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Original Contribution

EXPERIMENTAL PLEURAL EMPYEMA – PATHOLOGIC CHANGES

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ABSTRACT

Pleural empyema remains a significant medical problem today because of significant morbidity, prolonged hospitalisation, and increased risk of death. Our **aim** in this study was to examine macroscopic and microscopic changes that develop in the pleural space, underneath the lung and thoracic wall, as empyema progresses. We used our developed model of empyema in the rabbit. A group of twelve 2,5 to 3 kg New Zealand white rabbits was used for the study. Rabbits were sacrificed at sequential time points in the course of the emyema. We explored empyema gross score, the thickness of pleural peel and visceral pleura, as well as fibroblast count. We established that on day 4 there was a microscopic sign of early fibrosis and after day 8 there were macroscopic and microscopic changes, typical of phase of organisation of pleural empyema.

Our data have therefore demonstrated the need for early, aggressive treatment of pleural empyema in humans.

Key words: fibroblast count , pleural fibrosis, pleural peel, visceral pleura

INTRODUCTION

Pleural empyema is defined as an infection of the pleural space associated with the formation of thick, purulent fluid. Even today pleural empyema is a cause for morbidity and mortality in patients of all ages Empyema most commonly occurs as a serious complication of bacterial pneumonia. Approximately 50 % of all cases of pneumonia are associated with the development of pleural effusions (parapneumonic effusion), which develop into an empyema approximately 5 % of the time (1, 2, 3).

According to the American Thoracic Association, pleural empyema has three phases of progress, namely: acute (exudative), fibrinopurulent (transitional) and chronic (organising) phases (4). As empyema progresses, fibrosis of the pleural space develops. If fibrosis continues, a fibrothorax may ultimately develop. The most common causes for chronic empyema and pleural fibrosis are late diagnosis and treatment of empyema, as well as its unsuitable treatment during the acute and transitional stages (3). After development of pleural fibrosis, more invasive methods become the choice procedure for treatment (5, 6).

Our aim in this study was to examine macroscopic and microscopic changes that develop in the pleural space, underneath the lung and thoracic wall, as empyema progresses. We focus attention on pleural fibrosis because of its idiopathic nature. We hypothesised that pleural fibrosis develops relatively early in the course of pleural empyema.

We chose to use our developed model of empyema in the rabbit. This model enables us to sacrifice rabbits at determined time points as empyema progresses.

MATERIALS AND METHODS

Empyema induction

A group of twelve 2.5 to 3 kg New Zealand white rabbits was used for the study. The rabbits were anaesthetised using ketamine-(35mg/kg). The right anterior chest was shaved and then scrubbed with Betadine. A skin incision (2 cm) into 5th right intercostal space was made using a scalpel. A specially

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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 1 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. 2 ml of 10^6 *S. pneumoniae* in sterile normal saline solution was injected through the catheter into the pleural space. The injections of bacteria culture were again followed by a 0.5-ml flush of normal saline solution.

Sacrificing of Rabbits

Rabbits were sacrificed on days 2, 4, 6, 8, 10, 12 (two rabbits a day) after empyema induction. In this study we were mindful of the provisions of the "Guide of the Care and Use of Experimental Animal Care". Before sacrificing the rabbits, we injected a lethal dose of thiopental through the marginal ear vein.

Tissue specimen preparation: The thorax was dissected en block and right and left hemithoraces were examined. 1-cm thick tissue specimen of the right lung was taken from all 12 rabbits. The tissue specimens were placed for 5 days in a 10% formalin solution. After that, the tissue specimens were fixed in paraffin and stained with haematoxylin and eosin. From days 10 and 12 paraffin sections were also stained, using Van Giesen method, to display collagen filaments.

Gross empyema score: We determined a gross empyema score by number only as follows:

0= normal pleural space, 1=pleural effusion with minimal pleural peel, 2= exudative material with moderate pleural peel, 3= pleural peel with adhesions between visceral and parietal pleura, 4=extensive pleural peel and adhesions between visceral and parietal pleura.

We also made digital photography of the thorax before and after its dissection.

Measuring and proving the pleural fibrosis: Microscopically, the degree of pleural fibrosis was assessed in three ways.

First, the thickness (in microns) between the mesothelial cell layer and the basal layer of the visceral pleura was measured using a microscope with a calibrated micrometer eyepiece. Five sites were chosen from each specimen. The mean thickness was then reported as the microscopic pleural score. We also measured microscopically the fibropurulent pleural peel above the mesothelial cell layer with micrometer. The pleural peel thickness was obtained by calculating the mean of five measurements of the thickness (in microns) of the pleural peel at five different sites.

Second, the number of fibroblasts was counted from photomicrographs of haematoxylin and eosin-stained specimens of five sites of the visceral pleura. The mean count was then reported.

Third: Using Van Giesen method of staining of visceral pleura-specimens, for establishing collagen filaments as an evidence of pleural fibrosis.

Statistical analysis

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

Pleural empyema /characteristics/	***Days of empyema progression***					
	2	4	6	8	10	12
empyema gross score(mean number)	1.5	2	2.5	3.5	4	4
visceral pleural tchickness(μm)	33.7 (3.9)	101.3 (12.3)	181.3 (8.3)	315.3 (12.1)	372 (1.02)	393.7 (15.9)
fibropurulent pleural peel tchickness (μm)	108 (6.6)	278 (17.9)	380 (29.3)	410 (27.8)	x	x
fibroblast count	3.3 (0.3)	13.03 (0.8)	23.56 (0.9)	28.83 (1.2)	x	x

Table 1. *End point meassures from the days of empyema progression (Data are presented as mean or mean +/-SEM).*

Comparisons between results of measuring the pleural peel, thickness of visceral pleura and fibroblast count were made by alternative analysis (a paired t-test). Significance was accepted at $p<0,05$.Correlation studies on fibroblast count and visceral pleural thickness were done using Pearson's coefficient (R) correlation analysis.

RESULTS

Empyema gross score

Establishing empyema gross was by number and expressed as gross score. It is obvious that macroscopic changes in the pleural space became more severe in empyema progression (**Table 1**).

On day 10 and 12 the gross score number is the same. Because of pleural fibrosis in rabbits sacrificed on day10 and 12, the right intercostals spaces were retracted and the right hemithorax had smaller size versus the left (**Figure 1**). There were extensive pleural peel and adhesions between visceral and parietal pleura (**Figure 2**). In two rabbits (one on day10 and one on day 12 of empyema progression) was established spread of the infection along the catheter in the soft tissues of the thoracic wall. In these cases soft tissues of the thoracic wall were involved in the purulent inflammatory process. There were also extensive adhesions between parietal pleura and pericardium.

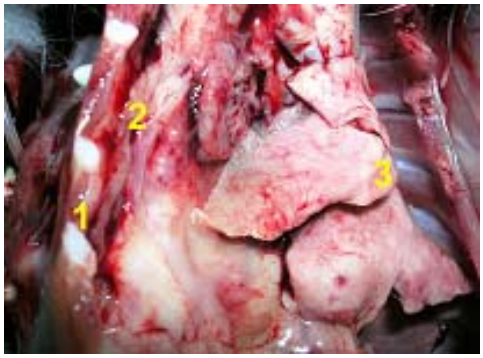

Figure 1. *Photograph on 10 day of empyema progression. A thorax is bisected coronally, the heart is removed and right and left hemi thoraces are displayed. The right intercostals spaces are retracted (1) and right hemithorax(2) is smaller versus left(3).*

Measures of fibropurulent pleural peel, visceral pleural thickness and fibroblast count

Measuring by micrometer was establishing that the fibropurulent pleural peel thickness increased daily and peaked at days 6 and 8 (**Table 1**). The values from day 2 and 4, as well as from day 4 and 6 were significantly different ($p< 0.05$). There was not significant difference in pleural peel thickness on day 6 and 8 ($p>0.05$).

The median microscopic visceral pleural thickness versus days-after- empyema induction is shown in **Table 1**. The most significant difference in pleural thickness was established between day 2 and 4 ($p<0.001$). The median values from day 10 and day12 were not significantly different ($p>0.05$).

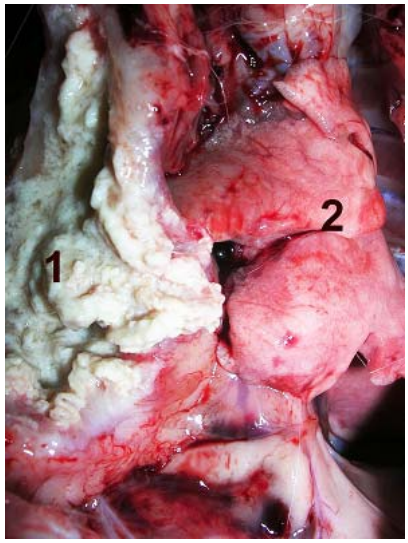

Figure 2. *A gross pathology specimen of a rabbit thorax after coronal bisection at autopsy on day 12 of empyema progression. The right hemithorax contains significant gross pus (1) in contrast of normal left hemithorax (2).*

As well as increasing the thickness of visceral pleura, there is a marked influx of fibroblasts into the expanded visceral pleura. They were chosen based on their cell characteristics: up to 12 microns in length, spindle-shape form, round to ovoid nucleus. Care was taken to exclude mesothelial cells and endothelial cells from the fibroblasts. The median fibroblast number versus days-after-empyema induction is shown in **Table 1**. The most significantly difference in fibroblast number also was established between day 2 and 4 ($p<0.001$). A significant correlation ($R=0.69$), in progression of empyema, was found between fibroblast number and visceral pleural thickness as shown in **Figure 3**.

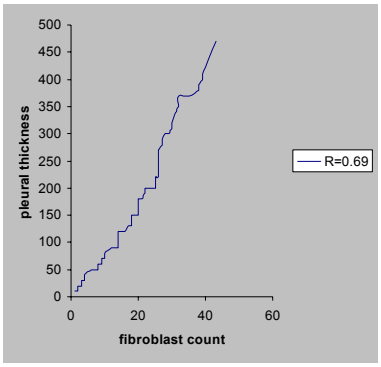

Figure 3. *Fibroblast count versus visceral pleural thickness (μm) in empyema progression. /coefficient of correlation (R) =0, 69 /.*

DISCUSSION

It was possible to investigate the pathologic changes and course of the pleural empyema with this animal model of experimental empyema.

At first pleural fluid was with low viscosity. After that fluid became more turbid and fibrin was deposited on both pleural surfaces and formed a fibropurulent peel that began to trap and fix the lung. Finally, we established very viscous fluid, pleural adhesions and fibrosis involving the thoracic wall and the lung. **Figure 1** and **Figure 2**).

Microscopically, we established that fibropurulent pleural peel was a reflection of the amount of exudative, extracellular empyematous material and cells of acute inflammatory process (polymorphonuclear leukocytes), outside of the mesothelial cell layer of the visceral pleura (**Figure 4**).

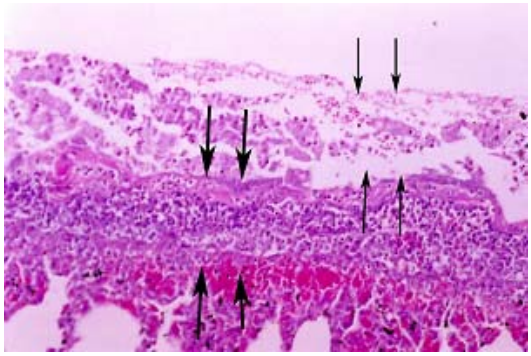

Figure 4. *Photomicrograph of pleural peel and thickened visceral pleura on day 4 of empyema progression (X10). Small arrows show pleural peel and large arrows show thickened visceral pleura.*

In comparison with the normal thin pleura (**Figure 5**), the thickened visceral pleura at first was a result of acute inflammatory process between mesothelial layer and lamina elastica interna. With progression of the disease, there was a fibroblast proliferation, deposition of collagen filaments, in-growth of capillaries and mononuclear cells (lymphocytes, monocytes) infiltration in the thickened visceral pleura (**Figure 6**).

The day 4 after onset of the disease represented the time point, at which the first signs of early fibrosis of the visceral pleura began to occur microscopically (**Figure 4**). Evidences of this were most significantly seen as the increasing of pleural peel thickness, visceral pleural thickness and fibroblast count between day 2 and 4 day of empyema progression that we established (**Table 1**). Our data were comparable with those obtained by Scott Sasse in his experimental study of pleural fibrosis (7, 8, 9).

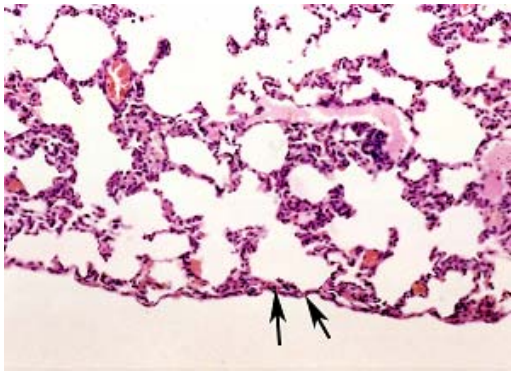

Figure 5. *Photomicrograph of normal rabbit visceral pleura(X 20). The arrows point to the thin mesothelial layer of visceral pleura.*

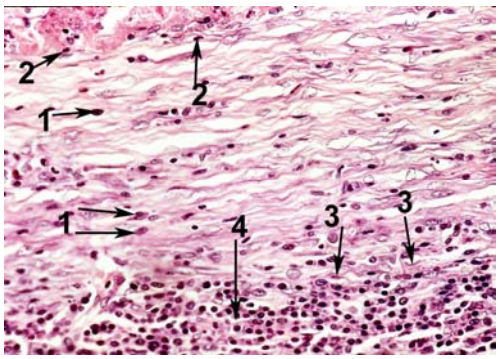

Figure 6. *Photomicrograph of thickened visceral pleura and underneathed lung on day 10 of empyema progression(X 40). Number 1 point to fibroblasts, 2 - point to mesothelial cells, 3- point to endothelial cells and 4 point to mononuclear cells (monocytes, lymphocytes) infiltration of the pleura and the lung.*

We established that after day 8, there was expansion of fibroblasts and collagen filaments in the pleural peel and the lung. Because of this there was no clearly separated zone of pleural peel above the visceral pleura. That was the reason for not measuring and reporting the pleural peel thickness on days 10 and 12 (**Table 1**). From the photomicrograph of thickened visceral pleura on the day 10 (**Figure 6**), it was impossible to determine and count exact fibroblasts numbers because of extensive collagen filaments growth. That is why we did not count and report the fibroblasts on the day 10 and 12 of empyema progression (**Table 1**).

Using Van Giesen method of staining we showed the existence of collagen fibres in the thickened visceral pleura, as well as in the pleural peel and the lung, on day 10 and 12 of empyema progression (**Figure 7**).

Intense fibroblasts growth with collagen filaments production and growth of capillaries in the visceral pleura, as well as expansion of fibroblasts and collagen filaments in the pleural peel and the lung, established after day 8 of the onset of the disease, we consider

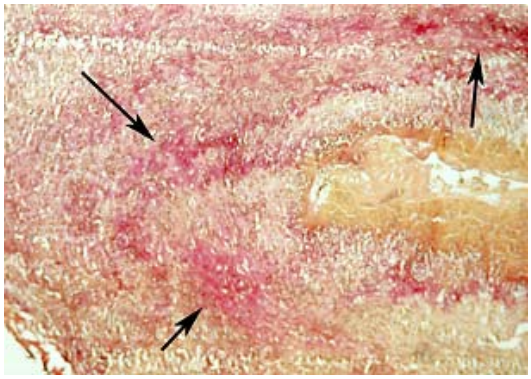

Figure 7. *Photomicrograph of Van Gieson's staining of visceral pleura on day 12 of empyema progression (X 20) . Extensive pleural fibrosis is shown. The arrows point to the collagen filaments with red staining.*

as signs of phase of organisation.

The comparability of this animal model to humans with empyema is attested to by the similarities of the pathologic changes. At this point our first steps of studying the pathologic changes in pleural empyema progression, especially pleural fibrosis, were made with recent study.

CONCLUSION

1. In conclusion, we have shown that pleural fibrosis begins to develop relatively early in course of pleural empyema. On the day 4 there is sign of early fibrosis, established microscopically. After day 8 there are macroscopic and microscopic changes, typical for phase of organisation the pleural empyema.
2. The comparability of our animal model to humans with empyema, as well as our results of studying pathologic changes, have demonstrated the need of early, aggressive treatment of pleural empyema in humans.

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