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Alveolar macrophages and the diagnosis of drowning

P. Betz*[a], A. Nerlich[b], R. Penning[a], W. Eisenmenger[a]

[a]*Department of Legal Medicine,* [b]*Department of Pathology, University of Munich, Frauenlobstr. 7a, 80337 Munich, Germany*

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Abstract

In the present study, we examined the number of alveolar macrophages in lung tissue from 17 cases of fresh water drowning, 22 cases of acute death and 6 cases of lung emphysema. When counting only the number of alveolar macrophages per alveolus without consideration of the alveolar size we found no relevant differences between the groups investigated. To exclude any influence of the alveolar size on the results the surface density of the alveolar macrophages and interstitial tissue was estimated and compared in the different groups. In cases of drowning, the lungs showed significantly lower values in both categories. The ratio of 'alveolar macrophages/interstitial tissue' was also reduced in cases of drowning in comparison to the other groups, however, without significant differences. These morphometrical results characterizing the 'emphysema aquosum' with almost 'empty' and dilated alveoli could be explained by a wash-out effect of the drowning fluid leading to a partial removal of the macrophages from the alveoli. This hypothesis was confirmed by the detection of alveolar macrophages in the drowning froth by immunohistochemical analysis. Even though alveolar macrophages were unambiguously identified in advanced putrefied lungs in HE-stained sections as well as by immunohistochemical staining, an estimation of the number of these cells cannot provide further information for the diagnosis of drowning in putrefied corpses due to the autolytic destruction of the lung architecture providing no reliable values.

Key words: Alveolar macrophages; Drowning; Morphometry; Immunohistochemistry

* Corresponding author.

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1. Introduction

The diagnosis of death by drowning is a major medicolegal problem and is usually based on particular macroscopic and histomorphological changes of the lungs. Various light microscopic signs are complemented by ultrastructural parameters [1,2,6]. The existence of specific morphological changes in the lung, however, is still controversial [4].

Since some authors underlined the meaning of an analysis of alveolar macrophages [see Ref. 9 in Dérobert et al.], the present study was designed to prove whether an estimation of the amount of alveolar macrophages can be used as an indicator of death by drowning in putrefied and non-putrefied lungs.

2. Materials and methods

In this study, 17 cases of fresh water drowning were investigated (individual age ranging between 3 and 90 years, on average 40 years). The control group comprised 22 individuals (age between 6 and 40 years, on average 25 years) who had died acutely of electrocution, strangulation (hanging), traumatic events (without involvement of the lungs) or natural diseases (intracerebral bleeding, epileptic seizures, gastrointestinal bleeding) showing no relevant alterations of the cardiopulmonary system. No cases of intoxication were included. Furthermore, the lungs of 6 patients with morphological features of chronic lung emphysema (individual age ranging between 49 and 92 years, on average 64 years) were investigated.

Lung tissue specimens were obtained at autopsy (postmortem interval <2 days) from peripheral parts of the right upper and lower lobe as well as from central parts of the left lower lobe. The specimens were fixed in 4%-PBS-formaldehyde solution and after embedding in paraffin, sections of 2–3 μm thickness were prepared. The sections were stained with H&E and in selected cases the alveolar macrophages were additionally specifically identified by use of a monoclonal antibody recognizing the CD 68 surface antigen of macrophages (Fa. Dako, Hamburg, Germany) and the alkaline phosphatase-anti-alkaline phosphatase (APAAP) method [3] to confirm the results obtained from the H&E-stained sections. The numbers of alveolar macrophages were counted in 40 randomly selected alveoli in 10 cases of drowning and in 15 cases of the control group.

In addition, a morphometrical analysis of the surface density (proportional area of the alveolar macrophages on the totally evaluated area in %) of alveolar macrophages and interstitial tissue was performed in 20 randomly selected areas sized 0.000025 cm^2 of all specimens using a 100-point grid (original size 1 cm^2, magnification 200×). Lung areas showing extreme emphysematic changes besides numerous collapsed alveoli (dystelectases), major blood vessels or bronchiolar structures were not evaluated. To exclude a wash-out effect of the fixation solution on the number of alveolar macrophages, the respective values in specimens of smaller ($2 \times 1 \times 0.5$ cm^3) and larger ($5 \times 5 \times 5$ cm^3) tissue volumes (each in central parts) were compared.

In order to investigate the occurrence of alveolar macrophages in the froth of drowned individuals found on the external air ways smears were prepared and stain-

ed with the May-Giemsa-Grünwald stain as well as by immunohistochemistry as described above.

In addition, the effect of putrefaction on the amount of alveolar macrophages was examined. Therefore, 10 left lungs were stored at room temperature for 7 days and specimens were removed every day from corresponding regions of the upper and lower lobe, prepared and evaluated as described.

3. Results

An estimation of the number of the alveolar macrophages without consideration of the size of the alveoli revealed a considerable intra- and interindividual variability of the values thus providing no relevant differences between the drowning cases and the controls. This indicates that such an analysis is not useful for the diagnosis of drowning. The individual amounts ranged between 0.41 and 2.21 cells/alveolus on average (maximum value 10 cells) in cases of drowning and between 0.10 and 5.67 cells/alveolus (maximum value 46 cells) in the control group.

Using an advanced morphometrical analysis, however, relevant differences could be observed. Thus, the surface density of the alveolar macrophages and of the interstitial tissue in the drowning group showed (except for the specimens obtained from peripheral parts of the right upper lobe) significantly reduced amounts (signifi-

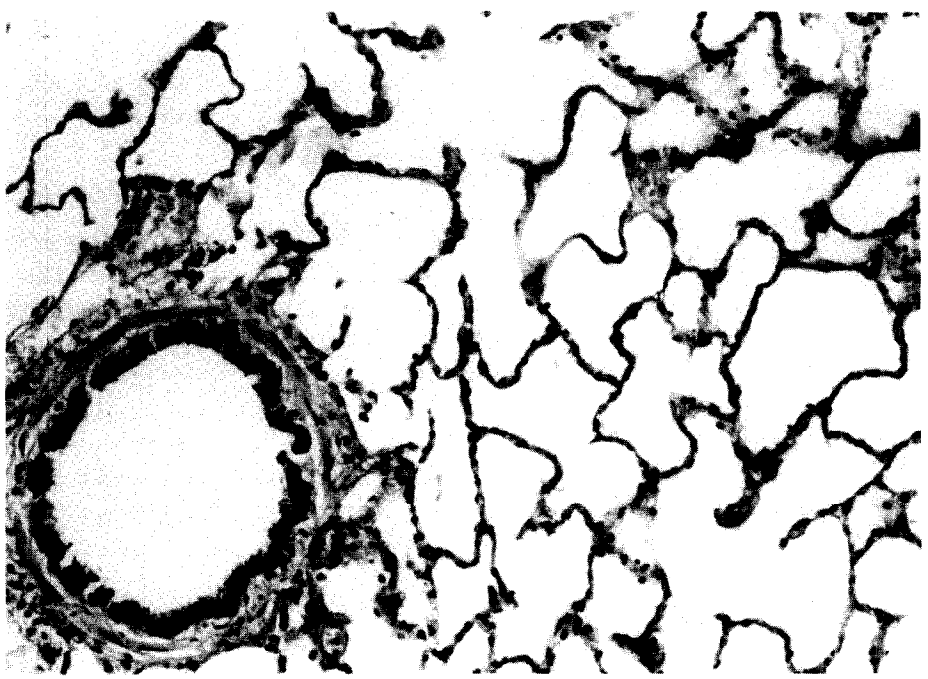


Fig. 1. Drowning lung (male, 22 years): typical signs of the emphysema aquosum with dilated alveoli and thinning of the alveolar septa; 'empty' alveoli and terminal bronchiolus (H&E, 190 ×).

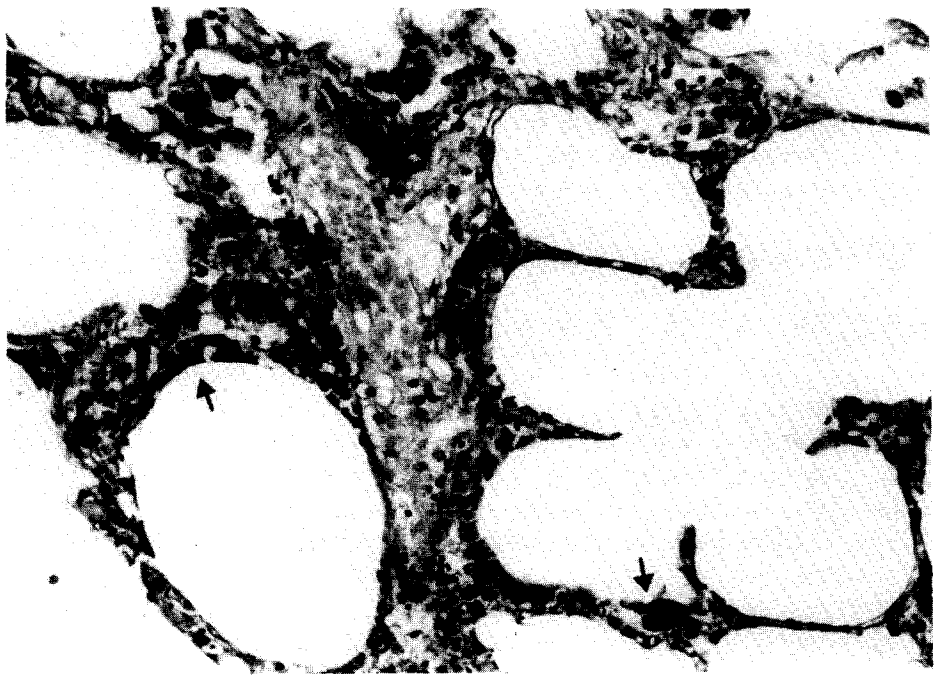


Fig. 2. Lung of a drowned female (38 years) showing numerous alveolar macrophages (see arrows) attached to the alveolar walls, but also almost 'empty' alveoli (H&E, 190 ×).

cance level: 95%; $P \leq 0.05$) when compared to the control values and the emphysema group. Only in one case of drowning (38-year-old female, suicide) a surface density of alveolar macrophages exceeding 1% was found (2.75%). In this instance, the macrophages were, however, not observed in the central parts of the alveoli, but exclusively in corners or closely attached to the alveolar wall. This leads also to the feature of almost 'empty' alveoli which was predominantly observed in the other drowning lungs (Figs. 1 and 2). The ratio of 'alveolar macrophages/interstitial tissue' also showed reduced values in cases of drowning, but significant results could only be observed in specimens obtained from the left upper lobe.

The results are listed in detail in the Tables 1–3.

Table 1
Surface density of alveolar macrophages (alveolar macrophage surface/total surface of the evaluated area

Lung region	*Drowning*	*Controls*	*Lung emphysema*
Right upper lobe	0.29 ± 0.22 **	0.88 ± 0.91	1.35 ± 0.63
Left lower lobe	0.37 ± 0.31 **	1.16 ± 1.14	1.33 ± 0.37
Right lower lobe	0.61 ± 1.09 ns	1.01 ± 1.08	1.74 ± 0.88
Mean value	0.42 ± 0.55 **	1.01 ± 1.05	1.74 ± 0.62

**$P \leq 0.05$.
ns, differences not significant.

Table 2
Surface density of the interstitial tissue (interstitial tissue surface/total surface of the evaluated area

Lung region	*Drowning*	*Controls*	*Lung emphysema*
Right upper lobe	23.36 ± 4.49**	33.90 ± 4.71	25.27 ± 4.26
Left lower lobe	27.77 ± 4.64**	37.54 ± 4.51	32.19 ± 3.38
Right lower lobe	26.69 ± 4.88**	38.34 ± 4.31	30.09 ± 4.15
Mean value	25.94 ± 4.69**	36.60 ± 4.53	29.18 ± 3.95

**$P \leq 0.05$.

Table 3
Ratio of surface densitiy alveolar macrophages/interstitial tissue

Lung region	*Drowning*	*Controls*	*Lung emphysema*
Right upper lobe	0.012 ± 0.011 ns	0.026 ± 0.035	0.053 ± 0.037
Left lower lobe	0.013 ± 0.010**	0.031 ± 0.029	0.041 ± 0.013
Right lower lobe	0.023 ± 0.048 ns	0.026 ± 0.028	0.058 ± 0.036
Mean value	0.016 ± 0.025 ns	0.028 ± 0.029	0.050 ± 0.029

**$P < 0.05$.
ns, differences not significant.

Table 4
Surface density of the alveolar macrophages in dependency on the postmortem interval

Postmortem interval (days)	*Case 1 (myocardial infarction, male, 46 years)*	*Case 2 (hanging, male, 25 years)*
0	1.42	1.27
1	2.13	0.81
2	1.58	3.06
3	1.11	2.96
4	2.33	0.86
5	2.05	0.90
6	1.89	1.23
7	1.99	1.45

There was no significant difference between the values obtained in specimens of smaller or larger volumes indicating that no wash-out effect of the fixation solution has occurred which might have influenced the results.

In the smears prepared from the froth localized in the external openings of the air ways besides numerous respiratory epithelial cells alveolar macrophages could be identified by both the routine Giemsa-staining and by immunohistochemistry (macrophage antibody CD 68).

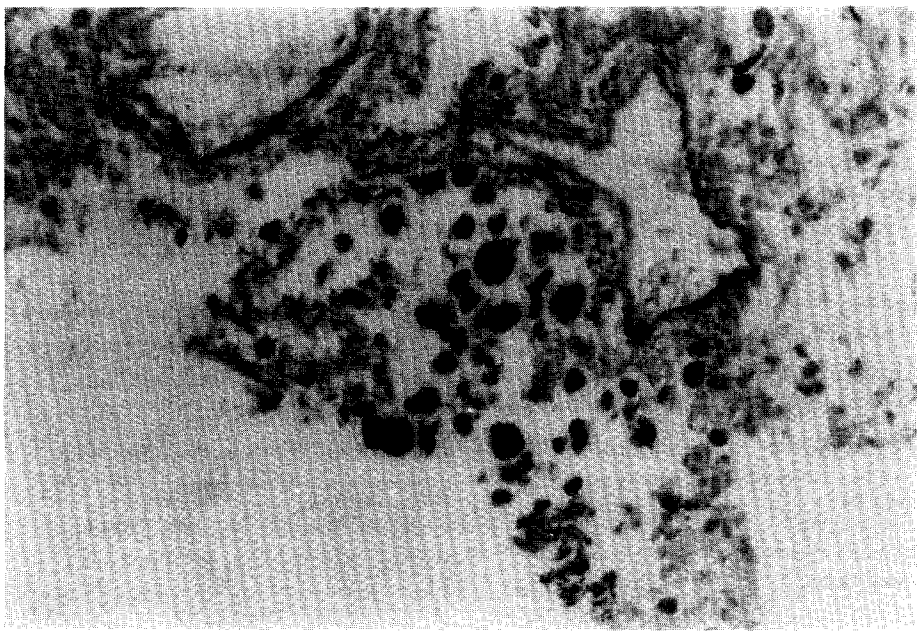
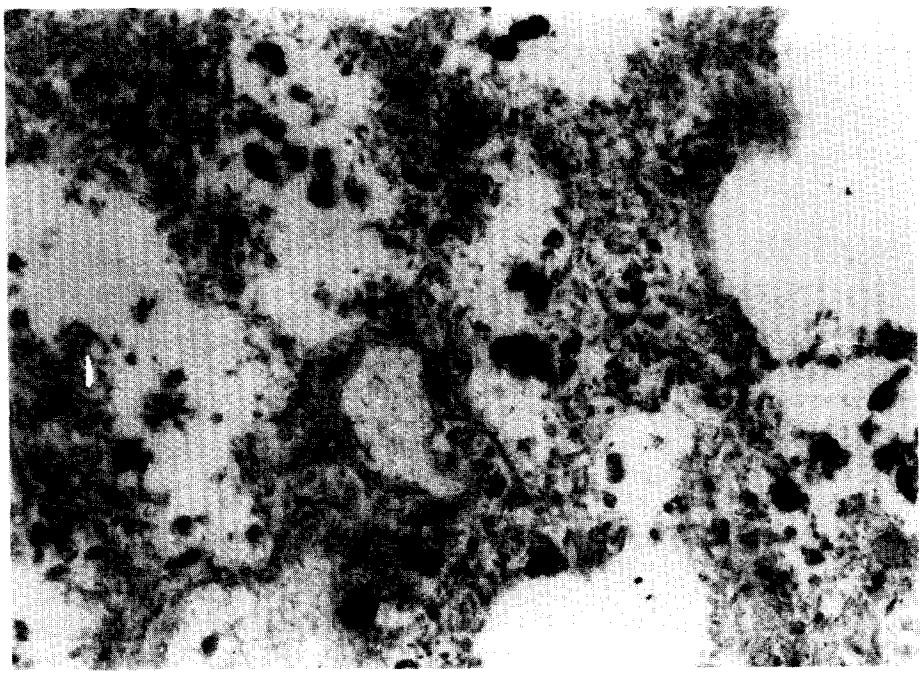


Fig. 3. Putrefied lung (postmortem interval 7 days): advanced signs of putrefaction, but alveolar macrophages still detectable: (a) H&E staining (190 ×); (b) immunohistochemical staining (APAAP-method, 190×).



An evaluation of the specimens obtained from lungs with artificial putrefaction revealed no evidence for time-related changes in the amount of the alveolar macrophages with regard to the postmortem interval which would have exceeded the considerable intraindividual variability.

Two representative examples are listed in Table 4.

4. Discussion

The 'emphysema aquosum' of the drowning lung is characterized by various ultrastructural and light microscopical criteria like dilation of the alveoli with extension, elongation, and thinning of the septa and compression of the alveolar capillaries [1,2,4,6]. Besides changes in the structure of the alveolar walls [7,8] an 'activation' of alveolar type II cells and an increase in the number of alveolar macrophages have been described [2,6,9]. Up to now, however, no numerical investigations have been performed.

An estimation of the number of alveolar macrophages per alveolus revealed in our series a considerable variability providing no relevant differences between the cases of drowning and the controls. In particular, no increased amount of alveolar macrophages was seen in the lungs of drowned individuals. This observation may be explained by the fact that this evaluation technique does not include differences in alveolar size (for example in atelectasis) indicating no use of this technique for the diagnosis of drowning.

Therefore, a more detailed morphometrical analysis of the surface densities of the alveolar macrophages and the interstitial tissue was performed and by estimation of the ratio of 'alveolar macrophages/interstitial tissue' such an size-dependent influence could be compensated.

In the drowning group, the mean value of the surface density of the interstitial tissue was significantly lower than that of the control group due to the distension of the lung architecture which is characteristic for the 'emphysema aquosum'. In comparison to the cases of lung emphysema, the values were — as expected — similar. With regard to the mean value of the surface density of the alveolar macrophages the drowning group showed similarly significantly lowered ones in comparison to the control group and in particular when compared to the cases of lung emphysema. The elevated amount of alveolar macrophages in the emphysematous lungs when compared to normal can easily be explained by chronic (inflammatory?) alterations of the pulmonary system. Only one case of drowning (38 female, suicide) was characterized by an elevated surface density of alveolar macrophages. These cells, however, were not found in central parts of the alveoli, but mostly in corners of the alveolar walls leading to a similar feature of almost 'empty' alveoli as found in the other cases of drowning.

The ratio of 'alveolar macrophages/interstitial tissue' was also decreased in the drowning group, however without statistically significant differences.

These findings are in contrast to previous reports of other authors [2,6,9]. We believe, however, that they can easily be explained by a wash-out effect by the drowning fluid. It seems to be out of discussion that considerable amounts of water reach the alveoli during the process of typical drowning [5]. In the dyspnoic phase,

the water is thoroughly mixed with the alveolar air leading to the 'emphysema aquosum'. In addition, the mixture of water and alveolar air reaches the trachea leading to the development of a typical froth in the tracheal system of drowned individuals which can also be detected in the external openings of the air ways. This fact raises the assumption that parts of the alveolar content may be removed as it occurs in the artificial system of BAL (bronchiolo-alveolar lavage). This hypothesis was confirmed by the microscopic examination of smears prepared from the drowning froth showing numerous respiratory epithelial cells and alveolar macrophages which could unambiguously be identified by immunohistochemistry. Therefore, it seems probable that alveolar macrophages are passively mobilized and removed from the alveoli during drowning leading to the decreased surface densities of these cells.

Additionally to our morphometrical results the meaning of the number of alveolar macrophages for the diagnosis of drowning was investigated in putrefied lungs. Even though these cells could be identified unambiguously in advanced putrefied lung tissue by HE-staining and by immunohistochemistry, the decay-related destruction of the lung architecture restricts the value of this parameter to this regard. This is based on a considerable variability in the number of alveolar macrophages with high values of these cells also occasionally found in non-putrefied lungs of drowned individuals as described in one of our cases even though the alveolar macrophages were not localized in central parts of the alveoli. On the other hand, it seems probable that the destruction of the lung architecture leads to an 'release' of the macrophages from the alveolar corners or to a detachment from the alveolar wall and/or the alveolar macrophages are washed out by putrefaction fluid. These possible mechanisms are presumed to considerably influence the number of the alveolar macrophages as confirmed by our results obtained from putrefied lungs, showing neither constant values or a clear tendency to an increase or an decrease with advanced postmortem interval. Therefore, this parameter cannot be used for the diagnosis of drowning in putrefied corpses.

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