) | 1.8 (1.4–2.8)† |
| LDH (U/L)* | 125 (98–203) | 1081 (403–1935)† | 208 (155–394) | 79 (63–96)† |
| Glucose (mg/dL)* | 118 (104–131) | 62 (4–146) | 115 (108–139) | 137 (119–211) |
| Exudative* | 8 (73) | 11 (100) | 18 (100) | 0 (0)† |

Data are presented as median (IQR) or number (percentage).

*P<0.05 as determined by Kruskal-Wallis test or χ^2 test.

†P<0.016 versus lupus pleuritis as determined by Mann-Whitney U test or χ^2 test based on adjustment for multiple comparisons.

LDH, lactate dehydrogenase.

(V.9.3; MedCalc Software, Ostend, Belgium). Youden index was calculated to set the optimal cut-off point. We also used Mann-Whitney U test to compare between patients with lupus pleuritis and SLE patients with fluid overload. Statistical significance was set at a two-sided p value of <0.05.

RESULTS

Demographic data and clinical characteristics of patients with lupus pleuritis and patients without SLE and with pleural effusion of other aetiologies

Patients with infection-related pleural effusion were the oldest, with a median age of 71 years, whereas patients with lupus pleuritis were the youngest, with a median age of 28 years (table 1). The group of patients with lupus pleuritis was predominantly female (82%), whereas the group of patients with infection-related pleural effusion was predominantly male (82%). Patients with infection-related pleural effusion had the highest white cell count, the highest percentage of neutrophils, as well as the highest level of lactate dehydrogenase in the pleural fluid.

Levels of HMGB1, sRAGE, ADA, IL-17A, TNF- α , ANA, C3 and C4 in the pleural fluid from patients with lupus pleuritis and patients without SLE and with pleural effusion of other aetiologies

We found significant differences in the level of sRAGE, ADA activity, IL-17A, C3 and C4, and in the proportion of ANA positivity, among the different groups with pleural effusion (table 2 and figure 1). The proportion of ANA positivity was higher whereas the level of C4 was lower in the lupus pleuritis group when compared with the infection-related pleural effusion group. There were lower levels of C3 and a trend towards lower ADA

activity and levels of IL-17A in the lupus pleuritis group when compared with the infection-related pleural effusion group. The proportion of ANA positivity was higher whereas the level of C4 was lower in the lupus pleuritis group when compared with the malignant pleural effusion group. There appeared a trend towards higher levels of sRAGE (p=0.059) but lower levels of C3 in the lupus pleuritis group when compared with the malignant pleural effusion group. The proportion of ANA positivity was higher and the level of C3 tended to be higher (p=0.080) in the lupus pleuritis group than the fluid overload-related pleural effusion group. The most frequent ANA pattern was fine speckled (90%) in lupus pleuritis, which was not different from pleural effusion due to other aetiologies.

Diagnostic performance of HMGB1, sRAGE, ADA, IL-17A, TNF- α , ANA, C3 and C4 in the pleural fluid to discriminate lupus pleuritis from pleural effusion of other aetiologies

Diagnostic performance regarding pleural fluid levels of HMGB1, sRAGE, ADA activity, IL-17A, TNF- α , C3 and C4 is illustrated in table 3 and figure 2. C3 and C4 levels both had good diagnostic performance in differentiating between lupus nephritis and infection-related pleural effusion (AUC: 0.81 and 0.82, respectively). C4 levels also had good diagnostic performance in differentiating between lupus pleuritis and malignant pleural effusion (AUC: 0.83). The sensitivity and specificity of ANA and C3/C4 at different cut-off points and their combinations are shown in table 3 and online supplemental table S1. The sensitivity and specificity of ANA were both good in differentiating between lupus pleuritis and pleural effusion of other aetiologies. A parallel combination of C3 (<24 mg/dL) and C4 (<3 mg/dL) had good diagnostic

Table 2 Level of potential biomarkers in the pleural fluid

| Potential markers | Lupus pleuritis (n=11) | Infection-related pleural effusion (n=11) | Malignant pleural effusion (n=18) | Fluid overload (n=14) |
|---------------------------------|------------------------|---|-----------------------------------|-----------------------|
| Immunological markers | | | | |
| HMGB1 (ng/mL) | 0.48 (0.39–18.02) | 4.24 (0.48–30.5) | 1.03 (0.62–2.53) | 0.54 (0.43–0.77) |
| sRAGE (pg/mL)* | 4232 (1256–5096) | 3030 (315–4721) | 3020 (1900–3806) | 4496 (3915–4956) |
| ADA (U/L)* | 13 (7–28) | 37 (19–87) | 12 (10–20) | 8 (6–11) |
| Proinflammatory cytokines | | | | |
| IL-17A (pg/mL)* | 3.07 (1.54–3.74) | 4.46 (2.4–15.33) | 2.31 (1.79–2.84) | 3.43 (1.79–6.81) |
| TNF- α (pg/mL) | 5.99 (2.21–21.56) | 5.93 (4.31–20.69) | 7.08 (4.89–8.65) | 4.37 (2.91–8.09) |
| SLE-related markers | | | | |
| ANA titre \geq 1: 80, n (%)* | 10 (91) | 2 (18)† | 4 (24)† | 1 (7)† |
| Pattern | | | | |
| Fine speckled | 9 (90) | 2 (100) | 2 (50) | 1 (100) |
| Homogenous | 6 (60) | 1 (50) | 1 (25) | 1 (100) |
| Coarse speckled | 3 (30) | 0 (0) | 0 (0) | 0 (0) |
| ANA titre \geq 1: 160, n (%)* | 9 (82) | 2 (18)† | 2 (12)† | 0 (0)† |
| ANA titre \geq 1: 320, n (%)* | 8 (73) | 1 (9)† | 1 (6)† | 0 (0)† |
| C3 (mg/dL)* | 22.3 (17.1–40.0) | 44.2 (24.1–78.8)† | 38.4 (33.2–53.0) | 14.0 (5.5–25.3) |
| Protein-adjusted C3*‡ | 6.56 (3.82–11.36) | 13.00 (8.31–18.7) | 11.15 (9.50–12.41) | 7.49 (6.11–9.47) |
| C4 (mg/dL)* | 2.5 (0.6–6.0) | 8.5 (6.1–13.9)† | 8.3 (6.4–10.0)† | 3.4 (1.2–4.7) |
| Protein-adjusted C4*‡ | 0.73 (0.16–2.35) | 2.83 (1.27–4.27)† | 2.27 (1.64–3.14)† | 1.53 (0.88–2.07) |

Data are presented as median (IQR) or number (percentage).

* $P < 0.05$ as determined by Kruskal-Wallis test or χ^2 test.

† $P < 0.016$ versus lupus pleuritis as determined by Mann-Whitney U test or χ^2 test based on adjustment for multiple comparisons.

‡C3 or C4 levels divided by pleural fluid levels of protein and then multiplied by 1000.

ADA, adenosine deaminase; HMGB1, high-mobility group box 1; IL-17A, interleukin 17A; sRAGE, soluble receptor for advanced glycation end products; TNF- α , tumour necrosis factor- α .

performance in differentiating lupus pleuritis versus infection-related pleural effusion and malignant pleural effusion.

Levels of HMGB1, sRAGE, proinflammatory cytokines and potential biomarkers in the pleural fluid between lupus pleuritis and pleural effusion of other aetiologies in patients with SLE

As shown in online supplemental table S2, nine (56%) patients with SLE had an active disease (SLEDAI \geq 4). The fluid overload group had a higher proportion of lupus nephritis and nephrotic range proteinuria than the lupus pleuritis group. We demonstrated levels of HMGB1, sRAGE, proinflammatory cytokines and potential biomarkers, including pleural effusion/serum ANA, C3 and C4 ratios,^{21 22} between lupus pleuritis and pleural effusion of other aetiologies in patients with SLE. We only found a trend towards higher C3 levels in lupus pleuritis than fluid overload-related pleural effusion in patients with SLE (online supplemental figure S1).

DISCUSSION

Pleural effusion is a common manifestation of SLE.^{2 3} Given multiple causes of pleural effusion, it is useful to

identify biomarkers for discriminating lupus pleuritis from pleural effusion of other aetiologies. We found in the present study that pleural fluid levels of ANA, C3 and C4 are potentially useful in discriminating lupus pleuritis from pleural effusion of other aetiologies.

Circulating levels of HMGB1 were elevated in RA and SLE.^{9 11 23} Upregulated HMGB1 is also present in patients with infectious diseases. Several studies have reported markedly elevated serum levels of HMGB1 in patients with sepsis²⁴ or severe sepsis,²⁵ and a positive correlation between plasma levels of HMGB1 and organ dysfunction in septic shock.²⁶ A prior study also reported elevated levels of HMGB1 in malignant and inflammatory pleural effusion compared with transudative pleural effusion.²⁷ Although the median level of HMGB1 appeared higher in infection-related pleural effusion than lupus pleuritis in the present study, the elevated level was not statistically significant. sRAGE acts as a suppressor of the inflammatory response in the RAGE axis. Circulating levels of sRAGE were decreased in patients with RA and SLE.^{10 12 13 28} A previous study reported lower levels of pleural fluid sRAGE in patients with bacterial pneumonia compared with those with tuberculosis or lung cancer.²⁹ We only observed a trend of higher levels of pleural fluid

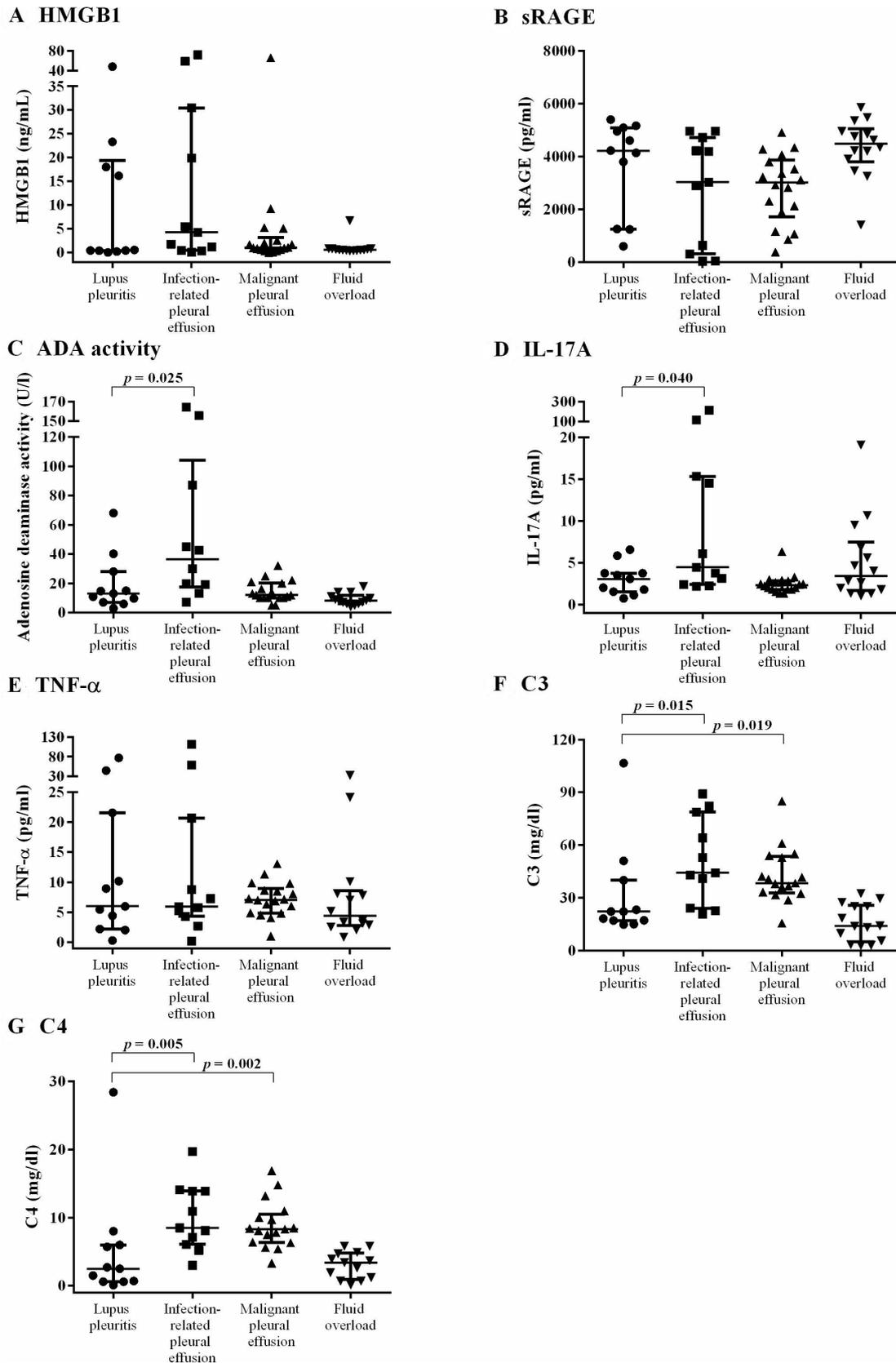


Figure 1 Pleural fluid level of potential biomarkers, including (A) HMGB1, (B) sRAGE, (C) ADA activity, (D) IL-17A, (E) TNF- α , (F) C3, and (G) C4. ADA, adenosine deaminase; HMGB1, high-mobility group box 1; IL-17A, interleukin 17A; sRAGE, soluble receptor for advanced glycation end products; TNF- α , tumour necrosis factor- α .

sRAGE with lupus pleuritis when compared with malignant pleural effusion.

ADA catalyses the deamination of adenosine, which is a crucial suppressor of the inflammation.³⁰ Moreover,

Table 3 Diagnostic performance of potential biomarkers

| | Lupus pleuritis vs infection-related pleural effusion | Lupus pleuritis vs malignant pleural effusion | Lupus pleuritis vs fluid overload |
|--|---|---|-----------------------------------|
| AUC (95% CI) for numerical variables | | | |
| Immunological markers | | | |
| HMGB1 | 0.62 (0.38 to 0.82) | 0.53 (0.33 to 0.72) | 0.55 (0.34 to 0.75) |
| sRAGE | 0.67 (0.44 to 0.85) | 0.71 (0.52 to 0.86) | 0.58 (0.37 to 0.78) |
| ADA activity | 0.72 (0.55 to 0.93) | 0.52 (0.33 to 0.71) | 0.70 (0.48 to 0.86) |
| Proinflammatory cytokines | | | |
| IL-17A | 0.70 (0.47 to 0.87) | 0.63 (0.43 to 0.80) | 0.59 (0.38 to 0.78) |
| TNF- α | 0.53 (0.31 to 0.74) | 0.52 (0.33 to 0.71) | 0.57 (0.36 to 0.73) |
| SLE-related markers | | | |
| C3 | 0.81 (0.58 to 0.94) | 0.77 (0.57 to 0.90) | 0.71 (0.49 to 0.87) |
| C4 | 0.82 (0.60 to 0.95) | 0.83 (0.64 to 0.94) | 0.52 (0.31 to 0.73) |
| Sensitivity/specificity for binary variables | | | |
| ANA titre \geq 1: 80, % | 91/82 | 91/76 | 91/93 |
| ANA titre \geq 1: 160, % | 82/82 | 82/88 | 82/100 |
| ANA titre \geq 1: 320, % | 73/91 | 73/94 | 73/100 |

Data are presented as AUC (95% CI).

ADA, adenosine deaminase; AUC, area under the receiver operating characteristic curve; HMGB1, high-mobility group box 1; IL-17A, interleukin 17A; sRAGE, soluble receptor for advanced glycation end products; TNF- α , tumour necrosis factor- α .

ADA is involved in the differentiation and maturation of the immune cells such as lymphocytes.³¹ Elevated serum ADA activity was found in patients with SLE.^{31,32} A previous study reported elevated ADA activities in tuberculous pleurisy compared with lupus pleuritis. Similarly, we observed a trend towards higher ADA activity in infection-related pleural effusion compared with lupus pleuritis. Taken together, ADA activity in the pleural fluid was upregulated in infection-related pleural effusion. Regarding pleural fluid cytokines, we observed a trend of

a higher level of IL-17A in infection-related pleural effusion compared with lupus pleuritis. This finding is in line with its known role in bacterial infection.³³

The significant biomarkers for lupus pleuritis included the higher proportion of ANA positivity and lower levels of C4. Notably, ANA positivity achieved a high sensitivity of 91%, a specificity of 83%, a positive predictive value (PPV) of 59% and a high negative predictive value (NPV) of 97% in discriminating lupus pleuritis from pleural effusion of all other aetiologies combined (data not shown).

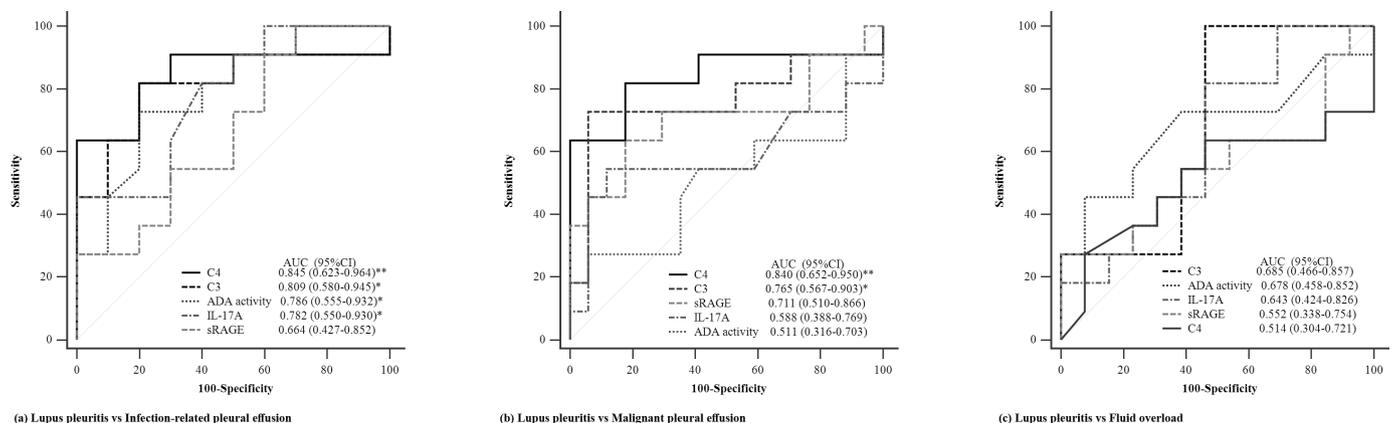


Figure 2 Receiver operating characteristic curve for pleural fluid level of potential biomarkers with respect to (a) lupus pleuritis vs. infection-related pleural effusion, (b) lupus pleuritis vs. malignant pleural effusion, and (c) lupus pleuritis vs. fluid overload. One patient without SLE with malignant pleural effusion did not undergo C3 level determination. One patient without SLE and with malignant pleural effusion, and one without SLE and with fluid overload did not undergo C4 level examination. One patient without SLE and with infection-related pleural effusion did not undergo ADA activity examination. ADA, adenosine deaminase; AUC, area under the receiver operating characteristic curve; IL-17A, interleukin 17A; sRAGE, soluble receptor for advanced glycation end products.

Our results are consistent with previous reports on high titre ANA ($\geq 1:160$) in pleural fluid being a sensitive but less specific indicator of lupus pleuritis,^{21 22 34 35} although we found the positivity for a low titre ANA ($\geq 1:80$) having a better diagnostic performance over the other titre thresholds. Resonated with our findings, the newly updated diagnostic criteria for SLE have taken a low titre ANA ($\geq 1:80$) as the entry criterion to improve its sensitivity.^{36 37} To be noted, 89% of patients with lupus pleuritis had pleural fluid/serum ANA ratio ≤ 1 (data not shown), which implied the origin of pleural fluid ANA being the circulating blood.

In agreement with previous studies,^{38–41} we revealed lower pleural fluid levels of C3 and C4 in patients with lupus pleuritis. Our results showed that either C3 or C4 level had good diagnostic performance in discriminating lupus pleuritis from infection-related or malignant pleural effusion. Furthermore, a parallel combination of C3 (<24 mg/dL) and C4 (<3 mg/dL) showed better diagnostic performance than either biomarker alone. This combination achieved a sensitivity of 82%, a specificity of 89%, a PPV of 75% and a high NPV of 93% in differentiating between lupus pleuritis and exudative pleural effusion (infection-related and malignant pleural effusion combined; data not shown). To be noted, the lupus pleuritis group had lower pleural fluid levels of C4 when compared with infection-related and malignant pleural effusion. This may be partly explained by the concomitant genetic deficiency of C4 in these patients with SLE.⁴² On the contrary, we demonstrated higher pleural fluid levels of C3 in lupus pleuritis when compared with fluid overload-related pleural effusion in patients both without and with SLE. The finding differed from earlier studies which had reported lower C3 levels in lupus pleuritis.^{38–41} However, most of these studies recruited few patients with SLE (<10), and fluid overload-related pleural effusion was under-represented in the control group. Besides, another study reported lower C4 levels in pleural effusion due to heart failure when compared with parapneumonic and malignant pleural effusion.⁴¹ Notably, we found lower serum levels of C3 in patients with SLE with fluid overload than the lupus pleuritis group (44.25 (IQR 33.05–61.25) mg/dL vs 89.8 (IQR 62.2–119) mg/dL; data not shown), which might be the result of a higher proportion of nephrotic range proteinuria in the fluid overload group. This partly explains the lower pleural fluid C3 levels in our patients with SLE with fluid overload. It was also likely that the complement components in the blood had entered the affected tissue (eg, the pleural space) only under inflammation like in exudative pleural effusion.

In our 11 patients with lupus pleuritis, only 1 (10%) had pleural fluid/serum C3 and C4 ratios >1 . In addition, as demonstrated in [table 2](#), pleural fluid levels of C3 and C4 adjusted by protein levels appeared lower in the lupus pleuritis group. Furthermore, most (78%) of them had lower protein-adjusted pleural fluid levels of C3 and C4 when compared with protein-adjusted serum levels of C3 and C4 (data not shown). These observations are in

line with the results of previous studies which suggested activation of the complement cascade locally in lupus pleuritis.^{38 40 41} Interestingly, we found a significantly lower pleural fluid levels of C3 (14.2 (IQR 9.9–25.1) mg/dL vs 40.7 (IQR 24.1–53.0) mg/dL) and C4 (2.85 (IQR 0.95–4.80) mg/dL vs 8.0 (IQR 5.4–10.9) mg/dL) between exudative and transudative pleural effusion in our 59 patients (both $p<0.001$; data not shown). Their diagnostic performance in comparison with traditional Light's criteria should be explored in the following studies.

There are some limitations to our study. First, our study is limited by the small number of patients with pleural effusion. Patients with lupus pleuritis are difficult to recruit owing to the few number of these cases in clinical practice. A larger multicentre study is required to validate our findings on biomarkers for lupus pleuritis. Nevertheless, we have recruited patients with pleural effusion due to different common aetiologies. We have also comprehensively analysed an array of potential biomarkers in these pleural fluid samples. Second, we did not recruit enough patients with SLE presenting with pleural effusion of other aetiologies. Therefore, our findings cannot be extrapolated to differentiation between autoimmune pleuritis and pleural effusion of other aetiologies in patients with SLE.

CONCLUSIONS

Our results showed that ANA positivity and levels of C3 and C4 in the pleural fluid could help discriminate lupus pleuritis from pleural effusion of other aetiologies with a high NPV. If we analysed exudative pleural effusion only (exudative lupus pleuritis vs infection-related or malignant pleural effusion; online supplemental table S3), the diagnostic performance of ANA would be slightly better, whereas those of C3 and C4 would not change. We have proposed a diagnostic algorithm (online supplemental figure S2). More studies are needed to validate our findings.

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Table S1. The diagnostic performances of C3 or C4 levels with selected cut-off points, or parallel/serial combinations of potential biomarkers in discriminating lupus pleuritis from infection-related or malignant pleural effusion.

| Sensitivity/Specificity | Lupus pleuritis vs. infection-related pleural effusion | Lupus pleuritis vs malignant pleural effusion |
|--|--|---|
| C3 (cut-off: < 23 mg/dl) | 64%/82% | 64%/94% |
| C3 (cut-off: < 24 mg/dl) | 73%/82% | 73%/94% |
| C4 (cut-off: < 3 mg/dl) | 64%/100% | 64%/100% |
| Parallel combinations | | |
| C3 (cut-off: < 23 mg/dl) and ANA | 64%/100% | 64%/94% |
| C3 (cut-off: < 23 mg/dl) or ANA | 91%/64% | 91%/76% |
| C3 (cut-off: < 24 mg/dl) and ANA | 64%/100% | 64%/94% |
| C3 (cut-off: < 24 mg/dl) or ANA | 100%/64% | 100%/76% |
| C4 [‡] and ANA | 64%/100% | 64%/100% |
| C4 [‡] or ANA | 91%/82% | 91%/78% |
| C3 (cut-off: < 23 mg/dl) and C4 [‡] | 55%/100% | 55%/100% |
| C3 (cut-off: < 23 mg/dl) or C4 [‡] | 73%/82% | 73%/94% |
| C3 (cut-off: < 24 mg/dl) and C4 [‡] | 55%/100% | 55%/100% |
| C3 (cut-off: < 24 mg/dl) or C4 [‡] | 82%/82% | 82%/94% |
| Serial combinations | | |
| ANA and C4 [‡] | 64%/100% | 64%/100% |
| ANA or C4 [‡] | 91%/82% | 91%/76% |

[‡]cut-off: < 3 mg/dl

ANA, antinuclear antibodies titer \geq 1:80; C3, complement 3; C4, complement 4.

Table S2. Levels of HMGB1, sRAGE, pro-inflammatory cytokines and SLE-related markers in the pleural effusion of recruited SLE patients.

| | Lupus pleuritis (n=11) | Malignant pleural effusion (n=1) | Fluid overload (n=4) |
|--|---------------------------|--|-------------------------|
| Characteristics | | | |
| Age (years) | 28 (23, 44) | 26 | 40 (30, 50) |
| Female sex (%) | 9 (82%) | 1 (100%) | 3 (75%) |
| Disease duration (years) | 3 (0, 8) | 6 | 10 (3, 24) |
| Lupus nephritis* | 3 (27%) | 1 (100%) | 4 (100%) |
| Disease activity (SLEDAI) | 7 (3, 15) | 0 | 19 (9, 25) |
| Active disease (SLEDAI \geq 4) | 6 (55%) | 0 (0%) | 3 (75%) |
| Nephrotic-range proteinuria (\geq 3.5 gm/day)* | 0 (0%) | 0 (0%) | 2 (50%) |
| Immunological markers | | | |
| HMGB1 (ng/mL) | 0.48 (0.39, 18.02) | 0.79 | 5.30 (2.95, 8.22) |
| sRAGE (pg/ml) | 4232 (1256, 5096) | 4457 | 3562 (2053, 4555) |
| Adenosine deaminase activity (U/l) | 13 (7, 28) | 11 | 6 (5, 12) |
| Cytokines | | | |
| IL-17A (pg/ml) | 3.07 (1.54, 3.74) | 1.64 | 2.20 (1.34, 2.39) |
| TNF- α (pg/ml) | 5.99 (2.21, 21.56) | 6.02 | 9.98 (6.35, 16.82) |
| SLE-related markers | | | |
| Antinuclear antibody (ANA) titer \geq 1: 80, n (%) | 10 (91%) | 0 (0%) | 3 (75%) |
| Pattern | | | |
| Fine speckled | 9 (90%) | 0 (0%). | 3 (100%) |
| Homogeneous | 6 (60%) | 0 (0%) | 3 (100%) |
| Coarse speckled | 3 (30%) | 0 (0%) | 0 (0%) |
| ANA titer \geq 1: 160, n (%) | 9 (82%) | 0 (0%) | 3 (75%) |

| | | | |
|--|-------------------|--------|-------------------|
| ANA titer \geq 1: 320, n (%) | 8 (73%) | 0 (0%) | 3 (75%) |
| PE/serum ANA ratio \geq 1 [‡] | 4 (44%) | 0 (0%) | 3 (75%) |
| C3 (mg/dl)* | 22.3 (17.1, 40.0) | 42.5 | 12.2 (8.05, 14.6) |
| PE/serum C3 ratio | 0.21 (0.18, 0.51) | 0.31 | 0.21 (0.16, 0.45) |
| C4 (mg/dl) | 2.5 (0.6, 6.0) | 4.1 | 2.9 (2.0, 5.5) |
| PE/serum C4 ratio | 0.22 (0.09, 0.34) | 0.18 | 0.16 (0.11, 0.60) |

C3, complement 3; C4, complement 4; HMGB1, high mobility group box 1; IL-17A, interleukin-17A; PE, pleural effusion; SLE, systemic lupus erythematosus; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; sRAGE, soluble receptor for advanced glycation end products; TNF- α , tumor necrosis factor- α

[‡]Two patients did not have serum ANA examinations.

* $p < 0.05$ between lupus pleuritis and fluid overload as determined by the Mann-Whitney U test.

Table S3. Diagnostic performances of the potential biomarkers in patients with exudative pleural effusion[‡].

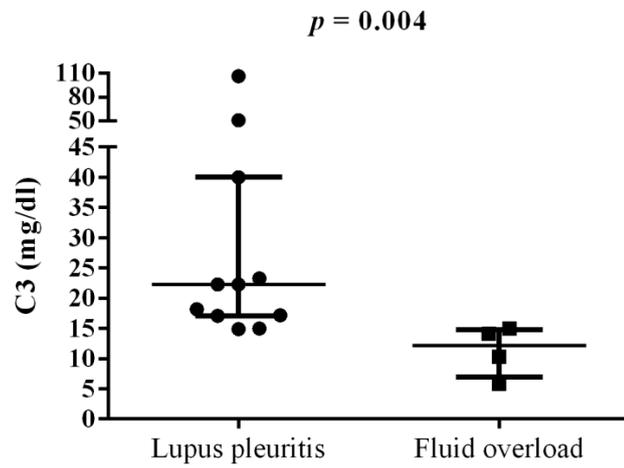
| | Lupus pleuritis vs. infection-related pleural effusion | Lupus pleuritis vs malignant pleural effusion |
|--|--|---|
| The area under the receiver operating characteristic (95% CI) for numerical variables | | |
| Immunological markers | | |
| HMGB1 | 0.64 (0.33, 0.90) | 0.51 (0.18, 0.87) |
| sRAGE | 0.69 (0.40, 0.91) | 0.76 (0.50, 0.98) |
| ADA activity | 0.73 (0.44, 0.93) | 0.58 (0.30, 0.87) |
| Pro-inflammatory cytokines | | |
| IL-17A | 0.80 (0.49, 0.95) | 0.50 (0.16, 0.86) |
| TNF- α | 0.50 (0.21, 0.80) | 0.52 (0.18, 0.81) |
| SLE-related markers | | |
| Complement 3 | 0.78 (0.48, 1.00) | 0.75 (0.47, 1.00) |
| Complement 4 | 0.81 (0.49, 1.00) | 0.80 (0.51, 1.00) |
| The sensitivity/specificity for binary variables | | |
| ANA titer \geq 1: 80 | 100%/82% | 100%/76% |
| ANA titer \geq 1: 160 | 88%/82% | 88%/88% |
| ANA titer \geq 1: 320 | 75%/91% | 75%/94% |

[‡]Data are presented as AUC and 95% confidence interval;

ADA, adenosine deaminase; ANA, antinuclear antibody; AUC, the area under the receiver operating characteristic curve; HMGB1, high mobility group box 1; IL-17A, interleukin-17A; SLE, systemic lupus erythematosus; sRAGE, soluble receptor for advanced glycation end products; TNF- α , tumor necrosis factor- α .

Figure S1. Pleural fluid levels of C3 in SLE patients with lupus pleuritis and fluid

overload.

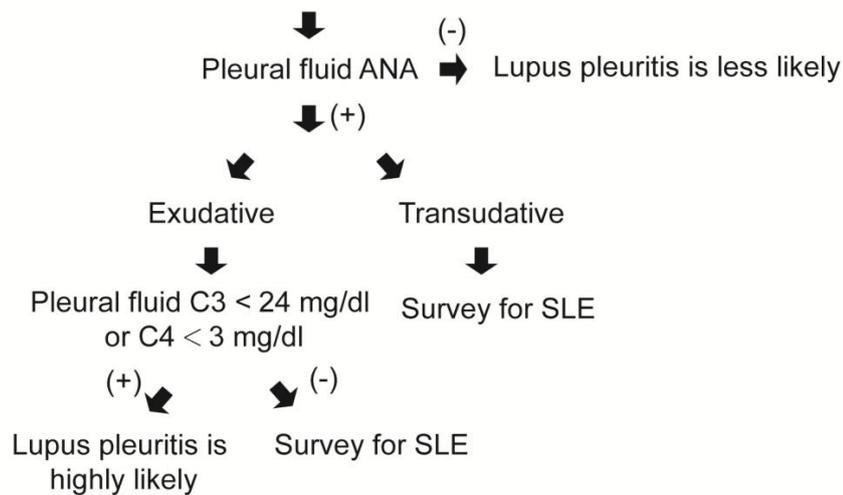


C3, complement 3, SLE, systemic lupus erythematosus.

Figure S2. The proposed diagnostic algorithm in patients with suspected lupus

pleuritis.

Pleural effusion of unknown etiology with features suggestive of lupus pleuritis, e.g. young age, female sex, accompanying symptoms/signs, etc. in patients without previous diagnosis of SLE



SLE, systemic lupus erythematosus.