Int J Leg Med (1993) 106:156–159

International Journal of
Legal Medicine

Pulmonary giant cells and their significance for the diagnosis of asphyxiation

P. Betz[1], A. Nerlich[2], R. Penning[1], and W. Eisenmenger[1]

Departments of Legal Medicine[1] and Pathology[2], University of Munich, Frauenlobstrasse 7a, D-80337 Munich, Germany

Received March 8, 1993 / Received in revised form June 7, 1993

Summary. This study was performed to prove whether the detection of polynuclear giant cells in lungs is useful for the diagnosis of asphyxiation due to throttling or strangulation. Therefore, lung specimens of 54 individuals with different natural and unnatural causes of death were investigated. In most lungs examined numerous alveolar macrophages with 1–2 nuclei were found. Polynuclear giant cells, which were arbitrarily defined as alveolar macrophages containing 3 or more nuclei, were observed in all groups investigated except in the cases of hypoxia due to covering the head with plastic bags. Apparent differences between the other groups in particular an increased number in cases of throttling or strangulation, could not be observed. Immunohistochemical investigations confirmed the hypothesis that the observed polynuclear giant cells were derived from alveolar macrophages. The immunohistochemical analysis of the proliferation marker antigen Ki 67 revealed no positive reaction in the nuclei of polynuclear giant cells indicating that these cells had not developed shortly before death by endomitosis as an adaptative change following reduction in oxygen supply. The results provide evidence that the detection of pulmonary polynuclear giant cells cannot be used as a practical indicator for death by asphyxiation due to throttling or strangulation.

Key words: Alveolar macrophages – Giant cells – Asphyxiation – Immunohistochemistry

Zusammenfassung. Um die Wertigkeit des lichtmikroskopischen Nachweises von pulmonalen Riesenzellen für die Diagnose Erwürgen bzw. Erdrosseln zu überprüfen, wurden die Lungen von 54 Individuen mit unterschiedlichen natürlichen und nicht-natürlichen Todesursachen untersucht. In den meisten Lungen waren zahlreiche Alveolarmakrophagen mit 1–2 Zellkernen vorzufinden. Darüber hinaus waren in 20–30% aller untersuchten Präparate Riesenzellen, die als Alveolarmakrophagen mit 3 oder mehr Zellkernen definiert wurden, nachweisbar, lediglich bei durch Rückatmung in eine Plastiktüte Verstorbenen konnten derartige Zellen nicht beobachtet werden. Auffällige Unterschiede zwischen den übrigen, nach Todesursachen zusammengefaßten Gruppen ließen sich nicht feststellen, insbesondere fand sich keine erhöhte Zahl von Riesenzellen in Lungen Erwürgter bzw. Erdrosselter. Aufgrund immunhistochemischer Markierung konnte gezeigt werden, daß es sich bei den beobachteten Riesenzellen um Alveolarmakrophagen handelte. Durch Darstellung des Proliferations-Markers Ki 67 ließ sich zudem feststellen, daß sich sämtliche Riesenzellen nicht im Proliferationsstadium befanden und somit keine Bildung in engem zeitlichen Zusammenhang mit dem Todeseintritt durch Endomitose im Rahmen adaptiver Vorgänge anzunehmen war. Aufgrund der Ergebnisse scheint der Nachweis pulmonaler Riesenzellen nicht geeignet, die Diagnose Erwürgen oder Erdrosseln zu unterstützen.

Schlüsselwörter: Alveolarmakrophagen – Riesenzellen – Erstickung – Immunhistochemie

Introduction

The mobilisation of alveolar cells and transformation into giant cells have been reported to be histological signs of death by slow asphyxiation [4–8]. Since the appearance of pulmonary giant cells is also known in a variety of other conditions, the present study was performed to check whether the detection of these cells can be used as a practical parameter to confirm the diagnosis of asphyxiation due to throttling or strangulation.

Materials and methods

In this study, at least 2 specimens (approximately $1 \times 1 \times 0.5\,cm^3$) obtained from peripheral and central areas of lungs of 54 (male and female) individuals aged between 7 months and 92 years (see Tables) were investigated. The subjects could be divided into the following groups according to the cause of death:

1. Drowning: $n = 7$ (individual ages: 25–61 years; 2 cases of resuscitation).

Correspondence to: P. Betz

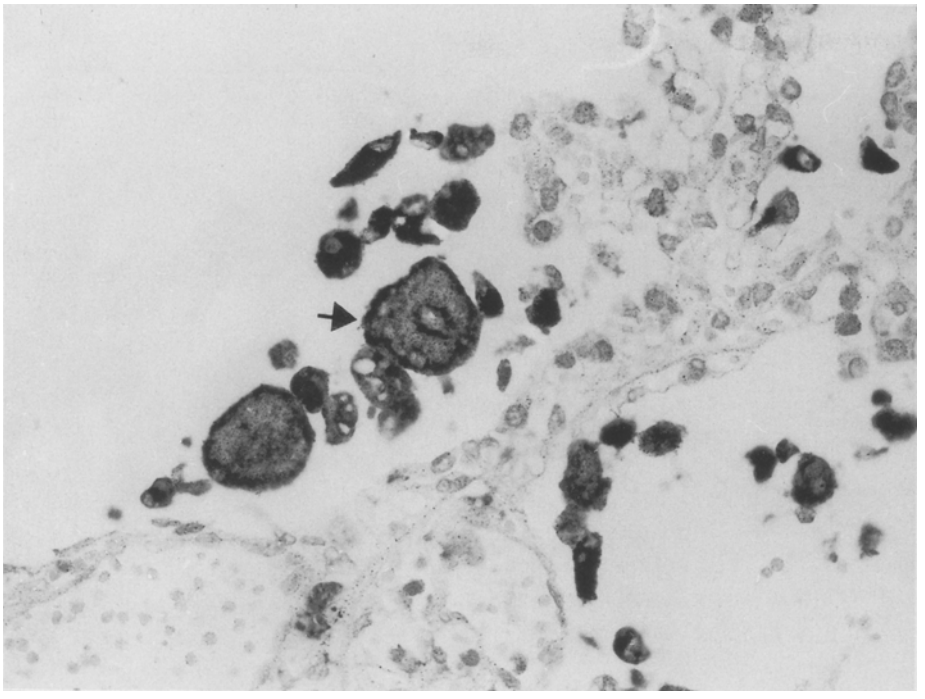

Fig. 1. 23-year-old male, cerebral aneurysma bleeding: positive staining of alveolar macrophages and polynuclear giant cells with a monoclonal, macrophage-specific antibody (CD 68) (paraffin, APAAP-method, 380 ×)

2. Severe head injury: $n = 9$ (individual ages: 7 months–82 years; survival time: a few minutes; no resuscitation).
3. Sudden infant death syndrome: $n = 10$ (individual ages: 1–10 months; 3 cases of resuscitation).
4. Other causes of death (bleeding, hypothermia, epileptic seizures, pulmonary embolism, intoxication, myocardial infarction): $n = 17$ (individual ages: 3–92 years; 5 cases of resuscitation).
5. Strangulation/throttling: $n = 7$ (individual ages: 11–62 years; no resuscitation).
6. Hypoxia (due to covering the head with plastic bags): $n = 4$ (individual ages: 22–60 years; no resuscitation).

No clinical therapy or intensive care was peformed apart from resuscitation. All groups (with the exception of the SIDS) included smokers and non-smokers, but in some cases a differentiation was impossible due to the lack of anamnestic data or specific alterations to the lungs.

The specimens were obtained at autopsy (postmortem interval less than 3 days), fixed in 4% PBS-formaldehyde solution and embedded in paraffin. Sections 2–3 µm thick were prepared and stained with HE. Pathological changes of the lung which could have induced the development of giant cells, such as aspiration or in particular apparent pulmonary alterations due to viral or other infections, were excluded by anamnestical data or morphological criteria. In order to identify hemosiderin-positive alveolar macrophages or giant cells, the Prussian-Blue reaction was performed. Cases with positively reacting alveolar macrophages were excluded. Alveolar macrophages were also identified using a monoclonal antibody (CD 68, Fa. Dako, Hamburg, Germany) according to the APAAP-method [2]. Furthermore, the Ki 67-antigen, a marker which is selectively expressed during cell proliferation, was visualized using a monoclonal antibody (MIB 1, Fa. Dianova, Hamburg, Germany) to prove whether polynuclear giant cells may have developed in the lungs by (endo)mitosis shortly before death.

All sections (approximately 1 cm^2) were evaluated by light microscopy and the different etiological groups were compared with regard to the presence of alveolar macrophages showing more than 2 nuclei ("giant cells"). Therefore, a semi-quantitative analysis was performed and a number of 5 or less polynuclear giant cells per specimen was regarded as "+", a number of more than 5 cells as "++".

Results

In almost every lung investigated numerous alveolar macrophages showing 1–2 nuclei could be found. Furthermore, isolated giant cells (containing 3 or more nuclei) were observed in most specimens with the exception of the cases of hypoxia ($n = 4$). A few individuals (6 out of 54 cases) contained no polynuclear giant cells or alveolar macrophages showing at least 2 nuclei. The percentage of cases showing giant cells (see Table 2) was comparable in the different etiological groups and ranged from 22% (severe head injury) up to 30% (sudden infant death syndrome). Apparent differences between the groups (in particular an increased number in cases with strangulation/throttling or hypoxia) could not be observed and in our series all cases showed only 5 or less polynuclear giant cells per specimen.

Since only isolated giant cells were found in the specimens a more extensive numerical analysis was not performed.

The results are listed up in detail in the following tables.

The immunohistochemical reactivity of the monoclonal antibody CD 68, which specifically stains macrophages, was restricted only to alveolar macrophages, to the giant cells which could be found in the alveoli and to some cells in the interstitial tissue which have to be regarded as resident macrophages. Alveolar type I or type II cells revealed no positive reaction.

A positive staining for the proliferation antigen Ki 67 was occasionally observed in alveolar type II cells, in some lymphocytes of peribronchiolar follicular lymphatic infiltrates and in few cells of the respiratory epithelium of bronchioles. The nuclei of very few alveolar macrophages (containing only 1 nucleus) also stained positively. However, no reaction was seen in cells containing more than 1 nucleus or in giant cells.

Table 1. Number of nuclei in alveolar macrophages in the different etiological groups (+: 5 or less polynuclear giant cells per specimen, ++, more than 5 polynuclear giant cells per specimen, s = smoker, ns = non-smoker, r = resuscitation

Severe head injury

Individual age	2 nuclei	3 nuclei	More than 3 nuclei
7 months (ns)	++	+	–
6 years (ns)	++	+	+ (7 nuclei)
17 years (ns)	+	–	–
21 years (s)	++	–	–
30 years	+	–	–
39 years	+	+	–
53 years (ns)	+	+	–
74 years (s)	++	+	+ (4 nuclei)
82 years	+	–	–

Sudden infant death syndrome

Individual age (months)	2 nuclei	3 nuclei	More than 3 nuclei
1	+	–	–
3 (r)	+	–	–
3	++	+	+ (4 nuclei)
3	+	+	–
3	+	–	–
3 (r)	++	–	+ (4 nuclei)
3	+	–	–
5 (r)	+	–	–
9	++	+	+ (5 nuclei)
10	–	–	–

Drowning

Individual age (years)	2 nuclei	3 nuclei	More than 3 nuclei
25 (s, r)	+	+	+ (4 nuclei)
34 (ns)	+	+	+ (4 nuclei)
38 (r)	+	–	–
47 (s)	+	–	–
50	+	–	–
53	+	–	–
61	+	–	–

Table 1 (continued)

Other causes of death

Cause of death	Individual age (years)	2 nuclei	3 nuclei	More than 3 nuclei
Intoxication	16 (ns, r)	–	–	–
Intoxication	18 (s, r)	++	–	–
Intoxication	25 (s)	++	+	+ (5 nuclei)
Intoxication	33	++	+	–
Intoxication	56 (ns)	++	–	–
Carbon monoxide	18	++	–	–
Carbon monoxide	42 (s)	+	–	–
Myocardial infarction	53 (s)	+	–	–
Myocardial infarction	58 (s, r)	+	–	–
Myocardial infarction	92	++	–	–
Cerebral bleeding	19 (r)	++	–	+ (4 nuclei)
Cerebral bleeding	23	++	+	+ (4 nuclei)
Exsanguination	23 (s)	+	–	–
Exsanguination	43	++	+	+ (4 nuclei)
Exsanguination	56	–	–	–
Hypothermia	89 (ns)	–	–	–
Epileptic seizure	3 (ns, r)	+	–	–

Strangulation/throttling

Individual age (years)	2 nuclei	3 nuclei	More than 3 nuclei
11 (ns)	+	–	–
12 (ns)	–	–	–
30 (s)	++	–	+ (4 nuclei)
31	++	+	+ (4 nuclei)
46	++	–	–
53 (ns)	++	–	–
62	–	–	–

Hypoxia

Individual age (years)	2 nuclei	3 nuclei	More than 3 nuclei
22 (ns)	+	–	–
42	++	–	–
45 (s)	+	–	–
60	++	–	–

Discussion

In 1963 Janssen [5] reported 4 cases of slow asphyxiation (in particular one case of lethal throttling, the exact duration of the asphyxiation process, however, is not known) of young individuals without any other pathological changes of the lungs and found an "alveolar cell proliferation" with transformation into giant cells which he assumed to be an indicator for slow asphyxiation. This hypothesis was confirmed in an experimental model and the animals were killed by hypoxia and CO_2 increase with asphyxiation intervals up to 12 hours [5, 6]. In experimental animals dying of prolonged hypoxia "changes of alveolar cells" (swelling of the cells and hyperchromasia of the nuclei) were observed as well as polynuclear giant cells which had not been found in cases of hypoxia of shorter duration. These results were interpreted as adaptive changes of the alveolar cells to the reduction of oxygen. In 1977 Janssen described the localization of polynuclear giant cells as a useful indicator for death by slow asphyxiation following the exclusion of natural developmental processes [7].

To elucidate the practical meaning of the detection of polynuclear giant cells as a sign for asphyxiation, in particular for lethal throttling or strangulation, the individual variability in the number of polynuclear alveolar

Table 2. Incidence of giant cells in the different etiological groups

Group	Positive cases (%)
Drowning ($n = 7$)	2 (29%)
Severe head injury ($n = 9$)	2 (22%)
Sudden infant death syndrome ($n = 10$)	3 (30%)
"Other causes of death" ($n = 17$)	4 (24%)
Strangulation/throttling ($n = 7$)	2 (29%)
Hypoxia ($n = 4$)	0 (0%)

macrophages in lungs of individuals with other causes of death has to be investigated. The interpretation of the results of Janssen [5] without inclusion of human controls and only based on findings obtained from experimental animals [5, 6] is critical since the problem remains whether experimental data can be transferred to human conditions under forensic aspects. So it seems generally possible that the morphological alterations of the lungs described in the 4 cases reported by Janssen [5] are – at least partly – not necessarily related to the cause of death, i.e. with asphyxiation.

In our series (48 out of 54 cases) alveolar macrophages containing at least 2 nuclei were observed in almost every lung. In every group, with the exception of the cases of hypoxia, giant cells were found which were arbitrarily defined as cells showing 3 or more nuclei, but no increased numbers of these cells occurred in cases of strangulation/throttling in comparison to the other groups. A considerably longer duration of asphyxia, at least in the case of throttling reported by Janssen [7] which could easily explain an increased number of polynuclear giant cells when compared to our series, seems improbable since the exact interval of throttling is unknown in the case of Janssen as well as in our series. Furthermore, the individuals investigated in the study of Janssen [5] showed no morphological pulmonary abnormalities and therefore a contribution of any major disease to the giant cell formation which would explain the differences seems to be excluded.

On the other hand, since an increase in the number of alveolar macrophages can be induced by various stimuli such as smoking, dust inhalation etc. [1] and with regard to a mean lifespan of these cells of 81 days [9], it is easily conceivable that a considerable individual variability in the number of polynuclear giant cells as observed in our series can be found and the occurrence of these cells is also possible in (now) healthy, functionally normal lungs. This hypothesis can be confirmed by the findings of Reh [8] who described "alveolar epithelial cells" with 2–5 nuclei in, for example, cases of coma diabeticum, chronic alcoholism and epileptic seizure. In this study, the occurrence of such giant cells was also interpreted as a sign of prolonged asphyxiation, but the connection between these diseases and death by asphyxiation seems difficult to conceive except if most causes of death can be regarded as some kind of asphyxiation. In this instance, giant cell formation would be expected to occur in most cases of prolonged heart failure whatever the cause. It is much more likely to expect that increased numbers of these cells are caused by previous stimuli. This assumption can be confirmed by our results concerning the immunohistochemical analysis of Ki 67 positive cells. The Ki 67-antigen is a nuclear protein which can be found in proliferating cells [3] and in our series only the nuclei of very few mononuclear alveolar macrophages stained positively. Alveolar macrophages containing more than 1 nucleus and particularly the polynuclear giant cells remained unstained indicating that these cells were not actively proliferating and have therefore probably not developed shortly before death by endomitosis as an indicator of adaptive changes following, for example, oxygen deficiency. The Ki 67-staining pattern was similar in the different groups investigated and could therefore confirm the assumption that the observed giant cells have developed following previous stimuli, even though a development by the mechanism of cell fusion also seems possible.

On the other hand, the experimental results of Janssen [5] and Janssen and Bärtschi [6] can be regarded as a strong argument for the development of pulmonary giant cells during prolonged asphyxiation, but under forensic aspects a comparison with morphological findings in lungs obtained from individuals with prolonged asphyxia, for example causes of death due to compression of the chest, should be performed. The evaluation of our human control cases, however, provides evidence that polynuclear giant cells also occur in the lungs of non-asphyxiated individuals and not in increased numbers in cases of lethal throttling or strangulation. Therefore, the detection of these cells seems not to be useful for confirming the diagnosis of (at least rather short) asphyxiation due to throttling or strangulation.

References

1. Cottier H (1980) Pathogenese, Bd 1. Springer, Berlin Heidelberg New York
2. Cordell IL, Falini B, Erber WN, Ghosh AK, Abdulaziz Z, MacDonald S, Pulford AF, Stein H, Mason DY (1984) Immunoenzymatic labeling of monoclonal antibodies using immuno complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP-complex). J Histochem Cytochem 32: 219–229
3. Gerdes J, Lemke U, Baisch H, Wacker H-H, Schwab U, Stein H (1984) Cell cycle analysis of a cell proliferation human nuclear antigen defined by the monoclonal antibody Ki 67. J Immunol 133:1710–1715
4. Henssge C (1990) Beweisthema todesursächliche/lebensgefährliche Halskompression: Pathophysiologische Aspekte der Interpretation. In: Brinkmann B, Püschel K (eds) Ersticken – Fortschritte in der Beweisführung. Springer, Berlin Heidelberg New York, pp 3–13
5. Janssen W (1963) Riesenzellenbildung bei Erstickung. Dtsch Z Ges Gerichtl Med 54:200–210
6. Janssen W, Bärtschi G (1964) Vitale und supravitale Reaktionen der Alveolarzellen nach prothrahiertem Sauerstoffmangel. Dtsch Z Ges Gerichtl Med 55:47–60
7. Janssen W (1977) Forensic histopathology. Springer, Berlin Heidelberg New York
8. Reh H (1965) Die Verfettung der Alveolarepithelien beim plötzlichen Tod. Beitr Gerichtl Med XXIII:236–242
9. Thomas ED, Ramberg RE, Sale GE, Sparkes RS, Golde DW (1976) Direct evidence for a bone marrow origin of the alveolar macrophage in man. Science 192:1016–1018