Predictive value of Thomsen-Friedenreich antigen activation for *Streptococcus pneumoniae* infection and severity in pediatric lobar pneumonia

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**KEYWORDS**  
Empyema; Parapneumonic effusion; Pediatric; *Streptococcus pneumoniae*; Thomsen-Friedenreich antigen

**Abstract**  
Background: Most cases of complicated pneumonia in children are caused by pneumococcal infections. Thomsen-Friedenreich antigen (TA) is present on erythrocytes, platelets and glomeruli, and it can be activated during pneumococcal infection. The aim of this study was to investigate the predictive value of TA activation for pneumococcal infection and association with the severity of complicated pneumonia.  
Materials and methods: Patients with lobar pneumonia were routinely tested for TA at the Department of Pediatrics, Mackay Memorial Hospital from January 2010 to December 2015. We retrospectively reviewed and analyzed their charts and data including age, sex, etiology of infection, chest tube insertion or video-assisted thoracoscopic surgery, length of hospital stay, TA activation, white blood cell count and level of C reactive protein.  
Results: A total of 142 children with lobar pneumonia were enrolled, including 35 with empyema, 31 with effusion, 11 with necrotizing pneumonia and four with lung abscess. *Streptococcus pneumoniae* was the most commonly identified pathogen. Twenty-two patients (15.4%) had activated TA, all of whom were infected with *S. pneumoniae*. TA activation had 100% specificity and 100% positive predictive value for pneumococcal infection. In the multivariate analysis in lobar pneumonia, TA activation (OR, 15.8; 95% CI, 3.0–83.5; *p* = 0.001), duration of fever before admission (OR, 1.2; 95% CI, 1.1–1.5; *p* = 0.013) and initial CRP level (OR, 1.1; 95% CI, 1.0–1.1; *p* = 0.004) were independent predictors of empyema.
Introduction

Community-acquired pneumonia (CAP) is the leading cause of morbidity and mortality in children worldwide. Complications of CAP include parapneumonic pleural effusion (PPE), empyema, necrotizing pneumonia, and lung abscess. Pediatric PPE have been reported in up to 50% of patients who require hospital admission for typical bacterial pneumonia. These complications have been reported to result in prolonged hospitalization, the need for surgical intervention, and a higher risk of serious and irreversible sequelae in children.

Streptococcus pneumoniae is the most common bacterial pathogen involved in pediatric CAP. However, conventional microbiological culture methods have low sensitivity, usually because of antimicrobial pretreatment before sampling of a sterile site. The Thomsen-Friedenreich antigen (TA) is covered by N-acetyl-neuraminic acid, which can cleave N-acetyl-neuraminic acid and expose the normally hidden TA present on erythrocytes, platelets, and glomeruli, which in turn reacts with anti-TA antibodies normally present in the plasma resulting in "TA activation". TA activation induced by bacteria may occur quickly in vivo and last a few days or weeks, rarely persisting for months, and it may not be affected by antibiotic pretreatment. We hypothesized that since TA activation is an indirect measure of neuraminidase activity, it may help in the diagnosis of S. pneumoniae infections and may also indicate the severity of the infection. Therefore, the aim of this study was to investigate the predictive value of TA activation with regards to the identification of S. pneumoniae infection and its severity in children with CAP.

Materials and methods

Study population

We enrolled pediatric patients (aged from 1 to 18 years) who were admitted to the Department of Pediatrics, Mackay Memorial Hospital, from January 2010 to December 2015, with the diagnosis of lobar pneumonia with or without PPE. Children were excluded if they had proven immunodeficiency or immunosuppression in previous medical records. The Ethics Committee of Mackay Memory Hospital approved this study (IRB: 16MMHIS061e).

Conclusions: TA activation is a specific marker for pneumococcal pneumonia and might indicate higher risk for complicated pneumonia.

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into five groups according to the degree of agglutination as follows: trace, 1+, 2+, 3+, and 4+. We retrospectively reviewed and analyzed the age, sex, etiology and pathogen involved in the infection, duration of fever before and after hospitalization, chest tube insertion only or VATS, duration of hospitalization, intensive care unit (ICU) stay, and antimicrobial therapy. Laboratory data included T antigen, hemoglobin (Hb), white blood cell (WBC) and platelet counts, and C-reactive protein (CRP). Pathogens were detected in bacterial cultures from blood, pleural effusion, ear, throat and sputum specimens. Rapid antigen assays were used for \textit{S. pneumoniae}, \textit{H. influenzae type b}, respiratory syncytial virus, adenovirus and influenza virus, with throat or nasopharyngeal viral cultures, and serology studies for \textit{Mycoplasma pneumoniae}. The diagnostic criteria for pneumococcal infection were: (1) isolation of \textit{S. pneumoniae} from cultures of blood, ear, throat or pleural effusion; (2) positive results of pneumococcal antigen in urine or pleural effusion. \textit{S. pneumoniae} sepsis was defined as isolation of \textit{S. pneumoniae} from at least one set of blood cultures. \textit{M. pneumoniae} enzyme-linked immunoassays were performed. Mycoplasmal infection was diagnosed if the immunoglobulin M (IgM) level was \( \geq 10.0 \) index or there was a \( 4 \)-fold rise in immunoglobulin G (IgG) titer in measurements two weeks apart. Hemolytic uremic syndrome (HUS) was diagnosed in the presence of thrombocytopenia (platelet count \( < 150,000/mm^3 \)), acute onset of anemia with microangiopathic changes in blood smears, and renal injury evidenced by hematuria, proteinuria, or an elevated serum creatinine level.11

Statistical analysis

Continuous variables were expressed as mean \( \pm \) standard deviation (SD) and compared using the Student’s t test. Categorical variables were expressed as absolute and relative frequencies and compared using the chi-square or Fisher’s exact test. We then performed univariate and multiple logistic regression analysis to identify independent predictors of the occurrence of empyema. Variables were entered into the regression model if they were significantly associated with empyema in the univariate analysis. Pearson correlation was used to evaluate the degree of the relationship between the titer of TA activation and the severity of complicated pneumonia. A \( p \) value of \( \leq 0.05 \) was considered to be significant. All probabilities were 2-tailed. Odds ratios (ORs) and their 95% confidence intervals (CIs) were also determined. All analyses were performed using SPSS statistical software (SPSS Inc., Chicago, IL, USA).

Results

Epidemiology

A total of 142 children with lobar pneumonia were enrolled, including 30 with pleural effusion, 22 with empyema, 10 with necrotizing pneumonia, four with lung abscesses, and 76 patients with lobar pneumonia only. The female-to-male ratio was 1.02 and the mean age was 57.2 \( \pm \) 31.7 months. A total of 37 patients (26%) underwent VATS, and eight needed thoracostomy only. The length of hospital stay was 10.6 \( \pm \) 8.1 days. Twenty-nine patients (20.4%) were admitted to the ICU, and the mean length of ICU stay was 7.2 \( \pm \) 5.7 days. Twenty-two patients (15.4%) had TA activation, and their mean age was 41.9 \( \pm \) 15.0 months.

Clinical characteristics and laboratory data

The clinical characteristics and laboratory data of the patients with and without TA activation are listed in Tables 1 and 2, respectively. The hospitalized children with TA activation were younger (average age: 41.9 months vs. 60.0 months; \( p = 0.013 \), more likely to be \( < 2 \) years old (18.2% vs. 5.8%, \( p = 0.046 \)), less likely to be \( \geq 5 \) years old (9.1% vs. 33.3%, \( p = 0.022 \)), and had a higher peak WBC count, higher initial and peak bands, higher initial and peak CRP levels, longer hospital stay, higher rate of ICU admission, higher rates of VATS and thoracostomy, longer total duration of fever, and higher rates of \textit{S. pneumoniae} sepsis, \textit{S. pneumoniae}-related pneumonia and HUS than those without TA activation. There was no significant difference in sex. Pneumococcal infections were confirmed in 71 patients by positive cultures of pleural fluid (eight patients), blood (19 patients) or by the detection of pneumococcal antigen in pleural fluid or urine (54 patients). In pneumococcal pneumonia, culture proven from pleural effusion (22.7% vs. 6.1%, \( p = 0.041 \)) and positive results of pneumococcal antigen in pleural effusion (59.0% vs. 12.2%, \( p < 0.001 \)) had statistically significant difference in TA activation group.

Etiology and serotype

An etiologic diagnosis was established in 22 (100%) of the patients with TA activation and 84 (70%) of those without TA activation. The most commonly identified pathogen was \textit{S. pneumoniae} in 71 (50%) patients. TA activation had 100% specificity, 100% positive predictive value, 59.1% negative predictive value and 30.9% sensitivity for pneumococcal infection. In the TA activation group, dual infections with \textit{S. pneumoniae} and \textit{M. pneumoniae} were the proven etiology in two patients, and the other 20 patients were diagnosed with pneumococcal pneumonia only (Table 3). The serotypes of \textit{S. pneumoniae} in the TA activation group included serotype 19A in 12 (54.5%), serotype 3 in three (13.6%), serotype 6A in one (4.5%), and unknown in six (27.2%) (Table 4). Among the proven serotypes of \textit{S. pneumoniae}, serotype 19A was the most common in both the patients with (12/16, 75%) and without (17/21, 80.9%) TA activation. There was no statistically significant difference in serotype distribution (Table 4).

There were 14 (9.8%) virus-related cases, and all of them were without TA activation (Table 3). Nine patients had virus only, including adenovirus in five (55.6%), parainfluenza virus in two (22.2%), respiratory syncytial virus in one (11.1%) and influenza virus type A in one (11.1%). Dual infections with virus and \textit{M. pneumoniae} were in three patients, and the etiology was adenovirus, respiratory syncytial virus and influenza virus type B, respectively. Two patients had triple infection with virus, \textit{M. pneumoniae} and \textit{S. pneumoniae}. One was parainfluenza virus with \textit{S. pneumoniae} proven by throat culture, and another was
multivariate analysis in all lobar pneumonia, TA activation (OR, 6.9; 95% CI, 1.8–25.5; p = 0.005) and duration of fever before admission (OR, 5.2; 95% CI, 1.4–19.4; p = 0.015) were independent predictors of empyema. In the multivariate analysis in pneumococcal infection, TA activation (OR, 2.9; 95% CI, 1.1–7.4; p = 0.032) and duration of fever before admission (OR, 4.8; 95% CI, 1.3–16.9; p = 0.013) were independent predictors of empyema.

**Discussion**

The presence of pleural effusion is significantly associated with bacterial pneumonia. In this study, the most common pathogen was *S. pneumoniae*, which is consistent with previous studies. TA activation is a specific marker for pneumococcal pneumonia and might indicate higher risk for complicated pneumonia. Neuraminidase produced by...
In our study, three patients had HUS, all of whom had a highly specific diagnostic tool for pneumococcal infection. It has been suggested that the detection of neuraminidase activity is a highly specific diagnostic tool for pneumococcal HUS. In our study, TA activation was detected in 100% of the patients with pneumococcal HUS, and in 69% of the patients with invasive pneumococcal disease. This indicates that TA activation can be detected in patients with severe pneumococcal infection without HUS. The pathophysiology of pneumococcal HUS is proposed to involve both bacterial and host factors, such as host genetics factors, host immunity or environmental factors which influence patients to develop HUS.

In our patients with lobar pneumonia with or without PPE, positive TA activation had 100% specificity and positive predictive value of 100% for pneumococcal infection. Antimicrobial treatment before sampling pleural fluid frequently results in negative cultures. Penicillin-sensitive *S. pneumoniae* has been reported in over 70% of culture-negative empyema fluids using a molecular diag-

**Table 2** Hematological findings of the 142 pediatric patients with lobar pneumonia.

<table>
<thead>
<tr>
<th>Laboratory data</th>
<th>All (N = 142)</th>
<th>Pneumococcal infection group (N = 71)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TA⁺ (Mean ± SD)</td>
<td>TA⁻ (Mean ± SD)</td>
</tr>
<tr>
<td>Initial Hb (g/dl)</td>
<td>10.4 ± 1.9</td>
<td>11.5 ± 1.1</td>
</tr>
<tr>
<td>Lowest Hb (g/dl)</td>
<td>8.3 ± 1.8</td>
<td>11.1 ± 1.3</td>
</tr>
<tr>
<td>Initial WBC count (×10³/µL)</td>
<td>12.109 ± 8486</td>
<td>15.683 ± 9096</td>
</tr>
<tr>
<td>Peak WBC count (×10³/µL)</td>
<td>22.095 ± 8577</td>
<td>16.902 ± 9235</td>
</tr>
<tr>
<td>Initial band (%)</td>
<td>15.8 ± 23.0</td>
<td>3.5 ± 4.8</td>
</tr>
<tr>
<td>Peak band (%)</td>
<td>16.5 ± 16.8</td>
<td>4.4 ± 5.5</td>
</tr>
<tr>
<td>Initial platelet (10³/µL)</td>
<td>206.0 ± 153.0</td>
<td>287.0 ± 120.1</td>
</tr>
<tr>
<td>Lowest platelet (10³/µL)</td>
<td>159.0 ± 144.2</td>
<td>273.7 ± 107.6</td>
</tr>
<tr>
<td>Initial CRP (mg/dL)</td>
<td>31.9 ± 13.4</td>
<td>15.7 ± 12.6</td>
</tr>
<tr>
<td>Peak CRP (mg/dL)</td>
<td>33.4 ± 11.0</td>
<td>16.6 ± 12.5</td>
</tr>
</tbody>
</table>

**Table 3** Etiologies of the 142 pediatric patients with lobar pneumonia.

<table>
<thead>
<tr>
<th>Proven etiology</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TA⁺ (N = 22)</td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>(90.9%)</td>
</tr>
<tr>
<td><em>Mycoplasma pneumoniae</em></td>
<td>22 (18.3%)</td>
</tr>
<tr>
<td>Virus</td>
<td>9 (7.5%)</td>
</tr>
<tr>
<td><em>S. pneumoniae</em> and <em>M. pneumoniae</em></td>
<td>10 (8.3%)</td>
</tr>
<tr>
<td><em>S. pneumoniae</em> and virus</td>
<td>2 (1.7%)</td>
</tr>
<tr>
<td>Virus and <em>M. pneumoniae</em></td>
<td>3 (2.5%)</td>
</tr>
<tr>
<td>Virus and <em>M. pneumoniae</em> and <em>S. pneumoniae</em></td>
<td>2 (1.7%)</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>36 (30%)</td>
</tr>
</tbody>
</table>

**Table 4** Proven serotypes of *Streptococcus pneumoniae* infection.

<table>
<thead>
<tr>
<th>Proven Serotype</th>
<th>TA⁺ (N = 16)</th>
<th>TA⁻ (N = 21)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA⁺: Thomsen-Freidenreich antigen positive; TA⁻: Thomsen-Freidenreich antigen negative.</td>
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</table>

*S. pneumoniae* can remove N-acetyl-neuraminic acid from cell membrane surfaces and expose the TA present on erythrocytes, platelets, and glomerular capillary walls. It has been suggested that the detection of neuraminidase activity is a highly specific diagnostic tool for pneumococcal HUS. In our study, three patients had HUS, all of whom had TA activation and underwent hemodialysis. In our previous study, TA activation was detected in 100% of the patients with pneumococcal HUS, and in 69% of the patients with invasive pneumococcal disease. This indicates that TA activation can be detected in patients with severe pneumococcal infection without HUS. The pathophysiology of pneumococcal HUS is proposed to involve both bacterial and host factors, such as host genetics factors, host immunity or environmental factors which influence patients to develop HUS.

In our patients with lobar pneumonia with or without PPE, positive TA activation had 100% specificity and positive predictive value of 100% for pneumococcal infection. Antimicrobial treatment before sampling pleural fluid frequently results in negative cultures. Penicillin-sensitive *S. pneumoniae* has been reported in over 70% of culture-negative empyema fluids using a molecular diagnosis. In addition, the prevalence of culture-negative empyema has increased since the introduction of heptavalent pneumococcal conjugate vaccine. TA test results can be obtained within several hours, and treatment can be
Starting accordingly. In addition to molecular methods, TA activation appears to be a useful predictor that can assist in the early and prompt detection of pneumococcal infection, especially in culture-negative pleural effusions.

In this study, TA activation was associated with higher peak WBC count, higher peak band and peak CRP level, longer durations of fever and hospital stay, admission to the ICU, complicated pneumonia, VATS and thoracostomy. All of the patients with TA activation had complicated pneumonia with at least parapneumonic effusion, and all received interventions of VATS or thoracostomy. The titer of TA activation was moderately correlated with the severity of complicated pneumonia in our study. Interventions to drain the pleural space are often warranted to expedite the resolution of complicated pneumonia in addition to antibiotics, and conservative management with antibiotics alone may prolong hospitalization.21 Patients with empyema have a significantly higher rate of morbidity than patients with effusion alone. However, treating empyema can be a challenge in children, because of the possible need for a more aggressive approach.28

Previous studies have suggested that VATS, early thoracotomy or insertion of a chest tube with instillation of fibrinolytic agents can result in the best outcomes as measured by hospital length of stay.21,22 Thoracostomy with intrapleural fibrinolytic agent treatment is not performed in our hospital. Light et al. reported that treatment should be performed within the first 10 days of hospitalization for the best outcomes.24 In our study, TA activation was a significant predictor for empyema in the multivariate analysis, suggesting that TA activation could act as a predictor of the severity of empyema in children with parapneumonic effusion, and that early interventions should be considered, especially in patients with higher titers of TA.

All serotypes of S. pneumoniae are capable of unmasking the TA through the production of neuraminidase.23 In this study, TA activation had no statistically significant difference in serotype distribution. In our previous study performed from 2000 to 2004, the most predominant serotype in patients with HUS was serotype 14, with five isolates in 10 patients in the TA activation group.11 Serotype 14 was the most frequently isolated serotype reported in the literature at that time.24–26 Serotype 19A was also the most predominant serotype identified among children less than five years old with invasive pneumococcal disease in Taiwan from 2007–2013.27–29 In the current study, serotype 19A was the most common in both patients with and without TA activation, there was no specific serotype with higher tendency towards TA activation in our studies.

TA activated RBC may be cleared more quickly from the circulation due to the paucity of membrane sialic acid residues.5 TA activation can represent current infection rather than previous infection before this hospitalization. Although influenza virus also possesses neuraminidase,5 in our study, only two patients had proven etiology of influenza virus, and both of them had no TA activation.

There are several limitations to this study. First, the sample size in studies of pneumococcal serotypes related to TA activation are typically quite small, as evidenced in this and previous studies.11 In addition, the findings may have been affected by the predominant serotype of invasive pneumococcal disease of a particular year. As a result, further studies are needed with more cases, and serotype identification may provide further evidence to elucidate the relationship between serotypes and TA activation. Second, it is difficult to detect the exact timing of TA activation because frequent blood sampling from children is problematic at our hospital. Therefore, the number of cases of TA activation in the patients with complicated pneumonia may have been underestimated. Serial measurements of TA titers may be helpful in evaluating disease progression or clinical response.

In conclusion, TA activation is a specific marker for pneumococcal infection and is also associated with the severity of complicated pneumonia. TA activation may be a useful predictor of empyema and be helpful in the early and rapid detection of empyema, especially in patients with culture-negative parapneumonic effusions.

Conflict of interest

The authors have no conflicts of interest to declare or sources of funding.

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11. Huang DT, Chi H, Lee HC, Chiu NC, Huang FY. T-antigen activation for prediction of pneumococcus-induced hemolytic


