INFLAMMATION AND POST-STROKE DEPRESSION

by

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Alexander Kusnecov

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Acute ischemic stroke (AIS) has been associated with elevations in circulating inflammatory cytokines. In addition, a subset of AIS patients can develop clinically significant depression (post-stroke depression [PSD]), which can compromise recovery and increase recurrence of stroke. The cytokines, interleukin-1β (IL-1β), tumor necrosis factor α (TNFα), and interleukin-6 (IL-6) are known to have neuromodulatory effects, including the induction of behavioral changes similar to depressive symptomatology. Moreover, increased circulating levels of these cytokines – in particular, IL-6 - have been observed in depressed individuals. Therefore, it is possible that the development of PSD may similarly be associated with increased levels of inflammatory cytokines. In the current study, we recruited 25 AIS patients and assessed executive cognition, clinical depression, and circulating IL-1β, TNFα and IL-6. Each of these variables was measured at three time points following admission for AIS: (A) 1-2 days, (B) 5-7 days, and (C) 90 days. Depression was assessed throughout using the Hamilton Depression Scale and Beck Depression Inventory, as well as a structured diagnostic interview for depression (SCID) on Day 90. Cognitive functioning was measured using the RBANS. Additional repeated measures of functional and neurological status were
obtained using the modified Rankin Scale (mRS) and the National Institute of Health Stroke Scale (NIHSS). Of those patients that completed all three time points (n=22), six showed detectable levels of plasma IL-6 within seven days of AIS. A further three patients (13.6%) showed evidence for PSD at Day 90, but none of these had detectable IL-6 at any time point. Contrary to expectations, no patients, at any time point, had detectable plasma levels of TNFα or IL-1β. Based on evidence that IL-6 may be neuroprotective in animal studies of stroke, the current findings, although based on a small cohort of patients, lend themselves to the novel hypothesis that failure to generate plasma IL-6 elevations after AIS is associated with PSD and poor cognitive recovery.
Acknowledgements

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Introduction

A stroke, or cerebrovascular accident (CVA), is a sudden loss or reduction of the blood supply to the brain, or parts of the brain. If the blood supply is not restored quickly, permanent damage occurs, resulting in varying degrees of functional loss, depending on the part of the brain affected. Because stroke affects the arteries leading to and within the brain, stroke is considered a type of cardiovascular disease.

In 2010, worldwide prevalence of stroke was 33 million, with 16.9 million being first-time stroke. Stroke is the leading global cause of death behind heart disease, accounting to 11.8% of deaths worldwide. Over the past 10 years, death from stoke has dropped; however, stroke remains the leading cause of preventable disability.\(^1\) Up to half of all surviving patients fail to regain independence and require long-term health care.\(^2\) With people living longer than ever, we are currently faced with an aging population, and the prevalence of stroke survivors is projected to increase as a result of this.\(^1\) Projections show that from 2012 to 2030 there will be a 20.5% increase in the prevalence of stroke.\(^1\) Research is currently needed that addresses the functional and psychological challenges facing stroke survivors.

There are many known risk factors for stroke, which are broadly classified into modifiable and non-modifiable risk factors. Non-modifiable risk factors include family history and race; however, age is by far the most impactful non-modifiable
risk factor. Although stroke can affect individuals of any age, risk increases dramatically with aging, such that for each decade after 55 years of age, the risk for stroke approximately doubles. Age of stroke also negatively impacts prognosis, with elderly individuals often having poorer outcomes than their younger counterparts. Modifiable risk factors include smoking, physical inactivity, unhealthy diet, high cholesterol, high blood pressure, and abnormal blood sugar.

Stroke may be caused when a clot occludes the blood vessel, resulting in an ischemic stroke, or when a blood vessel ruptures, leading to both a loss of blood flow and bleeding, resulting in a hemorrhagic stroke. Although hemorrhagic strokes are more likely to be fatal, ischemic strokes are far more prevalent, with approximately 87% of strokes in the United States being of this type. Given the large literature on strokes, the focus of what follows will be on ischemic stroke, and how it can result in clinical depression.

**Depression and Stroke.** Major depressive disorder is characterized by depressed mood and/or diminished interest of pleasure in previously-enjoyed activities (anhedonia), in addition to symptoms such as sleep disturbances, eating disturbances, fatigue, feelings of worthlessness or guilt, inability to concentrate, and suicidal ideation. Twelve-month prevalence of major depressive disorder in the US is about 7%, with adolescents and females being at greater risk.
Depression is a known risk factor for several cardiovascular diseases (CVDs) \(^6\text{–}^9\), including stroke.\(^9\text{–}^{11}\) A 2007 meta-analysis of 11 studies concluded that depressed mood increased the risk for a wide range of CVDs, including stroke. The association was strongest when depression was measured by means of a clinical interview; however, the association remained significant when depression was defined only by the presence of depressive symptoms.\(^9\) One study found that individuals with high levels of depressive symptomology had a 50 – 160% increased risk of stroke in a 22 year follow-up compared to individuals with low levels of depressive symptomology. This increase in risk was on par with the increase in risk observed from a 40 point increase in baseline systolic blood pressure. The relationship between depression and stroke was not confined to purely clinically relevant levels of depressive symptomology, as even intermediate levels increase risk of stroke 20 – 40% in a 22 year follow-up.\(^10\) This association between depression or depressive symptomology and stroke is independent of changes in health behaviors that are common in depression and which are, on their own, risk factors for cardiovascular illness (for example, increased levels of smoking and decreased levels of physical activity).\(^10,11\) As depression has been shown to be a risk factor for stroke, it is then possible that after stroke, the development of depression may be a threat to recovery. Evidence from the literature has shown this to be the case, showing that subsequent to stroke, a subset of patients fail to adapt and develop what has come to be called post-stroke depression (PSD), which is associated with several adverse outcomes.
The development of PSD is one of the most common psychiatric consequences of stroke. Prevalence rates of depressive disorders following stroke vary wildly, from less than 25% \(^{12}\) to more than 60% \(^{13}\), with exact prevalence rates depending on the selection of patients, time course, and diagnostic criteria. However, most studies describe a prevalence rate for major depression of about one-third.\(^{14-17}\) When minor depression is taken into account, the prevalence rises to about 50% of stroke patients.\(^{15,18}\) Depression can occur immediately after stroke, in which case this is reactive to the CVA and the neurological symptoms experienced by the patient. However, depression is more problematic as a mood disorder more distal to the CVA (e.g., 3 months post-admission) and can compromise recovery.\(^{14,20}\) Generally, 60% of those who have acute (immediate) depression recover by 12 months post-stroke, whereas those who do not are at high risk of chronic depression, which may persist for years.\(^{14}\)

Post-stroke depression is associated with a range of adverse clinical outcomes, including limiting physical, cognitive, and neurologic recovery. A survey of deficits in Activities of Daily Living (ADLs), which include work, leisure, and self-care (e.g. grooming, dressing) activities, showed that PSD is associated with both increased length of hospital stay and increased risk of dependency following discharge.\(^{15,21,22}\) One study found that patients with PSD had significantly less recovery in ADLs than non-depressed patients with comparable baseline impairments and demographic variables.\(^{23}\) Numerous studies have found that the severity of depression following stroke positively and independently correlated with severity of cognitive impairment as measured by the mini-mental state...
examination (MMSE). This cognitive impairment was attenuated with improved mood. Furthermore, cognitive recovery occurs in both spontaneous remission of PSD and remission following active treatment, and is maintained at 2 years post-stroke. Another study has shown that severity of PSD positively correlates with neurologic impairment as measured by the NIH Stroke Scale (NIHSS).

The onset of PSD also adversely influences mortality, both in the short-term (12 – 24 months) and long-term (5 – 10 years). For example, in a 10 year follow-up study of 103 patients, those diagnosed with either major or minor depression post-stroke were 3.4x more likely to have died. This association held after controlling for age, marital status, ADL, MMSE score, social class, social ties, social functioning, alcohol use, medical comorbidity, type of stroke, lesion location, volume of lesion, and severity of impairment. Presently, the mechanism by which PSD influences mortality is not known.

**The Immune System and Stroke.** The relevance of neuroinflammation to the association between stroke and PSD has been proposed given the neural impact of immune-derived cytokines. There is a long-standing literature showing that the central nervous system (CNS) is influenced by immunological processes, especially cytokines released by activated immune cells. Inflammatory signaling is involved in every stage of an ischemic event – from the initial damage to the eventual tissue repair. Although it was once thought that the inflammatory response following stroke was merely a reaction to tissue damage,
it is now recognized as a key contributor to the pathophysiology of stroke, and is an active participant in both brain injury and recovery following stroke. Ischemic damage results from a cascade of cellular and molecular events triggered by a sudden loss of blood flow. Within minutes of an ischemic event, pro-inflammatory signals are rapidly generated by cells of the immune system. Specifically, microglia in the brain parenchyma, and mast cells and macrophages in the perivascular space produce pro-inflammatory cytokines, such as IL-1β and TNFα. These pro-inflammatory signals alter the permeability of the blood-brain barrier, which can facilitate ingress of leukocytes from the periphery several hours after a CVA. These cells can be seen in regions of acute infarction and cell necrosis. Finally, additional T cell infiltration may mediate a delayed inflammatory response to stroke, occurring up to 3 days after cerebral infarction.35

As this inflammatory cascade progresses, injured, dying, and dead cells release “danger signals” (eg., ATP) that further activate immune cells, such as microglia, which will engage in phagocytosis and remove necrotic cells, and release pro-inflammatory cytokines. Furthermore, surface proteins on neurons induce a resting phenotype in microglia, and after neuronal death, the loss of cell-to-cell signaling between neurons and microglia leads to microglial activation. Therefore, following stroke there is both a loss of normal immunosuppressive mechanisms, as well as the activation of pro-inflammatory mechanisms.

Following brain injury, the immune system may also be directly involved in tissue remodeling and neuronal repair.36 The resolution of inflammation and eventual
tissue repair are not passive processes – that is, they are not merely the result of the cessation of pro-inflammatory signaling. Rather, they involve the active suppression of the inflammatory response.\textsuperscript{37} As dead cells are removed by microglia and infiltrating macrophages, an anti-inflammatory milieu is established, primarily by the release of TGFβ by microglia and macrophages and IL-10 by several cell types, including T cells. Both TGFβ and IL-10 have anti-inflammatory actions after stroke, as well as direct cytoprotective effects. Furthermore, growth factors, such as IGF-1 (insulin-like growth factor 1 by microglia) and VEGF (vascular endothelial growth factor by reactive astrocytes) can be secreted by immune cells, promoting neurogenesis and angiogenesis.\textsuperscript{37} Interestingly, the major pro-inflammatory cytokines produced by microglia and astrocytes and by cells of the immune system – viz., tumor necrosis factor (TNFα), interleukin-1 (IL-1), and IL-6 – have been implicated in both neuronal degeneration and neuroprotection, with the latter occurring during neurotraumatic conditions.\textsuperscript{38,39}
**Figure 1. A time line of immune involvement in stroke.**

**ACUTE PHASE** (minutes – hours)
- microglia, astrocytes, and mast cells are activated within the brain
- reactive oxygen species (ROS) and pro-inflammatory cytokines (e.g., IL-1β and TNFα) are released from activated cells, as well as from injured tissue
- expression of adhesion molecules on endothelial cells and blood leukocytes is increased

**SUBACUTE PHASE** (hours – days)
- due to altered blood brain barrier permeability and increased expression of adhesion molecules, circulating leukocytes from the periphery infiltrate the brain
- dying and dead cells release “danger signals” that further activate immune cells
- immune cells destroy necrotic cells and release pro-inflammatory cytokines

**CHRONIC PHASE** (days – weeks)
- microglia activation may persist for 2 weeks post-CVA
- infiltrating leukocytes may remain for at least 1 week post-CVA

**The Immune System and Depression.** Considerable evidence exists that TNFα, and IL-1β produce anorexia, anhedonia, increased somnolence, reduced social exploration, reduced locomotion and increased anxiety. In the realm of infection and immunity, these behaviors are known as “sickness behaviors.” Sickness behaviors are adaptive – they enable an individual to cope better with illness. However, there is overlap between sickness behaviors and the clinical criteria for depression. Whereas TNFα, and IL-1β have been directly linked to producing sickness behaviors, another major pro-inflammatory cytokine, IL-6, may modulate TNFα and IL-1β to exacerbate these behaviors.

Immune theories of depression posit that pro-inflammatory cytokines, such as TNFα, IL-1β, and IL-6, may become dysregulated and contribute, along with
psychosocial factors, to the development of clinical depression in vulnerable individuals. Evidence for these theories comes from studies which show that depressive symptoms occur in autoimmune disease or after immunotherapeutic treatment with cytokines.\textsuperscript{41} Even in healthy humans, stimulation of the immune system produces cytokine-mediated mood and memory disturbances.\textsuperscript{42} Moreover, clinical depression involves cognitive deficits\textsuperscript{43,44}, and cytokines, such as IL-1, have been shown to modify learning and memory\textsuperscript{45}. This is consistent with observations of cognitive dysfunction in autoimmune diseases like multiple sclerosis.\textsuperscript{46} Lastly, pro-inflammatory markers, such as IL-6 and TNFα, have been found to be elevated in clinically depressed populations.\textsuperscript{47} For a comprehensive review of immune theories of depression, including potential causal mechanisms, see Dantzer et al, 2008.\textsuperscript{33}

As dysregulation of the immune system, specifically pro-inflammatory signaling, may play a causal role in the development of major depression, and pro-inflammatory signaling is a consequence of stroke, the immune system and/or cytokines may precipitate depression in neurotraumatic conditions, therefore accounting for the high rate of depression after acute ischemic stroke (that is, PSD).

\textbf{Cytokines and PSD}. There are reports that a higher incidence of depression occurs in stroke patients than in orthopedic patients with similar levels of disability.\textsuperscript{48} This suggests that PSD involves more than just a psychological response to acquired disability. One hypothesis is the influence of cytokines,
given that during the acute stage of stroke, pro-inflammatory cytokines are heavily upregulated.\textsuperscript{37,49,50} This should normally resolve, but in some patients, such resolution of a pro-inflammatory state is incomplete or insufficient. This then results in PSD.

Elevated levels of IL-1 have been reported in both the brains of rodents and the cerebrospinal fluid (CSF) of humans following stroke.\textsuperscript{51-53} In mice, IL-1 may mediate post-stroke development of anhedonia, a key feature of PSD. Anhedonia, measured by sucrose consumption, is observed following an induced cerebral occlusion. However, sucrose consumption can be reinstated by IL-1Ra, an IL-1 receptor antagonist.\textsuperscript{54} This suggests that the inflammatory response associated with stroke, and specifically IL-1, contributes to anhedonia, and possibly other symptoms of PSD. In humans, one study observed that at the time of a diagnosis for PSD, patients had higher blood levels of the pro-inflammatory cytokines IL-6, TNFα, and IFNγ, as well as a higher ratio of pro-inflammatory to anti-inflammatory cytokines (IL-6:IL-10, TNFα: IL-10).\textsuperscript{55} This evidence implies that the etiology of PSD is related to immune dysregulation as demonstrated by a cytokine imbalance.

**Lesion Location.** Although the neuroanatomical relationship between cerebral infarct location and PSD is unclear \textsuperscript{56,57}, left anterior lesions may be important.\textsuperscript{20,58} Several studies with immediate or early time points have observed a significant association between lesion location and prevalence of PSD.\textsuperscript{13,14,59} A meta-analysis conducted by Narishima et al found a significant inverse
correlation between severity of depression and distance of the anterior border of the lesion from the frontal pole of the left hemisphere.\textsuperscript{58} This association was only significant in the left hemisphere. Other studies conducted 3 or more months post-stroke have failed to show a difference in prevalence rates of PSD in left versus right hemispheric stroke patients.\textsuperscript{14,60} This suggests that time since stroke is a crucial variable in moderating the effect of lesion location on the chance of developing PSD.

Some evidence suggests that there is a lateralization of brain-immune interactions in both animals and humans. This is one potential mechanism that may explain the relationship between PSD and stroke lesion location. Left-handedness has been, in some studies, associated with a higher incidence of immune disorders.\textsuperscript{61,62} One study has shown that brain lesions caused by stroke can lead to a lateralization of a cutaneous inflammatory response in a location- and time-dependent manner.\textsuperscript{63} In mice, left and right cortical lesions may have differential and even opposite effects on several immune parameters. For example, left cortical lesions resulted in a depression of T lymphocyte proliferation whereas right cortical lesions show enhancing effects.\textsuperscript{64} In humans, surgery on either the language dominant or language nondominant hemisphere in patients with epilepsy differentially affected lymphocyte number, with surgery in the language dominant hemisphere resulting in a reduction in lymphocytes.\textsuperscript{65} Although this evidence points to the left hemisphere to be an important part of at least a T-cell mediated immune response, each hemisphere may be heterogeneous in its immunoregulatory functioning, with the exact immune
outcome being dependent on both the size and specific location of the lesion. There may also be a lateralization of the communication pathway from the immune system to the brain. One study found that immune stimulation with bacillus Calmette-Guetin, norepinephrine levels increased more in the right hemisphere than the left.\textsuperscript{66}

**Current Study.** In this project, I proposed to prospectively correlate markers of systemic inflammation to the development of PSD in ischemic stroke. Furthermore, lesion location was evaluated and compared to the incidence of both PSD and the magnitude of the immune response. Lastly, it was determined whether the development of PSD and/or the magnitude of the immune response could relate to cognitive and neurological recovery. Currently, screening for PSD is not part of standard post-stroke care. Should our hypothesis be correct, the opportunity to explore the molecular basis and treatment approaches to this problem will prove significant in the management of depression and prognosis among stroke patients.

**Methods**

**Patient Recruitment.** Acute ischemic stroke (AIS) patients were recruited through the Stroke service at Robert Wood Johnson Medical School and RWJ University Hospital (RWJUH). Patients were recruited within 48 hours of admission if they showed evidence of neurological dysfunction due to cerebral ischemia. All patients were given time-dependent standard therapies on
admission (eg., administered tPA [tissue plasminogen activator] as required).

The first blood draw was completed at the time of enrollment. This early consent, enrollment, and evaluation is consistent with previous NIH funded and IRB-approved acute stroke trials in which Dr. McKinney is a site-principal investigator (CLEAR-ER – NCT00894803; POINT – NCT00991029), and causes no potential harm, since the initial blood is already drawn for clinical evaluation. Consent was sought from the patient, if deemed lucid and capable of comprehending the consent form, and/or consenting surrogate. If surrogate consent was received first, it was only to collect the first blood draw. This and other study-related information was destroyed if at any time the patient negated the surrogate consent. If surrogate consent was received and then at a later point an investigator determined that the subject had regained capacity to provide consent on their own, the subject was able to give consent by signing the “enrolled under prior surrogate” consent form.

**Time Points of Testing.** As shown in Table 1, subjects had peripheral blood samples taken within 48 hours of symptom onset, hospital day 7 (+/- 3 days), and day 90 (+/- 7 days). Establishing consistent sampling time points across patients standardized assessments of the duration of inflammatory cytokine responses. Neurological and physical function were assessed at enrollment, throughout the hospital course, and at 90(+/-7) days following enrollment (see Table 1).
**Subject Number.** The study enrolled a total of 25 subjects, which was the maximum number approved by the IRB. Three subjects did not complete the third visit; incomplete data was not included in the final analysis.

**Data Collection.** Routine clinical and neurological assessments (see Table 1) were conducted by medical personnel and two trained graduate students. Experimenter-delivered tests were used to measure (i) depression severity, (ii) cognitive function, and (iii) immunological parameters. Depression (section 1.3.3) was continuously monitored through validated instruments and at day 90(+/- 7 days) after stroke a structured clinical interview for diagnosis of depression (SCID) was conducted.

**Screening Evaluation.** Potential subjects were identified by vascular neurologists in the UMDNJ-RWJMS, Department of Neurology in conjunction with study personnel at RWJUH. Diagnosis of acute (<48 hours) cerebral infarction, patient history, neurological examination, and neuroimaging (CT or MRI) was obtained. A urine pregnancy test was used to exclude pregnant women. Complete physical exams and baseline laboratory testing with a complete blood count (CBC), comprehensive metabolic panel (CMP), coagulation studies, chest x-ray, and urinalysis (U/A) was performed on all patients as part of routine clinical care.

**MRI/CT Review.** All MRI and CT data were collected by trained technicians at RWJUH. MRI data was collected on three machines, all of which used Tesla software. For one patient, a CT scan was done in place of an MRI scan, as an
MRI was not possible due to medical concerns. Lesion volume estimation was computed by Dr. Igor Rybinnik, MD, using the well-accepted formula listed below.

\[ \frac{A \times B \times C}{2} \]

Where \( A \) is the longest diameter in the Y axis, \( B \) is the longest diameter in the X axis, and \( C \) is the Z dimension (slice thickness [in all cases 3mm] multiplied by the number of slices where lesion is visible).

**Baseline Evaluation and Enrollment into Study**

Baseline evaluation included patient demographic information, history of stroke and pre-stroke level of disability, CT/MRI review, concurrent medical therapy, past medical history, vital signs, laboratory tests, and recording of the subjects baseline neurological status (NIHSS) and language ability (LAST score). Upon presentation of a potential subject, selection was determined as follows: (1) A study patient’s eligibility as determined by site personnel; (2) Qualified site personnel completed a screening form, which included protocol specific eligibility; (3) Written informed consent documented on the approved consent form; (4) Baseline clinical evaluation; (5) Peripheral blood sample obtained for baseline biomarker assay.

**Inclusion/Exclusion Criteria**

*Inclusion Criteria.* The subjects were male and female patients with acute ischemic stroke. Vascular risk factors, including diabetes, hypertension, and
coronary artery disease, were expected to be common, and were recorded.

Patients were included if:

- Aged at least 18 years of age
- Neuroimagining or clinical symptoms were consistent with an acute ischemic stroke (AIS)
- There were no alternative explanations for symptoms (eg, tumor, witnessed seizure, history of complicated migraine headache, hypoglycemia (blood sugar (BS) < 50 mg/dL) or hyperglycemia (BS > 400 mg/dL)
- Subject was able to be enrolled and have blood samples drawn (Note: inability to provide a sample within 48 hours does not preclude inclusion if consent was provided after this time and patient could provide blood at subsequent time points)
- Subject was able to provide informed consent for participation in this research study

Exclusion Criteria. Patients were excluded from the study if they showed the following:

- Other known severe/terminal illness which limited life expectancy to < 90 days, sepsis, disseminated intravascular coagulopathy (DIC), ineffective endocarditis, metastatic, cancer, or cerebral vasculitis
- Current diagnosis of or treatment for major depressive disorder
- Women who were pregnant at the time of stroke, since pregnancy alters inflammatory markers
- History of substance abuse and other relevant psychiatric conditions
- Autoimmune, current or recent infection, hematological disorders, use of immune modulating drugs
- Communication problems due to aphasia (indicated by a score of <15 on the LAST), inability to speak English

**Follow-up Days for Data Collection**

After admission and enrollment in the study (visit A), subjects had follow-up visits B (day 7+/− 3 days) and C (day 90 +/- 7 days). During these visits blood samples were collected, neuropsychological and neurological tests were completed, and measures of depression were taken (described below).

**Neurological Assessments**

**(A) National Institutes of Health Stroke Scale (NIHSS).** The NIHSS is a standard method developed by the National Institutes of Health to quantitate the severity of stroke. Scores range from 0 (“normal”, no deficiency) to 30 (maximal level of deficiency). Domains tested include level of consciousness, oculomotor/sensory functions, facial palsy, limb movement and ataxia, language comprehension, communication, speech, and self-awareness/recognition.
(B) Modified Rankin Scale (mRS). The mRS is used for measuring the degree of disability or dependence in the daily activities of people who have suffered a stroke. The scale runs from 0 – 6 (perfect health [0] to death [6]). A copy of the mRS and the NIHSS is provided in the Appendix.

Neuropsychological Assessments

(D) Language Screening Test (LAST). The LAST has shown good internal consistency and has been externally validated with the Boston Diagnostic Aphasia Evaluation. The LAST can be administered in approximately two minutes at bedside, has equivalent alternate forms and does not require a speech and language therapist. This test was used as a prescreen to determine participants’ ability to complete the neuropsychological testing battery (see RBANS below). Subjects who scored <15 (maximum score = 15) were excluded from the study.

(E) Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). The RBANS is a brief neuropsychological test battery developed for clinical use, and assesses language, visuospatial/constructional perception, attention, and immediate and delayed memory. A numerical score is obtained within each category, with a high score indicating increased proficiency. Quantification can be done within and across categories by establishing an index score of overall cognitive function. The RBANS has been used in ischemic stroke patients. It has good construct validity against other measures of similar functions and was found to predict functional status 12 months post-stroke.
Administration requires about 30 minutes and was completed at bedside.

Administration and interpretation of the RBANS and executive function tests was conducted by trained interviewers. Training was supervised by Dr. Nancy Fiedler, a clinical neuropsychologist.

**Diagnosis of Depression**

Continuous measures of clinician-rated depression severity and potentially co-morbid anxiety were measured with the Hamilton Depression Scale (Ham-D). This is a widely used and well-validated rating scale that has been used in a variety of patient populations, and was supplemented for thoroughness with the Beck Depression Inventory. The presence and history of depression and anxiety disorders was assessed using the Structured Diagnostic Interview for Axis I DSM-IV Disorders depression and anxiety modules (SCID). The SCID is a diagnostic semi-structured interview designed to assess and diagnose mental illnesses as defined by the DSM-IV (American Psychiatric Association, 2000). Trained graduate research assistants administered these instruments under the supervision of Dr. Nancy Fiedler and Dr. Richard Contrada. Subjects identified and diagnosed with depression were recommended to undergo a formal psychiatric evaluation with treatment as indicated.

**Biological Measures: Inflammatory Cytokines.**

Approximately 10 mL of blood was collected in endotoxin-free EDTA-treated vacutainer tubes. Tubes were stored at 4°C, then centrifuged at 2500 rpm. Plasma was collected and stored at -70°C until time of assay. Commercial
immunoassays were used to measure plasma levels of IL-1β, TNFα, and IL-6 (BD Biosciences, San Jose, CA). Detection ranges were 3.9 – 250 pg/mL for IL-1β, 7.8 – 500 pg/mL for TNFα, and 4.7 – 300 pg/mL for IL-6.

Table 1. Time points of testing.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Visit A (within 48 hours of stroke onset)</th>
<th>Visit B (day 7 +/- 3)</th>
<th>Visit C (day 90 +/- 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrollment/Demographics</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CT/MRI review</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood draw</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>LAST</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>NIHSS</td>
<td>X</td>
<td>X</td>
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</tr>
<tr>
<td>mRS</td>
<td>X</td>
<td>X</td>
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</tr>
<tr>
<td>RBANS</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>HAM-D &amp; BDI</td>
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<td></td>
<td>X</td>
</tr>
<tr>
<td>SCID</td>
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</tbody>
</table>

Results

Demographic Information. Twenty-two patients completed the present study. Only those who completed the study were included in analysis. The mean age of patients at the time of enrollment was 56, with ages ranging from 28 to 80. Patients were 16 males and 6 females; 12 Caucasian, 7 African American, and 3 of Hispanic descent. Of the 22 patients who completed the study, three (13.6%) developed PSD, as diagnosed by a clinical interview.

Cytokine Analysis. The levels of IL-1β and TNFα levels were not detectable for any patient at any time point. Interleukin-6 (IL-6) was detectable in five patients at Visit A, two patients at Visit B, and one patient at visit C. Six patients (27.2%) had detectable IL-6 at any time point. All six patients who had an IL-6 response had detectable levels of IL-6 within 7 days post-stroke. The median levels of IL-6
were 44.66 pg/mL (n=5, range: 10.78 to 238.34 pg/mL) at Visit A and 31.97 (n=2, range: 12.75 – 51.18 pg/mL) at Visit B. At Visit C, one patient continued to have an IL-6 response of 17.27 pg/mL.

A chi-square analysis showed that those patients who developed PSD were not more likely to have had detectable levels of IL-6 (x2=1.303, p=.254). Interestingly, although 31.6% (6 of 19) of patients who did not develop PSD had detectable levels of IL-6 on at least one time point, none (0 of 3) of those who did develop PSD had detectable IL-6 at any time point.

**Lesion Location.** Two of three patients who developed PSD had a left-hemisphere stroke. In contrast, 8 of 19 non-PSD patients had left-hemisphere strokes, 9 of 19 had right-hemisphere strokes, and 2 of 19 had bilateral damage. All three PSD-positive patients had a stroke in the anterior circulation, whereas 12 of 19 non-PSD patients had anterior circulation strokes, and 7 of 19 had a posterior circulation stroke (see Table 2).

**Table 2. Lesion information for those who did and did not develop PSD. Lesion volume is presented as median (min, max).**

<table>
<thead>
<tr>
<th></th>
<th>Hemisphere of Lesion</th>
<th>Affected Circulation</th>
<th>Lesion Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SCID (+)</strong></td>
<td>2 left 1 right</td>
<td>3 anterior 0 posterior</td>
<td>1.3 (0.35, 4.49)</td>
</tr>
<tr>
<td><strong>SCID (-)</strong></td>
<td>8 left 9 right 2 bilateral</td>
<td>12 anterior 7 posterior</td>
<td>4.5 (0.17, 158.36)</td>
</tr>
</tbody>
</table>

Median lesion volume for those who developed PSD was 1.3 (mean=2.0). Median lesion volume for those who did not develop PSD was 4.5 (mean=21.8)(see Table 2). However, three outliers were identified by inspection
of a boxplot, all subjects who did not develop PSD. When the three outliers were removed from the dataset, those who did not develop PSD had a median lesion volume of 2.1 (mean=5.7).

Two of six patients who had an IL-6 response within 7 days post-stroke had a left hemisphere lesion and four of six had a right hemisphere lesion. Of those who did not have an IL-6 response within 7 days post-stroke, eight of 16 had a left hemisphere lesion, six of 16 had a right hemisphere lesion, and two of 16 had bilateral damage. It therefore appears that there is no relationship between likelihood of IL-6 response and which hemisphere incurred the ischemic lesion.

Table 3. Lesion information for those who did and did not have detectable levels of IL-6 within 7 days post-stroke. Lesion volume is presented as median (min, max).

<table>
<thead>
<tr>
<th>Hemisphere of Lesion</th>
<th>Affected Circulation</th>
<th>Lesion Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (+)</td>
<td>2 left 4 right</td>
<td>4.9 (0.43, 158.36)</td>
</tr>
<tr>
<td>IL-6 (-)</td>
<td>8 left 6 right 2 bilateral</td>
<td>2.08 (0.17, 95.76)</td>
</tr>
</tbody>
</table>

Median lesion volume for those who had an IL-6 response was 4.9 (mean=30.1). Median lesion volume for those who did not have an IL-6 response was 2.08 (mean=14.1). When the three outliers were removed, median lesion volume was 1.3 (mean=2.48) for those who had an IL-6 response and 4.49 (mean=2.48) for those who did not have an IL-6 response.

Disability Scores. Level of disability was assessed at all three visits. At Visit A, the NIHSS alone was used. At visits B and C, the NIHSS was supplemented with
the mRS. For both the NIHSS and mRS, higher scores indicated increased level of disability. A one-way ANOVA was run for each visit (ie, for Visit B and Visit C), with the mRS and NIHSS as dependent variables. Those who developed PSD did not differ from those who did not develop PSD on level of disability at any time point (see Table 4). Surprisingly, at all time points, for both the mRS and NIHSS, median disability scores were lower in those who developed PSD than those who did not.

Those who had an IL-6 response within 7 days post-stroke did not differ from those who did not on level of disability at any time points (see Table 4).

**Cognitive Functioning Scores.** Cognitive functioning was assessed using different versions of the RBANS at Visits B and C. A composite RBANS score was calculated for each patient, and the percentile rank determined based on age. A repeated-measures ANOVA was run. Those who developed PSD did not differ from those who did not develop PSD in cognitive functioning at either time point, as measured by the RBANS (see Table 4).

There was a trend for those who had an IL-6 response 7 days post-stroke to have better cognitive functioning scores, as measured by the RBANS (p=.09; see Table 5). Those who had an IL-6 response within 7 days post-stroke had a median score of more than twice that of those who did not have an IL-6 response within 7 days post-stroke at both time points.

**Depressive Symptomatology Scores.** Depressive symptomatology was measured with the Ham-D and the BDI. For both the Ham-D and BDI, higher
scores indicate increased depressive symptomatology. A one-way ANOVA was run for each visit (ie, for Visit B and Visit C), with the Ham-D and BDI as dependent variables. For Visit C, one score was determined to be an extreme outlier by visual inspection of a boxplot. This score was removed from analysis, as it was irregular compared to the patient’s Ham-D score and the results of the patient’s diagnostic interview (ie, the patient had an extremely high BDI score, but a low Ham-D score, and was not flagged for depression during the diagnostic interview). It is possible that the patient misunderstood the questionnaire. Those who developed PSD were more likely to have higher Ham-D and BDI scores at Visit C (p=.000), but not at Visit B (p=.574 and .082, respectively) (see Table 4). In addition, depressive symptomology increased between 7 and 90 days post-stroke only for those who developed PSD, as indicated by a significant diagnosis * time point interaction (p=.003).

Although not significant, those who had an IL-6 response within 7 days post-stroke had lower BDI scores at Visit B and C, and lower Ham-D scores at Visit C by a factor of at least two (see Table 5). In addition, there was a trend toward the magnitude of the IL-6 response negatively correlating with BDI scores at 90 days post-stroke (p=-.375, p=.094). To test if the effect of IL-6 was limited to those with a clinically relevant levels of depressive symptomatology (ie, those with a diagnosis of PSD), the three patients who developed PSD were removed from the analysis, and the ANOVA was run again at Visit C. Interestingly those with an IL-6 response within 7 days post-stroke still had lower median BDI and Ham-D scores at this time point.
Table 4. Median (min, max) scores on measures of disability (mRS and NIHSS), measures of cognitive functioning (RBANS), and measures of depressive symptomology (Ham-D and BDI) at three time points for those who did and those who did not develop PSD.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group</th>
<th>Visit A</th>
<th>Visit B</th>
<th>Visit C</th>
<th>F value (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRS</td>
<td>SCID (+)</td>
<td>1.0 (0.3)</td>
<td>1.0 (0.1)</td>
<td>.66 (.430)</td>
<td>1.16 (.290)</td>
</tr>
<tr>
<td></td>
<td>SCID (-)</td>
<td>2.0 (0, 5)</td>
<td>1.0 (0, 3)</td>
<td>.44 (.516)</td>
<td>1.29 (.270)</td>
</tr>
<tr>
<td>NIHSS</td>
<td>SCID (+)</td>
<td>2.0 (0, 5)</td>
<td>1.0 (0.2)</td>
<td>.00 (0, 0)</td>
<td>.44 (.516)</td>
</tr>
<tr>
<td></td>
<td>SCID (-)</td>
<td>3.0 (0, 15)</td>
<td>2.0 (0, 9)</td>
<td>.00 (0, 4)</td>
<td>.44 (.516)</td>
</tr>
<tr>
<td>RBANS</td>
<td>SCID (+)</td>
<td>14.0 (1.0, 42.0)</td>
<td>18.0 (7.09)</td>
<td>.05 (.832)</td>
<td>.05 (.832)</td>
</tr>
<tr>
<td></td>
<td>SCID (-)</td>
<td>5.0 (0.1, 47.0)</td>
<td>14.0 (0.1, 63.0)</td>
<td>.05 (.832)</td>
<td>.05 (.832)</td>
</tr>
<tr>
<td>Ham-D</td>
<td>SCID (+)</td>
<td>8.0 (7, 11)</td>
<td>16.0 (13, 26)</td>
<td>.33 (.574)</td>
<td>22.48 (.000)*</td>
</tr>
<tr>
<td></td>
<td>SCID (-)</td>
<td>6.0 (0, 18)</td>
<td>2.0 (0, 16)</td>
<td>.33 (.574)</td>
<td>22.48 (.000)*</td>
</tr>
<tr>
<td>BDI</td>
<td>SCID (+)</td>
<td>8.0 (7, 12)</td>
<td>26.0 (23, 29)</td>
<td>3.36 (.082)</td>
<td>19.13 (.000)*</td>
</tr>
<tr>
<td></td>
<td>SCID (-)</td>
<td>3.0 (0, 17)</td>
<td>3.0 (0, 17) ◊</td>
<td>3.36 (.082)</td>
<td>19.13 (.000)*</td>
</tr>
</tbody>
</table>

◊ One outlier removed from analysis
* Significant value at p<.05
Table 5. Median (min, max) scores on measures of disability (mRS and NIHSS), measures of cognitive functioning (RBANS), and measures of depressive symptomology (Ham-D and BDI) at three time points for those who did and those who did not have detectable levels of IL-6 within 7 days post-stroke.

<table>
<thead>
<tr>
<th></th>
<th>Visit A</th>
<th>Visit B</th>
<th>Visit C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>mRS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 (+)</td>
<td>1.5 (0, 5)</td>
<td>2.0 (0, 5)</td>
<td>1.0 (0, 3)</td>
</tr>
<tr>
<td>IL-6 (-)</td>
<td>2.0 (0, 5)</td>
<td>1.0 (0, 3)</td>
<td></td>
</tr>
<tr>
<td>F value (p-value)</td>
<td>.14 (.716)</td>
<td>.09 (.762)</td>
<td></td>
</tr>
<tr>
<td><strong>NIHSS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 (+)</td>
<td>3.0 (0, 8)</td>
<td>1.5 (0, 7)</td>
<td>0.0 (0, 3)</td>
</tr>
<tr>
<td>IL-6 (-)</td>
<td>2.5 (0, 15)</td>
<td>2.0 (0, 9)</td>
<td>0.0 (0, 4)</td>
</tr>
<tr>
<td>F value (p-value)</td>
<td>.06 (.817)</td>
<td>.01 (.936)</td>
<td>.14 (.716)</td>
</tr>
<tr>
<td><strong>RBANS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 (+)</td>
<td>13.0 (1.0, 47.0)</td>
<td>33.0 (0.5, 63.0)</td>
<td></td>
</tr>
<tr>
<td>IL-6 (-)</td>
<td>3.5 (0.1, 42.0)</td>
<td>13.5 (0.1, 37.0)</td>
<td></td>
</tr>
<tr>
<td>F value (p-value)</td>
<td>3.20 (.090)</td>
<td>3.20 (.090)</td>
<td></td>
</tr>
<tr>
<td><strong>Ham-D</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 (+)</td>
<td>7.0 (0, 16)</td>
<td>1.0 (0, 16)</td>
<td></td>
</tr>
<tr>
<td>IL-6 (-)</td>
<td>7.0 (0, 18)</td>
<td>6.5 (0, 26)</td>
<td></td>
</tr>
<tr>
<td>F value (p-value)</td>
<td>.07 (.792)</td>
<td>1.19 (.288)</td>
<td></td>
</tr>
<tr>
<td><strong>BDI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 (+)</td>
<td>2.5 (0, 8)</td>
<td>2.0 (0, 6)</td>
<td></td>
</tr>
<tr>
<td>IL-6 (-)</td>
<td>5.0 (0, 17)</td>
<td>4.0 (2, 29)</td>
<td></td>
</tr>
<tr>
<td>F value (p-value)</td>
<td>2.23 (.151)</td>
<td>2.31 (.15)</td>
<td></td>
</tr>
</tbody>
</table>

◊ One outlier removed from analysis

**Discussion**

It has been well-established that acute ischemic stroke (AIS) results in a subset of individuals developing clinically significant depression, a phenomenon referred to as post-stroke depression (PSD). The purpose of this study was to determine whether PSD develops after mild AIS, in which individuals retain language skills and receive moderate scores on clinical ratings of stroke. More importantly, it was also the goal of this study to determine whether PSD is associated with
increased pro-inflammatory cytokine production, since cytokines like IL-1, TNFα, and IL-6 have been associated with depressive-like symptomatology. It was found that of the 22 patients who completed the study, three, or 13.6% developed PSD, as diagnosed by a clinical interview. Other studies cite the prevalence rate of PSD at about 30%, which is much higher than was observed. There are two possible explanations for why the present study found lower levels of PSD than others. Firstly, the present study had strict exclusion criteria, namely, that no aphasia could be present at Visit A. This exclusion criterion resulted in those being admitted to the study having had mild to moderate strokes. Similar studies have included much more severe strokes (e.g. 14 - 16). Therefore, the present study demonstrates that even with mild to moderate stroke, PSD can still develop, and at a rate that is twice the national 12-month prevalence for adults. However, it remains possible that PSD is more common in those who experience more severe strokes. Secondly, three patients dropped out of the study prior to Visit C. These patients were dropped from the analysis, as there was no way to determine if they developed PSD. It is possible, if not likely, that those who developed PSD are more likely to discontinue participation in a study such as this. Follow-ups to this study should seek to incentivize the third visit to alleviate this potential confound.
PSD was not associated with disability scores or cognitive functioning scores

Level of disability was assessed at all three visits. Those who developed PSD did not differ from those who did not develop PSD on level of disability at any time point. Interestingly, at all time points, for both the mRS and NIHSS, median disability scores were lower for those who developed PSD, compared to those who did not. Those who did and did not develop PSD did not differ on the RBANS, a measure of cognitive functioning, which was assessed at only Visits B and C. This finding was not consistent with previous studies, which have found that PSD is negatively associated with cognitive recovery. However, as mentioned above, previous studies have included incidences of much more severe strokes.

TNFα and IL-1β were not detectable at any time point

In the present study, neither TNFα nor IL-1β were detectable at any time point for any patient. This was surprising, as we originally hypothesized that increases in proinflammatory cytokines would be associated with ischemic stroke, and specifically with worse cognitive and emotional prognosis. The lack of detectable IL-1β was especially surprising due to evidence that IL-1β is associated with poor prognosis in rodent models of stroke and PSD. For example, striatal injections of IL-1β in rats exacerbates ischemic brain lesions. Furthermore, IL-1 has been implicated in post-stroke anhedonia in mice, which can be reversed to the level of controls with an IL-1 receptor antagonist. However, it is possible that IL-1β may
play a role in the development of rodent, but not human, emotional dysfunction following stroke. One study in humans also found mostly undetectable levels of IL-1β, supporting this hypothesis. However, this same study also found elevated levels of IL-6 in patients who developed PSD, which the present study did not find.55

**IL-6 as a potential neuroprotective factor**

IL-6 was the only cytokine for which detectable levels were found at any time point. Six patients (27.2%) had detectable levels of IL-6 within 7 days post-stroke. Except for one patient, those who had an IL-6 response had the highest levels at Visit A, with lower or undetectable levels at Visit B. All but one patient had undetectable levels of IL-6 at Visit C.

Although those who developed PSD were not significantly more likely to have had detectable levels of IL-6, none of those who did develop PSD had detectable levels of IL-6 at any time point, whereas 31.6% (6 of 19) of patients who did not develop PSD had detectable levels of IL-6 at at least one time point. Similarly, those who had an IL-6 response within 7 days post-stroke had lower BDI scores at Visit C and D, and lower HAM-D scores at Visit D by a factor of at least two. Furthermore, those who had an IL-6 response within 7 days post-stroke had a median score of more than twice that of those who did not have an IL-6 response within 7 days post-stroke on the RBANS, which measures cognitive functioning, at both 7 days and 90 days post-stroke.
Taken together, this data presents a pattern wherein those who had a detectable IL-6 response within 7 days post-stroke tended to have a better emotional and cognitive prognosis, suggesting that IL-6 may be playing a neuroprotective role in the early post-stroke period. This was contrary to our original hypothesis that higher levels of inflammatory cytokines would be a risk factor for PSD and poor cognitive recovery. This original hypothesis was based on evidence that immune dysregulation plays a role in the pathophysiology of depression. However, there is evidence that IL-6 may play a neuroprotective role specifically in response to ischemic injury.

The glutamatergic ionotropic receptor-mediated excitotoxic cascade is a major contributing factor for neuronal death in ischemic conditions.\textsuperscript{73} Evidence suggests that IL-6 may attenuate this excitotoxic damage and thus have a neuroprotective role post-stroke. In vitro treatment with IL-6 protects cerebellar granule cells and neuroblastoma cells against glutamate-induced excitotoxicity and oxidative damage.\textsuperscript{74,75} In mice, inhibition of IL-6 signaling leads to more severe ischemic cerebral injury \textsuperscript{76}, and IL-6 deficient mice have increased degeneration and apoptotic cell death after excitotoxic stress or brain cryoinjury.\textsuperscript{77,78} In contrast, transgenic mice that overexpress IL-6 have an elevated resistance to neuronal damage and cell death after brain injury.\textsuperscript{79} In rat models of stroke, intracerebroventricular injections of IL-6 significantly reduced ischemic brain damage, as measured by lesion volume being 52 – 65\% of controls after 24 hours\textsuperscript{80}. 
One study found that GFAP-IL6 mice, which have increased cerebral expression of IL-6, had an increased number of infiltrating immune cells at the lesion site 1–6 days post-lesion, but lower numbers of infiltrating immune cells, along with increased tissue repair at 10 days post-lesion. At 20 days post-lesion, GFAP-IL6 mice had increased lesion healing and increased revascularization compared to controls. Furthermore, GFAP-IL6 mice showed fewer oxidative stress markers post-lesion.\textsuperscript{79} This suggests that the recovery timeline post-stroke is modulated by IL-6.

Elevated levels of IL-6 may specifically be a response to, and a defense against, NMDA receptor mediated calcium influx. In a paradigm of excitotoxicity, cortical neurons which were incubated with NMDA (a NMDA receptor partial agonist) or ionomycin (a calcium ionophore) had elevated levels of IL-6 mRNA. Recombinant IL-6 dose-dependently protected neurons from this NMDA-induced excitotoxicity, and this effect was blocked with an IL-6 competitive inhibitor. However, IL-6 was ineffective against AMPA- or kainate-induced excitotoxicity, as well as non-excitotoxic induced apoptosis, namely, serum deprivation and staurosporine (a nonspecific protein kinase inhibitor) induced apoptosis.\textsuperscript{81} One potential mechanism for the excitotoxic protection conferred by IL-6 is through the upregulation of STc-1, a glycoprotein that protects against hypercalcemia.\textsuperscript{82}

Taken together, evidence suggests that IL-6 may be an important endogenous inhibitor of neuronal death during cerebral ischemia. Specifically, the upregulation of IL-6 induced by cerebral ischemia may be an endogenous
neuroprotective mechanism against NMDA receptor mediated excitotoxic injury. However, it is important to note the same transgenic mice that overexpress IL-6 used in many of these studies have chronic neuroinflammation that leads to significant brain damage, especially later in life. Many studies point to IL-6 as having negative effects in both rodent models and in humans. It is possible, then, that IL-6 may play opposing roles, either neuroprotective or neurodegenerative, depending on the physiological context.

Alternatively, the source of IL-6 may be important in determining the role it plays in post-stroke recovery. Although IL-6 is primarily thought of in the context of the immune system, it may also be produced by cells in the neuroendocrine and endocrine systems, such as the hypothalamus, anterior pituitary, and adrenal cortex. IL-6 is produced by the adrenal cortex in response to physical and psychological stressors, even as this stress suppresses immune-cell-derived IL-6. Future studies should seek to determine the origin of IL-6 post-stroke. This could be done by stimulating PBMC collected shortly after stroke (within 7 days) with an immune stimulus such as LPS.

Limitations of the present study

The present study was very limited by the low number of patients. Many of the trends noticed in the data analysis did not reach statistical significance, but may have if the power were increased. Similar studies have enrolled 100 – 200 patients. Follow-up studies should seek to increase enrollment. This will
serve to provide additional confirmation of whether lack of an IL-6 response is more likely to be associated with PSD.

Another limitation of the study was the lack of a control group. To elucidate the role of IL-6 in post-stroke recovery, a control group of orthopedic patients would be appropriate. In the future, a follow-up may be conducted with orthopedic patient controls. In these orthopedic controls, which might be matched for level of disability, we would expect much lower rates of depression than in those who have had a CVA.
References


5 American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders (5th ed.).


