Childs Nerv Syst (2009) 25:925–931
DOI 10.1007/s00381-009-0888-5

ORIGINAL PAPER

Demonstration of fluid channels in human dura and their relationship to age and intradural bleeding

W. Squier · E. Lindberg · J. Mack · S. Darby

Received: 24 March 2009 / Published online: 10 April 2009
© Springer-Verlag 2009

Abstract
Purpose This paper aims to make a systematic study of human dura to establish the presence of fluid transport channels and their relationship to age.
Methods Samples of parasagittal dura from autopsy cases from mid-gestation to the ninth decade were examined by light microscopy.
Results We have demonstrated the presence of unlined rounded spaces, uncommon in the fetus and neonate but increasingly evident after 30 weeks of postnatal life. We have shown that intradural bleeding is inversely correlated with the presence of these channels and with age.
Conclusions We suggest that dural maturation, involving the development of arachnoid granulations, may be related to dilatation of intradural fluid channels, allowing them to be identified histologically. The risk of reflux of blood into the dura appears to reduce with age.

W. Squier (✉)
Department of Neuropathology,
West Wing, John Radcliffe Hospital,
Oxford OX3 9DU, UK
e-mail: waney.squier@clneuro.ox.ac.uk

E. Lindberg
Department of Pathology, Lund University Hospital,
Box 117, 221 00 Lund, Sweden

J. Mack
Department of Radiology, Penn State Hershey Medical Center,
30 Hope Drive, Suite 1800, P.O. Box 859, Hershey,
PA 17033-0859, USA

S. Darby
Clinical Trial Service Unit,
Nuffield Department of Clinical Medicine, University of Oxford,
Oxford, UK

Keywords Dura · CSF transport · Infant · Dural bleeding · Arachnoid granulations

Introduction

Transfer of cerebrospinal fluid (CSF) across the arachnoid membrane and through the dura has been demonstrated as one of the pathways of CSF resorption [1–3]. If it occurred over an extensive area of the arachnoid membrane, vesicular transport of CSF would be capable of contributing considerably to CSF drainage. This pathway might be particularly relevant in the infant prior to maturation of arachnoid granulations (AG).

A system of fluid-containing channels, which could be filled by dye injected into the dura, was described as long ago as 1875 [4]. The absence of an endothelial lining led the early authors to conclude that these were not the same as systemic lymphatics and they were termed "juice channels". In 1950, Balo [5] described a sponge-like appearance of the dura, due to multiple rounded holes without an endothelial lining and most noticeable in the parasagittal dura and around the confluence of sinuses. He noted bleeding into the parasagittal dura and in some cases the sponge was filled with red blood cells. The concept of a "dural sponge" has received scant attention and most pathologists dismiss this appearance as due to artifact. Recently, using resin injections into the superior sagittal sinus (SSS), an extensive network of endothelial lined channels has been described in the parasagittal dura in intimate relationship to the arachnoid granulations [6–8]. These represent the parasagittal venous plexus whose vessels coalesce to form the lateral lacunae of the SSS. Fox considered the parasagittal venous plexus to participate in uptake of CSF which is delivered into the SSS. This

explains the observation that blood clot is not described in the lateral lacunae at autopsy even when the SSS is thrombosed [6, 9]. It is not clear whether this system of channels is continuous with either the unlined juice channels described by Christensen or with the holes described by Balo. Tracers injected into the subarachnoid fluid pass into rounded spaces in the dura and thence into the SSS [10] indicating that the systems are connected.

Arachnoid granulations do not develop until 7–8 months after birth [11, 12]. The concept that they represent a major pathway for bulk resorption of fluid from the subarachnoid space was refuted by Dandy [13] who showed CSF to be resorbed by capillaries and it now seems that AG may resorb CSF only when intracranial pressure is raised, as an adjunct to other routes of CSF absorption [14]. The experimental studies of Welch [15] suggested an alternative role for AG as one-way valves allowing outflow of CSF into the dural sinuses and preventing backflow of blood into the meninges. The intimate relationship of AG to the intradural channels as they enter the lateral lacunae of the SSS suggests they may have a role in controlling flow in these channels.

If the arachnoid granulations are acting as valves, they would, when functioning, regulate forward flow of CSF drainage from the dural channels into the sagittal sinus. Any increase in hydrostatic pressure within the channels would then cause the channels to dilate and appear as rounded spaces between the fibers of the dura. If AG are not present, or are not functioning, the channels would be collapsed and would not be visible by routine microscopy.

In addition to regulating forward flow of CSF in the channels, the AG may function as a valve preventing retrograde flow of blood from the sagittal sinus. Bleeding into the dura is well-recognized in neonates and young infants [16–18]. In the absence of functioning AG, blood in the sinuses could more easily reflux into the valveless dural channels. Therefore, infants without AG would be more prone to reflux of blood from the dural sinuses into the dural channels. Therefore, the AG may have two related functions: regulating forward flow of CSF from the channels into the sinus and preventing retrograde flow of blood from the SSS into the parasagittal channels.

We have made a systematic study of human dura from mid-gestation to the ninth decade of life to describe the presence and distribution of fluid-containing channels and their relationship to age and intradural bleeding.

Materials and methods

Cases in our Departmental Archive between 2005 and 2008 where dura had been sampled as part of the routine diagnostic autopsy examination were reviewed. This study was authorized by the Chairman of the Local Research Ethics Committee as a case series presentation and not requiring specific research ethics approval.

Those cases where samples of dura had been taken coronally to include a part of the superior sagittal sinus were selected for study. The site of origin in the antero-posterior axis was not known. Sections were taken from brains fixed routinely in 10% formalin for a minimum of 7 days (fetal samples) and for 3 weeks (all other samples). In fetal samples, the dura was removed with a thin sagittal strip of the cranium and fixed, sampled, and embedded intact. Samples of dura taken from subjects from late gestational age onwards were separated from the skull at autopsy.

Sections were stained with hemotoxylin and eosin. In selected cases, sections were stained with antibodies against a range of endothelial markers (CD31, CD34, and factor VIII) (DAKOCYTOMATION, Ely, UK).

Sections were independently examined by two pathologists (WS, EL). Where there was disagreement between these observers the samples were re-examined and assigned by consensus to one of the four groups. The presence of intradural "holes", i.e. rounded spaces in the dural tissues, was assessed on an ordered scale 0, +, ++, or +++ and each assessment was assigned a score of 1, 2, 3, or 4, respectively. The samples were then classified according to age in groups of approximately five individuals. The boundaries for these age-groups were chosen to be round numbers and were chosen without reference to the scores. The association between the number of holes and age group was then examined using linear regression and a two-sided significance test for trend was carried out. Fresh bleeding into the dura was scored in a similar way. The association between bleeding and age was also examined using linear regression. The association between the scores for intradural holes and intradural bleeding was also examined directly. Examples of dura showing the assessment 0–+++ are shown in Fig. 1.

Results

A total of 58 samples of dura were examined. The mean score for intradural holes for all 58 samples was 2.5 and the corresponding mean score for intradural bleeds was 2.8 (Table 1). The scores for intradural holes were related to age and the mean score increased, on average, by 0.21 for each increase in age-group. This trend was highly significant statistically (p=0.000006). The scores for intradural bleeding were also significantly related to age (p=0.0005) but, in contrast to the scores for intradural holes, the mean score decreased, on average, by −0.12 for each increase in age-group (Fig. 2). The association between the scores for

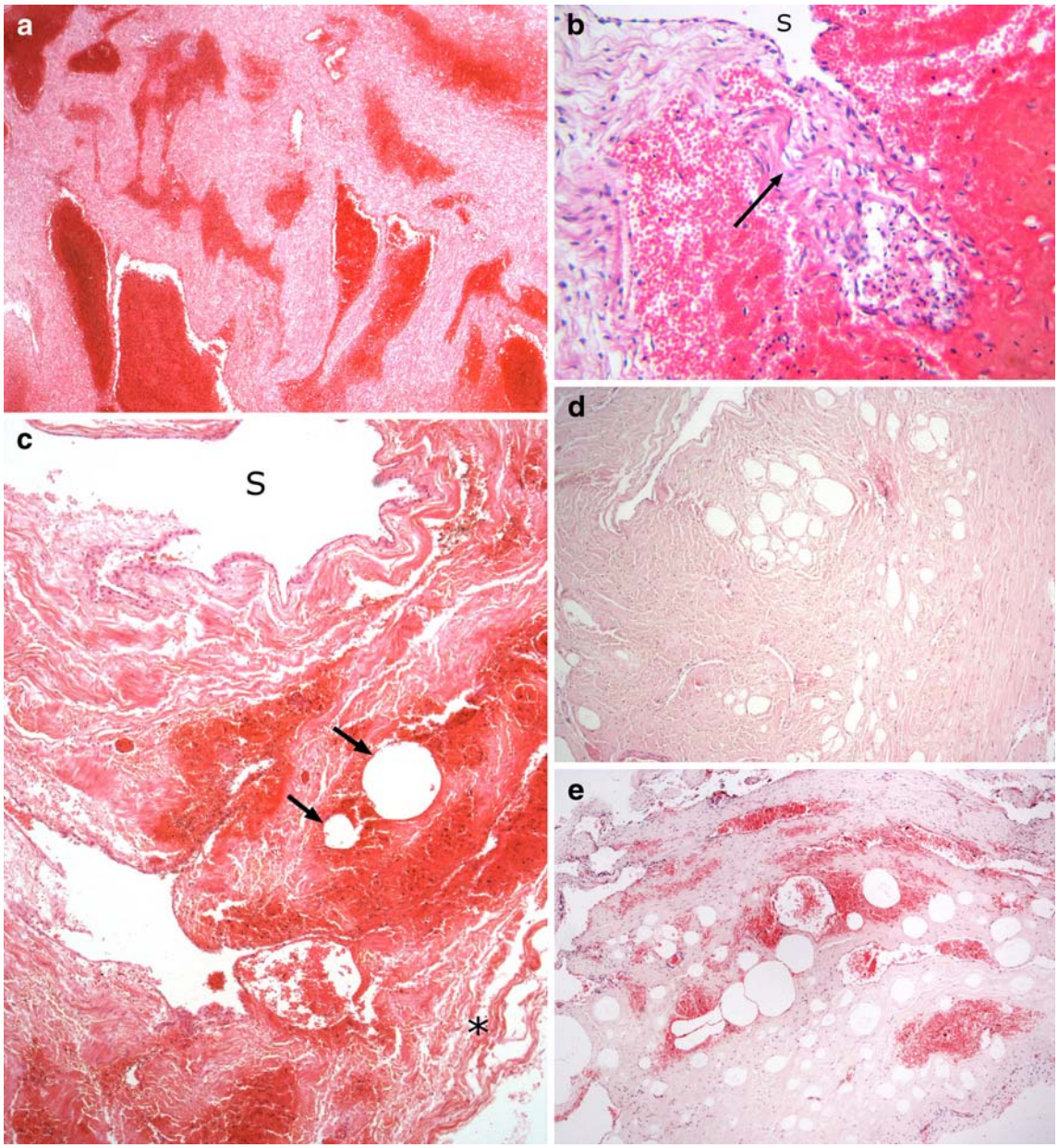

Fig. 1 Semiquantitative assessment of dural holes and bleeding. **a** Holes 0; intradural bleeding ++; fetus 25 weeks. Note patchy bleeding. The intervening dura is edematous with small irregular spaces between the fibroblasts. **b** Holes 0; intradural bleeding +++; fetus 22 weeks. There is extensive bleeding into the dura beneath the endothelium of the SSS (*S*). Note a large nerve fiber close to the endothelium (*arrow*). **c** Holes +; intradural bleeding ++; infant 17 weeks postnatal life. Two holes are marked (*arrows*). There is patchy intradural bleeding. The cells of the subdural compartment are separated by fluid filled clefts (*). These were not counted in this study. *S*=SSS. **d** Holes ++; intradural bleeding 0; 46 years. Clusters of rounded holes in fibrous dura. No bleeding is seen. **e** Holes +++; intradural bleeding +; 5 years. Clusters of rounded holes and a small amount of bleeding

intradural holes and intradural bleeds was not significant statistically ($p=0.09$).

In fetal dura the tissues were often very loose and edematous with irregular spaces between the collagen of the dura (Fig. 1a). Rounded spaces were very rare; only one such space was seen in a fetus of 25 weeks gestation. In older patients the rounded spaces could be very numerous and closely packed, forming a honeycomb appearance (Fig. 3). An endothelial lining could not be identified, even with the use of specific immunocytochemical markers. However, it was common to see spaces clustered around vessels with an endothelial lining and in some cases the lined vessel formed an indentation into round spaces (Fig. 3c, d). In some cases there were also enlarged slit-like spaces immediately beneath the deep dural border (Fig. 1c). These were considered to represent enlargement

Table 1 Scores for the presence of intradural "holes" (i.e. rounded spaces in the dural tissues) and intradural bleeds for 58 samples, categorized by age

	Age group	Number of samples	Presence of holes				Mean score for holes	Presence of bleeds				Mean score for bleeds
			0 (score 1)	+ (score 2)	++ (score 3)	+++ (score 4)		0 (score 1)	+ (score 2)	++ (score 3)	+++ (score 4)	
Before birth	20–30 weeks	6	6	0	0	0	1.0	0	1	3	2	3.2
	30–40 weeks	4	3	1	0	0	1.3	0	1	0	3	3.5
After birth	<1 month	6	4	1	1	0	1.5	0	0	1	5	3.8
	1–3 months	5	1	1	2	1	2.6	0	2	3	0	2.6
	4–6 months	4	1	1	1	1	2.5	0	0	2	2	3.5
	7–11 months	6	0	1	3	2	3.2	0	3	3	0	2.5
	1–2 years	5	1	0	0	4	3.4	0	2	3	0	2.6
	3–14 years	5	0	0	3	2	3.4	0	1	3	1	3.0
	15–49 years	6	1	0	3	2	3.0	2	2	2	0	2.0
	50–59 years	7	1	2	2	2	2.7	2	2	2	1	2.3
	60+years	4	0	1	2	1	3.0	0	1	3	0	2.8
Total number of samples		58					2.5	4	15	25	14	2.8
Average change in mean score for each increase in age group							+0.21					−0.12
p-value for trend in score with increasing age group							0.000006					0.0005

of the normal intercellular spaces of the dural border cell layer and were not counted in this study.

Intradural bleeding was often most marked close to the intradural sinuses, often immediately beneath the endothelium of the superior sagittal sinus (Fig.1b). It was not possible to define the source of intradural bleeding. Sometimes blood was seen leaking into tissues around a vessel with an endothelial lining or around part of a sinus. In some cases small rounded patches of blood were seen in the dura with no evidence of an associated endothelial lined vessel (Fig. 4). While it is tempting to speculate that this bleeding originated in the dural channels, this could not be established with any certainty with the methods used.

Discussion

Identification of dural fluid channels

This study has shown that rounded intradural channels were very infrequent before 6 weeks of postnatal life, but were

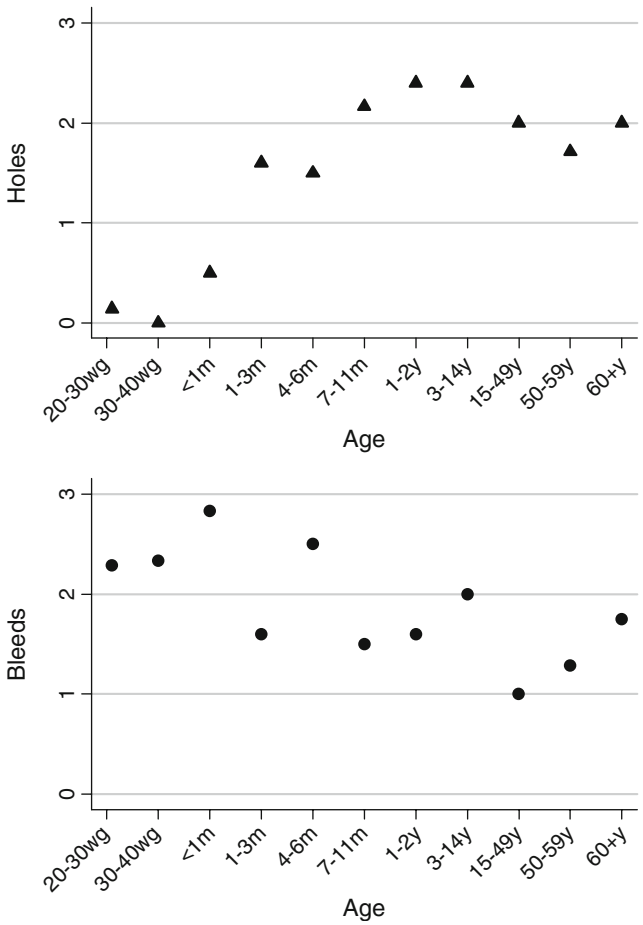

Fig. 2 Mean scores for the presence of intradural "holes" (i.e. rounded spaces in the dural tissues) and intradural bleeds for 58 samples, according to age

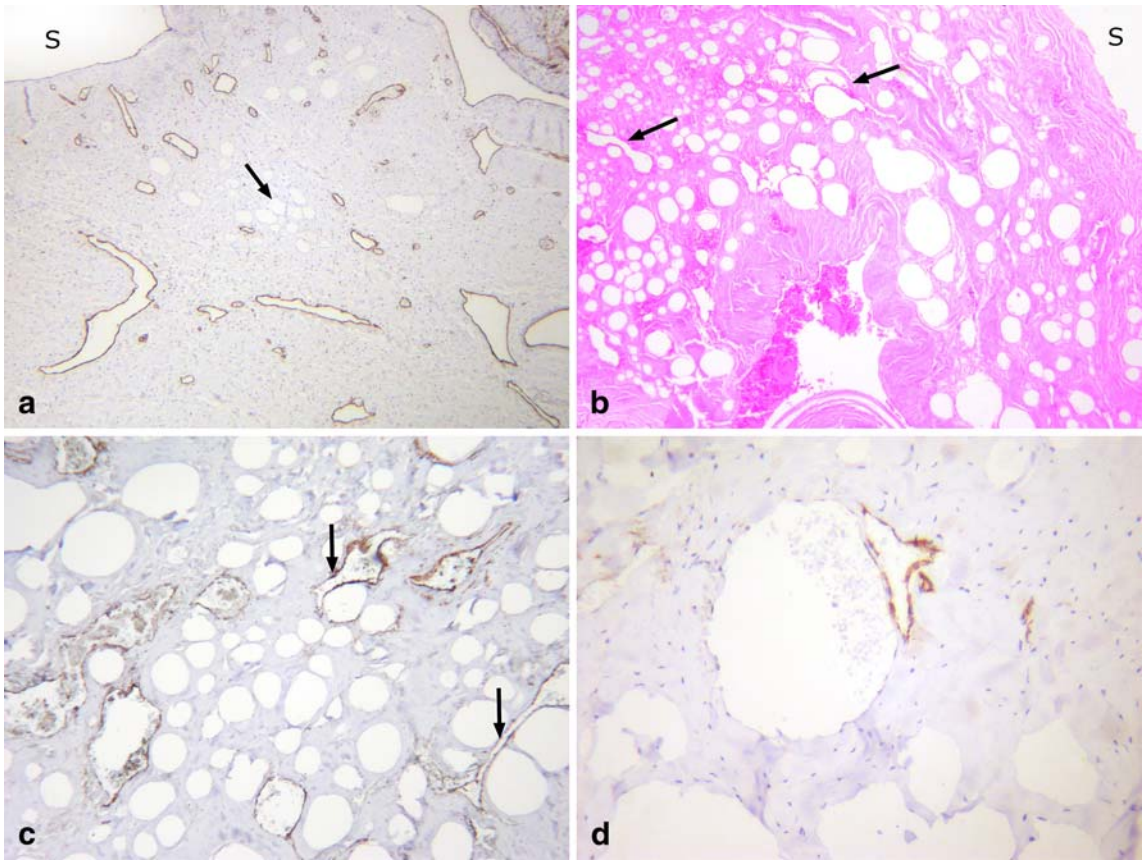

Fig. 3 **a** Six weeks; section stained with endothelial marker CD 31 shows the rounded holes (*arrow*) close to the sagittal sinus(s). They are quite distinct from vessels clearly lined by endothelium. **b** Two years; many intradural rounded holes. Some are close to or indenting vessels lined by endothelium (*arrows*). **c** Relationship of holes to vessels is seen more clearly in adjacent section from the same case stained with the endothelial marker CD 34. **d** Twenty-one months; higher power image showing relationship between unlined holes and vessels with an endothelial lining

increasingly common thereafter, particularly after the first 30 weeks of postnatal life.

The fluid channels are unlined and if collapsed would not be identified among the fibers of the dura. The existence of an intradural CSF transport system in this age group has been demonstrated by studies of Papaconaimou in neonatal lambs [10]. Our failure to identify these channels in our youngest cases does not mean that they do not exist, but indicates that they are collapsed and not readily identified with routine microscopy at this age. We suggest that they become evident when they are distended with fluid, forcing them to adopt a rounded profile.

If the AG have a role as valves, they may, when active, regulate the forward flow of CSF from dural channels, causing their dilatation; a hypothesis supported by our finding that rounded dural channels are infrequent before 30 weeks of postnatal life, about the time when AG are said to develop [12].

The relationship between these intradural rounded channels and the parasagittal venous plexus is intriguing. Multiple authors have shown that direct injection of resin or dye into the dura results in eventual filling of the sagittal sinus, indicating a connection between the interstitium of the dura and the venous sinuses. How they are connected remains unclear. We have shown a system of unlined intradural channels distinct from the endothelial lined parasagittal plexus. However, we did observe collections of unlined channels in intimate relationship with endothelial lined vessels of the parasagittal plexus. This may represent a mechanism for transfer of fluid from the dural interstitium to the SSS.

The rounded channels we describe have not been studied in the last 60 years and may be considered by some to be artifact. The relationship to age that we have shown is significant and a more detailed quantitative study, taking into consideration their distribution in other parts of the dura, is indicated.

We did not consider smaller slit-like spaces in the dura in this study. These were seen particularly in fetal dura, but slit-like spaces in the deep border layer of the dura were common at all ages. We have previously regarded this as artifact. In the light of a new appreciation of the role of the dura in fluid handling and the anatomy of the dural border layer, which consists of loosely adherent cells with fluid

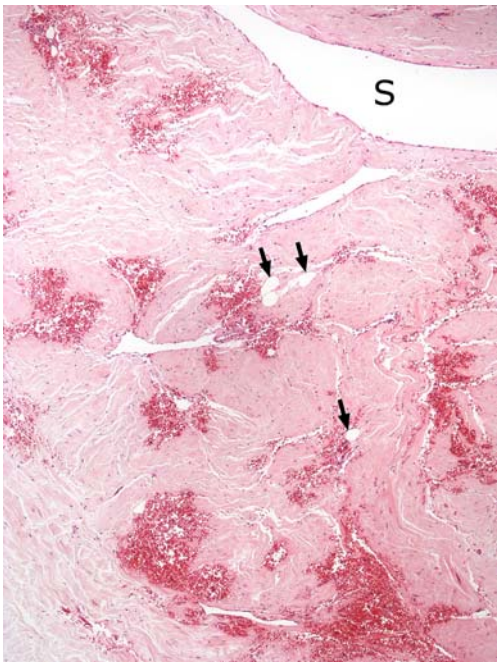

Fig. 4 Ten months; patchy bleeding is seen in the dura not apparently related to any kind of vessel. Three rounded holes are marked (*arrows*)

spaces between them [19, 20], these findings may reflect a specific fluid transport function of the dural border layer and also deserves further study.

Intradural bleeding

Intradural bleeding is common at all ages but is more severe in fetal life and the first 20 weeks of postnatal life, declining between 20 and 50 weeks. The numbers of samples studied at each age do not allow a more definite conclusion on the age at which the severity of intradural bleeding begins to decline.

Intradural bleeding in neonates is well-recognized [16–18] but its causes are little understood. There are very extensive venous sinuses of the dura at this age which regress during the first year of life [21]. Increased pressure within these sinuses due to compression of the chest during childbirth may be one explanation.

We consider another explanation for intradural bleeding in early life may be immaturity of AG. The extent of intradural bleeding close to the superior sagittal sinus in very early life, before the arachnoid granulations develop, would support a role for AG as valves preventing reflux of blood from the sinuses into the dura as shown by Welch [15].

Pressure in the cranial venous sinuses may be elevated in circumstances such as reduced venous drainage from the head or raised intrathoracic pressure, for example during childbirth, cardiopulmonary resuscitation, or mechanical ventilation. The clinical circumstances of our patients were not addressed in this series.

Two possible causes of artifactual bleeding must be considered.

The fetal cases in our study were all medical terminations of pregnancy. In these fetuses it is not uncommon to see bleeding throughout the brain and in the ventricles due to the termination procedure. In these fetuses we cannot exclude the possibility that some of the bleeding was related to the termination procedure, however, it remains of note that the bleeding was extensive and often very closely related to the dural sinuses.

It has been suggested that intradural bleeding is an artifact caused by stripping the dura from the skull at autopsy. In our youngest cases the dura was processed with the overlying skull so that it did not need to be subjected to this trauma. The fact that these were the cases with most bleeding suggests that the bleeding was not the result of post-mortem handling.

Arachnoid granulations

Arachnoid granulations were not specifically sought in this study. They are not usually identified in fetal life but first become apparent after 7–8 months of postnatal life [12, 22]. Arachnoid granulations are traditionally believed to be responsible for bulk reabsorption of CSF. However, they have a thick multilayered cap of cells which becomes thicker and more fibrous with age and a core of arachnoid, cells, dural neurothelium, and fibroblasts in a spongy arrangement [11, 23]. Their structure does not resemble other tissues involved in fluid transfer such as choroid plexus or renal glomerulus. Furthermore, arachnoid granulations are innervated with nerve terminals resembling mechanoreceptors which respond to changes in CSF pressure [24]. These features support a role as valves rather than in fluid absorption.

Le Gros Clark noted that AG become more prominent in adults with raised intracranial pressure, an observation noted recently in neonatal lambs [10]. It may be that changes in intracranial pressure cause AG to enlarge and thus modulate flow within the dural channels. We did not have any data concerning the intracranial or intravenous pressure in the terminal hours of life in our patients.

While this study provides no direct evidence on the function of AG it does demonstrate that dilated intradural fluid channels are more evident after the time at which they are expected to mature and that intradural bleeding is more extensive before this time.

Conclusion

We have demonstrated the presence of intradural spaces in human dura which may correspond to the fluid channels described over 100 years ago. We have also demonstrated

that bleeding into the dura is inversely correlated to the presence of the spaces, being common and extensive in younger age groups. We hypothesize that these two findings are related to the development of arachnoid granulations which function as valves controlling forward fluid flow in the dura and preventing reflux of blood from the dural sinuses into the dural tissues.

This case series highlights the need to undertake further research into the detailed distribution and function of fluid channels within the dura. In particular, the relationship to mode of death, premortem intracranial pressure, and presence or absence of cardiorespiratory resuscitation is indicated.

What is clear is that there is a complexity of dural anatomy and function that has not been previously recognized.

Acknowledgements We would like to thank the technical staff at the Department of Neuropathology, John Radcliffe Hospital, for careful preparation of these tissues for study. We are indebted to the families of the patients studied for their generosity in donating tissues for research.

Conflict of interest statement The authors declare that they have no conflict of interest.

References

1. De La Motte DJ (1978) Removal of horseradish peroxidase and fluorescein-labelled dextran from CSF spaces of rabbit optic nerve. A light and electron microscope study. Exp Eye Res 27:585–594
2. Johnston M, Armstrong D, Koh L (2007) Possible role of the cavernous sinus veins in cerebrospinal fluid absorption. Cerebrospinal Fluid Res 4:3
3. Zenker W, Bankoul S, Braun JS (1994) Morphological indications for considerable diffuse reabsorption of cerebrospinal fluid in spinal meninges particularly in the areas of meningeal funnels. An electronmicroscopical study including tracing experiments in rats. Anat Embryol (Berl) 189:243–258
4. Christensen E (1944) Studies on chronic subdural haematoma. Acta Psychiatr Scand 19:69–148
5. Balo J (1950) The dural venous sinuses. Anat Rec 106:319–324
6. Fox RJ, Walji AH, Mielke B, Petruk KC, Aronyk KE (1996) Anatomic details of intradural channels in the parasagittal dura: a possible pathway for flow of cerebrospinal fluid. Neurosurg 39:84–90
7. Han H, Tao W, Zhang M (2007) The dural entrance of cerebral bridging veins into the superior sagittal sinus: an anatomical comparison between cadavers and digital subtraction angiography. Neuroradiol 49:169–175
8. Grzybowski DM, Herderick EE, Kapoor KG, Holman DW, Katz SE (2007) Human arachnoid granulations Part I: a technique for quantifying area and distribution on the superior surface of the cerebral cortex. Cerebrospinal Fluid Res 4:6
9. O'Connell JEA (1934) Some observations on the cerebral veins. Brain 57:484–503
10. Papaiconomou C, Zakharov A, Azizi N, Djenic J, Johnston M (2004) Reassessment of the pathways responsible for cerebrospinal fluid absorption in the neonate. Childs Nerv Syst 20:29–36
11. Gros Clark WE (1920) On the Pacchionian bodies. J Anat 55:40–48
12. Oi S, Di Rocco C (2006) Proposal of "evolution theory in cerebrospinal fluid dynamics" and minor pathway hydrocephalus in developing immature brain. Childs Nerv Syst 22:662–669
13. Dandy W (1929) Where is cerebrospinal fluid absorbed? J of the Am Med Assoc 92:2012–2014
14. Johanson CE, Duncan JA III, Klinge PM, Brinker T, Stopa EG, Silverberg GD (2008) Multiplicity of cerebrospinal fluid functions: new challenges in health and disease. Cerebrospinal Fluid Res 5:10
15. Welch K, Friedman V (1960) The cerebrospinal fluid valves. Brain 83:454–469
16. Friede RL FR (1989) Hemorrhages in Asphyxiated Premature Infants. Dev. Neuropathol. Gottingen: Springer, 44–58
17. Smith C, Bell JE, Keeling JW, Risden RA (2003) Dural haemorrhage in nontraumatic infant deaths: does it explain the bleeding in 'shaken baby syndrome'? Geddes JE et al. A response. Neuropathol Appl Neurobiol 29:411–412
18. Kibayashi K, Shojo H, Sumida T (2005) Dural hemorrhage of the tentorium on postmortem cranial computed tomographic scans in children. Forensic Sci Int 154:206–209
19. Orlin JR, Osen KK, Hovig T (1991) Subdural compartment in pig: a morphologic study with blood and horseradish peroxidase infused subdurally. Anat Rec 230:22–37
20. Haines DE, Harkey HL, al Mefty O (1993) The "subdural" space: a new look at an outdated concept. Neurosurgery 32:111–120
21. Browder J, Kaplan HA, Krieger AJ (1975) Venous lakes in the suboccipital dura mater and falx cerebelli of infants: surgical significance. Surg Neurol 4:53–55
22. Turner L (1961) The structure of arachnoid granulations with observations on their physiological and pathological significance. Ann R Coll Surg Engl 29:237–264
23. Weller RO (2005) Microscopic morphology and histology of the human meninges. Morphologie 89:22–34
24. von During M, Andres KH (1991) Sensory nerve fiber terminals in the arachnoid granulations of non-human primates. Neurosci Lett 127:121–124