Journal of Microbiology, Immunology and Infection (2015) 48, 483–489

Available online at www.sciencedirect.com

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ORIGINAL ARTICLE

# The value of total protein in guiding management of infectious parapneumonic effusion by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

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Received 18 July 2013; received in revised form 25 October 2013; accepted 19 November 2013
Available online 21 February 2014

**KEYWORDS**
Biomarkers;
Complicated parapneumonic effusion;
Surgical intervention

*Background/Purpose:* Infectious parapneumonic effusion (PE) contains proteins originating from circulation as well as proteins locally released by inflammatory pulmonary cells. The purpose of this study was to investigate the value of total protein analysis in guiding management of infectious PE by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.
*Methods:* Fifty-seven children with pneumonia followed by PE were consecutively enrolled into our study. Protein profiles generated by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry after fractionating samples with functionalized magnetic

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http://dx.doi.org/10.1016/j.jmii.2013.11.013

beads (C8) were used for differentiating complicated PE (CPE) from non-CPE. A training set was used to generate classification models and the clinical efficacy of these models in detecting CPE and the need for intervention was then evaluated in an independent set.
*Results:* The MS spectra derived from PE were analyzed, and classification models were constructed in the training set. A total of 123 mass/charge ($m/z$) values were identified and 23 $m/z$ values which were significant with $p < 0.05$ were used as classifiers. An optimized genetic algorithm model containing enforced selection of three significant downregulated $m/z$ values (2127, 2232, and 2427) was able to classify CPE with 100% positive predictive value and predict the need of aggressive therapeutic intervention with 77% positive predictive value.
*Conclusion:* A diagnostic model construction comprising three potential biomarkers can predict CPE and need for surgical intervention rapidly and precisely. Pleural fluid proteins downregulated during the progression of pneumonia could potentially guide the management of infectious PE.


## Introduction

Community-acquired pneumonia is a common infectious illness in children and complicated parapneumonic effusion (CPE) is a well-recognized complication of bacterial pneumonia.[1] Failing adequate therapy to control the pleural inflammation may lead to progressive pleural thickening and fibrin deposition, resulting in organizing empyema. The therapeutic strategies for parapneumonic effusion (PE) vary from a conservative approach with adequate antibiotic therapy, expedient drainage including thoracentesis, tube thoracostomy, or surgical drainage.[2] However, the optimal time of intervention for PE and empyema in pediatric patients remains a challenge in clinical practice.

Infectious pleural fluid accumulation is posited to be a continuing process of pleural inflammation and is mainly a result of inflammatory response caused by pneumonia. Clinically, biochemical analysis of pleural effusion plays an important role in the management of pleural effusions.[3] In general practice, pleural infection is indicated by acidosis associated with raised lactate dehydrogenase (LDH) and low glucose levels. The indicators of pH $\leq$ 7.2, LDH $\geq$ 1000 U/L and glucose $\leq$40 mg/dL are characteristics of CPE that are more likely to require aggressive interventions.[3,4] Despite this, several inflammatory mediators such as tumor necrosis factor-$\alpha$, interleukin-1$\beta$, and interleukin-6 have been reported to have a significant role in pyogenic infections in the pleural space.[5–7] A recent study has also shown that not only pleural fluid proinflammatory cytokines but also anti-inflammatory cytokines are accurate in discriminating complicated effusions.[8] It is therefore believed that proteins in pleural fluids could potentially be useful as markers of CPE for guiding clinical management.

Use of proteomics techniques to identify disease-specific protein biomarkers is a powerful tool for defining prognosis of disease.[9,10] The discovery of biomarkers, proteins that change in concentration or state in association with a specific biological process can help to gain deep insights into disease mechanisms in which proteins play a major role. Several studies have shown that pleural effusion contains proteins originating from circulation as well as proteins locally released by inflammatory or epithelial cells.[11] The pleural exudates from a patient with severe pneumonia have been investigated and contain proteins of potential diagnostic and therapeutic value.[12] A systematic identification of the dynamic changes of proteins involved in inflammation in the pleural cavity, however, has not been well clarified. An improved understanding in the pattern recognition of proteins in pleural fluids is potentially able to classify CPE for clinical management. The aim of this study was to investigate the proteomics profiling data of infectious parapneumonic effusions obtained using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), hopefully to obtain some potential diagnostic value and even guide the timing of surgical intervention.

## Materials and methods

### Patients and supernatant specimens of pleural fluid

The study population consisted of patients aged <18 years who had community-acquired pneumonia requiring hospitalization in Chang Gung Children's Hospital (Taoyuan, Taiwan) between January 2005 and January 2007. Patients with PE were consecutively enrolled into our study and all underwent real-time chest sonography and thoracentesis irrespective of size of pleural effusion. This study was approved by the Ethic Committee of Chang Gung Memory Hospital. Informed written consent was obtained from the parents of all study participants.

Pleural fluid was collected using a standard thoracocentesis technique after chest sonography prior to intervention procedures. Pleural fluid was immediately analyzed for pH, total cell counts, and differential cell count, and for protein, glucose, and LDH concentrations. Pleural fluid for pH analysis was collected anaerobically with heparin and measured in a handheld analyzer (i-STATA Portable Clinical Analyzer; i-STATA Corporation; East Windsor, NJ, USA). A 4-$\mu$L sample of specimen was mixed with 3.2% sodium citrate solution in a ratio of 9:1 pleural fluid to citrate, which were immediately immersed in ice separately and centrifuged at 1500*g* for 10 minutes. The cell-free supernatant from each sample was stored