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ORIGINAL PAPER

Shaken baby syndrome: re-examination of diffuse axonal injury as cause of death

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Abstract The discussion surrounding shaken baby syndrome (SBS) arose from the lack of evidence implicating diffuse axonal injury (DAI) as a cause of death. It was assumed instead that injury to the cervical cord, medulla, and nerve roots played a causal role. The present pathomorphological study examines 18 selected infants (<1-year-old) whose deaths were highly suspicious for SBS, exhibiting the classical SBS triad of acute subdural hemorrhage (SDH), retinal bleeding, and encephalopathy. Gross autopsy and microscopic findings of these infants were compared with those of 19 victims of sudden infant death syndrome (SIDS; control group 1) and of 14 infants who died of disease or injuries/violence not involving the head, neck or eyes (control group 2). Symptoms of mechanical impact to the head were evident in seven of the SBS infants, but in none of the control infants. DAI was not detected in either the SBS or control cases. Localized axonal injury (AI) was regularly present in the brains of the SBS infants surviving longer than 1.5–3.0 h, but only occasionally in the craniocervical junction and within the nerve roots of the upper cervical cord; it was never present in the medulla. Epidural hemorrhage of the cervical cord was seen in four of the ten examined SBS cases, but in none of the control cases. Based on the absence of DAI in the brain and of signs of generalized cervical cord or nerve root injuries, we conclude that the cause of death in the SBS victims was a global cerebral ischemia secondary to SDH, focal vasospasm, trauma-induced transitory respiratory and/or circulatory failure.

Keywords Shaken baby syndrome · Subdural hemorrhage · Encephalopathy · Retinal bleeding · Diffuse axonal injury · Cervical cord · Global brain hypoxia/ischemia

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Introduction

Subdural hemorrhages (SDH) associated with retinal bleeding and encephalopathy in infants with little external evidence of mechanical impact and an inadequate and/or inconsistent history given by the parents or other caregivers are considered pathognomonic for shaken baby syndrome (SBS) [3]. This association is generally but not universally accepted. The event producing the forces sufficient to induce these injuries is seldom witnessed by a non-interested third party and cannot be reproduced experimentally. It is generally accepted, though, that the shaking episode causing the head to flop back and forth will generate rotational, shear, and oscillatory loadings within the infant's intracranial space and eyes. The term for this type of injury is "infantile whiplash shaking syndrome" [8, 28].

The general agreement regarding this explanation of SDH was challenged in the mid-1980s by the studies of Duhaime et al. [9, 43]. Geddes et al. [7, 17, 18] examined unselected cases of non-accidental head injuries involving

mixed types of trauma and symptoms in the context of abuse of infants and children. They highlighted the lack of real evidence [20] of a causal link between shaking and intracranial hemorrhages, retinal bleeding, and encephalopathy. They suggested that a primary spontaneous disease or non-inflicted hypoxic brain damage rather than intentional forces may account for SDH [19], a hypothesis that has since been refuted by Dr. Geddes [12]. The same authors also discussed injuries of the cervical spinal cord and of the nerve roots as possible causes of the encephalopathy, without, however, providing any real evidence. They finally declared the cause of death from shaking to be hypoxic–ischemic injury, since their case material revealed no indications of diffuse axonal injury (DAI). Their observation of a lack of DAI in SBS, however, contrasts sharply with the findings of the teams of Shannon [49] and Gleckman [22], who described DAI as the cause of death in nearly all of their SBS victims.

In the present study, we systematically compared the morphological findings in 18 selected victims of SBS (age < 1 year) characterized by the triad SDH, retinal bleeding, and encephalopathy with those in 33 control cases in order to discern the causes of the injury and death. Though the authors are aware of the wide spectrum of possible differential diagnoses for the individual components of the triad (SDH, retinal bleeding, encephalopathy), they agree with the now extensive scientific literature [8, 13, 24] indicating that the combination of these symptoms in a single infant is nearly pathognomonic for SBS, especially in cases with additional symptoms and/or a corroborating history.

The possibility of an additional impact besides shaking as the primary traumatic event has led some authors to avoid the term "shaken" in favor of "non-accidental head injury" (NAHI). We use the term "shaken" in the present study because "shaking" was the main traumatic event in all 18 cases of SBS that we studied.

Materials and methods

SBS cases: inclusion criteria

Eighteen selected cases of SBS were studied [12 males, 6 females; mean age 107.5 days (range: 37–235 days; three infants aged <50 days, seven aged 50–100 days, eight aged 100–360 days); mean survival time 375.3 h (range: 0–120 days; five infants survived <3 h, six survived 3–48 h, seven survived >48 h)] (Table 1). The following selection criteria were applied:

- Postmortem findings of the triad, SDH, retinal bleeding, and severe brain swelling;
- Age < 1 year;
- Sudden, unexpected neurologic disturbances (seizures, unconsciousness, etc.) prior to death, with or without transitory cardiac or respiratory arrest;
- Clinical suspicion of SBS in cases of delayed death ($n = 13$).

As far as possible, infants exhibiting the following phenomena were excluded:

- Neonatal childbirth-related SDH by clinical examination (not by MRI);
- SDH secondary to infection or primary or secondary coagulation disorders;
- Old or fresh intracerebral or extracerebral injuries of known accidental origin;
- Severe extracranial injuries caused by abuse;
- Preexisting seizures and other severe diseases;
- Toxic influences.

The diagnosis of SBS was based on the presence of the otherwise unexplained triad encephalopathy, SDH, and retinal bleeding supported by information from police protocols (details of crime scene, social circumstances, statements of witnesses, including eyewitness of the traumatic event, and the attitude of the perpetrator, especially vis a vis the victim), medical records (unexplained injuries such as bruises or soft tissue hemorrhages with relevant localization [47], exclusion of a history of unintentional violence), and by morphological and clinical data (to exclude diseases, coagulation disorders, or toxic influences). In 11 cases, an isolated shaking (without impact) was established by statements of the perpetrator and by autopsy. In 14 cases, the perpetrator was convicted in court of manslaughter; in four cases, the perpetrator could not be identified.

All legal autopsies were performed in Lübeck, Göttingen, or Heidelberg, Germany, followed by a legal inquiry and, in 15 cases, by a trial. In all cases, a forensic neuropathological examination of brain, eyes, and the upper cervical cord was carried out. Each case was examined regarding the character and extent of all components of the injury by two forensic pathologists, who also evaluated the medical history and results of criminal investigations.

Control cases

The 33 control cases were divided into two groups comprised of infants in whom mechanical violence to the head, neck or eyes was excluded. Group 1 (Table 2) consisted of 19 victims of sudden infant death syndrome (SIDS) diagnosed according to the definition of Krous et al. [30] [12 males, 7 females; mean age 102.8 days (range: 28–183 days; three infants aged <50 days, six aged 50–100 days, ten aged 100–360 days)]. Group 2 (Table 3)

Table 1 Personal data and postmortem findings in 18 victims of shaken baby syndrome

Case no.	Personal data				Postmortem findings									
	Sex	Age (days)	ST (h)	Traumatic event	Scalp haemo	Neck haemo	Thoracal bruises	Skull fracture	SDH	SAH	Brain mass (g)	Edema	Eyes examined	Cervical spine examined
1	m	37	2,160	Shak + impa	+	–	+	–	+	+	570	+	Clin	+
2	m	64	18	Shak + impa	+	–	–	–	+	+	650	+	Clin	n.e.
3	m	123	264	Shaking	–	–	–	–	+	+	710	+	Clin	n.e.
4	f	87	0	Shaking	–	+	–	–	+	+	805	+	Histol	n.e.
5	m	39	0	Shaking	–	+	–	–	+	–	570	+	Histol	n.e.
6	m	229	1,167	Shaking	+	+	–	–	+	–	600	+	Clin	n.e.
7	m	111	>34	Shaking	–	–	+	–	+	+	790	+	Histol	n.e.
8	m	208	>29	Shak + impa	+	–	–	–	+	–	950	+	Clin	+
9	m	74	0	Shaking	–	–	–	–	+	–	580	+	Histol	+ (EDH)
10	f	94	50	Shak + impa	+	–	–	+	+	+	800	+	Histol	+ (EDH)
11	f	95	4	Shaking	–	–	+	–	+	+	730	+	Histol	+ (EDH)
12	m	52	48	Shaking	–	–	–	–	+	–	610	+	Clin	+
13	f	60	0	Shaking	–	–	–	–	+	+	580	+	Histol	+
14	f	39	79	Shak + impa	+	–	+	+	+	+	490	+	Histol	+
15	m	108	20	Shaking	–	+	–	–	+	+	690	+	Histol	+
16	m	235	>3	Shaking	–	–	–	–	+	+	930	+	Histol	+ (EDH)
17	f	103	0	Shak + impa	+	–	–	+	+	+	570	+	Histol	+
18	m	177	2,880	Shak + strang	–	+	+	–	+	+	920	+	Clin	n.e.

m male, *f* female, *ST* survival time, *Shak + impa* shaking and impact, *Shak + strang* shaking and strangulation, *hemo* hemosiderin-containing macrophages, *SDH* subdural hemorrhage, *SAH* subarachnoidal hemorrhage, *EDH* epidural hemorrhage, *Clin* clinical examination, *Histol* histological examination, *n.e.* not examined, + positive findings, – no findings

comprised of 14 victims who died of natural diseases or of intentional or unintentional violence not involving the head, neck or eyes [nine males, five females; mean age 154.3 days (range: 27–357 days; three infants aged <50 days, one aged 50–100 days, ten aged 100–360 days)]. The causes of death in group 2 were acute asphyxia, drowning or bolus death ($n = 6$), infection or pneumonia ($n = 3$), cardiac failure due to heart and vessel malformation ($n = 3$), exsiccosis or starvation ($n = 2$). The survival times varied widely due to the different primary diseases. Secondary hypoxic/ischemic brain damage could not be excluded. Infants dying of mechanical brain injury were not included because the association between non-intentional traumatic SDH, retinal bleeding, encephalopathy, and DAI has been extensively evaluated by other authors [8, 13, 24, 27]. It is known that only a very small percentage of cases will be characterized by the simultaneous presence of all these symptoms especially of SDH in combination with retinal bleeding.

Tissue sampling and preparation

Brains including the upper cervical spine and dura were removed from the infants at autopsy and fixed in 4% formalin for 2–4 weeks. In most SBS cases ($n = 11/18$), and in some control cases ($n = 9/33$), both eyes and the cervical spine including cervical cord were removed and also fixed in formalin for 2–4 weeks. In SBS victims, the eyes were removed only in those cases with no prior ophthalmologic proof of retinal petechiae by clinicians, allowing documentation of retinal bleeding in all SBS victims. Removal and pathomorphological examination of the cervical spine and of the eyes in the control cases was at the discretion of the forensic pathologist performing the autopsy.

The macroscopic findings were documented by protocol, including photography in some cases. Blocks of dura mater from the hemorrhagic area, eyes, ten different brain areas, especially the frontal cortex including the white matter, corpus callosum, pons, medulla oblongata, caudatum, pallidum, putamen, thalamus, cerebellum, the upper cervical cord at the level of the craniocervical junction, and various cervical cord segments in selected cases, were dehydrated and embedded in paraffin. Sections (5 μm) were routinely stained with hematoxylin and eosin (H&E) and other staining techniques (see below) for histological evaluation.

The removed cervical spine was prepared as described by Oehmichen et al. [40] for detecting bony fractures,

Table 2 Control group 1: personal data and postmortem findings in 19 victims of sudden infant death syndrome (SIDS)

Case no.	Personal data			Postmortem findings			
	Sex	Age (days)	Diagnosis	Brain mass (g)	Edema	Eyes examined	Cervical spine examined
1	m	136	SIDS	640	+	+	+
2	m	101	SIDS	600	+	n.e.	n.e.
3	m	65	SIDS	610	+	n.e.	n.e.
4	f	28	SIDS	440	+	n.e.	n.e.
5	f	104	SIDS	670	+	n.e.	n.e.
6	m	138	SIDS	660	+/−	n.e.	n.e.
7	f	102	SIDS	560	+	n.e.	n.e.
8	f	72	SIDS	500	−	n.e.	n.e.
9	f	167	SIDS	630	+	n.e.	n.e.
10	f	39	SIDS	480	+	n.e.	n.e.
11	m	176	SIDS	850	+	n.e.	n.e.
12	m	76	SIDS	650	+	n.e.	n.e.
13	m	29	SIDS	630	+	+	+
14	m	54	SIDS	590	+	+	+
15	m	50	SIDS	570	+	n.e.	n.e.
16	m	183	SIDS	620	+	+	n.e.
17	m	183	SIDS	910	+	n.e.	n.e.
18	f	164	SIDS	600	+	n.e.	n.e.
19	m	87	SIDS	670	+	+	+

m male, *f* female, *n.e.* not examined, + positive findings, − no findings

Table 3 Control group 2: personal data and postmortem findings in 14 victims of natural diseases and non-intentional or intentional violence not involving the head, neck or eyes

Case no.	Personal data			Postmortem findings				
	Sex	Age (days)	Traumatic event	Brain mass (g)	Edema	Eyes examined	Cervical spine examined	Remarks
1	m	27	Asphyxia	540	+	n.e.	n.e.	
2	f	104	Pneumonia	565	−	n.e.	n.e.	
3	f	209	Tumor of the heart	820	+	n.e.	n.e.	
4	f	105	Starvation, exsiccosis	540	−	n.e.	n.e.	
5	m	206	Infection	900	+	n.e.	n.e.	
6	m	30	Asphyxia	550	+	+	+	
7	m	151	Asphyxia	750	−	+	+	Atrophic brain
8	m	73	Isthmus stenosis	580	+	+	+	
9	m	114	Isthmus stenosis	680	+	n.e.	+	
10	f	338	Infection	890	+	n.e.	+	
11	f	128	Bolus	570	+	+	n.e.	
12	m	357	Starvation, exsiccosis	800	+	n.e.	n.e.	
13	m	285	Drowning	1,100	+	n.e.	n.e.	
14	m	33	Asphyxia	460	+	n.e.	n.e.	

m male, *f* female, *n.e.* not examined, + positive findings, − no findings

ruptures of disks or ligaments, etc. The spinal cord and spinal dura were excised for evaluation of dural hemorrhages. The isolated spinal cord was cut into multiple cross sections and the cut surfaces were macroscopically examined before being prepared for histological evaluation.

Staining techniques and immunohistochemistry

After deparaffinization and rehydration, tissue sections from all specimens were stained with H&E, and subsequently stained with Prussian blue for demonstration of hemosiderin, and with naphthol AS-D chloroacetate esterase for polymorphonuclear leukocytes [33].

Immunohistochemistry was performed according to standard methods [31] and the following primary antibodies were applied:

CD68: Monoclonal mouse antihuman antibody, clone KP-MI, code no. M 0876 (Dako Co., Carpinteria, CA), dilution 1:750

GFAP (glial fibrillary acid protein): Monoclonal mouse antihuman antibody, clone 6F2, code no. M 0761 (Dako Co.), dilution 1:100

β-APP (β-amyloid precursor protein): Monoclonal mouse antihuman antibody, clone 22c11, code no. MAB348 (Chemicon Int., Temecula/CA), dilution 1:750.

Results

SBS victims

Macroscopic examination (Table 1)

Unilateral ($n = 6$) or bilateral ($n = 11$) SDH (Fig. 1) were detected in all 18 SBS infants in accordance with the definition of this group. The SDH were predominantly small and not space-occupying. A quantitative evaluation was not possible as the fresh clots were too liquid. Associated subarachnoid hemorrhages (SAH) were found in 13 (72%) cases. All infants exhibited bilateral retinal bleeding (Fig. 2) in the form of different-sized bleeding spots in the sclera, choroid, retina or vitreous, as documented by clinicians or at autopsy (Fig. 2a, b). Retinal detachment was detected in two cases by fundoscopy and in two cases at autopsy. Diagnosis of brain edema was based on the brain weight, a tightened dura mater, flattened gyri, and compressed ventricles, which were present in all 18 cases. An indication of herniation of the cerebellar tonsils was found in one case (Fig. 3a). No case exhibited cortical hemorrhages.

Bruises of the scalp were detected in seven of the 18 SBS cases (39%), and skull fractures in three cases (17%). Hemorrhages within different neck muscles and muscle insertions—especially the sternomastoid muscles—were seen in 5/18 cases (28%). Inspection of the cervical spine in 11/18 cases disclosed EDH in four cases (36%). Thoracic bruising was documented in 5/18 cases (28%) with no evidence of additional injuries although old rib fractures in one case indicated prior abuse. All SBS infants appeared anemic.

Histological examination (Table 4)

Dura mater: Five of the 12 cases with a survival time <48 h showed extravasal red blood cells without any cell reaction, while two cases were marked by a granulocytic reaction and a further four by a combined granulocyte–macrophage reaction (Fig. 1d). Erythrocyte- (Fig. 1e) and hemosiderin-containing macrophages (Fig. 1f) were seen within the bleeding zone in two of all 18 cases (55%) and in five of the six cases (83%) with a survival time >48 h.

Eyes: Histological examination of the eyes in 11 autopsy cases revealed bilateral superficial (Fig. 2b), intraretinal, or subretinal bleeding. Perineural hemorrhage of the optic nerve (Fig. 2e) was detected in five of nine cases, axonal injury (AI) within the optic nerve in one case, hemosiderin-containing macrophages (Fig. 2c, d) in three infants, only one of whom survived >48 h.

Brain tissue: With the sole exception of brain edema, no additional histological alterations could be detected in seven of the 18 SBS cases (39%), while isolated focal macrophage aggregations (Fig. 3b, c) were seen in five cases (28%). A combined reaction by macrophages and hemosiderin-containing macrophages was demonstrable within the subarachnoid space (SAS) in 4/13 cases (30%), combined focal reactions by macrophages, astrocytes (Fig. 4c–e) and occasionally by hemosiderin-containing macrophages (Fig. 3d) in another 4/18 cases (22%). In 11 (61%) cases of delayed death (survival time >10 h), the following hypoxic–ischemic features were demonstrated in the white matter and/or white matter atrophy and porencephaly (Fig. 5a–f): edema (Fig. 3a), diffuse microglial reaction (Fig. 3c), perineuronal microglia nodes (Fig. 3b), a local aggregation of hemosiderin-containing macrophages (Fig. 3d), ischemic nerve cell injury, with occasional neuronal loss (Fig. 4a, b), micoglial (Fig. 4c) and astroglial aggregations (Fig. 4d, e).

Axonal injury: β-APP-reactivity has been shown to begin no earlier than 1.5–3 h after a traumatic event [38]; 13 of our SBS cases obviously survived longer than 3 h and, therefore, could exhibit AI. AI was demonstrated in the pons alone in 2/13 (16%) cases (Fig. 4f), in both the pons and corpus callosum (Fig. 5f) in 5/13 (38%), in the craniocervical junction in 2/13 (16%) cases, and within the radices at the level of the craniocervical junction in one case (8%)(Fig. 4g). No AI at all was detected in the medulla oblongata in our SBS victims. DAI could not be demonstrated in any of our SBS cases, neither in the brain, nor in the cervical cord or nerve roots.

Cervical spine and spinal cord: Macroscopic evaluation established an EDH (Fig. 6a, b) localized in the lower cervical spine at the transition to the thoracal spine in 4/11 cases. Microscopy revealed a slight macrophage reaction confined to the SAS in two cases, but no cell reaction in the

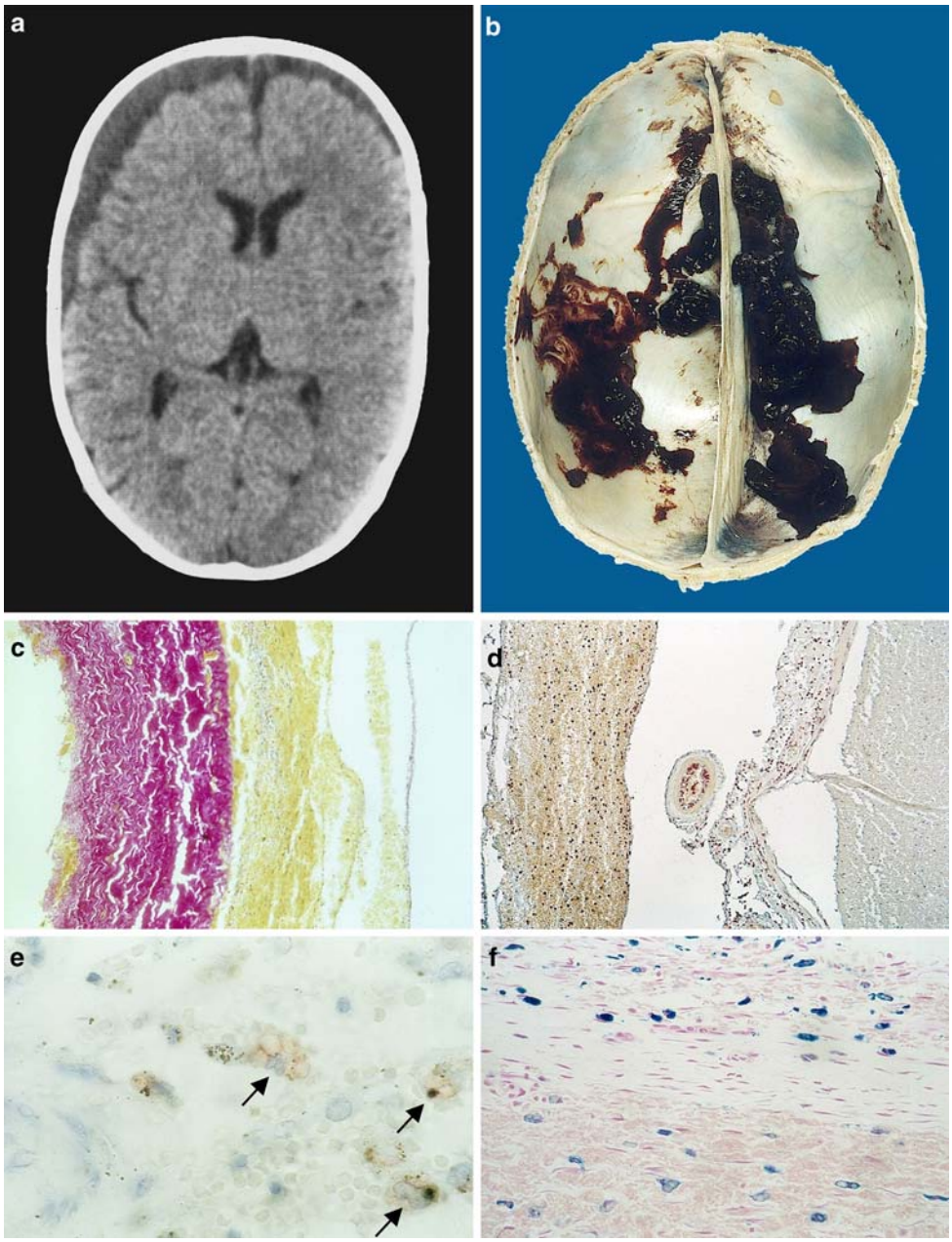

Fig. 1 Subdural hemorrhage in victims of shaken baby syndrome (SBS): **a** magnetic resonance tomography, **b** autopsy, **c**–**f** histology. **c** Hemorrhage—survival time 18 h (trichrome stain, ×50); **d** granulocytic-macrophage reaction—survival time 18 h (naphthol AS-D chloroacetate esterase, ×100); **e** macrophages and erythrocyte-containing macrophages indicated by *arrows*—survival time 4 h (CD68, ×1,000); **f** hemosiderin-containing macrophages—survival time 4 h (Prussian blue reaction, ×500). **e**, **f** Indicate a second, older hemorrhage

dura spinalis or spinal parenchyma. No indications of AI were detected below the craniocervical junction.

Control cases

SIDS group (group 1)

As per definition, no cause of the acute death could be found in the 19 SIDS infants. In all cases, a slight brain edema was documented, but no EDH or SDH of the brain. The histology of the brain produced no evidence of any pathological event or process. No AI was detected in either the brain or craniocervical junction. The four cases in which the cervical spine and the spinal cord were examined displayed no pathological features. The five pairs of eyes examined revealed no evidence of pathological changes.

Disease/violence group (group 2)

In 11 of the 14 cases in this group, the brains were marked by edema of varying extent. Histological evaluation of the brain parenchyma established partly disseminated, partly localized aggregations of microglia and/or astrocytes plus neuronal loss in two cases, and single β-APP reactive axons in the corpus callosum in one case, each due to prolonged, diminished brain perfusion secondary to cardiac or vessel malformation. None of the cases exhibited any hemosiderin-containing macrophages in the dura mater, brain

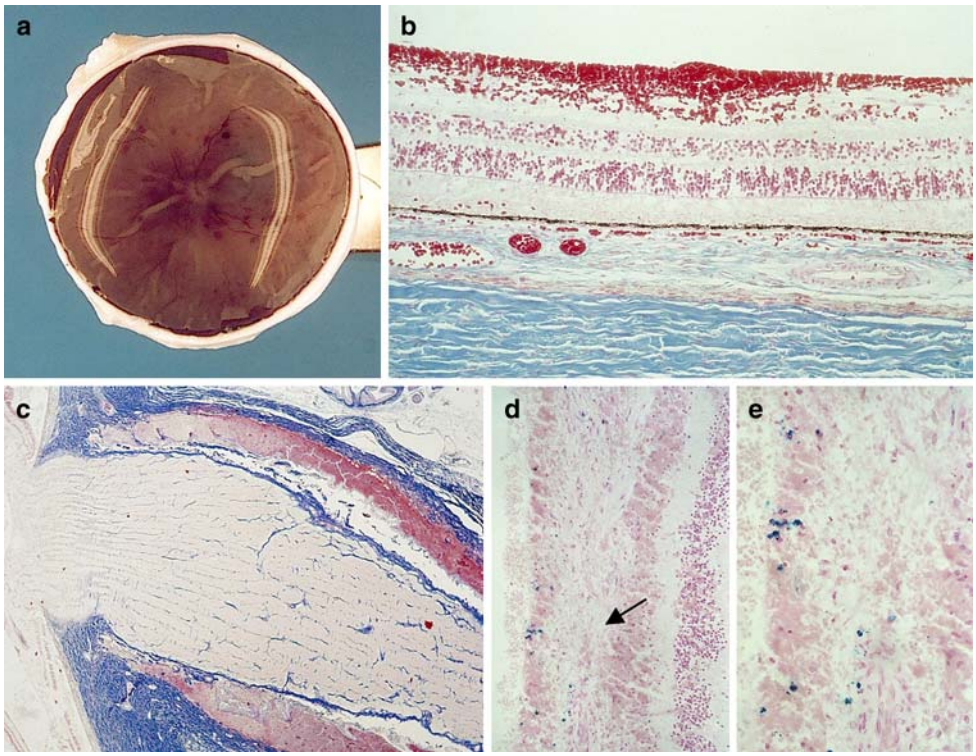

Fig. 2 Retinal bleeding in SBS victims: **a** macroscopic view of the eye ground; **b** retinal hemorrhages—survival time >3 h (trichrome stain, ×100); **c** perineural hemorrhage of the optic nerve—survival time >3 h (trichrome stain, ×50); **d**, **e** hemosiderin-containing macrophages in the retina—survival time 0 h (Prussian blue reaction, **d** ×100, **e** ×500)

parenchyma, or leptomeninges. Examination of four pairs of eyes disclosed no signs of a fresh or old hemorrhage. Examination of the cervical spine in five cases revealed neither macroscopic nor microscopic pathological changes.

Discussion

The spectrum of macroscopic and microscopic findings in the intracranial and intraspinal spaces and eyes of our SBS cases was highly diverse compared with the control cases. In accordance with our selection criteria, all of the present SBS victims were characterized by the morphological triad of SDH (sometimes in association with SAH), retinal bleeding, and early severe and diffuse brain swelling. In the control groups, SIDS victims (group 1) were characterized by a slight brain edema, while disease/violence victims (group 2) exhibited various discrete alterations that could be explained by the lethal disease or extracerebral injuries. None of the controls showed retinal or intraspinal alterations.

With few exceptions that can be excluded by autopsy and/or by clinical check up [5], SDH is usually caused by rotational stress secondary to acceleration and deceleration [21]. Ischemic brain damage may result from space-occupying SDH or from transitory cardiac or respiratory failure or arrest. Retinal bleeding and bleeding in the coverings of the optic nerve are explained [35] by the traction of the vitreous on the underlying retina as the vitreous "swirls" within the eye, or by the elevated venous pressure secondary to the elevated intracranial pressure and/or chest compression caused by squeezing of the baby during shaking.

Traumatic alterations of the scalp and/or skull as vestiges of a mechanical impact were absent in most (11/18) of our SBS victims. The evidence of subcutaneous hematomas of the scalp and skull fractures in the remaining seven cases was indicative of an impact in addition to the rotation. Such contact injury occurs when the infant's head impacts on a soft or hard surface during shaking, or if the infant is thrown or dropped after shaking. Moreover, the absence of signs of impact does not exclude impact. As mentioned above, however, contact injuries of the head are rarely associated with retinal bleeding [27].

Although the occurrence of AI cannot be entirely ruled out, it could not be detected by immunohistochemistry. The evidence for AI was not reliable after a certain survival time. We could establish, however, that extensive hypoxia–ischemia and a lack of cerebral perfusion due to brain swelling were present in nearly all of our cases. Since fast axonal transport is a highly energy-dependent process, the presence of severe oxygen depletion could impede the transport and accumulation of β-APP such that it might be difficult to detect by immunohistochemistry. Unfortunately, in SBS cases it is almost impossible to determine at which point hypoxia–ischemia occurred relative to the survival time and thus to assess its impact on axonal transport.

In the SBS cases presented here, the postmortem findings confirming the diagnosis were supported by statements

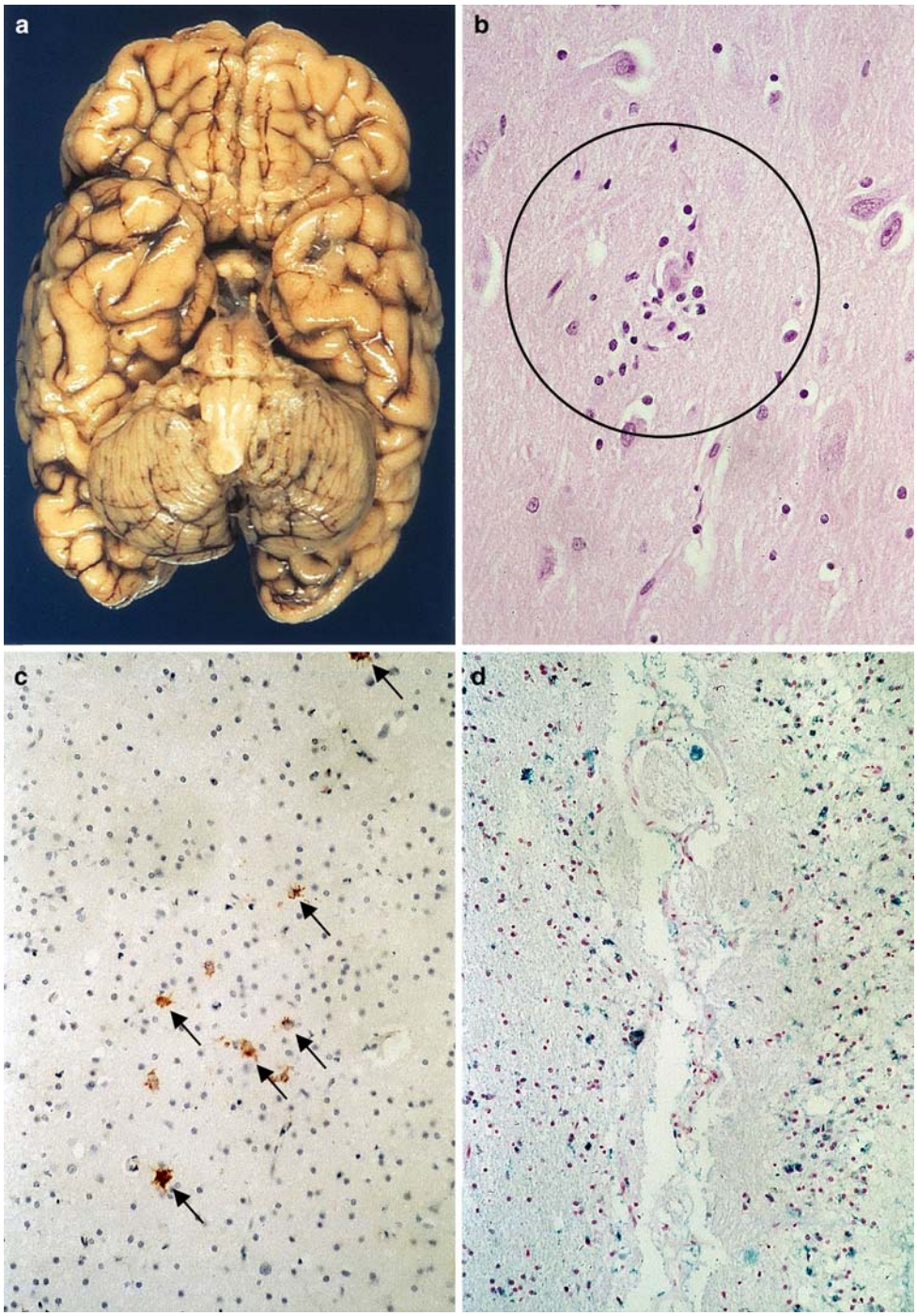

Fig. 3 Encephalopathy in SBS victims: **a** brain edema indicated by a slight herniation of the cerebellar tonsils—survival time >0 h; **b** neuronophagy as an indication of ischemia—survival time 79 h (H&E, ×1,000); **c** combined glial and macrophage reaction—survival time 50 h (CD68, ×300); **d** hemosiderin-containing macrophages in the brain parenchyma—survival time 48 h (Prussian blue reaction, ×300)

regarding the perpetrator's intention, by witness accounts, the psychopathology underlying the perpetrator's attitude, by inappropriate or inconsistent histories regarding the fatal event, and by crime scene investigations. They were also backed by gross autopsy findings of additional symptoms otherwise not explainable, including hemorrhages within tissues of the neck, insertional hemorrhages of the muscles, EDH in the cervical spinal cord, symptoms explained by traction forces generated by shaking, fingertip bruises on the chest and shoulders indicating grasping during shaking, and the presence of non-fatal multiple hematomas scattered over the surface of the whole body suggestive of additional abuse.

The absence of any cytological reaction in the dura mater (SDH and EDH), SAS and the injured brain parenchyma in five cases was interpreted as being symptomatic of acute cardiac arrest coinciding with the traumatic event. The presence of macrophages, by contrast, was associated with delayed death occurring 12–20 h after the traumatic event ($n = 10$), the presence of hemosiderin-laden macro-

Table 4 Histological findings in 18 victims of shaken baby syndrome

Case no.	Dura mater			Brain					β-APP		Cervical cord				Eyes	
	NAS	CD68	Hemosiderin	NAS	CD68	Hemosiderin	GFAP	Ischemia	Pons	Corpus callosum	CD68		β-APP		RBC	Hemosiderin
											Ccj	Cco	Ccj	Cco		
1	–	+	+	–	+	+	+	+	+	+	–	–	–	–	n.e.	n.e.
2	–	+	+	–	–	–	–	–	–	–	–	n.e.	–	n.e.	n.e.	n.e.
3	+	+	+	+	+	+	+	–	+	+	–	n.e.	–	n.e.	n.e.	n.e.
4	–	–	+	–	+	+	+	–	–	–	–	n.e.	–	n.e.	+	–
5	–	–	–	–	–	–	–	–	–	–	–	n.e.	–	n.e.	+	–
6	–	+	+	–	+	–	+	+	+	–	–	n.e.	–	n.e.	n.e.	n.e.
7	–	+	–	–	–	–	–	–	+	+	–	n.e.	–	n.e.	+	–
8	+	+	+	–	+	–	+	–	–	–	–	–	+	–	n.e.	n.e.
9	–	–	–	+	–	–	–	–	–	–	–	–	–	–	+	–
10	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	–
11	+	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–
12	–	+	+	–	+	+	+	+	–	–	–	–	–	–		n.e.
13	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
14	+	+	+	+	+	–	+	+	+	–	–	–	–	–	+	+
15	–	–	–	–	+	–	+	+	+	–	–	–	+	–	+	+
16	+	–	–	–	–	–	–	–	+	+	–	–	–	–	+	–
17	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+
18	–	+	+	–	+	+	+	+	–	–	–	n.e.	–	n.e.	+	–

NAS naphthol AS-D chloroacetate esterase as a leukocyte marker, *CD68* a macrophage marker, *Hemosiderin* hemosiderin containing macrophages, *GFAP* glial fibrillary acid protein, an astrocytic marker, β-APP β-amyloid precursor protein, a marker of axonal injury, *Ccj* craniocervical junction, *Cco* cervical cord, *RBC* red blood cells, *n.e.* not examined, + positive findings, – no findings

phages with delayed death occurring 72–100 h after onset of hemorrhages ($n = 10$) [40]. The demonstration of hemosiderin-laden macrophages in the dura, SAS or brain within an estimated survival time <48 h may be an indication of repeated traumatic incidents, including reiterated shaking [10]; in individual cases, however, a birth trauma could not be excluded. These findings may agree with the observations of others that 30–40% of newly diagnosed SBS victims had previously undiagnosed head injury [1, 15, 28].

AI, as demonstrated by local aggregations of β-APP-reactive axons in white matter, was seen in the corpus callosum and pons in five cases, and in the pons alone in two cases. The AI was due in part to a vasogenic event, and in part to traumatic impact. While vascular AI exhibited a zigzag, wavy, or focal pattern, traumatic AI revealed a diffusely scattered pattern or groups of AI along the axis of the axon [25, 46]. A discrimination was not possible in every case, because the phenomena often appeared to be of combined origin. DAI—characterized by a diffuse distribution of axonal lesions within the white matter of the whole brain—could not be demonstrated in any of our SBS cases. Unlike Shannon et al. [49], we observed no injured axons in the medulla oblongata. In two cases, injured axons were established at the level of the craniocervical junction, in one of these cases additionally within the nerve roots.

Global hypoxic–ischemic brain alterations (eosinophilic neurons, focal microglial, and astrocytic reactions, especially in the hippocampus, but also in the frontal cortex and Purkinje cell layer) were established in 13 of our SBS cases with delayed death, sometimes in association with a reduced number of neurons in victims surviving >24 h. AI and hypoxic–ischemic pathology in victims of disease/violence (control group 2) were detected only in infants who suffered prolonged diminished brain perfusion secondary to cardiac or vessel malformation.

In 4 of the 11 examined cervical spinal cords of SBS victims, a fresh EDH was detected, none with a cell reaction. Histological examination of the SAS of the spinal cords revealed a macrophage reaction in two cases. In further two cases, localized AI was detected at the level of the craniocervical junction.

Histological examination of the eyes revealed retinal bleeding in all SBS cases, in four cases associated with retinal detachment (established by clinical as well as histological evaluation). In 3 of 11 histologically examined cases, hemosiderin-bearing macrophages were found, representing a further sign of repeated traumatic events [37]. As red blood cells regularly disappear within 4–6 weeks after onset of hemorrhages [4, 14, 16], the retinal bleeding in our SBS victims could not have been induced by birth trauma.

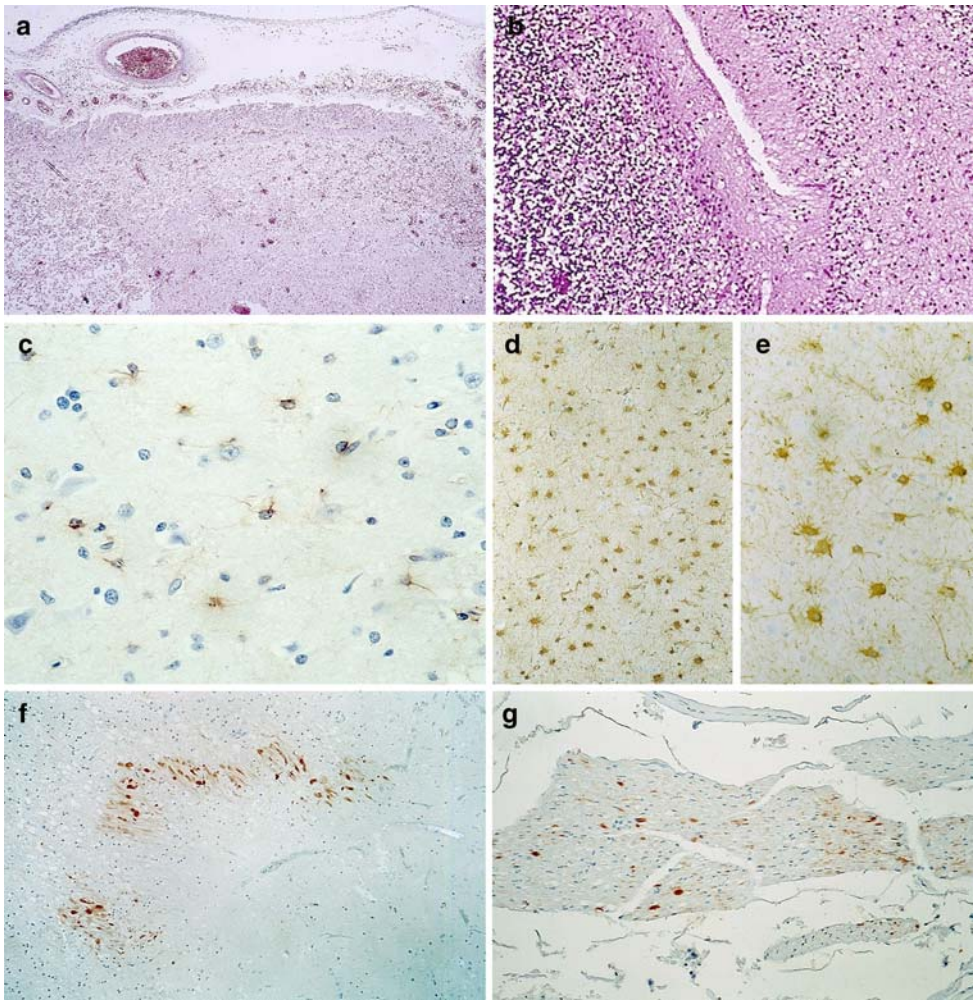

Fig. 4 Hypoxic–ischemic brain alterations in SBS victims: **a** loss of cerebral neurons in the cortex—survival time 90 days (H&E, ×100); **b** Purkinje cell loss in the cerebellar cortex—survival time 90 days (H&E, ×100); **c** microglial activation—survival time >4 h (CD68, ×500); **d**, **e** astrocytic reaction—survival time >0 h (GFAP, **d** ×100, **e** ×500); **f** hypoxia/ischemia induced axonal injury in white matter—survival time 79 h; **g** axonal injury in the radices of peripheral nerves—survival time 79 h (β-APP, ×300)

Geddes et al. [18] could establish DAI in only two cases, while we did in none. While locally aggregated injured axons (AI) could be demonstrated by that team [18] in nearly one-third of their cases, we could establish AI in half of our cases (9/18). We could detect AI in the nerve roots of only one case, namely at the level of the upper cervical cord, a finding that refutes the above mentioned Geddes hypothesis [18]. In contrast, AI resulting from mechanical loading and/or ischemic events, especially to the brain stem and/or cervical cord, was detected in most of their cases by the teams of Shannon [49] and Gleckman [22]. These findings may be attributable to a misinterpretation of diffuse hypoxic AI, as discussed by Geddes et al. [18]. In both studies, these changes were held to be responsible for the encephalopathy and death.

In the literature, EDH of the cervical spinal cord is described only in single cases [17, 34, 39, 40]. It may be underreported due to a lack of systematic morphological analysis. Spontaneous cervical EDH in infants [2, 23] or toddlers [41] is extremely rare. They are highly suspicious for shaking, especially when associated with cerebral SDH, retinal bleeding, and encephalopathy. But even in cases of isolated cervical spine, EDH shaking must be discussed [42]. EDH in our cases (4/11) was localized at the lower cervical spine, that is, at the level of the cervical–thoracal junction.

But what exactly is the cause of death in SBS victims? The space-occupying effect of SDH does not adequately explain death in all cases of SBS, and mechanically induced DAI can be excluded as a cause of death in our cases. Hypoxic–ischemic brain injury as a cause of death is supported by our present findings (comp. Fig. 6), and the findings of Geddes et al. [18]. Hypoxic–ischemic brain injury may be caused by a multifactorial process: slight space-occupying SDH possibly strengthened by accompanying vasospasms resulting from an associated SAH; a primary medullary stress due to whiplashing, which may entail a reflectory cardiac or respiratory disturbance secondary to overextension of the cervical cord [28, 29, 36, 48] associated with secondary reduction or transitory arrest of respiratory or cardiac function. Recent experimental studies have suggested that severe encephalopathy can result from repeated mild head injuries owing to the vulnerability of the immature brain [6, 26, 32, 44]. Another

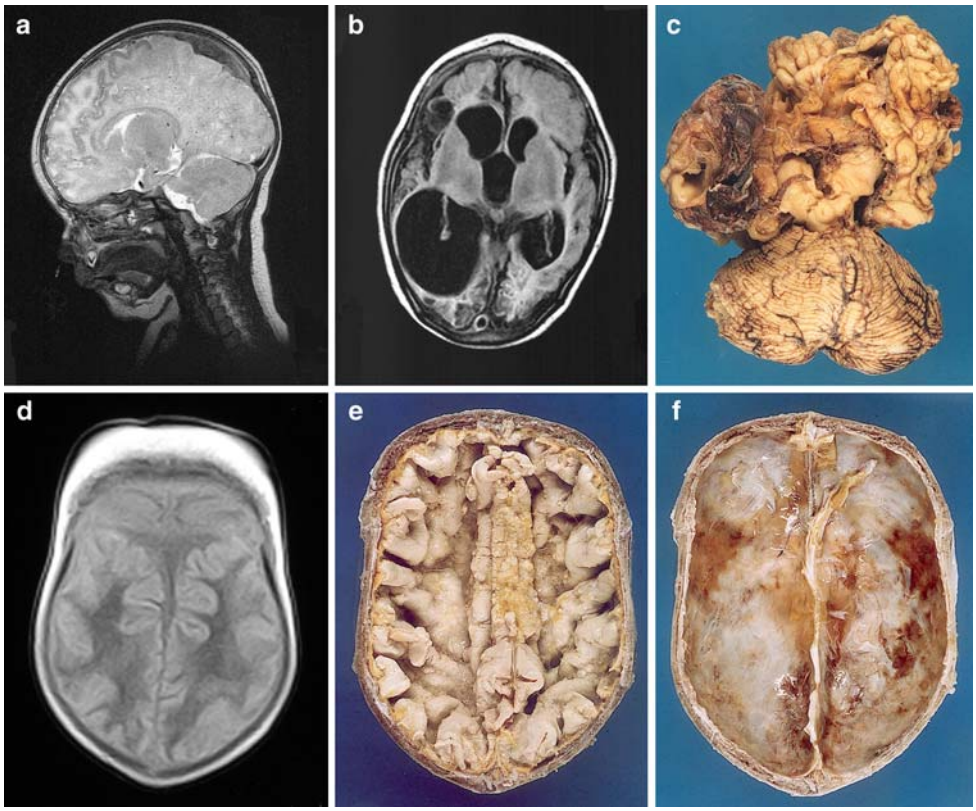

Fig. 5 Delayed cerebral alterations resulting from hypoxia and ischemia in SBS victims: **a**–**c** the same case at different intervals after shaking; **a** acute subdural hemorrhage (SDH) (magnetic resonance imaging, MRI); **b** about 6 months after trauma (MRI), **c** brain, 18 months after trauma (postmortem findings); **d**–**f** the same case as demonstrated by different methods: **d** acute SDH (MRI), **e** brain, 120 days after trauma (postmortem finding), **f** view of the inner surface of the dura mater indicating reabsorbed subdural hemorrhages, 120 days after trauma

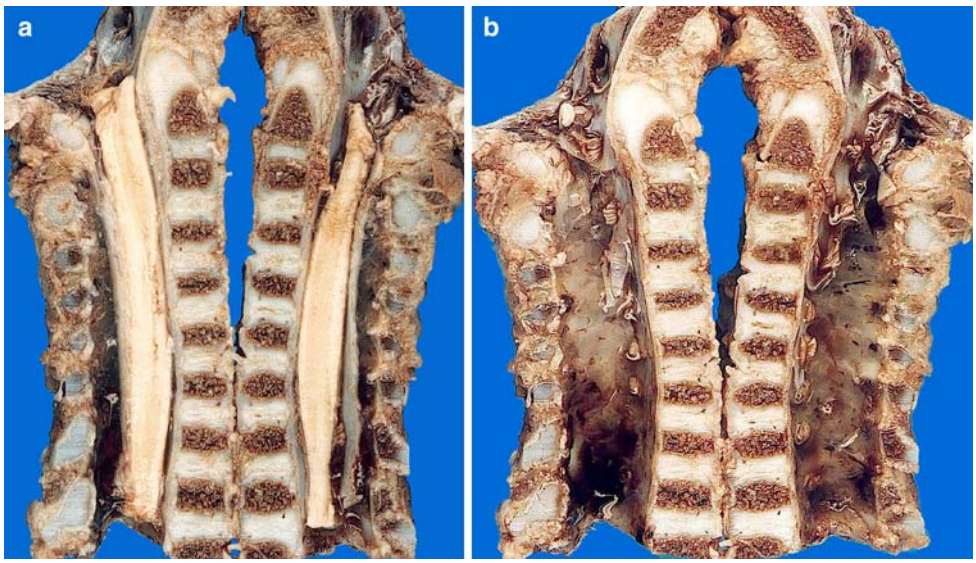

Fig. 6 Acute epidural hemorrhage of the cervical cord in an SBS victim (survival time 0 h) as demonstrated on a section of the cervical spine after formalin fixation: **a** cervical spine with the cord, **b** cervical spine after removal of the cord

experimental study appears to suggest that possible mechanical and neurotoxic effects of subdural blood to the immature brain [11] may play a causal role.

Summarizing the results of our study we can state the following:

1. The diagnosis of SBS must be based on thorough analysis of each case, including the macroscopy of a whole body autopsy, a neuropathological examination including the cervical cord and eyes as well as microscopy of these organs supported by histological demonstration of AI, granulocytes, macrophages, and hemosiderin-laden macrophages.
2. SBS brains are not regularly characterized by DAI, but often by AI (confirming Geddes et al. [25]).
3. AI is not generally observed in the cervical spinal cord, medulla oblongata, or in the concomitant nerve roots.
4. In nearly half of our cases, we detected an epidural hemorrhage of the cervical spine as an additional diagnostic criterion of shaking as a traumatic event.

5. The cause of death was a global brain ischemia induced by a multifactorial process.

The absence of DAI is not a certain indication of a misdiagnosis in cases in which SBS is diagnosed after detailed analysis, including neuropathological examination. The process of death is primarily caused by a shaking trauma that triggers a hypoxic–ischemic process as the final and deadly event.

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