



*Original Contribution*

## CELL TYPES IN THE NORMAL PLEURAL FLUID FROM RABBITS

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### ABSTRACT

Our knowledge of the cell types from normal pleural fluid of laboratory animals forms the basis of our experimental investigation of the inflammatory diseases of the pleura in humans. We sought to standardise the various cell numbers in normal pleural fluid of rabbits as well as assess the use and power of aspiration and pleural lavage as experimental tools. Fluid from the left and right pleural spaces was aspirated from three of six white rabbits, while the remaining three produced irrigated fluid. Cell types seen in prepared smears from these procedures comprised the following: monocytes, lymphocytes, macrophages, mesothelial cells (intact and degenerative), polymorphonuclear leukocytes and others that could not be identified definitively. The only significant difference between pleural lavage and aspiration was in the number of mesothelial cells obtained from both procedures. The similarity in different cell counts obtained in the rabbit and human pleural fluids thus makes the rabbit a useful model for investigating inflammatory diseases of the pleura in humans. Lavage and aspiration were useful tools in these experiments.

**Key words:** aspiration, cellular count, pleural lavage, pleural smear

### INTRODUCTION

Previous studies of various cell types found in the normal pleural fluid in laboratory animals formed the basis for studying the inflammatory diseases of the pleura under experimental conditions. Most of the investigators used the rabbit as model for laboratory investigations into pleural diseases (1, 2). Rabbits have two separated pleural cavities as in humans and these have same volume (0.1-0.3ml/kg), cellular contents and morphology of normal pleural fluid

Reports on cell counts from rabbit pleural fluids have always been markedly different, comprising the following findings: 9% to 70% mesothelial cells, 28% to 70% macrophages, 2 to 11% lymphocytes and 0% to 2% polymorphonuclear leukocytes. The results have been found very similar to those in humans (1, 3, 4, 5).

Based on the aforementioned we set out

to actualise the following aims: standardisation of the cellular content of the normal pleural fluid in rabbits in order to use this as a base for interpreting results from laboratory studies of pleural diseases in the human. We intend to use pleural lavage and aspiration as methods for obtaining the pleural fluid for the laboratory investigations.

### MATERIALS AND METHODS

Six normal 2.5-3.5 kg New Zealand white rabbits were used. The animals were anaesthetised with intramuscular Kalipsol (25mg/kg) and intravenous Thiopental (50mg/kg). With the animals in the prone position, the thorax was shaved and the right and left pleural spaces were opened with care to avoid bleeding. The ribs were retracted and fluid bathing the pleural surface was allowed to collect by gravity in the ventral costo-diaphragmatic sulcus.

Fluids were aspirated gently through a nylon catheter (1.0 mm diameter) from three rabbits. One drop of fluid was smeared on a clean, dry glass slide after that.

Pleural lavage was made after

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dissecting the thorax of the remaining three animals. The entire pleural surface was thoroughly irrigated with 10 ml of sterile saline. The solution was aspirated and centrifuged (1000 revolutions per minute) for five minutes. The sediment was smeared on a dry, clean glass slide too, after removing the supernatant.

All smears were prepared using a May-Grunwald-Giemsa stain. Cells were examined with a light microscope at 100, 200 and 400 X magnifications.

The study was done in line with the Guide of the Care and Use of Experimental Animal Care.

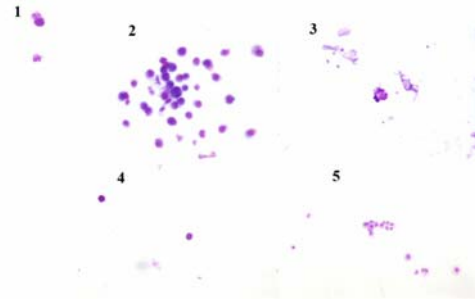
### STATISTICAL ANALYSIS

Comparison between results of differential cell count in lavage and aspiration was made by alternative analysis (a paired t-test) with level of significance set at  $p < 0.05$ .

### RESULTS

We established the presence of the following cell types in the normal pleural fluid: monocytes, lymphocytes, macrophages, mesothelial cells (intact and degenerative) and polymorphonuclear leukocytes. Some other cells were found but could not be identified definitively. They were thus grouped as

“unidentified cells”. The characteristics of the observed cells in the pleural smear were identical with those in human. **Figure 1** shows a pleural smear with different kind of cells.



**Figure 1:** Smear of normal pleural fluid: 1. Macrophage X400, 2. Complex of mesothelial cells and single macrophages X200, 3. Two monocytes and two leucocytes X400, 4. two monocytes and two lymphocytes X200, 5. Complex of mesothelial cells X100

Differential cell counts of right and left pleural spaces were made on fluids from all animals irrespective of the method used (lavage or aspiration). The general conclusions from the results are presented on **Table 1** and **Table 2**.

Kind of Cells	Left Pleural Space	Right Pleural Space	t	p
	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$		
monocytic cells	120.67 ± 0.60	120 ± 0.14	0.91	>0.05
lymphocytes	19.33 ± 0.64	19.67 ± 0.39	0.96	>0.05
macrophages	10.33 ± 0.32	9.67 ± 0.22	0.84	>0.05
intact mesothelial cells	19.67 ± 0.29	20.33 ± 0.23	0.83	>0.05
polymorphonuclear leukocytes	2.00 ± 0.14	2.00 ± 0	1.00	>0.05
unidentified cells	1.33 ± 0.08	1.33 ± 0.08	1.00	>0.05
degenerative mesothelial cells	26.67 ± 0.75	27.00 ± 0.31	0.96	>0.05

**Table 1:** Results of differential cell counts in pleural fluid obtained by lavage

Kind of Cells	Left Pleural Space	right pleural space	t	p
	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$		
monocytic cells	133.33 ± 0.58	132.67 ± 0.84	0.94	>0.05
lymphocytes	22.00 ± 0.31	22.33 ± 0.27	0.93	>0.05
macrophages	18.67 ± 0.39	18.33 ± 0.32	0.94	>0.05
intact mesothelial cells	15.67 ± 0.18	16.33 ± 0.23	0.94	>0.05
polymorphonuclear leukocytes	0.67 ± 0.04	0.33 ± 0.04	0.53	>0.05
unidentified cells	1.33 ± 0.04	1.33 ± 0.08	0.75	>0.05
degenerative mesothelial cells	8.33 ± 0.18	8.33 ± 0.25	1	>0.05

**Table 2:** Results of differential cell count in pleural fluid obtained by aspiration

Kind of Cells	Pleural Lavage			Aspiration			u	p
	count	%	Sp	count	%	Sp		
monocytic cells	120.33	60.17	3.46	133.00	66.50	3.24	1.32	>0.050
lymphocytes	19.50	9.75	2.10	22.17	11.08	2.22	0.44	>0.050
macrophages	10.00	5.00	1.54	18.50	9.25	2.05	1.66	>0.050
intact mesothelial cells	20.00	10.00	2.12	16.00	8.00	1.92	0.70	>0.050
polymorphonuclear leukocytes	2.00	1.0	0.70	0.50	0.25	0.35	0.95	>0.050
unidentified cells	1.33	0.67	0.58	1.50	0.75	0.61	0.10	>0.050
degenerative mesothelial cells	26.83	13.42	2.41	8.33	4.17	1.41	3.32	<0.001
total cell number	200	100.00		200	100			

**Table3:** Comparative results of differential cell counts in pleural fluid

We also compared the differential cell counts in pleural fluid obtained by aspiration and lavage. Data are presented on **Table 3**.

## DISCUSSION

Our results obtained from studying the cell types and their morphology in the normal pleural fluid in rabbits were similar to those found in other studies that included work on pleural fluid in humans (3, 4, 5).

Mononuclear cells (monocytic and macrophages) and mesothelial cells had the largest population, whereas lymphocytes, single polymorphonuclear leukocytes and unidentified cells occurred in smaller numbers. It was, however, difficult to differentiate between monocytic and macrophages as mononuclear cells because of their morphological similarity (1, 4 6, 7). Macrophages usually exist singly, have many small or big vacuoles within pale cytoplasm (1, 3). On the other hand it was difficult to identify mononuclear and mesothelial cells. Exact identification of the mesothelial cells was on the basis of their bigger size, oval or spindle shape, lower nucleo/cytoplasmic ratio, as well as frequent formation of cell complexes.

Because of their smaller size and higher nucleo/cytoplasmic ratio lymphocytes were well identified. Their origin in pleural fluid and those of the single leukocytes are unknown (1, 3, 5).

Lavage and aspiration did not show any significant differences between cell types in the left and right spaces.

The most important difference in differential cell count was observed in the number of mesothelial cells – greater in smear obtained by pleural lavage. The greater number was either in normal or in degenerated cells. Even sheets of mesothelial cells in the effusions were observed- the results of their mechanical damage. Mesothelial cells

exfoliated from the surface of the pleura and accumulated in the pleural fluid (1, 3). The greater number of mesothelial cells in the pleural fluid after lavage showed its role in the exfoliation; this was in agreement with the results given by other authors (1, 2). The formation of groups of mesothelial cells can give rise to their mechanical destruction (1, 3, 6).

It is very important that blood materials were excluded in this kind of investigation since blood also contains these cells.

Pleural fluid of rabbit is normally little ( $0.45\pm 0.12$  ml). That is why there are difficulties in obtaining it as well as a possible risk of rupture of the thin pleural septum between both spaces during aspiration with a catheter. All these make lavage technically easier than aspiration.

## CONCLUSION

There are great similarities in the differential cell count and morphology of the pleural fluid obtained by aspiration and lavage. It makes both methods useful in experimental study of inflammatory diseases of the pleura.

Identical morphology and similarity of differential cell count of pleural fluid of rabbits and humans make it possible to use the rabbit as experimental model in the investigation of the inflammatory diseases of pleura in the human.

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