VARIATION OF TOTAL ANTIOXIDANT STATUS AFTER ISCHEMIC STROKE

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ABSTRACT

There is strong evidence that oxidative stress appears to be a major contributor to ischemic brain injury.

Objective: To study dynamic evolution of Total Antioxidant Status (TAS) and other markers of oxidative stress after ischemic stroke and to find if there is any special correlation between them or with clinical status.

Methods: in a prospective study we measured markers of oxidative stress – TAS, uric acid, albumin, LDL-cholesterol and CRP – as inflammatory marker, in 24 patients, initial and 2 months after the onset of stroke, and try to find the evolution in time of these markers and if there is any correlation between them or with the neurologic deficit evaluated by NIHSS scale.

Results and conclusions: Levels of TAS and albumin were initial significant lower than in controls and 2 months after the stroke, higher than initial but still lower than in controls. We found a correlation between TAS and uric acid stronger and for CRP weaker at the onset than 2 months after the stroke. There was no correlation between NIHSS and TAS at both determinations.

Key words: oxidative stress, TAS, ischemic stroke, antioxidant

INTRODUCTION

There is strong indirect evidence that free radical production (oxidative stress) appears to be a major contributor to ischemic brain injury. Despite numerous defenses, the brain is vulnerable to oxidative stress resulting from ischemia/reperfusion. Excitotoxic stimulation of superoxide and nitric oxide production leads to formation of highly reactive products which are capable of damaging lipids, proteins and DNA, playing a critical role in initiation of apoptosis and providing additional mechanisms for oxidative damage and new targets for post-ischemic therapeutic intervention. During ischemia, high amounts of free radicals are formed by several mechanisms.

Increased free radical formation together with a reduced antioxidant defense causes oxidative stress that may play a pivotal role in the pathogenesis of stroke-associated neuronal injury. High plasma concentrations of antioxidants have been associated with a decreased risk of cerebral stroke in some epidemiological cross-sectional studies, but the antioxidant intervention trials to reduce the risk for stroke have not been all encouraging (1). There is also some recent evidence of an association between oxidative damage and tissue inflammation as measured by C-reactive protein (CRPs) in subjects with stroke disease (2). A number of individual components which are present in serum have been shown to possess antioxidant capacity, including albumin, uric acid, bilirubin and protein thiols, vitamin C and E, minerals known to be involved in antioxidant enzyme activation (selenium, iron, copper and zinc).

Total antioxidant capacity considers the cumulative effect of all antioxidants present in blood and body fluids. Measurement of total antioxidant sta-

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tus (TAS) is regarded as more physiologically representative than individual antioxidants and is believed to be a useful measure of how much the antioxidants present can protect against oxidative damage (3).

PATIENTS AND METHODS

In a prospective study we included 24 patients with acute ischemic stroke. We compare them with 19 controls, subjects aged, between 41-69 years, defined as being free of major medical or surgical illness within 5 years and leading an active and independent life. We excluded from the stroke group the patients who presented diseases that could modify the oxidative status: as sepsis, other inflammatory disease, cancer or acute renal failure, hepatitis, history of gout, other neurodegenerative disease or those taking supplemental vitamins or micronutrient supplementations. All stroke patients were clinical assessed by National Institutes of Health Stroke Scale (NIHSS) at the onset and 2 months after the stroke. Cerebral CT scan was done in the first 24 hours after the admission in the hospital to exclude hemorrhagic stroke.

Laboratory

Blood tests were done twice: first time – in the initial 72 hours after the onset of stroke and second time – 2 months after. Total Antioxidant Status (TAS) was measured spectrophotometrically, Randox Laboratories Ltd. U.K. reagents were used, standard TAS Lot. No. 224NX, 1.87 mmol/L concentrations, human serum control Lot 228NX, target value 1.88 mmol/L, range 1.50-2.26 mmol/L as per manufacturer's instructions. Uric acid, copper, total cholesterol, HDL, LDL, triglycerides, bilirubin, albumin, C-reactive protein, were analyzed with Hitachi 717 Boehringer Mannheim automatic analyzer, using Futura System (Italy) reagents.

Statistical analyses and validation of groups were performed by using STATISTICA Six Sigma version 8.0.

RESULTS

The results of our investigations are presented in tables 1 and 2.

Table 1. *Laboratory tests in controls*

	Range	Median	Mean±S.D.
	Control (n=19)		
NIHSS	0	0	0
Albumin (g/dL)	3,40-4,60	4,19	4,02±0.31
Uric Acid (µmol/L)	294,30-398,05	315,20	319,83±31,10
TAS (mmol/L)	1,40-1,80	1,60	1,61±0,14
CRP(mg/L)	5,45-9,50	6,50	6,89±1,35
LDL-Ch (mmol/L)	2,64-3,76	2,9	3,05±0,37

At the onset we obtain values, for TAS and albumin significant lower in stroke patients than in controls ($p<0,0001$). Uric acid presented similar values in both lots.

Two months after, levels for TAS, albumin and uric acid were higher than at the onset of stroke, but significant lower for TAS ($p<0.008$) and albumin ($p<0,04$) than in controls.

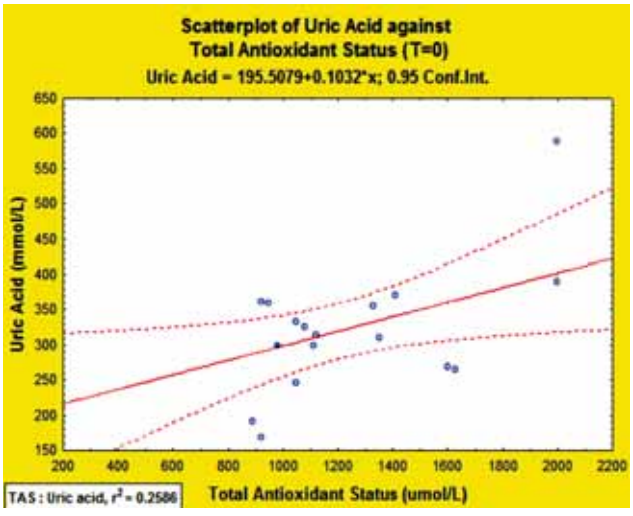
We found a linear correlation between uric acid and TAS stronger initial than two months after the stroke: $r^2 = 0,2586$ – initial vs. $r^2 = 0,1142$ – after 2 months (Fig. 1). Patients who presented significant improving in neurological deficit (decrease in NIHSS>7p) associated increasing in levels of uric acid and less in those of TAS. Minimal variation in levels of albumin correlated with minimal variation in levels of TAS, but this correlation disappeared for larger differences. There was no statistical correlation between NIHSS and TAS at both determinations.

We point out a correlation between TAS and CRP – all the patients presented lower levels of CRP and almost all, higher values of TAS after two

Table 2. *Laboratory tests in stroke patients*

	Range	Median	Mean±S.D.	Range	Median	Mean±S.D	P
	Initial (n=24)			2 months after (n=24)			
NIHSS	2.0 – 21.0	10.0	10.11± 4.71	0 – 14	4.00	5.44± 3.63	<0.001
Albumin (g/dL)	2.13 – 4.87	3.34	3.52 ±0.73	3.00 – 5.12	3.44	3.70 ±0,60	n.s.
Uric Acid (µmol/L)	168.74 –589.4	311.94	318.85±89.8	275 – 440.0	328.0	331.62± 47.4	n.s.
TAS (mmol/L)	0.89 – 2.00	1.12	1.25 ±0.35	1.05 – 2.18	1.26	1.38 ±0.34	n.s
CRP(mg/L)	8.74 – 15.20	10.26	10.95±1.84	4.25 – 14.32	9.71	9.65± 1.91	<0.01
LDL-Ch (mmol/L)	0.44 – 6.00	2.35	2.53 ±1.41	1.06 – 3.94	1.96	2.20±0.81	n.s

months; those with important decrease in levels of CRP presented important increase in TAS.



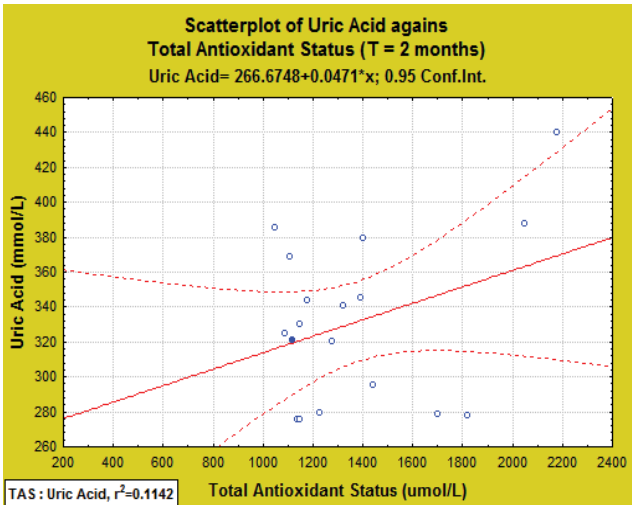
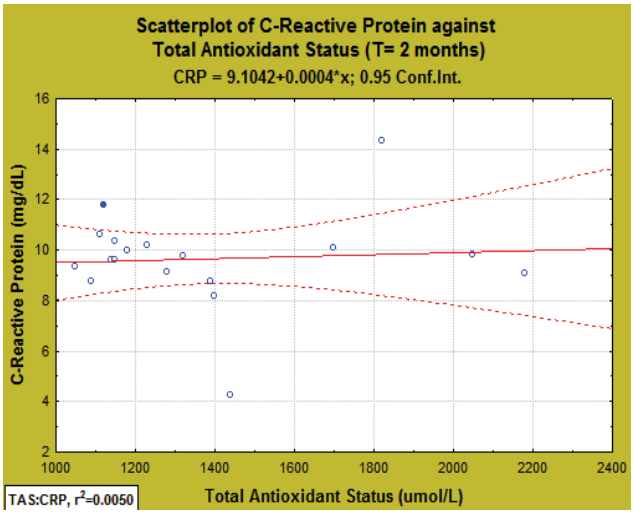


Figure 1. *Correlation between TAS and uric acid at the onset and 2 months after ischemic stroke*

Analyze of the whole lot showed a correlation between values of CRP and TAS, more evident – but not statistical significant – 2 months after, than at the onset of stroke (Fig. 2).

DISCUSSIONS

In this study, we found lower levels of TAS and albumin in patients immediately after acute ischemic stroke, similar to other studies (2, 3). Garibala and Shikrishna found also lower levels of TAS in serum (3,4) and Yildirim demonstrated decreasing of TAC (Total Antioxidant Capacity) from serum and CSF (cerebro-spinal fluid), in the first 48 hours after stroke (5). These appeared, probably, by consuming of antioxidants instantaneously after the onset of stroke, at the place of ischemic lesion. Two months later, levels of TAS and albumin were higher



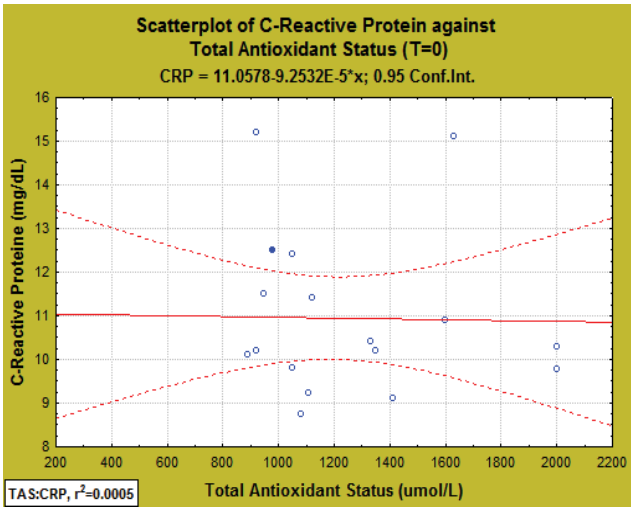


Figure 2. *Correlation between TAS and CRP at the onset and 2 months after ischemic stroke*

than at the onset of stroke, but still lower than in controls. Gariballa and col. found lower levels of TAS immediately after stroke, but with continuous decreasing of values till the seventh day after stroke (3). This could suggest that levels of TAS tend to decrease in the first days after the onset of stroke, and then increase. It is possible, in our study, that the transition to the normal levels of TAS and albumin will appear later than 2 months after the onset of stroke.

Evidence shows that uric acid plays an important role in acute ischemic stroke, as a consequence of its antioxidant properties and contributes as much as two-thirds of all free radical scavenging capacity in plasma (6). Uric acid could be particularly beneficial in situations of enhanced oxidative-mediated reperfusion injury, detaining an important neuroprotective role. Amaro demonstrated that increased uric acid serum levels associated with better outcome and significantly lower levels of uric acid with malignant middle cerebral artery infarction and parenchymal hemorrhage postthrombolysis, in pa-

tients with stroke treated with reperfusion therapies (7). We found similar values for uric acid in stroke patients comparing to controls, despite lower levels for TAS, which is considered to be represented mostly by uric acid. Gariballa and Shikrishna found, in the same sense, lower levels of TAS despite increased concentrations of uric acid in stroke patients compared with both control groups (3, 4).

Two months after, we observed levels of uric acid were higher – not significantly – than the levels immediately after the ischemic stroke. This appeared with concomitant increasing of TAS. Cherubini et al. found, also, lower levels of uric acid on admission, which showed a gradual increase over time, in patients with acute ischemic stroke (8).

Values of TAS correlated initial, almost linearly, with those of uric acid. The correlation was not so evident two months after the onset of ischemic stroke. A lot of previous studies highlighted a strong correlation between serum levels of TAS and uric acid and under multivariate analysis serum uric acid explained most of the variance in TAS during the studies` period (3, 4, 9).

We obtained no correlation between TAS and neurological deficit (NIHSS) at both determinations. There are divergent results regarding to association between total antioxidant capacity and severity of clinical status assessed by NIHSS. Several studies found an association between total antioxidant capacity and severity of neurological impairment, quite a few, not (5). Leinonen demonstrated that the total antioxidant activity of plasma is associated with the volume of ischemic cerebral infarction and the degree of neurological impairment that follows (10). Alexandrova et al. reported that the blood concentration of thiobarbituric acid-reactive material (TBARM) is an indicator of the severity of neurological deficit, and is associated with infarct size and the severity of stroke (11). Moreover, Polidori reported a significant negative correlation between lipid hydroperoxides and GCS (Glasgow Coma Scale) (12). On the other hand, Yildirim and col. didn't observe any correlation between serum and CSF TAC levels and neurologic deficit (5).

There are, also, some recent studies that demonstrate an association between oxidative stress and tissue inflammation as measured by C-reactive proteins (CRPs) in patients with acute ischemic stroke (3). C-reactive protein (CRP) has been demonstrated to induce blood-brain barrier disruption involving NAD(P)H-oxidase dependent oxidative stress (13). Recent studies showed, as well, that supplementation with antioxidants immediately after acute stroke increases plasma antioxidant capacity and reduce markers of acute inflammation (CRP) (3). In the same sense, we found in our study a correlation between TAS and CRP stronger 2 months after than at the onset and observed in patients who presented significant decrease in levels of CRP, important increase of TAS levels.

Further larger clinical studies are needed to confirm and sustain what we point out in our study regarding the sense of variation and the moment of transition to normal values of oxidative stress markers, the correlation between TAS and clinical status or between TAS and inflammation status and it's variation in time. These would be necessary to clarify the temporal relationship between antioxidant capacity and oxidative damage following ischemia and reperfusion and to form the starting point of appropriate antioxidant intervention strategies to minimize brain injury following cerebral ischemia.

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