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Discovery of four potential markers for the early diagnosis of stroke

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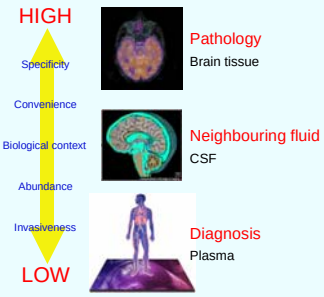
Introduction

Vascular cerebral accident, or stroke, is a leading cause of death and disability in industrialized countries. Currently, a diagnosis of stroke relies on physician's neurological examination and neuroimaging techniques such as brain CT scan and/or MRI. An early diagnostic plasmatic marker of stroke would allow more rapid and appropriate therapeutic interventions and may possibly reduce the extent of tissue damage, disability and risk of death. This marker should ideally be capable of discriminating between established versus transient ischemic attacks (TIA, which shows a complete recovery within 24 hours) and between ischemic and hemorrhagic stroke.

Strategy

Cerebrospinal fluid (CSF) is an ideal fluid to use in the search for biomarkers of brain injury. Owing to the close proximity of CSF to the brain, changes that occur in the protein composition of CSF often reflect changes that have occurred in the brain.



Methods

Here, we describe a proteomic strategy to discover specific protein markers of human *post-mortem* CSF (n=4) as a model of massive brain insult. The proteins were arrayed by two-dimensional gel electrophoresis, identified by mass spectrometry and validated in plasma of Caucasian (n=37 age and sex matched with controls) and North American (n=60 not matched with controls and n>600 age matched with controls) stroke patients by ELISA.

Results

Four protein spots found over-expressed on 2-DE gels ($p<0.05$) were identified by mass spectrometry as fatty acid binding protein (FABP) (Fig. 1a), ubiquitin fusion degradation protein (UFD1) (Fig. 2a), RNA-binding protein (RNA-BP) (Fig. 3a) and nucleoside diphosphate kinase A (NDKA) (Fig. 4a). Sandwich ELISA performed on plasma samples on three different cohorts of patients (one Caucasian and two North American) confirmed these four proteins (Fig 1b to 4b) as being significantly over-expressed in stroke patients ($p<0.0001$). Moreover, when tested on a large cohort encompassing more than 600 North American patients, RNA-BP and NDKA appeared significantly increased in the different types of stroke; hemorrhagic, TIA and ischemic (Fig 3c and 4c).

Table 1 reports the sensitivity (SE) and specificity (SP) associated with each biomarker in the three different studies. We demonstrated that it is possible to diagnose an ischemic stroke with SE and SP above 80% with age/sex matched Caucasian's patients and with 90% specificity with the large scale North American study.

Finally, Figures 5 clearly shows a significant increase, compared to controls, of UFD1 (Figure 5a), RNA-BP (Figure 5b) and NDKA (Figure 5c) even between 0 and 3 hours after the onset of the symptoms. Thus, this study demonstrated that it was possible to diagnose an ischemic stroke within the crucial first three hours when an effective treatment can still be considered.

Fig 1. Fatty acid binding protein

a) 2-DE discovery

Ante-mortem CSF Post-mortem CSF

b) Plasma ELISA validation

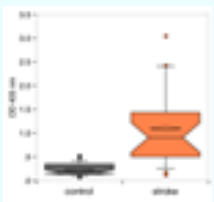


Fig 2. Ubiquitin fusion degradation protein

a) 2-DE discovery

Ante-mortem CSF Post-mortem CSF

b) Plasma ELISA validation

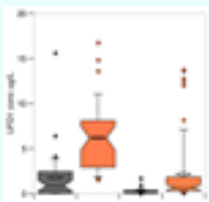


Fig 3. RNA-binding protein

a) 2-DE discovery

Ante-mortem CSF Post-mortem CSF

b) Plasma ELISA validation

c) Large scale validation

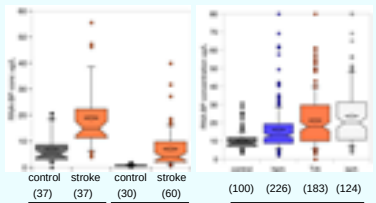
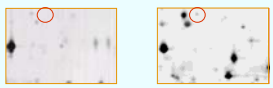


Fig 4. Nucleoside diphosphate kinase

a) 2-DE discovery

Ante-mortem CSF Post-mortem CSF



b) Plasma ELISA validation

c) Large scale validation

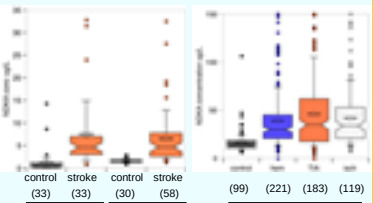
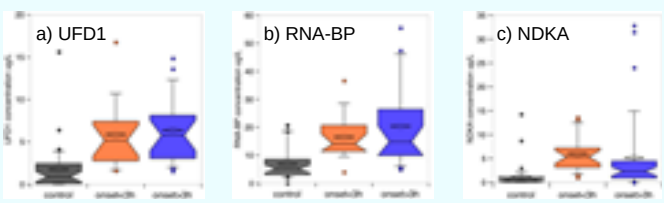


Table 1: Sensitivities and specificities obtained with Caucasian, North American 1 and 2 studies for the detection of H-FABP, UFD1, RNA-BP and NDKA.

	Caucasian		N. American1		N. American2*	
	SE%	SP%	SE%	SP%	SE%	SP%
H-FABP	68	100	-	-	-	-
UFD1	83.8	81	88.3	70	-	-
RNA-BP	86.5	81.1	86.7	96.7	61	90
NDK A	93.9	84.8	91.4	83.3	70	90

*Differentiation between control and established ischemic +TIA.

Fig 5. Stroke marker concentrations as a function of time after the onset of symptoms (Caucasian patients, controls, n=37, onset<3h, n=16 and onset>3h, n=21).



Conclusion

Validation in plasma samples of proteins found differentially expressed in *post-mortem* CSF demonstrated the value of this concept as a first step toward the discovery of plasmatic markers of cerebral injury. These markers were validated in plasma from the three different types of stroke but also within the crucial three first hours following the onset of symptoms, which is when an effective treatment can still be given to ischemic patients. Moreover, these results suggest that an extension to this study should be undertaken to assess the sensitivity and specificity of acute stroke diagnosis using a six-marker panel including H-FABP, UFD1, RNA-BP and NDK A as well as SAA and At-III fragment.

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