Int J Legal Med (1998) 112 : 31–34 © Springer-Verlag 1998

ORIGINAL ARTICLE

F. K. H. van Landeghem · B. Brinkmann
T. Bajanowski

Basement membrane thickness of the vocal cord in cases of sudden infant death

Received: 10 June 1998 / Received in revised form: 21 August 1998

Abstract In 1991, 1994 and 1997 Shatz et al. reported on a specific and pathognomonic basement membrane (BM) thickening of the vocal cord in cases of sudden infant death syndrome (SIDS). In 40 cases of sudden and unexpected infant death the histological examination of the larynx was performed to identify possible differences between SID (n = 26) and non-SID (n = 14) cases. The dissection technique of Hohmann (1963) and Maxeiner (1986) was modified for the infant's larynx. Since the normal range of the BM thickness of the vocal cord for the age group younger than 1 year had not yet been exactly defined, a reference interval had to be established (0.5 μm–2.0 μm). In 2 SID and 1 non-SID cases a mean BM thickness (BMT) of more than 2.0 μm could be estimated (2.38 μm–2.95 μm). The BMT in these cases appeared to be highly variable and not harmonically thickened. None of the investigated regions of the BM (cranial to caudal thirds, ventral to dorsal areas) seemed to be prefered thickened. There was no statistically significant difference between the two groups. A specific thickening of the BM of the vocal cord in SID cases could not be confirmed. Therefore, BMT cannot be used as a diagnostic postmortem marker for SID.

Key words Sudden infant death (SID) · Larynx · Vocal cord · Basement membrane thickness

Introduction

The macroscopic and histologic investigation of the respiratory tract including the larynx is a standard procedure in cases of sudden infant death. Besides inflammatory changes as reported by many authors [e.g. 1–4] Shatz et al. found [5–8] a BM thickening of the vocal cord in cases of sudden infant death syndrome (SIDS) and considered it to be specific and pathognomonic for SIDS. This BM thickening was neither correlated with any other pathological finding nor did it occur in infants who died suddenly and unexpectedly of known causes. Therefore, Shatz et al. suggested that the BMT of the vocal cord could be used as a postmortem marker in SIDS cases.

F. K. H. van Landeghem · B. Brinkmann (✉) · T. Bajanowski
Institute of Legal Medicine, Westfälische Wilhelms-Universität,
Von-Esmarch-Strasse 86, D-48149 Münster, Germany
email: brinkma@uni-muenster.de
Tel. (49) 251 8355 158; Fax (49) 251 8355 158

F. K. H. van Landeghem
Department of Neuropathology, Charité Campus Virchow,
Humboldt-Universität zu Berlin, Germany

Material and methods

Thorough postmortem (pm) examinations were performed on 26 cases of sudden infant death (SID) and 14 control cases in the age of 4 weeks to 14 months (Table 1) at the Institute of Legal Medicine in Münster, Germany. A standardized autopsy protocol including death scene investigation by the police, previous history, extensive histology, microbiology, virology and toxicology [9, 10] was used. The pm interval was 6–73.5 h (on average 26.5 h). In the SID group 57.7% were male and 42.3% female and 57.7% of the SID cases occured during the first 6 months of life.

Table 1 Distribution by sex and age of the SID and non-SID cases (d days, m months). * In two cases from category V the infant was older than one year (55 and 59 weeks)

Group	Total	Male	Female	Age	
	N	N	N	(on average)	(range)
SID cases (categories I–III)	26	15	11	5.5 m	44 d–10.5 m
Non-SID cases (categories IV,V)	14	10	4	5.25 m	28 d–14 m*

Table 2 Causes of death in the non-SID cases (natural and unnatural cause of death)

Non-SID cases (natural deaths) (category IV; n = 6)		Unnatural deaths (category V; n = 8)	
Interstitial pneumonia	4	Drowning	3
Bronchopneumonia	2	Suffocation	3
		Cocaine intoxication	1
		Embolism of pulmonary arteries due to injection of carrot juice	1

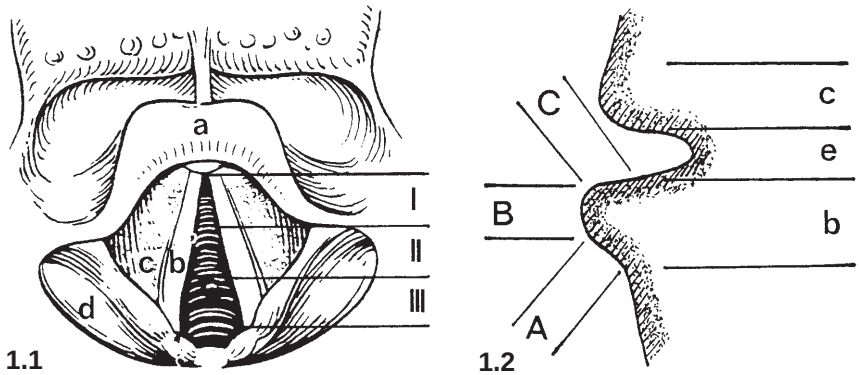

Fig. 1 Scheme for sampling and measurements. 1.1 – laryngoscopic view: a – epiglottis, b – vocal cord, c – vestibular fold, d – piriform recess; I – ventral, II – central, III – dorsal third of the vocal cord. 1.2 – Cross section through the larynx with the vestibular fold (c) and the vocal cord (b) and the glottis (e). Measurements were carried out in: A – caudal, B – frontal, C – cranial area of the vocal cord

The SID cases are not homogeneous and could be subdivided into three categories, and the non-SID group contains natural and non-natural deaths, in modification of [9,11]:

SID cases:

I: cases with inconspicious history and no pathological autopsy findings (n = 6, so-called "SIDS"),
II: minimal pathological autopsy findings and/or previous history of minor illness (n = 16, so-called "SIDS+"[9,12,13]),
III: intermediate pathological findings, potentially cause of death (n = 4, SID);

non-SID cases:

IV: cases of natural death (n = 6),
V: cases of non-natural death (n = 8).

In the non-SID group (categories IV and V) 71.4% of the infants were male 28.6% female and 57.1% were younger than 6 months of age when they died. The causes of death in the categories IV and V are given in Table 2.

The larynx was dissected following the method of Hohmann [14] and Maxeiner [15], modified for the infant's larynx. Three sagittal tissue samples each one from the ventral, central and dorsal third were taken from both vocal cords (with the vestibular folds, Fig. 1.1), fixed in buffered 5% formaldehyde and embedded in paraffin. One 3–4 μm thick section from each sample was stained with hematoxylin and eosin (H&E), luxol fast blue, Schiff's periodic acid and Heidenhain's azan. In each section 3 different localisations were chosen to measure the basement membrane (BM) thickness: 6 measurements were carried out in the cranial third, seven in the frontal and also 6 in caudal area (Fig. 1.2). A total of 120 measurements were made in each case using a Leica Quantimed Q600 S. The arithmetic as well as the harmonic mean barrier thickness were calculated. For statistical purposes the cases in categories 1–3 were grouped together as SID cases and compared with the cases of the categories 4 and 5 as non SID cases. Statistical calculations were performed using the U-test (Mann-Whitney-Wilcoxon).

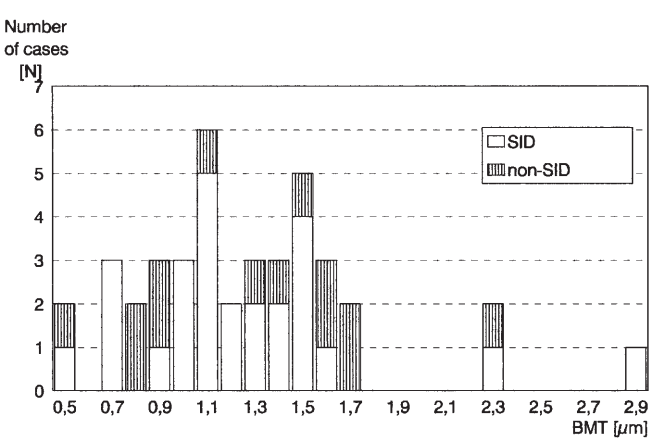

Fig. 2 BMT in SID and non-SID cases

Results

Because the normal range of the BMT of the vocal cord for the age group younger than 1 year had not yet been exactly defined, a reference interval had to be established. The curve of distribution of the BM measurements suggested a Gaussian curve for values between 0.5 μm and 2.0 μm as the reference interval in this group (Fig. 2). The mean BMT was not statistically different between the cases of categories 1–3 and the non-SID group (Table 3, Fig. 3a). The arithmetic mean of the BMT was slightly higher than the harmonic: In the SID group the factor was 1.05, in the non-SID group 1.06.

Age dependent differences of the BMT in the cases aged up to 14 months were not found. In three cases, two out of 26 SID cases (7.7%) and one out of 14 non-SID cases (7.1%), a BMT of more than 2.0 μm could be estimated (2.38 μm–2.95 μm, Figs. 2, 3b,c). The BM was not harmonically thickened in these cases, a preferred localization of the thickened BM could not be observed. In two

Table 3 Arithmetic thickness of the BM, standard deviation and ratio of the arithmetic and harmonic mean BMT in the categories investigated

Category	Arithmetic mean BMT (μm)	Range (μm)	Ratio of $\frac{\text{arithmetic mean BMT}}{\text{harmonic mean BMT}}$
I	1.14 ± 0.37	0.71–1.55	1.02
II	1.37 ± 0.65	0.70–2.95	1.06
III	1.32 ± 0.31	0.92–1.61	1.04
IV	1.10 ± 0.44	0.56–1.72	1.07
V	1.52 ± 0.45	0.98–2.38	1.05
SID cases (I–III)	1.31 ± 0.24	0.70–2.95	1.05
Non-SID (IV,V)	1.34 ± 0.34	0.56–2.38	1.06

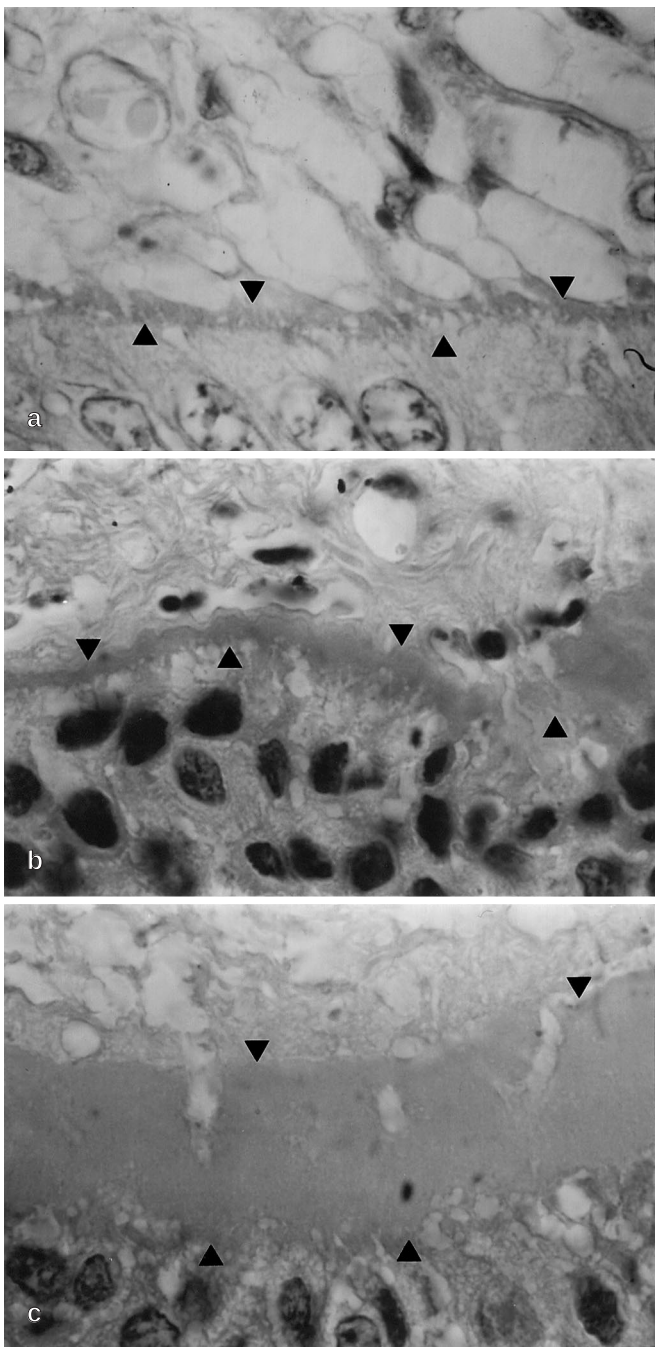

Fig. 3a–c Histology of the BM in different cases. **a** Normal thickness of the BM (<) in a SID case. PAS, × 1250. **b** Focally thickened basement membrane (>) in a SID case. PAS, × 1250. **c** Distinct thickening of the basement membrane (>)with subepithelially located edema in a SID case. PAS, × 1250

of these three cases the thickening was correlated with a distinct edema (Fig. 3c). Moderate signs of upper airway inflammation appeared in one of the two cases with distinct edema.

Furthermore, there were two SID and three non-SID cases with a focally thickened BM (average BMT 1.59 μm ± 0.36 μm, Fig. 3b). All of these cases revealed a distinct subepithelial edema, but signs of inflammation could not be seen.

None of the investigated parts of the vocal cord (cranial to caudal thirds, ventral to dorsal areas) seemed to be preferred for thickening, neither for the focal nor for the total BMT. The observed edema was not only located beneath the thickened BM, but appeared to be more obvious at these sites.

Discussion

Depending on the organ, the localization of measurement and the age of the infant the BM varies in thickness ranging from 0.05 μm–3 μm and may be particularly thick [16–18]. The BM, which appeared in the PAS reaction as a homogenic band because of the glycoprotein content [18], shows in electron microscopy an electrondense Lamina densa and less electrondense internal and external Lamina rara [18, 19]. The Lamina densa consists of a meshwork of type IV collagen fibres [20, 21]. Therefore, the results of measurements in the PAS reaction and in type IV collagen immunohistochemistry give comparable values.

In 1991 Shatz et al. [5] reported on a BMT with a harmonic mean of 4.7 μm ± 0.7 μm (range 2.7–11.0 μm) in the SIDS victims (n = 23), with no pathological condition other than the BM thickening. BM thickening could not be demonstrated in any of the 6 non-SIDS cases where the BMT was below the resolution of light microscopy (0.1–0.2 μm) [5]. For the measurements the authors used horizontal and sagittal section stained with H&E and PAS.

In a second study [6] these authors demonstrated a specific BMT of 5.98 μm ± 0.49 μm on average (range 1.34–16.7 μm) in SIDS cases. The BMT was correlated with the age of the infants at death (3 μm at 2 months, 11 μm at about 3–5 months, decline after the 5th month of life). The mean BMT in non-SIDS cases was less than 0.015 μm. A thickened BM could not be observed in these cases.

We established a reference interval for the BMT of the vocal cord in the age group of infants younger than one year between 0.5 μm and 2.0 μm, and could demonstrate that only in three of our cases a marked BMT with a mean thickness of 2.57 μm ± 0.89 μm occurred. In only one of the three cases a possible pathogenesis of the BM thickening could be supposed: the microscopic examination revealed moderate signs of upper airway inflammation with a distinct edema. In none of these cases was a positive smoking history of the parents during pregnancy and after birth documented. Rißе at al. [22] discussed recurrent laryngospasms and laryngeal stress by frequent spells of shrill crying as possible causes for fibrinoid necrosis and thickening of the BM.

Our measurements of the BMT were confirmed by Berry [23] and Krous et al. [24]. who estimated a mean BM thickness of 1.97 μm ± 0.03 μm (SID), and 2.0 μm ± 0.04 μm (non-SID) respectively which is in accordance with the postulated reference interval of 0.5–2.0 μm.

Possible reasons for the apparent difference of the BMT reported by Shatz et al. [5–8] could be due to the dissection of the larynx not only sagittally, but also horizontally. In the latter dissection technique a tangential section of the BM as well as a higher thickness of the sections could be a cause for BM measurements of 10 μm and more (Fig. 4).

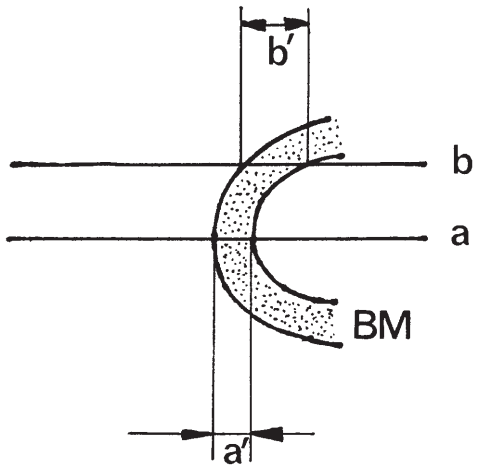

Fig. 4 Influence of the section plane in horizontal sections on the BM thickness. A sagital section of the BM of the vocal cord is shown. Two horizontal sections are performed: a in the midline, b offset from the midline a′ gives the real BM thickness measured in the midline and b′ shows a "thickened" BM caused by a measurement offset from the mideline there the BM is tangential cut

However, there are two apparent discrepancies which are difficult to resolve. In contrast to the findings of this study Shatz et al. [5] did not find BM thickening in the control group despite tangential cutting. The average thickness of BM in the control group was also much lower and was "below the resolution" of light microscopy (0.1–0.2 μm). This value is lower than given in literature for the average thickness of basement membranes (up to 1 μm [25]). Also, it can be understood from the work of Shatz et al. that the basement membranes have not been observed by these authors in the control cases. If this is true, it can only be speculated about the cause for the differences between both control collectives (Shatz et al. versus ours).

A specific thickening of the BM of the vocal cord in SID cases could not be confirmed in the present work. Therefore, BMT is not suitable as a diagnostic postmortem marker for SID.

References

1. Althoff H (1980) Sudden infant death syndrome (SIDS). Fischer, Stuttgart New York
2. Berry PJ (1992) Pathological findings in SIDS. J Clin Pathol 45: 11–16
3. Bajanowski T, Brinkmann B (1995) Pulmonary viral infection in SIDS. In: Rognum TO (ed) Sudden infant death syndrome. New trends in the nineties. Scandinavian University Press, Oslo Copenhagen Stockholm Boston, pp 203- 206
4. Hiller AS, Kracke A, Tschernig T, Kasper M, Kleemann WJ, Tröger HD, Pabst R (1997) Comparison of the immunohistology of mucosa-associated lymphoid tissue in the larynx and lungs in cases of sudden infant death and controls. Int J Legal Med 110: 316–322
5. Shatz A, Hiss J, Arensburg B (1991) Basement-membrane thickening of the vocal cords in sudden infant death syndrome. Laryngoscope 101: 484–486
6. Shatz A, Hiss Y, Hammel I, Arensburg B, Varend S (1994) Age related basement membrane thickening of the vocal cords in sudden infant death syndrome (SIDS). Laryngoscope 104: 865–868
7. Shatz A (1995) The significance of new anatomohistological findings in the upper airways of SIDS victims. In: Rognum TO (ed) Sudden Infant Death Syndrome. New trends in the nineties. Scandinavian University Press, Oslo Copenhagen Stockholm Boston, pp 203–206
8. Shatz A, Hiss J, Arensburg (1997) Myocarditis misdiagnosed as sudden infant death syndrome (SIDS). Med Sci Law 37: 16–18
9. Bajanowki T, Ortmann C, Hernandez M, Freislederer A, Brinkmann B (1997) Reaction patterns in selected lymphatic tissues associated with sudden infant death (SID). Int J Legal Med 110: 63–68
10. Brooks DR, Krous HF, Burton JL, Mc Kinley W (1994) Results of a pilot test of the Global Strategy Workshops's International Standardized Autopsy Protocol (SAP) in Georgia. In: Third SIDS international conference 1994, program and abstracts, Holstad Grafisk, p 155
11. Taylor EM, Emery JL (1990) Categories of preventable unexpected infant deaths. Arch Dis Child 65: 535–539
12. Huber J (1995) The pathologist's contribution to the prevention of cot death, and why it is important to abolish the concept of SIDS as a nosological entity. In: Rognum TO (ed) Sudden Infant Death Syndrome. New trends in the nineties. Scandinavian University Press, Oslo Copenhagen Stockholm Boston, pp 46–49
13. Wennergren G, Norvenius G, Alm B (1995) What symptoms and signs can we accept within the SIDS diagnosis ? A clinical view of the new use of the SIDS definition. In: Rognum TO (ed) Sudden infant death syndrome. New trends in the nineties. Scandinavian University Press, Oslo Copenhagen Stockholm Boston, pp 26–28
14. Hohmann M (1963) Über die Topographie und das Vorkommen von Fettgewebe im menschlichen Taschenband in Abhängigkeit von Alter, Gewicht und Krankheit. Anat Anz 112: 409–425
15. Maxeiner H (1986) Rechtsmedizinisch bedeutsame Kehlkopfbefunde (Prospektive autoptische Studie). Habilitationsschrift, Freie Universität Berlin
16. Kelly DE (1984) The epithelium. In: Kelly DE, Wood RL, Enders AC (eds) Bailey's textbook of microscopic anatomy, 18th end. Williams and Wilkins, Baltimore, p 138
17. Kuhn (III.) C (1995) Normal anatomy and histology. In: Thurlbeck WM, Churg AM (eds) Pathology of the lung, 2nd edn. Thieme, Stuttgart New York, pp 1–36
18. Williams PL, Warwick R et al. (1989) (eds) Gray's anatomy, 37th edn. Churchill Livingstone, Edinburgh
19. Merker H-J (1994) Morphology of the basement membrane. Microsc Res Tech 28:95–124
20. Leblond CP and Inoue S (1989) Structure, composition and assembly of basement membrane. Am J Anat 185:367–390
21. Martinez-Hernandez A and Amenta PS (1983) The basement membrane in pathology. Lab Invest 48:656–677
22. Rieße M, Weiler G, Glanz H, Dreyer T (1997) Histomorphologic studies of the glottic region to elucidate the pathogenesis and functional significance of fibrinoid vocal cord lessions in infants and young children. Int J Pediatr Otorhinolaryngol 42: 125–134
23. Berry J (1998) Cot death: Pathology after back-to-sleep. 5th SIDS International Conference, Rouen, France, 20.-23.04.1998, oral presentation
24. Krous HF, Valdes-Dapena M, McClatchey K, Herman SM, Filkins JA, Hoffman HJ, Hauck FR (1998) Laryngeal basement membrane thickening is not a reliable postmortem marker for SIDS: Results from the Chicago Infant Mortality Study. 5th SIDS International Conference, Rouen, France, 20.- 23.04.1998, oral presentation
25. Junqueira LC, J Carneiro (1996) Histologie. Zytologie, Histologie und mikroskopische Anatomie des Menschen unter Berücksichtigung der Histophysiologie. Translated by Schiepler TH, 4th edn. Springer, Berlin Heidelberg New York