Cognitive Decline After Stroke
Relation to Inflammatory Biomarkers and Hippocampal Volume

Efrat Kliper, MSc; Dafna Ben Bashat, PhD; Natan M. Bornstein, MD; Shani Shenhar-Tsarfaty, PhD; Hen Hallevi, MD; Eitan Auriel, MD; Ludmila Shopin, MD; Sivan Bloch, MD; Shlomo Berliner, MD, PhD; Nir Giladi, MD; Uri Goldbourt, PhD; Itzhak Shapira, MD; Amos D. Korczyn, MD, MSc; Einor Ben Assayag, PhD

Background and Purpose—Inflammation may contribute to cognitive impairment after stroke. Inflammatory markers are associated with hippocampal atrophy. We tested whether markers of inflammation, erythrocyte sedimentation rate (ESR), and serum levels of C-reactive protein are associated with reduced hippocampal volume and poor cognitive performance among stroke survivors.

Methods—We analyzed 368 consecutive cases from our prospective study of first-ever mild–moderate stroke patients. MRI, cognitive tests, and inflammatory markers were determined. Patients were reevaluated 6 and 12 months after the event.

Results—ESR remained unchanged in follow-up examinations, suggesting a chronic inflammation background in some patients. Higher levels of C-reactive protein and ESR were associated with worse performance in cognitive tests, particularly memory scores. This association was maintained for ESR (but not C-reactive protein) after adjustment for confounders (P=0.002). Patients with smaller hippocampi had inferior cognitive results. Moreover, in a multivariate regression model, higher ESR values (but not C-reactive protein) were related to reduced hippocampal volume (P=0.049).

Conclusions—This report shows a strong relationship between ESR and hippocampal volume, as well as with cognitive performance among poststroke patients. This could plausibly relate to incipient cognitive decline via hippocampal pathways. (Stroke. 2013;44:1433-1435.)

Key Words: erythrocyte sedimentation rate ■ hippocampus ■ inflammation ■ poststroke cognitive performance

Low-grade systemic inflammation occurs commonly in aging1 and has been implicated as an important mechanism underlying cognitive impairment in the elderly.2 Inflammatory markers are known to be associated with dementia, particularly Alzheimer’s disease (AD) and stroke,3 but (almost) no data are available in regard to their involvement in vascular cognitive impairment.4 The major risk factors for stroke, such as atherosclerosis, hyperlipidemia, and obesity, are associated with systemic as well as brain inflammation.5 Systemic inflammation may lead to a primed inflammatory environment in the brain prior to stroke occurrence,5 which could aggravate the consequences of ischemia. Additionally, central inflammation may adversely affect learning and memory through hippocampus remodeling.6,7

We hypothesized those subjects who exhibit elevated background inflammation may have smaller hippocampus, or have reduced brain reserve, and may be more prone to develop cognitive impairment after stroke.

Here, we report on the association between inflammatory markers, hippocampal volume, and cognitive functions.

Methods
Patients were consecutive eligible participants with mild–moderate stroke or transient ischemic attack in our prospective TABASCO (Tel Aviv Brain Acute Stroke Cohort) study.5 Approval was obtained from the local Ethics Committee, and participants signed informed consent forms.

Between April 2008 and February 2012, a total of 479 consecutive patients were admitted to our center within 72 hours after onset of symptoms with a diagnosis of transient ischemic attack or first-ever mild–moderate stroke and met the study inclusion/exclusion criteria. Neurological assessment includes verification of stroke pathogenesis and the NIH Stroke Scale. Participants who had MRI scans within 7 days from symptoms onset without temporal lobe infarcts and who had no evidence of inflammatory condition within 72 hours were included (n=255; see Figure I in the online-only Data Supplement).

All patients completed a baseline neuropsychological assessment (Montreal Cognitive Assessment (MoCA) and Minidrinks computized neuropsychological battery).9 Venous blood was drawn for erythrocyte sedimentation rate (ESR), serum C-reactive protein (CRP), plasma fibrinogen, and white blood cell count at baseline and repeated 6 and 12 months later.

To test the hypothesis that elevated ESR and CRP were associated with smaller hippocampal volume and cognitive scores, multiple linear regression models were carried out.

Detailed Methods are provided in the online-only Data Supplement.
Results

Table I in the online-only Data Supplement summarizes study population characteristics.

Associations Between Inflammatory Markers and Hippocampal Volume

The Table shows potential contributors to hippocampal volume at baseline. Among inflammatory markers, only ESR was associated with hippocampal volume. Multiple regression analyses confirmed this relationship ($P=0.049$, $R^2=0.543$; Table). Patients with smaller hippocampi (lower quartile, $6722.2+479.7$ mm$^3$) had significantly higher ESR than those with larger hippocampi (upper quartile, $8866.1+428.9$ mm$^3$) at all time points (Figure). Tests with Bonferroni correction analysis of variance revealed that lower and upper quartiles differed significantly across all time points ($P=0.002$). No association was found between CRP, white blood cell count, or fibrinogen and hippocampal volume.

Associations Between Inflammatory Markers and Cognitive Performance

Elevated ESR was associated with inferior performance in the computerized total cognitive ($r=–0.160$, $P=0.045$), memory ($r=–0.189$, $P=0.017$), visuospatial ($r=–0.164$, $P=0.042$), and the MoCA ($r=–163$, $P=0.023$) score. As hippocampus plays a key role in memory formation, we tested the association of ESR with memory scores after controlling for age, sex, hypertension, diabetes mellitus, total intracranial volume, presence of apolipoprotein E ε4 (ApoE ε4) allele, NIH Stroke Scale, years of education, hematocrit, body mass index, and time from symptom to blood sampling, in a multiple regression analysis ($\beta=0.451$, SE=6.023, $P=0.002$; see Table II in the online-only Data Supplement).

Elevated CRP levels were associated with decreased performance in the total cognitive ($r=–0.283$, $P<0.001$), memory ($r=–0.173$, $P=0.029$), visuospatial ($r=–0.259$, $P=0.001$), and verbal function scores ($r=–0.236$, $P=0.003$), but the association vanished after adjustment. No association was found between CRP and MoCA scores.

Decreased cognitive performance was associated with elevated white blood cell count in the total cognitive ($r=–0.180$, $P=0.024$) and the memory ($r=–0.179$, $P=0.025$) scores, but vanished after adjustment. No association was found between white blood cell count and MoCA scores.

Fibrinogen concentrations were related to lower MoCA and visuospatial scores ($r=–0.193$, $P=0.015$; $r=–0.202$, $P=0.024$, respectively).

Among the inflammatory markers, only ESR remained significant contributor to memory performance after adjustment.

Table. The Relation of Clinical Parameters to Hippocampal Volume

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unadjusted (Model 1)</th>
<th>Adjusted (Model 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>$-0.689$</td>
<td>$-0.575$</td>
</tr>
<tr>
<td>Sex</td>
<td>$-0.340$</td>
<td>$-0.065$</td>
</tr>
<tr>
<td>Intracranial volume</td>
<td>$0.417$</td>
<td>$0.164$</td>
</tr>
<tr>
<td>APOE ε4 allele</td>
<td>$-0.149$</td>
<td>$0.305$</td>
</tr>
<tr>
<td>NIHSS</td>
<td>$-0.019$</td>
<td>$0.01$</td>
</tr>
<tr>
<td>Education (years)</td>
<td>$0.136$</td>
<td>$0.124$</td>
</tr>
<tr>
<td>Log erythrocyte sedimentation rate</td>
<td>$-0.369$</td>
<td>$-0.550$</td>
</tr>
<tr>
<td>Log C-reactive protein</td>
<td>$-0.038$</td>
<td>$-0.550$</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>$0.305$</td>
<td>$19.76$</td>
</tr>
<tr>
<td>Body mass index</td>
<td>$0.124$</td>
<td>$19.76$</td>
</tr>
<tr>
<td>Time from symptoms to blood sampling</td>
<td>$-0.051$</td>
<td>$4.519$</td>
</tr>
</tbody>
</table>

**Note:**

ESR indicates erythrocyte sedimentation rate; NIHSS, NIH Stroke Scale; and SE, standard error. Significant results are shown in bold ($P<0.05$).
Our study only included mild–moderate stroke patients, who are expected to perform cognitive tests and be available for follow-up. We also acknowledge the lack of normal controls.

Future studies should focus on the specific inflammatory patterns associated with aging and brain atrophy to identify modifiable factors that may confer risk for accelerated poststroke cognitive aging.

Whether lowering inflammation and ESR can also prevent poststroke cognitive decline needs to be addressed in further clinical trials.

### Sources of Funding
This study is supported by grant 3000000-5062 from the Chief Scientist Office, Ministry of Health, Israel and grant RAG11482 from the American Federation for Aging Research.

### Disclosures
None.

### References
Cognitive Decline After Stroke: Relation to Inflammatory Biomarkers and Hippocampal Volume


Stroke. 2013;44:1433-1435; originally published online February 26, 2013; doi: 10.1161/STROKEAHA.111.000536

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/44/5/1433

Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2013/02/26/STROKEAHA.111.000536.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/
ONLINE SUPPLEMENT

Cognitive decline after stroke: relation to inflammatory biomarkers and hippocampal volume
Supplemental methods

Study population

Patients were consecutive eligible participants with mild-moderate stroke or transient ischemic attack (TIA) in the TABASCO (Tel Aviv Brain Acute Stroke Cohort) study. TABASCO is a prospective cohort study. Patients over 50 years of age within 72 h of symptoms onset were included in the study. Ethical approval was obtained from the Ethics Committee of the Tel Aviv Medical Center and participants gave written informed consent in accordance with the Declaration of Helsinki. Patients were excluded as previously described.

Between April 1 2008 and February 1 2012 a total of 479 consecutive patients were admitted to Tel Aviv Medical Center within 72-hours after onset of symptoms with a final diagnosis of TIA or mild-moderate stroke (NIHSS < 17). For each case, information was collected for demographic data, medical history and co-morbidities. Neurological assessment includes verification of stroke etiology and the NIH Stroke Scale (NIHSS). Participants who had magnetic resonance imaging (MRI) scan within 7 days from stroke symptoms onset were included in the present investigation (n=368). Participants who refused or were unable to undergo an MRI showed higher incidence of diabetes mellitus (33.3% vs. 20.7%, p=0.033), but did not differ significantly from those who had MRI in age (68.8 ± 10.2 vs. 66.7 ± 10.4, p=0.141), years of education (13.1 ± 3.6 vs. 13.2 ± 4, p=0.824), frequency of hypertension (71.4% vs. 58.2%, p=0.054) or dyslipidemia (60.3% vs. 52%, p=0.233).

Individuals, whose baseline inflammatory markers were not available and/or had evidence of inflammatory condition or infection within 72 hours from stroke symptoms onset, were excluded. This left a total of 255 subjects for complete analysis of baseline inflammatory markers, cognitive results and MRI data (Figure S1).
Cognitive assessments:
We included all participants who were reportedly free from cognitive decline before the event (determined by the IQCODE, Informant Questionnaire on Cognitive Decline in the Elderly\(^2\)) and completed a baseline neuropsychological assessment including the Montreal Cognitive Assessment (MoCA) and the Mindstreams computerized battery (Mindstreams®, NeuroTrax Corp., NY)\(^3\) within 72-96 hours from stroke symptoms onset.

Blood tests:
Venous blood samples were drawn within 72 hours from stroke symptoms onset (onset and collection time were recorded), and biomarkers were measured, including the erythrocyte sedimentation rate (ESR), concentrations of C-reactive protein (CRP) in the serum, plasma fibrinogen and white blood cell (WBC) count. Inflammatory biomarkers determinations were repeated at 6 and 12 months after the event.

MR protocol and image analysis:
Participants were scanned within 7 days from stroke symptom onset in a 3T General Electric scanner (GE Signa EXCITE, Milwaukee, WI, USA) using an 8-channels head coil. The protocol included: Axial fast spin-echo (SE), T2-weighted images (WI) (TR/TE 13000/110 =msec), fluid-attenuated inversion recovery (TR/TE/TI=10000/110/2500msec) and gradient echo T2* WI (TR/TE = 325/15msec). All axial slices were prescribed on the same orientation, covering the whole brain, with 4 mm slice thickness and no gap, aligned along the fourth ventricle-orbitofrontal orientation. Additionally high resolution 3D T1-WI Spoiled Gradient Recalled Acquisition (SPGR) acquired in axial and coronal orientation. Volumetric analyses of the hippocampal and intracranial volume were performed using the FreeSurfer V4.5 image analysis suite which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu). It is an automated well established software tool for all brain segmentation and cortical parcellation based on probabilistic
atlas and intensity values\(^4,5\). The complete FreeSurfer analysis pipeline was performed with manual intervention and quality assurance of the data.

**Statistical Analyses:**

Continuous variables were analyzed for normality and displayed as mean (+SD). The SPSS cross tabs and descriptive procedures were used to produce frequencies of categorical variables. Pearson or Spearman correlations were used to assess relationships between levels of inflammatory markers and demographic or clinical variables. As CRP and ESR were highly skewed, logarithmic transformation was used to partly normalize the distribution for statistical analysis.

To test the hypothesis that elevated ESR and CRP levels were associated with smaller hippocampal volume, 2 sets of multiple linear regression models were carried out. First, unadjusted regression (correlation) coefficients were calculated (model 1) analyzing demographic and clinical variables that could plausibly impact hippocampal volume: age, gender, hypertension, diabetes mellitus (DM), dyslipidemia, total intracranial volume (ICV), presence of APOE ε4 allele, stroke severity (NIHSS at admission), years of education, hematocrit, ESR, CRP, body mass index (BMI) and time from symptom to blood sampling. The second model further accounted for possible confounders by adjusting for factors associated with hippocampal volume in crude analyses (p < 0.10) with age, gender, total ICV, hematocrit and ESR entered as covariates. For this purpose, hippocampal volume was used as the dependent variable (model 2) (Table 1, Brief report).

To assess the relationship between inflammatory markers and cognitive domains, 2 sets of multiple linear regression models were carried out. First, unadjusted regression (correlation) coefficients were calculated (the same model 1 confounders mentioned above, data not shown). The second model further accounted for possible confounders by adjusting for factors associated with cognitive domains in crude analyses (p < 0.10), and
memory scores as the dependent variable (Table S2). Since the variable hypertension was highly correlated with the actually measured blood pressure at admission and the variable dyslipidemia was highly correlated with cholesterol, HDL and LDL levels, we only included hypertension and dyslipidemia in the regression model to avoid co-linearity. SPSS/WIN (version 19.0, SPSS, Chicago, IL, USA) software was used to carry out all statistical analyses.

**Supplemental results**

The etiologies of the patients were as follows: 114, lacunar stroke; 14, large-artery atherosclerotic stroke; 25, cardioembolic stroke; 28 stroke of other or undetermined etiology, 74 patients presented with TIA. No differences in inflammatory biomarkers were observed between stroke subtypes and they were therefore grouped together for further analyses.

**CRP**

Serum CRP concentrations were significantly higher in patients on admission, compared with their 6 and 12 months measures [median (interquartile range, IQR) of CRP concentration 2.8 mg/l (0.96-8.2) vs. 1.1 (0.4-4) and 1 (0.5-4.9) on admission, 6 and 12 month, p=0.004, p=0.011, respectively].

**WBCC**

Total WBC counts were elevated on admission compared with 6 and 12 month measures [WBC median (IQR) 8 X 10⁹/l (6.8-9.6) on admission vs. 6.9 (6-8.7) at 6 months and 6.8 (5.9-8.4) at 12 month, p<0.001, p=0.006, respectively.

**Fibrinogen**

Fibrinogen concentrations were similar at baseline, 6 and 12 months [mean ± SD 333.1 mg/dl ± 64.1 vs. 337 ± 70 and 329 ± 64) at admission, 6 and 12 month, p=0.597, p=0.556, respectively].
ESR

ESR values were similar at admission, 6 and 12 months [median IQR values 16 mm/H (9-26) 17.5 (9.3-27.5) and 16 (10-26) on admission, 6 and 12 month, p=0.278, p=0.752, respectively].
**Supplemental tables**

Table S1: Baseline Characteristics of the Study Group

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>368</td>
</tr>
<tr>
<td>Age, years (SD)</td>
<td>67.3 (10.1)</td>
</tr>
<tr>
<td>Gender, males (%)</td>
<td>206 (55.9)</td>
</tr>
<tr>
<td>Education, years (SD)</td>
<td>13.1 (4)</td>
</tr>
<tr>
<td>Body mass index, kg/m² (SD)</td>
<td>27.1 (4.4)</td>
</tr>
<tr>
<td>Current smokers, n (%)</td>
<td>85.3 (23.7)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>77 (21.4)</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>188 (52.3)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>220 (61.2)</td>
</tr>
<tr>
<td>Coronary artery disease, n (%)</td>
<td>35 (9.7)</td>
</tr>
<tr>
<td>Peripheral vascular disease, n (%)</td>
<td>29 (8)</td>
</tr>
<tr>
<td>APOE ε4 allele, n (%)</td>
<td>58 (16.1)</td>
</tr>
<tr>
<td>National Institutes of Health Stroke Scale, median (IQR)</td>
<td>2 (0-4)</td>
</tr>
<tr>
<td>Carotid intima-media thickness, mm (SD)</td>
<td>0.88 (0.17)</td>
</tr>
<tr>
<td>Total hippocampal volume, mm³ (SD)</td>
<td>7790.4 (1008.1)</td>
</tr>
<tr>
<td>MoCA score (SD)</td>
<td>23.6 (3.4)</td>
</tr>
<tr>
<td>The computerized total cognitive score (SD)</td>
<td>91.2 (14.3)</td>
</tr>
</tbody>
</table>

**Inflammatory biomarkers**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C- reactive protein, mg/L median (IQR)</td>
<td>3.13 (1.1-8.2)</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate, mm/H median (IQR)</td>
<td>16 (9.7-26)</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL (SD)</td>
<td>340 (78)</td>
</tr>
<tr>
<td><strong>Hematocrit, %</strong></td>
<td>41.7 (3.9)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Total white blood cell count, $10^3/\mu L$ median (IQR)</td>
<td>8 (6.8-9.6)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL (SD)</td>
<td>191 (43.6)</td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol, mg/dL (SD)</td>
<td>52.6 (13.8)</td>
</tr>
</tbody>
</table>

IQR indicates interquartile range
Table S2: Beta Coefficients and Standard Errors for the relation of inflammatory biomarkers to memory scores at baseline after adjustment for age, gender, hypertension, diabetes mellitus, dyslipidemia, presence of APOE ε4 allele, stroke severity (NIHSS at admission), total intracranial volume, hematocrit, time from symptoms to blood sampling, body mass index, use of anti-inflammatory drugs and years of education

<table>
<thead>
<tr>
<th>Inflammatory biomarkers</th>
<th>β</th>
<th>SE</th>
<th>p</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR</td>
<td>-0.451</td>
<td>6.023</td>
<td>0.002</td>
<td>0.300</td>
</tr>
<tr>
<td>CRP</td>
<td>-0.157</td>
<td>3.467</td>
<td>0.300</td>
<td>0.187</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>-0.220</td>
<td>0.037</td>
<td>0.156</td>
<td>0.245</td>
</tr>
<tr>
<td>WBCC</td>
<td>0.080</td>
<td>1.069</td>
<td>0.598</td>
<td>0.175</td>
</tr>
</tbody>
</table>
Supplemental Figure

Figure S1: Flow chart of study population that included in analyses.

Baseline enrolled stroke/TIA patients: n=479

No baseline MRI n=111 due to:
Medical contraindications n=72
Claustrophobia n=24
Participant refusal n=15

Eligible n=368

Excluded patients n=110 due to:
Infection, chronic or acute inflammatory condition within 72 hours from symptoms onset, n=70
No baseline inflammatory markers available: n=40

For inflammation and imaging analysis n=255

Temporal lobe infarcts: n=3
Remarks

This work was performed in partial fulfillment of the requirements for a PhD degree of E. Kliper, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.
References:


2. Jorm AF, Christensen H, Korten AE, Jacomb PA, Henderson AS. Informant ratings of cognitive decline in old age: Validation against change on cognitive tests over 7 to 8 years. *Psychol Med*. 2000;30:981-985

