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Endothelin and nitric oxide as cerebrospinal fluid biomarkers for cerebral vasospasm following subarachnoid haemorrhage

Endothelin und NO als Liquorbiomarker für cerebralen Vasospasmus nach Subarachnoidalblutung

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Abstract

Subarachnoid haemorrhage (SAH) is a neurological emergency. One of the major complications is the development of delayed cerebral ischaemia due to cerebral vasospasm (CV) that significantly affects clinical outcome and accounts for up to 23% of disability and mortality related to SAH. Here, we review the literature on endothelin and nitric oxide related components as potential cerebrospinal fluid (CSF) biomarkers and discuss their clinical relevance to act as surrogate for CV detection and for prediction of clinical outcome. We classified recommendations for these markers as levels A to C according to the scheme approved for the European Federation of Neurological Societies guidelines. We identified significantly elevated CSF endothelin-1 concentrations in patients with SAH, especially in those who developed CV, as well as the potential role of nitrite/nitrate for clinical outcome prediction. Owing to mainly methodological shortcomings, none of the above biomarkers reached a level of recommendation that would justify implementation in clinical routine. Further prospective studies under standardised conditions are required.

Keywords: biomarker; cerebral vasospasm (CV); cerebrospinal fluid (CSF); diagnosis; outcome; subarachnoid haemorrhage (SAH).

Zusammenfassung

Die Subarachnoidalblutung (SAB) ist ein neurologischer Notfall. Eine der wichtigen Komplikationen ist die Entwicklung cerebraler Ischämien aufgrund eines cerebralen Vasospasmus (CV), der das klinische Outcome signifikant beeinflusst und für bis zu 23% von Behinderung und Mortalität verantwortlich ist. In diesem Artikel geben wir einen Überblick über die Evidenz von Endothelin und Stickstoffmonoxid assoziierten Proteinen als Liquorbiomarker im Hinblick auf die Detektion von CV und Prädiktion von klinischem Outcome. Wir klassifizierten die Marker als Level A bis C gemäß dem Schema der European Federation of Neurological Societies. Es zeigte sich sowohl eine signifikant erhöhte Konzentrationen von Endothelin-1 im Liquor cerebrospinalis bei Patienten mit SAB, insbesondere wenn diese CV entwickelten, als auch eine potentielle Rolle von Nitrit/Nitrat zur Prädiktion von klinischem Outcome. Aufgrund vorwiegend methodologischer Probleme kann für keinen der beschriebenen Marker eine Empfehlung für die klinische Routine abgegeben werden. Prospektive Studien zur Bestätigung und genaueren Evalueriung unter standardisierten Rahmenbedingungen wären notwendig.

Schlüsselwörter: Biomarker; cerebraler Vasospasmus (CV); Diagnose; Liquor cerebrospinalis; Outcome; Subarachnoidalblutung (SAB).

Introduction

Subarachnoid haemorrhage (SAH) is a neurological emergency that occurs predominantly due to the rupture of arterial aneurysms. The fatality rate is approximately 50% [1, 2] including 10% to 15% of patients who die at onset of bleeding [3, 4]. Major complications of SAH comprise rebleeding, that can be prevented by surgical (clipping) or endovascular (coiling) treatment of the aneurysm, and the development of delayed cerebral ischaemia due to cerebral vasospasm (CV).

CV adversely affects outcome and accounts for up to 23% of disability and mortality related to SAH [5–8]. It can be detected by transcranial Doppler (TCD) sonography or angiography (AG) in up to 70% of patients during hospitalisation. Approximately 46% of these patients become clinically symptomatic [8, 9]. Ischemic stroke rates after SAH are estimated to be approximately 25% [10–13].

Although digital subtraction angiography (DSA) is still the gold standard for detection of vasospasm and TCD sonography is widely accepted for screening and monitoring of CV, both methods have their limitations. The DSA is an invasive

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procedure that requires the presence of a qualified neuroradiologist, often demands general anaesthesia, and is associated with risks such as contrast nephropathy and stroke. Additionally, DSA might not identify CV in up to 25% of patients with SAH who develop stroke [14]. TCD sonography is operator dependent and provides only restricted information in patients with poor temporal sonographic windows. Accordingly, sensitivity and specificity for detection of symptomatic CV and subsequent radiological infarction ranges from 70% to 80% [14–16].

Owing to the fact that CV, a potentially preventable complication, has a significant influence on morbidity and mortality in SAH patients and that existing methods for CV detection could be complemented by cerebrospinal fluid (CSF) biomarkers, particularly in patients who undergo CSF drainage, we performed a review of studies over the past two decades summarising the available data on potential CSF biomarkers for detection of CV.

Materials and methods

Search strategy

A Medline search using the search terms ''subarachnoid haemorrhage marker'' AND ''cerebrospinal fluid'', limited to the time between 1 January 1990 and 1 September 2010, returned 82 references. We focused on abstracts which reported on endothelins and nitric oxide (NO) related agents, because they are supposed to be strongly associated – either directly or indirectly – with the development of CV. Thus, abstracts which primarily did not deal with SAH, endothelins and NO related agents in the CSF of humans (e.g., ischaemic cerebrovascular disease, head injury, ventriculitis, experimental SAH in animals, other CSF markers) were excluded, resulting in four abstracts. In addition, articles identified in reference lists of individual papers were selected if considered appropriate. Only original articles written in English were considered for this review.

The level of evidence of single papers was assigned to classes I to IV (class I being the highest level) and recommendations regarding the diagnostic value of biomarkers were classified as levels A to C (level A being the strongest recommendation) according to the European Federation of Neurological Societies guidelines. When only class IV evidence was available this could be recommended as a good practice point if it appeared appropriate [17]. In the case of insufficient description of the study design, evidence was considered as class III or lower.

Results

Endothelins

Endothelins are a family of 21 amino acid peptides with predominantly vasoconstrictive effects. Three distinct isomers arising from three different genes have been identified: endothelin-1, -2, and -3. Endothelin-1 (ET-1) appears to be functionally the most important. After transcription of the endothelin-1 gene, cleavage of preproendothelin-1 generates the peptide big endothelin-1 (big ET-1) that is finally converted to ET-1 by means of an endothelin-converting enzyme. ET-1 is mainly produced by endothelial cells in response to ischaemia, although it can also be synthesised by neurons, astrocytes and activated leukocytes. It mediates its effects via two metabotropic receptors, ET_A and ET_B. The former is considered to be crucial in CV, predominantly located on smooth muscle cells where it mediates vasoconstriction via extracellular calcium influx [18, 19].

ET-1 concentrations in CSF were significantly elevated in SAH patients compared to control groups, the latter mainly consisting of healthy volunteers or patients with other neurological non-haemorrhagic diseases such as degenerative spinal disease [20–24]. In SAH patients who did not develop CV, ET-1 concentrations remained low or decreased gradually with time [24, 25]. In contrast, ET-1 levels significantly increased between days 5 and 7 after onset of bleeding in those patients who developed symptomatic CV and also differed significantly between both groups [24–27]. There is some temporal coincidence between the increase of ET-1 levels and the occurrence of CV [24, 27, 28]. However, whereas Mascia et al., in a prospective study, stated that the increase of ET-1 concentrations did not precede those of TCD velocities but collected CSF samples only on days 1, 4 and 7 [26], Suzuki et al., in a retrospective design, stated that ET-1 levels increased before the detection of CV on angiography and decreased prior to its disappearance, collecting CSF samples every day [24]. Although the correlation between ET-1 concentrations and the degree of angiographically detected vasospasm [26], as well as cerebral blood flow velocities (CBFVs), as measured by TCD sonography has been observed [20], it still remains inconclusive whether ET-1 levels differ between the CV and asymptomatic CV group [22, 24, 26, 29]. Finally, CSF ET-1 concentrations were significantly higher in older than in younger patients [25, 30] and did not correlate with the clinical outcome as determined by the Glasgow outcome scale (GOS) [20, 21]. All studies were generally performed in patients undergoing neurosurgical intervention and used either radioimmunoassay or enzyme-linked immunosorbent assay for measurement of CSF ET-1 levels (Table 1).

Fewer studies investigated the role of big ET-1 and endothelin-3 (ET-3) levels in CV patients. Although there is some evidence that big ET-1 levels are significantly elevated in SAH patients with CV [25, 31], findings on ET-3 remain inconsistent [25, 28]. Only one study described a parallelism between ET-1 and ET-3 concentrations in CV patients [28] (Table 1).

Nitric oxide (NO) related agents

Asymmetric dimethyl-L-arginine (ADMA) is an endogenous inhibitor of the NO synthase that catalyses the synthesis of NO – an important vasodilator – from the terminal guanidino nitrogen atom of the amino acid L-arginine and molecular oxygen [19]. One study measured the concentrations of ADMA in CSF and found that they were significantly higher in patients with SAH than in control subjects. Whereas CSF ADMA levels remained unchanged in patients without CV, there was a significant increase in the CV group only after day 3 peaking during days 7 and 9 with a subsequent decrease after day 12. Furthermore, ADMA levels were sig-

Table 1 Evidence of cerebrospinal fluid biomarkers in subarachnoid haemorrhage with regard to detection of cerebral vasospasm and prediction of clinical outcome.

Marker	Ref. no.	No. of patients		Specification of controls	Origin of CSF in SAH patients	Assay	SAH treatment	Detection of CV	Significance with				Temporal coincidence between CV and CSF marker	Outcome	Evidence class
		SAH	Co.						SAH	Symptomatic CV	Angiographic CV	CBFV			
ET-1	[24]	23	10	Healthy	c	RIA	s	AG+TCD	+	(+)	+		+ (↑ day 5)		III
	[27]	27	6	Healthy	c	RIA	s	AG	–	+			+ (↑ days 5–7)		III
	[20]	35	20	Non-meningitis/haemorrhage	v/lumbar	See ref.	s	TCD	+			+		–	III
	[29]	43	12	Unruptured aneurysms	c	RIA	s	AG+TCD	–		–				III
	[26]	20			v	ELISA	s/e	AG		+	–		(↑ day 7)		II
	[25]	22	15	Degenerative spinal disease	v	RIA	s	TCD	–	+					III
	[28]	7	20	Healthy	v	RIA	s	TCD		(+)			(+)		III
	[21]	20	6	Degenerative spinal disease	c	ELISA	s	TCD	(+)	–				–	II
	[22]	30	10	Healthy	Lumbar	ELISA		AG	+		+				II
	[23]	8	24	Healthy	c	RIA	s	AG	+	(+)			(+)		II
ET-3	[28]	7	20	Healthy	v	RIA	s	TCD		(+)			(+)		III
	[25]	22	15	Degenerative spinal disease	v	RIA	s	TCD	–	–					III
Big ET-1	[25]	22	15	Degenerative spinal disease	v	RIA	s	TCD	–	+					III
	[31]	44	4	Non-haemorrhagic hydrocephalus	v	ELISA	s/e/ot	See ref.	+	+					II
ADMA	[32]	18	12	Chiari malformation type I, ICH without SAH	v	HPLC	s/e	AG	+		+		(↑ day 4–9)		III
NOx	[33]	21			c	Griess reaction	s	TCD		+		+			III
	[34]	16	8	Headache, hydrocephalus	v	Griess reaction	s	TCD	+			–			II
	[35]	29	22	Degenerative spinal disease	c	Griess reaction	s	AG	+		+		(↓ days 7–9)		III
	[36]	15	11	Degenerative spinal disease	v	Griess reaction	s		+					–	II
	[37]	10	10	OND	v	Griess reaction			+					+	II
	[38]	10	20	OND	v	Griess reaction			+					+	II
Nitrite	[32]	18	12	Chiari malformation type I, ICH without SAH	v	Chemiluminescence	s/e	AG	–		+		(↓ days 4–6)		III

+, yes; –, no; blank cells denote that these parameters were not investigated. ET-1, endothelin-1; big ET-1, big endothelin-1; ET-3, endothelin-3; ADMA, asymmetric dimethyl-L-arginine; NOx, nitrite/nitrate; SAH, subarachnoid haemorrhage; CV, cerebral vasospasm; CBFV, cerebral blood flow velocity; CSF, cerebrospinal fluid; AG, angiography; TCD, transcranial Doppler sonography; ICH, intracranial haemorrhage; OND, other neurological diseases; v, ventricular; c, cisternal; RIA, radioimmunoassay; ELISA, enzyme-linked immunosorbent assay; HPLC, high performance liquid chromatography; s, surgery; e, endovascular treatment; ot, other treatment.

nificantly higher between days 4 and 9 in CV patients than in those without CV, and strongly correlated with the degree of arteriographic CV (Table 1) [32].

Nitrite and nitrate (NOx) are both oxidation products of NO. They reflect the amount of endogenous NO to a certain extent; however, they might also be considered as a storage pool for NO because NO can be reproduced by reduction of nitrite [39]. In SAH, CSF NOx levels were significantly elevated compared to other neurological non-haemorrhagic diseases such as multiple sclerosis, headache, hydrocephalus or degenerative spinal disease [34–38]. Regarding CV, findings on NOx in CSF remain inconclusive, probably because these molecules are complexly regulated and also correlate with tissue damage as an indirect result of CV. Woszczyk et al. found significantly higher CSF NOx concentrations in patients with symptomatic CV between days 2 and 8 after onset of bleeding as well as a significant correlation with CBFV [33]. However, Ng et al. did not find any association with CBFV [34] and Suzuki et al. even find a significant decrease of NOx levels in patients with angiographic determined CV between days 7 and 9 [35]. Another study measured only nitrite and did not find any differences between SAH patients and the control group. This could possibly be due to the inappropriate choice of control subjects including patients with intracranial haemorrhage. Nevertheless, significantly lower nitrite values were found in patients with angiographic CV between days 4 and 6 and they negatively correlated with the degree of CV [32]. Furthermore, a few studies found a significant correlation between NOx levels and outcome. Rejdak et al. measured significantly lower CSF NOx concentrations in patients with very good outcome (GOS 5) compared to those with worse outcome (GOS <5) [37]. Petzold et al. confirmed these findings by significantly lower NOx levels in survivors compared to non-survivors [38]. There is only one study that could not confirm these findings [36] (Table 1).

Conclusions

CSF ET-1 concentrations were significantly elevated in SAH patients compared to controls (Level A) [20, 22–24], as well as in patients with symptomatic CV compared to those without CV (Level B) [25–27]. Whereas ET-1 levels remained low or decreased gradually with time in non-CV patients, they increased between days 5 and 7 after onset of bleeding in the CV group (Level C) [24–27]. Furthermore, an association between ET-1 levels and radiologically detected CV was found (Level C) [20, 26].

Although it has been shown that CSF NOx concentrations were significantly increased in SAH patients compared to control subjects (Level A) [34–38], its relation to CV is undetermined. Patients with worse outcome showed significantly higher CSF NOx concentrations (Level C) [37, 38].

There is insufficient evidence for big ET-1, ET-3, ADMA and nitrite levels to give any recommendation.

Despite significant differences in CSF, several studies found that ET-1, big ET-1, NOx and ADMA levels did not differ between the CV group and non-CV group if they were measured in blood [25, 31, 32, 35]. However, there is not enough evidence to reach a level of recommendation.

CSF ET-1 appears to be more directly involved in development of vasospasm and therefore might serve as a marker for CV. CSF NOx is more related to outcome prediction, which might be related to its role in tissue destruction. So far, potential markers could only be detected in CSF, probably due to the close anatomical localisation of CSF to brain tissue, warranting further research in CSF rather than blood. Some clinical use of endothelins has been developed in that ET_A receptor antagonists are being tested in clinical trials, e.g., CONSCIOUS-2 (Clazosentan to Overcome Neurological iSChemia and Infarct OccUrring after Subarachnoid haemorrhage).

To clarify the issue of CV biomarkers or even surrogate markers, further studies in a prospective design are necessary to confirm the current findings and to evaluate the potential usefulness of these markers in a clinical setting. Basic information with regard to the performance of biomarkers such as sensitivity and specificity have not been assessed in any of the studies reviewed here and therefore need to be included in future research. Also, multicentre studies would be desirable rather than single centre investigations. The goal of such studies should be to enhance the diagnostic and prognostic performance of TCD.

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