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Chlamydia and sudden infant death syndrome. A study of 166 SIDS and 30 control cases

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Summary. Chlamydia inclusions could be demonstrated by an immunofluorescence assay in formalin-fixed lung sections in 32 of 166 cases (19.4%) of Sudden Infant Death Syndrome (SIDS) and in the lungs of only 1 of 30 infants with a known cause of death (3.3%). The difference is statistically significant ($P = 0.04$). *Chlamydia trachomatis* is an agent of pneumonia in 1–4 month-old infants who have acquired the disease from an infected cervix during birth, but other chlamydia species are also capable of causing pneumonia. The lung sections of the 32 chlamydia positive SIDS cases did not show typical histological signs of pneumonia. Even though chlamydia inclusions were detected in the lungs of 32 SIDS cases a causal relation between chlamydia infection and SIDS could not be demonstrated.

Key words: Sudden Infant Death Syndrome – *Chlamydia* – Bacteria – Diagnosis – Immunofluorescence

Zusammenfassung. *Chlamydia*-Einschlüsse konnten mit Hilfe eines Immunfluoreszenz-Ansatzes in formalinfixierten Lungenschnitten in 32 von 166 Fällen (19,4%) des Syndroms des plötzlichen Kindstodes und in lediglich einem von 30 Fällen von Kindern mit bekannter Todesursache (3,3%) festgestellt werden. Der Unterschied ist statistisch significant ($P = 0{,}04$). Chlamydia trachomatis ist ein Erzeuger von Pneumonien bei 1–4 Monate alten Kindern, die die Erkrankung während der Geburt aufgrund einer infizierten Cervix acquiriert haben, aber andere Chlamydia-Arten sind auch imstande, eine Pneumonie zu verursachen. Die Lungenschnitte von 32 Chlamydia-positiven SIDS-Fällen zeigten keine typischen histologischen Zeichen der Pneumonie. Obwohl jedoch Chlamydia-Einschlüsse in den Lungen von 32 SIDS-Fällen gefunden wurden, konnte eine kausale Beziehung zwischen Chlamydia-Infektion und SIDS nicht nachgewiesen werden.

Schlüsselwörter: Syndrom des plötzlichen Kindstodes – *Chlamydia* – Bakterien – Diagnose – Immunfluoreszenz

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Introduction

In Western countries about half of all postneonatal deaths are classified as Sudden Infant Death Syndrome (SIDS). The cause of SIDS is still unknown in spite of an increased public awareness and intensified research and its prevalence is unchanged [1]. In most of the SIDS cases the autopsy shows no pathological changes, but some cases have minor signs of infections in the respiratory organs insufficient to explain the cause of death. A number of the infants have had history of coughs and other symptoms of minor respiratory infection preceding death [2] but no causal relation between infectious agents and SIDS has until now been demonstrated.

Chlamydia trachomatis is among the most common agents of lower respiratory infections during the first three months of infancy [3, 4], but no studies of the relation between this microorganism and SIDS have ever been performed. In this paper a possible connection between chlamydia and SIDS was studied by determinating the occurrence of chlamydia inclusions in the lungs of these infants.

The genus *Chlamydia* consists of three species, *Chlamydia trachomatis, Chlamydia psittaci* and *Chlamydia pneumoniae. C. trachomatis* is a human pathogen causing oculogenital infections and pneumonia in 3–4 month-old infants [5]. *C. psittaci* is mainly an animal pathogen, but in man it produces ornithosis which occurs as a systemic infection with a severe pneumonia [5]. *C. psittaci* has also been associated with abortion in women [6, 7].

C. pneumoniae causes respiratory infections including pneumonia [8]. Chlamydia is an obligate intracellular bacteria with a biphasic life cycle. The characteristic chlamydia morphology is represented by the inclusion, which is the metabolically active part of chlamydia. We have previously described a method to detect chlamydia inclusions in postmortal formalin-fixed tissue with an immunofluorescence assay using two genus-specific monoclonal antibodies [9]. In this study the frequency of chlamydia inclusions in the lungs of 166 SIDS cases and a comparable control group of 30 infants which died of known causes is described and discussed.

Materials and methods

SIDS cases. A prospective study of all cases of sudden infant death was carried out in Denmark in 1987 and 1988. According to Danish law all cases of SIDS are submitted to a postmortal medico-legal investigation and in most cases a medico-legal autopsy is performed. The autopsy rate was 95% in the years 1987 and 1988. Two hundred and seven infants between the age of 1 week and 1 year died suddenly in 1987 and 1988. The cause of death was explained by autopsy in 24 cases. Eleven cases were not autopsied due to prohibition by the parents. From the 172 autopsied infants with no known cause of death, 166 cases were selected and were classified as SIDS according to the definition "The sudden death of any infant or young child which is unexpected by history and in which a thorough post mortem examination fails to reveal an adequate cause of death" [10]. One hundred and twenty-six cases had no pathological changes, but 5 infants with histologically minor signs of viral pneumonia insufficient to explain death and 35 infants with cellular inflammatory changes considered insignificant for the death, were included in the 166 SIDS cases selected.

The age interval of the selected SIDS group in this study was between 28 days and 1 year. The age distribution of the SIDS cases was from 1 to 10 months, with 2 month-old infants making up 28% of the total and 86% aged between 1 and 4 months.

Control cases. Thirty control cases aged between 28 days and 1 year were found in the files of the Danish Institutes of Forensic Medicine from January 1 1985 and December 31 1988.

Only infants with a known cause of death were selected and included 7 cases of previous undiagnosed congenital heart disease, 2 cases of bacterial meningitis, 4 cases of cerebral disorders (2

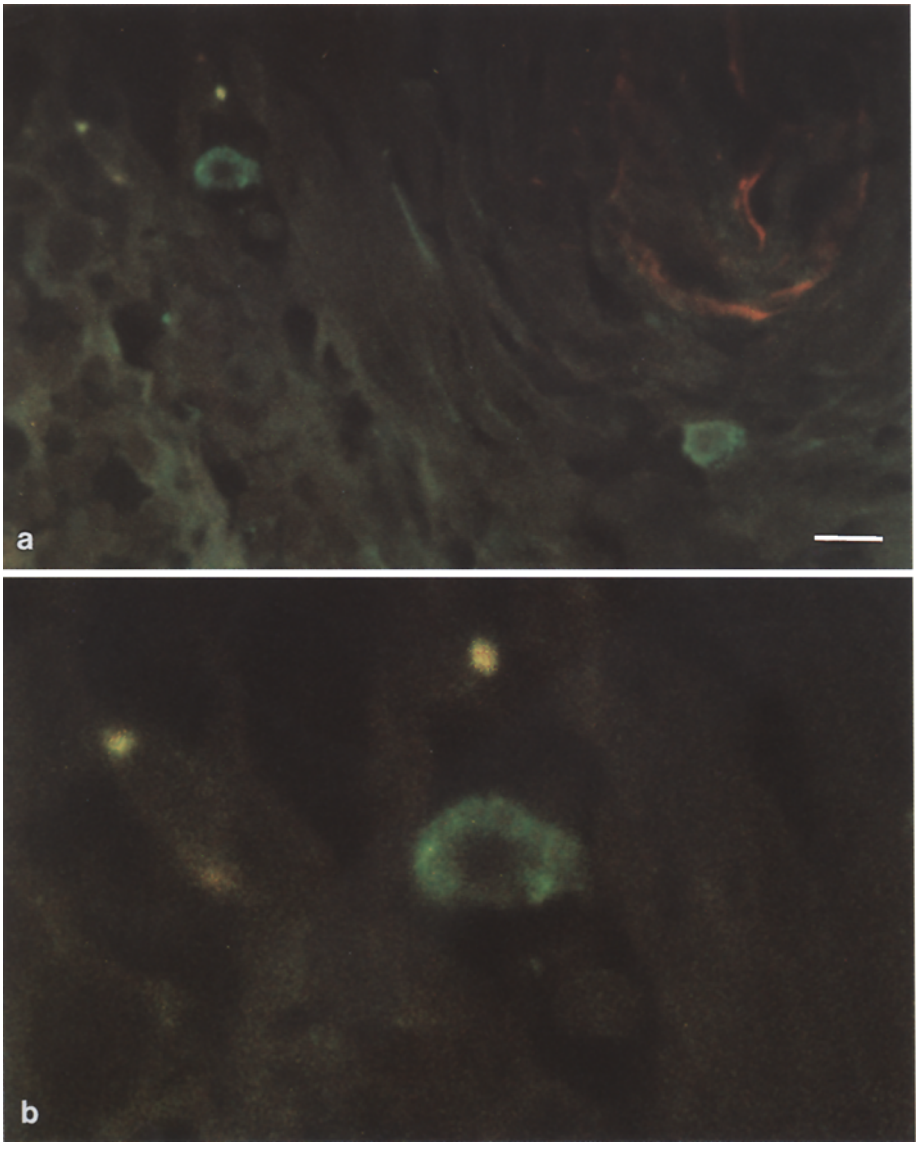

Fig. 1a Two chlamydia inclusions in the lung of one of the chlamydia positive SIDS cases. Original magnification × 500. Bar represents 10 μm. **b** The left upper inclusion from **a** magnified × 1500. The reticulate bodies are located in the cytoplasm around the nucleus of the host cell

thrombosis and 2 incarcerations), 5 major respiratory infections (with verified bacteriology), 1 abdominal haemorrhagic infarction, 8 accidents (domestic or traffic) and 3 homicides.

The age distribution of the infants in the control group was from 1 to 12 months, 66% were from 1–4 months old and 4 month-old infants making up 20% of the total.

Immunofluorescence method. Formalin-fixed lung sections from the SIDS and control cases were retrospectively examined for chlamydia inclusions as described by Lundemose et al. [9]. Two monoclonal antibodies 12.1 and 15.1 against the genus-specific chlamydia lipopolysaccharide (LPS) epitope were used [11]. These antibodies react only with the chlamydia-specific LPS epitope and no cross reactions occurs. Intracellular inclusions or LPS accumulated in the membrane of the infected host cell were visualized with immunofluorescence after reaction with fluorescein-conjugated anti-mouse antibody (IgG). Problems with background staining were avoided by using trypsin, rabbit serum and Evans-blue. Trypsin removes the non-specific antibody binding Fc-receptors in the tissue by digestion and the rabbit serum minimizes the non-specific antibody adsorption by competition with the specific antibody. Evans-blue was used as counterstain.

The positive immunofluorescence diagnosis in this study was exclusively based on the bright apple-green fluorescence and the characteristic morphology of the chlamydia inclusion as described earlier [9]. In all the chlamydia positive cases distinct and sharply demarcated inclusions were visualized (Fig. 1). The case was only considered positive if inclusions were seen in at least 2 of the 4 lung sections. LPS accumulated in the membrane of the infected host cells was sometimes visualized as a bright applegreen lining of the cell/epithelium, also called the alveolar lining.

Demonstration of chlamydia inclusions in the lung tissue by immunofluorescence. Four tissue sections, two from the central and two from peripheral parts of both lungs were examined for each case. The tissue sections were examined in the form of a blind trial and were all examined by the same individual. The results were reproducible in 100% of the cases when re-examined 1 day later (75% were re-examined). The positive controls were lung sections from a mouse artificially infected with *C. trachomatis* serovar L2. The negative controls were lung sections from a 45-year-old man who had died from suffocation with no histological signs of pneumonia.

Statistical analysis. Fisher's exact test was used for statistical analysis of the results observed in this study.

Results

Thirty-two of the 166 SIDS cases (19.4%) and one of the control cases (3.3%) had typical chlamydia inclusions in the lungs. The data was subjected to Fisher's exact analysis, and associations with P values below 0.05 were regarded as significant ($P = 0.04$).

All the chlamydia positive cases had 10 or more inclusions in each positive lung section, 10 cases had more than 25 inclusions and 3 cases more than 100 inclusions. The inclusions were usually seen in clusters and always in the peripheral parts of the lungs located in relation to the alveoli. In the 13 heavily (25 inclusions or more) infected cases accumulation of LPS in the membrane of the infected host cell (membrane/alveolar lining) was detected. The alveolar lining was nearly always located in the central parts of the lung.

Chlamydia positive cases were aged from 1–7 months, the first 4 months making up 84% with a peak at 1–2 months. The histological examination of the lung sections from the chlamydia positive cases did not show any signs of typical chlamydia pneumonia with inflammatory exudate in the alveoli and lymphocytic infiltration in the bronchial submucosa. There were no specific reports of symptoms of tachypnea, staccato cough and inspiratory stridor, although 6 of the 32 chlamydia positive infants were reported to have had colds and coughs immediately preceding death. One of the 3 chlamydia positive cases with more than 100 inclusions per positive lung section and 1 of the chlamydia positive cases with more than 25 inclusions per lung section had minor signs of viral pneumonia insufficient to explain death. In addition, 9 of the chlamydia positive infants with more than 25 inclusions per lung section showed non-specific lymphocytic infiltration in the lungs. None of these histological pictures were serious enough to explain the cause of death. The remaining 21 of the chlamydia positive cases showed no evidence of infectious diseases either in the medical history or in the histological examination. Forty of the 166 cases in the total SIDS group had inflammatory cellular changes in the lungs insufficient to explain death. Colds and coughs were reported in 43 of the 166 cases.

The single chlamydia positive infant in the control group was a 8 1/2 month-old female with Down's Syndrome. The girl had a severe congenital heart malformation, which was considered the cause of death. The histological examination of the lungs showed signs of bronchopneumonia. The immunofluorescence examination showed more than 10 chlamydia inclusions per positive lung section. Fourteen of the 30 cases in the control group had non-specific cellular infiltrations or slight focal bronchopneumonia in the lungs as a secondary finding to the primary cause of death.

Discussion

The occurrence of chlamydia inclusions in the lungs of a SIDS group has been examined and compared with a control group. Due to the high overall autopsy rate in cases of infant deaths in Denmark, the SIDS group as well as the control group was well-defined. A simple and easily performed immunofluorescence technique has been used which is applicable on formalin-fixed tissue sections [9]. It was found that 32 of the 166 SIDS cases examined were positive for chlamydia inclusions. Only 1 child was found positive in the control group. The difference between the SIDS group and the control group was statistically significant ($P = 0.04$). The P value was relatively higher than expected due to the low number of cases in the control group. However, we were not able to allocate more than 30 infants to the control group and could only have increased the number by expanding the age and time interval or by including material from ordinary pathological autopsies.

The immunofluorescence assay was selected as the diagnostic tool, since cultivation from autopsy material is difficult because of postmortal loss of viability of the chlamydia. Genus-specific monoclonal antibodies against LPS were selected instead of the species-specific monoclonal antibodies against the Major Outer Membrane Protein, because the non-protein origin of LPS allows

treatment with the background-reducing trypsin which is very important for the assay [9]. Furthermore LPS is in contrast to proteins, resistent to proteolysis and to postmortem autolysis [9]. The membrane lining observed in some sections is probably caused by LPS incorporated into the plasma membrane of the infected host cell during infection [12]. Birkelund et al. [13] have shown that chlamydial elementary bodies treated with monoclonal LPS antibodies liberate LPS from the surface into the surroundings. Thus an alternative explanation to the formation of the membrane lining could be that LPS is liberated from the infected cell during infection. The membrane lining seen in the sections from the central part of the lungs could thus represent free LPS removed by the alveolar macrophages.

Conjunctivitis and nasopharyngeal infections in infants of *C. trachomatis* infected mothers has been observed and it has been reported that this may result in pneumonia [14]. The disease is usually mild and does not require hospitalization, but it reportedly accounts for approximately one third of the cases of pneumonia in hospitalized infants from 1–6 months of age and may occasionally be life-threatening [15]. Impairment of lung function may persist for a longer period than after a viral pneumonia [16]. The chlamydia positive SIDS cases in our study were aged between 1–7 months, 86% between 1–4 months in which *C. trachomatis* pneumonia usually occurs. The age distribution of the SIDS group and the control group was almost identical.

Although *C. trachomatis* is the classic agent of chlamydia pneumonia in young infants, the 2 other chlamydia species may be responsible for some of the inclusions detected in the lung sections of the chlamydia positive cases. The applied immunofluorescence assay does not permit distinction between the 3 chlamydia species. As *C. pneumoniae* is a new chlamydia species capable of producing lower respiratory diseases transmitted by droplet infection, it might be considered a possible source of at least some of the inclusions observed in the lungs of the SIDS cases. *C. psittaci* in humans is associated with severe pneumonia, but has also been reported to produce acute placentitis and abortion in women, where both the mother and the fetus have been infected [17]. Therefore *C. psittaci* is difficult to exclude as a possible agent of some of the inclusions found in the lungs of the chlamydia positive cases.

In Denmark pregnant women are not routinely screened for genital chlamydia infections and gynaecological and obstetric data on the mothers, including venereal status, preceding the births of the infants included in this study are therefore not available. Several authors [for ref. see 15] have shown that approximately 7%–12% of cervices are infected with *C. trachomatis* before delivery. Approximately 2 out of 3 infants who are exposed to *C. trachomatis* acquire the infection, and pneumonia will develop in about 10%–20% [18]. Using these figures, the expected frequency of *C. trachomatis* pneumonia in this study should have been about 1% to 2%. In the present study we found 3.3% positive for chlamydia in the control group. The difference between the expected and the actual chlamydia frequency in the control group could either be caused be the presence of chlamydia inclusions from the other 2 chlamydia species or simply be due to the small number (30) in our control group. The high frequency of chlamydia in the SIDS cases could theoretically be regarded as a normal finding, explained by a 100% transmission rate from the infected mother to the infant, but since a similar frequency was not seen in the control group, the occurrence of chlamydia in the SIDS cases must be regarded as significant.

An association between chlamydia and SIDS has been noted before but was not further examined [19]. The chlamydia organism has been well studied and it seems odd that chlamydia infection in SIDS infants has been overlooked before. However, cultivation of chlamydia from autopsy material is, as already mentioned, difficult and a direct antigen detecting method applicable on postmortem formalin-fixed tissue, has not been described until recently [9].

We did not find any histologically verified signs of typical chlamydia pneumonia in the 32 chlamydia positive SIDS cases or in the 1 chlamydia positive control case. Nor did we find any cellular reactions in the lungs of these infants adequate to explain the cause of death. Twenty-one of the positive infants did not have any signs of pulmonary inflammation and 11 infants with more than 25 inclusions per lung section had only non-specific lymphocytic infiltration or minor histological signs of viral pneumonia. Based on this observation it could be speculated whether some infants who may be incapable of producing an adequate response to a microorganism could die of a chlamydia infection, without significant histological signs of inflammation.

Evidence of respiratory inflammation has been found in a considerable proportion of SIDS cases [20, 21]. Some investigators have sought the explanation in a specific microorganism. Püschel et al. [22] examined the parotid gland and/or the submandibular gland for Cytomegalovirus (CMV) in 255 SIDS cases by means of HE staining, immunohistochemical analysis, in situ hybridization and electron microscopy. Typical cytomegalic inclusions were recognized in 10% of the cases. A localized CMV infection of the salivary glands does not adequately explain the sudden death of these infants, but the investigators emphasize that cytomegaly may influence the immunological status of the organism. Telford et al. [23] found significantly higher rates of streptococcal and enterobacterial carriage in the nasopharyngeal flora in a SIDS group than in a matched living control group, indicating a disordered nasopharyngeal flora in SIDS cases. However, these studies do not deal with a control group of infants at the same age and dying in the same period as the SIDS group examined.

It is difficult to establish a causal relationship between certain microorganisms and SIDS. However, in support of a causal relation between chlamydia infection and SIDS, we have found a disseminated chlamydia infection in a SIDS infant who died in 1989. Chlamydia inclusions were found in the lungs, heart, liver and prostate gland, with an inflammatory response only in the prostate gland. We have not examined all the organs from the SIDS cases in this study, but are in the future planning to ex-

amine the degree of dissemination of the chlamydia infection in the 32 SIDS cases with chlamydia inclusions in the lungs. Whether a disseminated infection with chlamydia or other microorganisms is the cause of death in some SIDS cases as a result of an inadequate response to the infection needs further investigation.

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References

1. Peterson DR (1980) Evolution of epidemiology of sudden infant death syndrome. Epidemiol Rev 2:97–112
2. Lignitz E, Hirvonen J (1989) Inflammation in the lungs of infants dying suddenly. A comparative study from two countries. Forensic Sci Int 42:85–94
3. Stagno S, Brasfield DM, Brown MB, Cassell GH, Pifer LL, Whitley RJ, Tiller RE (1981) Infant pneumonitis associated with *Cytomegalovirus, Chlamydia, Pneumocystis,* and *Ureaplasma*: a prospective study. Pediatrics 68:322–329
4. Dworsky ME, Stagno S (1982) Newer agents causing pneumonitis in early infancy. Pediatr Infect Dis J 1:188–195
5. Schachter J (1978) Chlamydial infections. N Engl J Med 298: 428–435, 490–495, 540–549
6. Buxton D (1986) Potential danger to pregnant women of *Chlamydia psittaci* from sheep. Vet Rec 118:510–511
7. Johnson FWA, Matheson BA, Williams H, Laing AG, Jandial V, Davidson-Lamb R, Halliday GJ, Hobson D, Wong SY, Hadley KM, Moffat MAJ, Postlethwaite R (1985) Abortion due to infection with *Chlamydia psittaci* in a sheep farmer's wife. Br Med J 290:592–594
8. Grayston JT, Cho-Chou Kuo, San Pin Wang, Altman J (1986) A new *Chlamydia psittaci* strain, TWAR, isolated in acute respiratory tract infections. N Engl J Med 315:161–168
9. Lundemose AG, Lundemose JB, Birkelund S, Christiansen G (1989) Detection of *Chlamydia* in postmortal formalin-fixed tissue. APMIS 97:68–74
10. Bergman AB, Berkwith JB, Ray CC (1970) Sudden infant death syndrome. University of Washington Press, Seattle, pp 17–18
11. Birkelund S, Lundemose AG, Christiansen G (1988) Chemical cross-linking of *Chlamydia trachomatis*. Infect Immun 56:654–659
12. Karimi ST, Schloemer RH, Wilde III CE (1989) Accumulation of chlamydial Lipopolysaccaride antigen in the plasma membranes of infected cells. Infect Immun 57:1780–1785
13. Birkelund S, Lundemose AG, Christiansen G (1989) Immunoelectron microscopy of lipopolysaccaride in *Chlamydia trachomatis*. Infect Immun 57:3250–3253
14. Beem MO, Saxon EM (1977) Respiratory-tract colonization and a distinctive pneumonia syndrome in infants infected with *Chlamydia trachomatis*. N Engl J Med 296:306–310
15. Alexander ER, Harrison HR (1983) Role of *Chlamydia trachomatis* in perinatal infection. Rev Infect Dis 5:713–719
16. Harrison HR, Taussig LM, Fulginiti VA (1982) *Chlamydia trachomatis* and chronic respiratory disease in childhood. Pediatr Infect Dis J 1:29–33
17. Wong SY, Gray ES, Buxton D, Finlayson J, Johnson FWA (1985) Acute placentitis and spontaneous abortion caused by *Chlamydia psittaci* of sheep origin: a histological and ultrastructural study. J Clin Pathol 138:707–711
18. Schachter J (1989) Why we need a program for the control of *Chlamydia trachomatis*. N Engl J Med 320:802–804
19. Schachter J, Grossman M, Sweet RL, Holt J, Jordan C, Bishop E (1986) Prospective study of perinatal transmission of *Chlamydia trachomatis*. JAMA 255:3374–3377
20. Scott DJ, Gardner PS, McQuillin J, Stanton AN, Downham MAPS (1978) Respiratory viruses and cot death. Br Med J 2:12–13
21. Williams AL, Uren EC, Bretherton L (1984) Respiratory virus and sudden infant death. Br Med J 228:1491–1493
22. Püschel K, Hashimoto Y, Löning T, Lignitz E (1988) Cytomegalie der Kopfspeicheldrüsen bei SIDS. Z Rechtsmed 99: 281–289
23. Telford DR, Morris JA, Hughes P, Conway AR, Lee S, Barson AJ, Drucker DB (1989) The nasopharyngeal bacteria flora in the sudden infant death syndrome. J Infect 18:125–130