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Clinical Paper

Cerebrospinal fluid biomarkers in cardiac arrest survivors☆

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ABSTRACT

Aim: The aim of this study was to investigate the levels of various cerebrospinal fluid (CSF) biomarkers related to neuronal damage, inflammation and amyloid β (Aβ) metabolism in patients resuscitated after an out-of-hospital cardiac arrest (CA).
Methods: CSF levels of neurofilament light protein (NFL), total tau (T-tau), hyperphosphorylated tau (P-tau), YKL-40, Aβ38, Aβ40, Aβ42, soluble amyloid precursor protein α and β (sAPPα and sAPPβ) were measured in 21 patients approximately two weeks after CA and in 21 age-matched neurologically healthy controls. The biomarker levels were also compared between patients with good and poor long-term clinical outcome according to Glasgow Outcome Scale (GOS), activities of daily living (ADL) and mini-mental state examination (MMSE), measuring neurologic function, daily functioning and cognitive function, respectively.
Results: Patients with CA had a very marked increase in the CSF levels of NFL, T-tau and YKL-40 as compared with controls. The levels were increased at about 1200, 700 and 100%, respectively. NFL and T-tau were significantly higher in patients with poor outcome according to all three outcome measures. Patients with poor outcome according to GOS and ADL had higher levels of YKL-40. Levels of Aβ38, Aβ40, Aβ42, sAPPα and sAPPβ were lower in patients with a low MMSE score. P-tau was not significantly altered.
Conclusions: Biomarkers reflecting neuronal damage and inflammation, but not so much Aβ metabolism, were significantly altered in patients after a CA, and the changes were more pronounced in the groups with poor outcome. This calls for future larger studies to determine the prognostic potential of these biomarkers.

1. Background

Each year approximately 86 out of every 100,000 individuals in Europe receive medical care due to an out-of-hospital cardiac arrest (CA).[1] The prognosis for out-of-hospital CAs is poor; less than 10% survive.[2] Among the survivors, neurological sequelae due to hypoxic-ischaemic brain damage are common.[3] Early prediction of the neurological outcome is difficult, but nevertheless neurologists are often assigned this task. Clinical examination, electroencephalography, somatosensory evoked potential, as well as neuroimaging and biochemical markers may provide useful information. Biochemical markers offer a good way to objectively characterise molecular pathogenic processes. Since the brain is surrounded by cerebrospinal fluid (CSF), analysing this fluid gives a reflection of the biochemical status of the brain and the central nervous system (CNS). We hypothesised that CSF biomarkers related to neuronal damage and neuroinflammation are increased after CA.

Neurofilament light protein (NFL) is mainly present in large myelinated axons, and increased CSF levels have been found in acute conditions with neuronal damage such as cerebral infarction, cerebral haemorrhage and acute brain trauma.[4–6] Another marker of axonal degeneration is the microtubule associated protein tau, levels of which increase in the CSF after acute stroke, head trauma and also in the chronic neurodegenerative disorder Alzheimer's disease (AD).[6–9] The inflammatory protein YKL-40 is secreted by

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activated macrophages and increased levels have been found in the CSF of AD patients.[10,11] One study previously found that serum levels of AD-associated amyloid beta 1–42 (Aβ42) could predict poor outcome in CA patients.[12] The aim of this study was to investigate the CSF levels of the above mentioned biomarkers, in addition to the Aβ42-related analytes Aβ38, Aβ40, sAPPα, and sAPPβ, in out-of-hospital CA patients who survived the first two critical weeks and compare the levels in patients with good and poor long-term outcome.

2. Methods

2.1. Study population

Study participants were included between November 1994 and April 1999 at the Sahlgrenska University Hospital in Gothenburg, Sweden. Patients who had suffered from an out-of-hospital CA and been successfully resuscitated were included. The participants had to be >18 years of age and have a return of spontaneous circulation (ROSC) for at least 12 days. The study population has been described in detail previously.[13] Here, however, one of the 22 patients included in that study was excluded due to missing data. After admission to hospital, surviving patients were generally transferred to the intensive care unit and treated according to the general guidelines that apply for advanced cardiac life support and post resuscitation care. None of the patients at that time received hypothermia treatment. Patients who could not go through a lumbar puncture due to their clinical condition (e.g. raised intracranial pressure, brain death) or due to anticoagulant treatment were excluded. No patient was excluded on the basis of raised intracranial pressure, as assessed by fundus inspection. CSF from 21 age-matched individuals was used as control samples. The control subjects were patients who underwent lumbar puncture in preparation for spinal anaesthesia before knee surgery and had no history or signs of neurological disease. The study was approved by the Medical Ethics Committee at the University of Gothenburg. All patients gave informed consent to the study except in patients with unconsciousness, for whom relatives were consulted.

2.2. Definitions

The data of the arrests were collected from ambulance reports. A CA was defined as the ceasing of cardiac mechanical activity and was verified by coma, apnoea and the absence of a palpable pulse. The anoxia time was defined as the interval between collapse and the ROSC, and thus included both the no-flow (CA time) and low-flow (cardiopulmonary resuscitation [CRP] time) periods.

2.3. Neurological investigations

The intention was to examine the patients at day 2–4 (T1), day 12–14 (T2), day 45 (T3), 3 months (T4) and 1 year (T5). One of the authors (HR) performed the neurological examinations. The lumbar puncture was planned to be performed 12–14 days post arrest. Routinely 12 ml of CSF was collected, centrifuged and stored in aliquots in polypropylene tubes at −80 °C until analysed.

2.4. Laboratory methods

CSF levels of NFL were determined according to the manufacturer's instructions using a sandwich ELISA method (Uman Diagnostics AB, Umeå, Sweden). CSF Aβ1–42 was quantified using a sandwich ELISA (INNOTEST β-amyloid$_{1-42}$; Innogenetics, Ghent, Belgium) using a monoclonal antibody specific for the 42nd C-terminal amino acid on Aβ for capture, and a biotinylated monoclonal antibody specific for the first five N-terminal amino acids for detection, as described previously in detail.[14] CSF levels of total tau (T-tau) were determined with a sandwich ELISA using monoclonal capture antibodies (INNOTEST hTAU-Ag, Innogenetics), as presented previously.[15] Levels of tau phosphorylated at threonine 181 (P-tau) were measured with a sandwich ELISA (INNOTEST Phospho-Tau[181P], Innogenetics), as described previously in detail.[16] CSF levels of Aβx-38, Aβx-40 (called Aβ38 and Aβ40 in this study) and Aβx-42 was analysed using the MSD MULTI-SPOT Abeta Triplex Ultra-Sensitive Assay according to the instructions by the manufacturer (Meso Scale Discovery, Gaithersburg, Maryland, USA), by using C-terminal anti-Aβ antibodies for the different Aβ species for capture and SULFO-TAG 4G8 antibodies for detection. CSF concentrations of sAPPα and sAPPβ were determined using the MSD sAPPα/sAPPβ Multiplex Assay. As described previously in detail, this assay employs the 6E10 antibody to capture sAPPα and a neoepitope-specific antibody to capture sAPPβ.[17] Both isoforms are detected by the SULFO-TAG-labelled anti-APP antibody p2-1. CSF levels of YKL-40 were determined using a sandwich ELISA (R&D systems, Minneapolis, MN, USA). All measurements were performed in one round of analyses using one batch of reagents by board-certified laboratory technicians who were blind to the clinical characteristics of patients and controls. Intra-assay coefficients of variation were below 10%.

2.5. Assessment of outcome

The neurological outcome was determined with the Glasgow Outcome Scale (GOS) using the Pittsburgh cerebral performance modifications, at T2, T3, T4 and T5.[18] The result was dichotomised into good (good recovery and moderate disability [GOS 5 and 4, respectively]) or poor (severe disability, permanent vegetative state and death [GOS 3,2 and 1, respectively]). The patients' cognitive function was evaluated using the mini-mental state examination (MMSE) at T3, T4 and T5 and a score below 28 was considered low.[19] To determine the overall functional performance of the patients, the activity of daily living (ADL) according to Katz was used, and patients received a value from 0–6. They were then categorised as independent/dependent, where values <4 was regarded as dependent.[20] The best result of GOS, MMSE and ADL, respectively, was used in the outcome records since some patients awoke from the coma, but died later due to other causes than anoxic brain damage, mostly cardiac.

2.6. Statistical analysis

Pair-wise comparisons of group means were done using the non-parametric Mann–Whitney test. A p-value of <0.05 was considered statistically significant.

3. Results

In total, 21 CA patients were included in the study. Demographics and outcome of the study participants are shown in Table 1. All patients had a cardiac aetiology of the arrest. Upon follow-up, eleven patients had a poor outcome according to GOS. In the ADL assessment, ten patients were dependent. Twelve patients had a low MMSE score.

3.1. Biomarker results

Levels of NFL, T-tau and YKL-40 were significantly higher, and Aβx-42 lower, in CA patients compared with controls (Table 2). The increases in biomarker levels were substantial for NFL, T-tau and YKL-40, with elevations of 1200, 700 and 100%, respectively, in relation to control data. The levels of four biomarkers differed