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Original Contribution

EXPERIMENTAL PLEURAL EMPYEMA IN RABBITS – CELLULAR AND BIOCHEMICAL CHANGES

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ABSTRACT

Pleural empyema remains a significant medical problem today because of its significant morbidity, prolonged hospitalisation, and increased risk of death. The aim of this research was to share our experience in creating an animal model of pleural empyema. The model of pleural empyema appears suitable for studies on the different methods used in the treatment of empyema and in the investigation of the changes in pleural effusion. A group of nine New Zealand white rabbits, each weighing 2.5 to 3 kg, was used for the study. We produced a sterile pleural effusion and injected the *Streptococcus pneumoniae* into it. We then measured the glucose level below to 2.2 mmol/l and LDH value up to 1000UI/L. These values are typical for complicated parapneumonic effusions and pleural empyema in human. Pleural fluid WBC counts and different cell count, which we established, are also similar to those obtained in the human empyema. This model therefore mimics the course of parapneumonic effusions and empyema that occur in human, and is suitable for studies on the different methods used for the treatment of empyema.

Key words: pleural effusion, thoracentesis, pleural fluid analysis

INTRODUCTION

Pleural empyema remains a significant medical problem that has persisted till today Patients who develop empyema suffer significant morbidity, prolonged hospitalisation, and increased risk of death (1). Empyema can occur following these scenarios: (a) bacterial spread from the lungs, chest wall, diaphragm, or mediastinum; (b) bacterial spread from the bloodstream or lymphatics; or (c) as a result of direct inoculation during surgical procedures or in trauma (2). Pneumonia is responsible for most cases of empyema (1,2,3).

Surprisingly only few studies on experimental empyema currently exist. The reason for this could have been the difficulty in obtaining good animal models to mimic this human condition. The search for a suitable model therefore forms the bedrock of our investigation.

We first tried to create a model of empyema by inoculation of nutrient agar-grown *Pasteurella multocida* into the pleural space, as some authors had reported, but this attempt failed (4,5). The rabbits died rapidly of overwhelming sepsis. Our search led us to the creation of another model. Here the pleura was first injured by the injection of turpentine. It led to the formation of a sterile pleural effusion, into which bacteria were injected. We used as a bacterial agent *Streptococcus pneumoniae*, a Gram-negative organism associated with pneumonia in humans.

With the above realisation we established an aim which was to show our experience with creating pneumoccocal pleural empyema in rabbits. With serial pleural fluid analysis we would show that this model of thoracic empyema mimics the empyema that occurs in humans. In addition it would show various changes in pleural effusion and would evaluate the different methods of treatment of this condition in

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humans.

MATERIALS AND METHODS

Bacterial Preparation

S. pneumoniae was isolated from a patient with parapneumonic pleural empyema. Bacterial organisms had been growing in a blood agar for 24 h at 37^0C. 0.5 ml of 10^4 *S. pneumoniae* in sterile normal saline solution was injected into peritoneal space of white mice in order to increase bacterial virulence. After death of mice (up to 24 hours) suspension was prepared from their hearts and this was incubated in blood agar at 37^0C for 24 h for bacterial growth. It was performed twice. Finally bacterial culture of 10^6 *S. pneumoniae* in sterile normal saline solution was prepared. The concentration of organisms present in the solution was determined by serial dilution.

Study Protocol

A group of nine New Zealand white rabbits, each weighing 2.5 to 3 kg, was used for the study. The rabbits were anesthetised using ketamine-(35mg/kg). The right anterior chest was shaved and then scrubbed with Betadine. A skin incision (2-cm) into 5^{th} right intercostal space was made using a scalpel. A specially prepared 1mm catheter was then introduced into the pleural space. Through the catheter 0.3-ml turpentine was injected into the pleural space. The injections were followed by a 0.5-ml flush of normal saline solution. The distal portion of the chest tube was placed subcutaneously to avoid the risk of its mastication and a skin suture of the incision was made.

24 hours after that all animals had sterile pleural effusion. 2ml of $1x10^6$ *S. pneumoniae* in sterile normal saline solution was injected through the catheter into the pleural space. The injections of bacterial culture were again followed by a 0.5-ml flush of normal saline solution.

Diagnostic Thoracentesis and Pleural Fluid Analysis

After animals were lightly anaesthetised with 20mg/kg and 1% lidocaine locally, five milliliter of pleural fluid was removed through pleural catheter, at 24, 48 and 72h after bacterial injection. LDH and glucose values and leukocyte count were done and recorded in the clinical laboratory at our hospital. Differential cell count was made in our Department of Pathology. Pleural fluid was also Gram-stained, plated onto blood agar and incubated to show *S. pneumoniae* in fluids.

Care for animals and Killing of Rabbits

In this study we adhered to the "Guide of the Care and Use of Experimental Animal Care". The rabbits were administrated a lethal dose of thiopental through the marginal ear vein at 8^{th} day. At autopsy right and left hemithoraces were examined.

Statistical analysis:

A computer program was used for the analysis. Data were expressed as mean +/- SEM.

Comparisons between results of pleural fluid values were made by alternative analysis (a paired t-test). Significance was accepted at $p<0.05$.

Results

The mean pleural fluid glucose, LDH and leukocyte count obtained by serial thoracentesis of rabbits are shown on **Table 1**.

Table 1: *Mean (+/-SEM) Pleural fluid values over Time (Hours*)

	Hours		
	24	48	72
Glucose(mmol/l)	1.6(0.28)	1.3(0.3)	1.33(0.48)
LDH($x10^3$/L)	15.97(3.4)	20.8(4.2)	29.1(1.4)
WBC($x10^9$/L)	5.95(0.48)	5.4(0.41)	4.7(0.32)

The mean pleural fluid glucose level decreased as the experiment continued.

The values of glucose levels at 24h were significantly different from the values at 48 and 72h ($p<0.05$). Difference between glucose level at 48h and 72h was not significant ($p>0.05$). The results are shown in **Figure 1**.

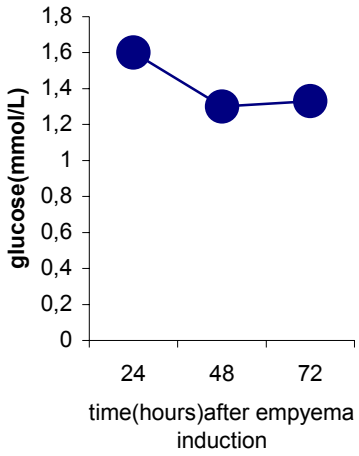

Figure 1: *Mean (+/-SEM) Pleural fluid glucose vs. time (hours)*

The mean pleural fluid LDH values increased during the experiment. LDH values were significantly different ($p<0.05$) between 24th h of pleural empyema and other two values (48th h and 72nd h of empyema).The results are shown in **Figure 2**.

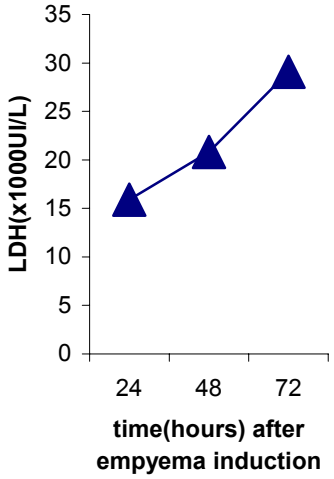

Figure 2: *Mean (+/-SEM) pleural fluid LDH vs. time (hours)*

The mean pleural fluid leukocyte count peaked at 5.95(+/-0.48)x10^9/l at 24h but then decreased to 4.7(+/-0.32)x10^9/l at 72h.The values at 24 and 48h were significantly different from the values at 72h. The results are shown at **Figure 3**.

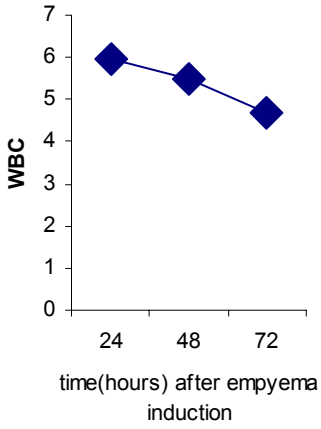

Figure 3: Mean (+/-SEM) pleural fluid leukocyte count(x10^9/L) vs. time (hours)

Pleural smears were prepared and different cell counts were made from pleural fluid aspirated at 48h. The results are shown in **Table 2**.

Gram's stains and pleural fluid bacterial cultures for growth at 24h were positive in all rabbits.

Discussion

Pneumonia remains one of the most common infections in both the community and the hospital, and it is associated with 36 to 47% incidence of pleural effusions (2, 3). Pleural effusions have been classified as parapneumonic effusions, complicated parapneumonic effusions and empyema (2, 3, 6). The main isolated agents are Gram-negative bacteria. One of the most common bacterial agents is *Streptococcus pneumoniae*. Because of that we have developed an animal model of empyema, which mimics the empyema that occurs in human. At first we had created a sterile pleural effusion (similar to parapneumonic effusions) and then created empyema using *S. pneumoniae*.

Table 2: *Result of different cell counts*

Sort of cells	Count / $\bar{x}$ /	%	Sp
monocytic cells	30.33	15.17	6.51
polymorphonuclear leukocytes	120.17	60.08	4.47
macrophages	11.83	5.92	6.86
intact mesothelial cells	10.5	5.25	6.88
lymphocytes	7.83	3.92	6.94
dystrophic mesothelial cells	12.83	6.42	6.84
eosinophils	6.5	3.25	6.96
total cell count	200	100 %	x

Another reason for comparability of this animal model of empyema with empyema in humans is similarity of the pleural fluid biochemical characteristics (7). We measured the glucose level below to 2.2 mmol/l and LDH value up to 1000UI/L. These values are typical for complicated parapneumonic effusions and pleural empyema in human (3, 7). The glucose level became progressively lower because of increased glycolysis from phagocytosis and bacterial metabolism. LDH values became progressively higher because of cell lysis. The glucose and LDH values in our model of empyema are typical for complicated parapneumonic effusions and empyema in human.

Pleural fluid WBC counts and different cell count, which we established, are similar to those obtained in human with empyema (8, 9). The polymorphonuclear leukocytes (neutrophils) are the most common (60.08%). We established decreasing leukocyte count in pleural fluid at 72h as a result of repeated thoracenteses with removal of bacteria and purulent debris (4, 10).

In our model of experimental empyema it was possible to aspirate pleural fluid up to the 3rd day (72h). After this period we could not aspirate pleural effusion. The likely explanations for that could be repeated thoracenteses - they may have been somewhat therapeutic with removal the pleural effusion.

The rabbits did not require parenteral antibiotics for survival. No animals died of overwhelming sepsis. They were alive up to 8th day after empyema induction.

CONCLUSION

The intrapleural injection of *S. pneumoniae* in sterile pleural effusions forms empyema in rabbits. This model mimics the course of parapneumonic effusions and empyema that occur in human.

It appears suitable for studies of the different methods used in the treatment of empyema.

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