Supplementary Material

Toast classification:

The TOAST classification criteria for stroke etiology includes five categories: 1) large-artery atherosclerosis, 2) cardioembolism, 3) small-artery occlusion (lacune), 4) stroke of other determined etiology, and 5) stroke of undetermined etiology. Diagnoses are based on clinical features and on data collected by tests such as brain imaging (CT/MRI), cardiac imaging (echocardiography, etc.), duplex imaging of extracranial arteries, arteriography, and laboratory assessments for a prothrombotic state. **Acute stroke of other determined etiology**: This category includes patients with rare causes of stroke, such as nonatherosclerotic vasculopathies, hypercoagulable states, or hematologic disorders. Patients in this group should have clinical and CT or MRI findings of an acute ischemic stroke, regardless of the size or location. Diagnostic studies such as blood tests or arteriography should reveal one of these unusual causes of stroke. (Adams HP, et al. Stroke. 1993 Jan;24(1):35-41).

Categorization of Stroke severity

The accepted scale to categorize the severity of stroke is the NIHSS. The NIHSS cutoffs were pre-specified based on both clinical judgment and scientific reports by others. Patients were categorized according to their admission score, as mild (0–6), moderate (7–15), or severe (16–38). (DeGraba TJ, et al. Stroke 1999;30:1208–12).

Cholinesterases Reflect Stroke Outcome

Enzyme measurements:

In general, AChE and BChE activities were determined from frozen serum samples using Ellman's Assay. Acetylthiocholine (ATCh, Sigma, 1 mM) or butyrylthiocholine (BTCh, Sigma, 10 mM) hydrolysis rates were measured by placing 10 μL 1:20 diluted serum in microtiter plate wells. The plate includes 96 wells and we randomly selected one well (4C) for re-measurements. The same well (4C) was re-measured in each plate, so that one sample was randomly selected for quality assurance. Since each 4C sample was analyzed in repeated measures, we have averaged two results, for this well.

Other Therapies

Thrombolytic therapy:

At the time of patient recruitment for this study, the use of thrombolytic therapy was still very limited in Israel. Intravenous tPA as a treatment for acute ischemic stroke was only approved at the end of 2004. Based on the National Acute Stroke Israeli Survey, only 1.3% of all patients with ischemic stroke were treated with tPA in 2007 (National Acute Stroke Israeli Survey Group. National survey of hospitalized patients, March-April 2007; Preliminary findings. 2007: 25).

Anticholinesterases:

None of the patients included in this study suffered from mild cognitive impairment or dementia, therefore none of them have received anticholinesterase medications.

Cholinesterases Reflect Stroke Outcome

SSRIs:

In an initial attempt to investigate the effect of medications on cholinesterase activity in stroke patients, we recruited an additional group of stroke patients who received selective serotonin reuptake inhibitor (SSRI) treatment between the acute phase of stroke and the 12 month follow up (14 patients, 5% of our studied group). SSRI-treated patients presented a trend toward better NIHSS scores and lower levels of inflammatory biomarkers when compared to untreated stroke patients. The observed differences were insignificant, likely due to the small sample size.

The detailed underlying pathways that link cholinergic and inflammatory stress responses clearly await further studies. Nevertheless, our promising preliminary results from the SSRI-treated stroke patients support the implementation of therapeutic modalities for augmenting anti-inflammatory reactions in stroke patients. SSRI treatment is frequently implemented post-stroke, as many of these patients develop clinical depression and/or anxiety symptoms in the aftermath of the acute insult (Barker-Collo SL. Arch Clin Neuropsychol. 2007 May;22(4):519-31). Consistent with previous in vitro observations (Muller TC et al, Biochim Biophys Acta. 2002 21;1587(1):92-8), our current findings also suggest that SSRI treatment may decrease the circulating ACh hydrolyzing capacity, and improve neurological recovery.

Cholinesterases Reflect Stroke Outcome

Supplementary Table

Supplementary Table 1: Subgroup analysis of the differences observed in the levels of inflammatory biomarkers between non-smokers and smokers in patients and controls

Variable	Non-smokers	Smokers	p Value
Stroke patients at admission	197	67	
hs-CRP, mg/L (SD)	8.3 (22.2)	9.9 (20.7)	0.84
ESR, mm/H (SD)	26.5 (18.2)	26 (17.4)	0.62
Fibrinogen, g/dL (SD)	332.8 (74.6)	361.9 (77.7)	0.01
AChE activity, nmol substrate hydrolysed /min*ml (SD)	287.27 (112.3)	291.3 (129.2)	0.82
Cholinergic Status total activity, nmol substrate hydrolysed /min*ml (SD)	1712.97 (353.6)	1652.26 (385.5)	0.262
Matched controls	247	17	
hs-CRP, mg/L (SD)	3.5 (4.5)	3.4 (2.5)	0.98
ESR, mm/H (SD)	19.8 (13.9)	19.9 (13.8)	0.95
Fibrinogen, g/dL (SD)	304.8 (60.9)	308.2 (48.8)	0.82
AChE activity, nmol substrate hydrolysed /min*ml (SD)	507.9 (125.7)	454.1 (127.4)	0.09
Cholinergic Status total activity, nmol substrate hydrolysed /min*ml (SD)	1626.74 (301.8)	1561.3 (318.2)	0.039

Cholinesterases Reflect Stroke Outcome

Of note, cigarette smokers usually show significantly elevated concentrations of most circulating inflammatory biomarkers. We included smoking in the multiple regression model, and therefore controlled for its influence on both inflammatory and cholinergic variables. Further, we performed a subgroup analysis of the differences observed in the levels of inflammatory biomarkers between non-smokers and smokers in patients and controls, which revealed no significant difference.