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ORIGINAL ARTICLE

Cytokines and sudden infant death

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Abstract
Background It has been hypothesised that inflammatory reactions could play an important role in the pathway(s) leading to sudden and unexpected death in infancy. On a molecular level, these reactions are regulated by various cytokines.
Methods To characterise the role of IL-1ß, IL-6 and TNFα more precisely, the concentrations of these cytokines were determined quantitatively using specific ELISA techniques in serum and cerebrospinal fluid (CSF) in 119 cases of sudden infant death. The infants were grouped into four categories (SIDS, SIDS with infection, natural death due to infection and unnatural death).
Results A good correlation was found between CSF and serum for IL-6 (Spearman correlation coefficients (SCC), 0.73) and also for TNFα (SCC, 0.57), although the CSF concentrations were lower than that from the serum. There were no significant differences between the categories of death for any of the serum or CSF cytokines. Compared with normal values, increased serum concentrations of IL-1ß, IL-6 and TNFα were found in 70%, 69% and 38% of the cases respectively, indicating possible agonal or post-mortem changes of cytokine concentrations. In three cases very high cytokine concentrations were found (mainly for IL-6). This may have contributed to the mechanism of death (cytokine storm) in two of the cases.
Conclusions In a small group of patients, very high cytokine concentrations are a possible explanation for the cause of death ("cytokine storm").

Keywords Cytokines · Sudden infant death · SIDS

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Introduction

In 1989 Guntheroth [1] suggested that respiratory tract infections and prolonged sleep apnea could be associated with death. He postulated the possible influence of interleukin-1 (IL-1) and muramylpeptide in infection, leading to a deeper sleep, with an increased body temperature, and activating an immune response. Stoltenberg et al. [2] gave evidence for a stimulation of the immune response prior to death in SIDS victims and postulated a viral infection as a possible cause of death. Vege et al. [3] measured IL-1ß, IL-6 and TNFα in cerebrospinal fluid obtained in SIDS cases, so-called borderline SIDS, deaths due to infection, and unnatural deaths, and found significantly higher IL-6 concentrations in the SIDS group. In 2005, Blackwell et al. in an overview of current finding hypothesised that sudden death could be the result of an overwhelming proinflammatory response to bacterial toxins [4].

A number of different functions of IL-1ß are known. In endothelial cells, it triggers the transduction of cyclooxygenase-2 and influences the synthesis of prostaglandin-E2 (PGE2) [5]. As a mediator of inflammatory reaction, it stimulates neurons of the hypothalamus to excrete a corticotropin-releasing hormone. Furthermore, it triggers IL-6 excretion, the formation of neutrophil granulocytes in bone marrow and the formation of CD14 [6].

IL-6 is part of the inflammatory pathway and takes a key position in the gateway from inborn to acquired immunity. In acute inflammation, soluble IL-6 receptor increases and limits the accumulation of neutrophil granulocytes as well as the immigration of CD3+ T lymphocytes [7]. This step marks the transformation from inborn to acquired immune reaction [8]. Further functions are known: the regulation of the apoptosis of leukocytes, the differentiation and proliferation of B lymphocytes, the IgG secretion of B lymphocytes and the differentiation of monocytes [9].

TNFα is mainly produced by macrophages as a response to lipopolysaccharide, other bacterial products and IL-1. It stimulates the phagocytotic activity of macrophages and the production of IL-1 oxidants and PGE2. A local increase in TNFα concentration causes typical signs of inflammation (heat, swelling, redness and pain). High concentrations of TNFα would be expected to induce shock-like symptoms [10–12].

The possible role of different cytokines in this process has not been fully investigated. The aim of this study was to evaluate the role of cytokines as a possible factor triggering or influencing the risk of sudden death of infants.

Materials and methods

The German Study on Sudden Infant Death (GeSID) comprised a full investigation of a total of 373 cases of sudden and unexpected infant death, using standardised protocols for evaluating the circumstances of death, plus autopsy findings, including histology, microbiology, virology, toxicology and previous history. The methods of the GeSID study have been described in detail previously [13]. All 119 infant deaths investigated in Hamburg and Hannover had serum and cerebrospinal fluid (CSF) taken during autopsy and stored at −20°C.

Classification of cases

All infants were classified according to the cause of death into four groups:

- Group 1: SIDS without infection, n=20; San Diego definition, category 1b [14]
- Group 2: SIDS showing infection not sufficient to explain the death, n=78; San Diego definition, category 1b or 2 [14].
- Group 3: explained natural death, due to infection, as previously described, n=13; (Table 1).
- Group 4: unnatural death, n=8; (Table 1).

Quantitative determination of cytokines

The concentrations of IL-1ß, IL-6 and TNFα were determined using specific ELISAs (Eli-pair, Diaclone, Cell Sciences, Canton, MA, USA) following the manufacturer's instructions. The calibration curves were each constructed using seven standard solutions of defined concentrations between 7.8 to 500 pg/ml for IL-1ß, 3.1 to 200 pg/ml for IL-6 and 12.5 to 800 pg/ml for TNFα in serial dilution. The

Table 1 Causes of death in explained natural deaths and unnatural deaths

Explained natural death (n=13)	Unnatural death (n=8)
Severe pneumonia (n=4)	Suffocation (n=4)
Septicaemia (n=3)	Drowning (n=1)
Malformation of the brain + respiratory tract infection (n=1)	Stab wounds + bacterial infection (n=1)
Congenital heart disease + respiratory infection (n=1)	Severe brain injury (n=1)
Generalised CMV infection (n=2)	Shaken baby syndrome (n=1)
Enteritis + dehydration (n=2)	

cytokine concentrations of the specimens were calculated by linear regression. The correlation coefficient was higher than 0.995 for all curves. In some cases, the fluids had to be diluted several times because the original concentrations were outside the calibration range.

Epidemiological data

To investigate groups 1–3 (n=111), comprising cases of natural death, a questionnaire was drawn up, containing 106 questions relating to all aspects of the infant's life (family and child history, sociodemographic factors, sleeping conditions, feeding, parents' cigarette and alcohol consumption, etc.) [13]. For the cases of unnatural death, this information was not available (Table 2).

Statistical analyses

Statistical analyses were performed using SAS 9.2. (SAS Institute Inc., Cary, NC, USA). For comparison of the groups, significances were calculated for categorical variables with χ^2 test and for continuous variables by Kruskal–Wallis test. To analyse the correlation between the levels of cytokines in serum and CSF, the Spearman correlation coefficient (SCC) was used.

Results

Epidemiological data

The age at death is very similar in the three groups of natural death (Table 2). The group of children with unnatural deaths were slightly older, one being older than a year. Comparing the main risk factors relating to SIDS (sex, sleeping position, mother's smoking behaviour during pregnancy and breast feeding), the three natural death groups did not display any statistically significant differences (data not shown). For the unnatural death group, no information about risk factors was available.

Cytokine concentrations

For all three cytokines, the CSF concentrations were lower than the corresponding serum concentrations (Table 3). There was a good correlation between IL-6 in serum and CSF in all infants (SCC=0.73) and also for TNFα (SCC=0.57). The correlation between IL-1ß in serum and CSF was lower (SCC=0.37). There were no significant differences between the groups for any of the serum or CSF cytokines. The cytokine concentrations did not differ by the presence or absence of known SIDS risk factors (results not shown).

The post-mortem interval (time between discovery of death and time of autopsy) varied between 7 and 47 h in all groups (arithmetic mean, 27 h). There was no relationship between the cytokines and risk factors for SIDS (results not shown).

Very limited data are available on normal values of cytokines for infants. For IL-6, normal values for infants are 0–9 pg/ml and for TNFα 0–3.6 pg/ml. For CSF there are no normal values available [15,16]. Compared to the "normal values", increased serum concentrations of IL-1ß, IL-6 and TNFα were found in 70%, 69% and 38% of the cases, respectively; there is no significant difference between the groups.

Case reports

Three cases showed very high cytokine concentrations, mainly for IL-6 (Table 4). These cases are presented in short case reports:

– Case 1: Male, sudden death at the age of 10 months, respiratory infection. Histology: tracheitis, bronchitis, mild interstitial pneumonia (no signs of bacterial superinfection) and mild gliosis of the brain. Toxicology negative. Cause of death: SIDS + infection.

Table 2 Epidemiological data given for the groups investigated

Variable	Group 1: SIDS without infection N=20	Group 2: SIDS + infection N=78	Group 3: explained natural death N=13	Group 4: unnatural death N=8
Mean age (days)	117	139	116	272
Min.–max. (days)	48–283	13–335	22–321	99–730[a]
Male sex of infants	81%	64%	44%	50%
Prone sleeping position (%)	55	39	46	n/a
Mother smokes (%)	60	54	38	n/a
Breast fed (%)	65	50	69	n/a

For unnatural deaths, the sleeping position, the type of feeding and, the smoking behaviour of the mother are unknown

[a] One child was older than 1 year

Table 3 Cytokine concentrations in serum and cerebrospinal fluid (CSF, in picogram per millilitre) in the four different groups of infant deaths

	IL-1ß (pg/ml)		IL-6 (pg/ml)		TNFα (pg/ml)	
	Serum	CSF	Serum	CSF	Serum	CSF
SIDS						
n	18	16	18	16	18	16
Median (range)	5.8 (0.0–46.4)	0.0 (0.0–5.0)	10.8 (4.4–380.0)	18.3 (0.7–113.3)	0.0 (0.0–77.6)	0.0 (0.0–8.6)
Mean (SD)	9.5 (11.6)	1.2 (1.8)	86.1 (136.7)	29.7 (35.4)	6.9 (18.6)	1.9 (3.1)
SIDS + infection						
n	72	70	72	65	72	69
Median (range)	3.5 (0.0–132.6)	0.0 (0.0–30.4)	22.4 (0.5–4,824.0)	12.9 (0.0–618.0)	0.0 (0.0–522.5)	0.5 (0.0–50.6)
Mean (SD)	15.2 (26.6)	2.4 (5.0)	194.7 (626.9)	43.2 (94.2)	33.3 (91.8)	6.8 (12.1)
Explained natural death						
n	10	12	10	12	10	12
Median (range)	3.0 (0.0–760)	0.5 (0.0–23.9)	50.7 (0.0–24,540.0)	20.9 (1.3–4,736.0)	2.8 (0.0–466.0)	0.0 (0.0–14.9)
Mean (SD)	107.3 (237.6)	4.3 (7.8)	2529.1 (7734.4)	489.8 (1353.1)	84.4 (162.9)	1.5 (4.3)
Unnatural death						
n	8	5	8	5	8	5
Median (range)	7.5 (0.0–2,336.0)	2.0 (0.0–38.1)	33.3 (0.0–179,590.0)	9.7 (3.4–177.0)	8.8 (0.0–1,701.0)	4.0 (0.0–7.9)
Mean (SD)	298.8 (823.2)	9.0 (16.4)	23,228.2 (6,3214.5)	42.3 (75.5)	222.6 (597.5)	3.5 (3.0)

- Case 2: Male, 5 months old. Aspiration of stomach contents (terminal event), brain oedema. Histology: Sialadenitis caused by CMV infection, virus associated myocarditis and hepatitis and interstitial nephritis. Toxicology negative. Cause of death: generalised CMV infection (explained cause of death).
- Case 3: Male, 12 months old, infanticide (stabbed by the father with very short survival time), respiratory tract infection. Histology: purulent laryngitis, purulent tracheitis, purulent bronchitis/bronchiolitis. Mild duodenitis; brain oedema. Toxicology negative. Cause of death: infanticide+infection (unnatural death).

Discussion

The investigation of sudden unexpected death in infants and mechanisms leading to SIDS have been the subject of research over the past decades [2–5] Several researchers have discussed the possible role of cytokines as a pathophysiological factor which could contribute to SIDS because cytokines are proteins which regulate immune responses in allergic reactions, virus infection and septicaemia [1–4,17–23]. This hypothesis was investigated as a part of the GeSID study [13]. Two different body fluids were collected to determine the cytokine concentrations (IL-1ß, IL-6 and TNFα). Serum was selected as a material which could be representative of "peripheral" effects while CSF could be a marker of central effects, indicating the function and or disturbances of the central nervous system.

In the present investigation, the cytokines concentrations in the serum and CSF did not vary by cause of death. This finding varies from that reported by Vege et al. [3] who found that the IL-6 CSF levels in cases of infectious death, heart/lung disease and SIDS with infection were significantly higher compared with "pure" SIDS cases. This difference may be due to heterogeneity of the cases showing various types of infection and/or due to

Table 4 Cytokine concentrations in serum and cerebrospinal fluid of three selected cases (pg/ml)

	Cause of death	IL-1ß		IL-6		TNFα	
		Serum	CSF	Serum	CSF	Serum	CSF
Case 1	SIDS + infection	80	n. m.	4,824	n.m.	20.8	n.m.
Case 2	Generalised CMV infection	102	9.1	24.540	302	300.4	0
Case 3	Infanticide + pre-existing infection	2,336	38.1	179,590	9.7	1,701	4

n.m. no material available

differences in diagnostic criteria used to classify the cases in both studies. In addition, it is possible that cytokine levels may have been influenced by differences in the post-mortem interval. Tsokos et al. [24] investigated the time course of post-mortem IL levels in a small number of adults found with increasing concentrations of IL-6 associated with an increasing post-mortem time interval, although in our study cytokine levels did not vary with post-mortem interval. The IL-6 levels did not exceed 1,500 pg/ml in those controls, which did not show signs of infection [24]. In their "sepsis group", IL-6 concentrations were higher than 1,500 pg/ml in all patients "in at least one serum sample" [24]. In a study performed by Mimasaka [25], increased levels of IL-1ß and IL-6 were found in serum obtained within 48 h of death from adults who had died from natural and unnatural causes without infection and without blunt trauma (average IL-1ß, 4–8 pg/ml; average IL-6, 3,000–8,000 pg/ml). Unfortunately, ranges of "normal" cytokine concentrations are available only for living infants and children [4]. For some parameters, the reference values were derived from laboratories where only small numbers of cases were investigated (Berdat et al.; n=25 younger than 1 year of age [15]). Decreased cytokine concentrations in individual cases can be explained by mild haemolysis, influencing test results.

Cytokine levels were higher than the normal range for the majority of cases (IL-1ß (70%), IL-6 (69%) and TNFα (38% of the cases)), indicating possible agonal or post-mortem changes of cytokine concentrations. A good correlation of serum and CSF concentrations was observed for IL-6 and TNFα but the correlation was not as good for IL-1ß. For all cytokines, the measured concentrations were lower in CSF compared with serum. A clear explanation of this phenomenon cannot be given at present.

Three cases showed markedly increased concentrations of IL-6 in serum (greater than 1,500 pg/ml). This could be the expression of an overwhelming activation of the cytokine system indicating a cytokine storm [15,24], which may explain the death in at least two cases. Such mechanisms (cytokine storm) have been described in influenza virus infection [5,17]. In two out of the three cases, signs of virus infection were found by histology. The third infant also showed very high cytokine concentrations, but this infant died due to haemorrhagic shock (unnatural death, stabbed by the father) and had a severe bacterial infection in both lungs.

Conclusions

Our study did not identify significant differences in cytokine levels between SIDS cases and non-SIDS cases. In a small group of infants, very high cytokine concentrations were found, which might have contributed to the mechanism of death.

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References

1. Guntheroth WG (1989) Interleukin-1 as intermediary causing prolonged sleep apnea and SIDS during respiratory infections. Med Hypotheses 28:121–123
2. Stoltenberg L, Vege A, Opdal SH, Saugstad OD, Rognum TO (1995) Does immunostimulation play a role in SIDS? In: Rognum TO (ed) Sudden infant death syndrome. New trends in the nineties. Scandinavian University Press, Oslo pp. 179–181
3. Vege A, Rognum TO, Aasen AO, Saugstad OD (1998) Are elevated cerebrospinal fluid levels of IL-6 in sudden unexplained deaths, infectious deaths and deaths due to heart/lung disease in infants and children due to hypoxia? Acta Paediatr 87:819–824
4. Blackwell CC, Moscovis SM, Gordon AE, Al Madani OM, Hall ST, Gleeson M et al (2005) Cytokine responses and sudden infant death syndrome: genetic, developmental, and environmental risk factors. J Leukoc Biol 78:1242–1254
5. Rivest S, Lacroix S, Vallieres L, Nadeau S, Zhang J, Laflamme N (2000) How the blood talks to the brain parenchyma and the paraventricular nucleus of the hypothalamus during systemic inflammatory and infectious stimuli. Proc Soc Exp Biol Med 223:22–38
6. Dinarello CA (2011) Blocking interleukin-1beta in acute and chronic autoinflammatory diseases. J Intern Med 269:16–28
7. Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G, Schaper F (2003) Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J 374:1–20
8. Jones SA (2005) Directing transition from innate to acquired immunity: defining a role for IL-6. J Immunol 175:3463–3468
9. Fischer CP (2006) Interleukin-6 in acute exercise and training: what is the biological relevance? Exerc Immunol Rev 12:6–33
10. Locksley RM, Killeen N, Lenardo MJ (2001) The TNF and TNF receptor superfamilies: integrating mammalian biology. Cell 104:487–501
11. Old LJ (1985) Tumor necrosis factor (TNF). Science 230:630–632
12. Wajant H, Pfizenmaier K, Scheurich P (2003) Tumor necrosis factor signaling. Cell Death Differ 10:45–65
13. Findeisen M, Vennemann M, Brinkmann B, Ortmann C, Rose I, Kopcke W et al (2004) German study on sudden infant death (GeSID): design, epidemiological and pathological profile. Int J Legal Med 118:163–169
14. Krous HF, Beckwith JB, Byard RW, Rognum TO, Bajanowski T, Corey T et al (2004) Sudden infant death syndrome and unclassified sudden infant deaths: a definitional and diagnostic approach. Pediatrics 114:234–238
15. Berdat PA, Wehrle TJ, Kung A, Achermann F, Sutter M, Carrel TP et al (2003) Age-specific analysis of normal cytokine levels in healthy infants. Clin Chem Lab Med 41:1335–1339
16. Laboratoriumsmedizin Dortmund. Available at http://www.labmed.de/de/de/laboratoriumsmedizin_dortmund-981.html. Accessed 13 July 2011.

17. Dinarello CA (1988) New concepts on the pathogenesis of fever. Rev Infect Dis 10:168–179
18. Kunkel SL, Streiter RM (1990) Cytokine networking in lung inflammation. Hosp Pract (Off Ed) 15:63–76
19. Sayers NM, Drucker DB, Grencis RK (1995) Cytokines may give insight into mechanisms of death in sudden infant death syndrome. Med Hypotheses 45:369–374
20. Reid GM (2001) Sudden infant death syndrome. Part 2: the response of the reticuloendothelial system to hypoxemia and infection. Med Hypotheses 57:1–5
21. Goldwater PN (2004) SIDS pathogenesis: pathological findings indicate infection and inflammatory responses are involved. FEMS Immunol Med Microbiol 42:11–20
22. Moscovis SM, Gordon AE, Al Madani OM, Gleeson M, Scott RJ, Roberts-Thomson J et al (2004) Interleukin-10 and sudden infant death syndrome. FEMS Immunol Med Microbiol 42:130–138
23. Rognum IJ, Haynes RL, Vege A, Yang M, Rognum TO, Kinney HC (2009) Interleukin-6 and the serotonergic system of the medulla oblongata in the sudden infant death syndrome. Acta Neuropathol 118:519–530
24. Tsokos M, Reichelt U, Jung R, Nierhaus A, Püschel K (2001) Interleukin-6 and C-reactive protein serum levels in sepsis-related fatalities during the early postmortem period. Forensic Sci Int 119:47–56
25. Mimasaka S (2002) Postmortem cytokine levels and the cause of death. Tohoku J Exp Med 197:145–150