Neuropathology of Post-Stroke Depression: Possible Role of Inflammatory Molecules and Indoleamine 2,3-dioxygenase

by

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ABSTRACT

The study evaluated whether the activity of the indoleamine 2,3 dioxygenase (IDO) enzyme is increased post-stroke and contributes to the development of post-stroke depression (PSD) via tryptophan (TRP) depletion and neurotoxic kynurenine (KYN) metabolite production. The activity of IDO was measured using the KYN/TRP ratio. Participants were assessed for depression severity using the Center for Epidemiological Studies Depression Scale (CES-D). Blood TRP, KYN, large neutral amino acids and cytokines were measured and compared. Fifty-four (mean age=69.9±15.2, male=52.7%, mean NIHSS=7.3±4.6) patients within 28.9±40.3 days of stroke were separated into two groups: non-depressed (n=38, CES-D=6.1±4.9) and those with significant depressive symptoms (n=16, CES-D=26.8±10.8). Higher mean KYN/TRP ratios were demonstrated in stroke patients with depressive symptoms (non-depressed=69.3±36.9 vs. depressive symptoms=78.3±42.0, F_{3,50}=4.61, p=0.006) after controlling for LNAA (p=0.026) and hypertension (p=0.039). As the KYN/TRP ratio reflects decreased TRP and increased neurotoxic KYN metabolites, both mechanisms may play an etiological role in PSD.
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1. INTRODUCTION

1.1 Statement of the Problem

Stroke is the third leading cause of death in Canada, with more than 50,000 strokes occurring annually\(^1\). This figure amounts to approximately one stroke for every 10 minutes of time that has elapsed. About 30\% of stroke victims do not survive the acute attack\(^1\). For the survivors, rehabilitation involves addressing the physical, cognitive, psychological and psychosocial impairments that are common post-stroke sequelae. One of the major psychological changes observed in post-stroke patients is depression.

Depression is a significant health problem among the elderly population leading to diminished quality of life\(^2\), impaired daily functioning\(^3\)\(^-\)\(^6\), greater health service use\(^7\), poorer perceived health\(^7\) and increased mortality risk\(^8\). With regards to stroke, depression occurs in approximately one-third of all reported cases\(^9\)\(^-\)\(^14\) and is the strongest predictor of quality of life (QoL)\(^15\)\(^-\)\(^18\). Consequentially, post-stroke depression (PSD) patients have increased functional disabilities\(^19\)\(^-\)\(^23\), increased health service utilization\(^24\),\(^25\), increased cognitive impairment\(^26\), higher mortality rates\(^27\)\(^-\)\(^31\) even after controlling for other factors such as associated illnesses and prior stroke\(^27\),\(^28\) and poorer rehabilitation outcomes\(^32\)\(^-\)\(^34\) compared to non-PSD patients. Moreover, stroke survivors are at elevated risk for clinically significant depressive symptoms even two years after stroke onset\(^35\).

Although antidepressant medications can be prescribed, the drugs are not efficacious in all depressed survivors despite the availability of different formulas acting upon distinct neurological pathways. Even when effective for depressive symptoms, post-stroke cognitive impairments frequently persist\(^36\). Additionally, response rates to antidepressant treatments are inconsistent between studies partly due to variable definitions of response. The high prevalence of depression post-stroke (about three times greater than the general population) combined with
the impact of these symptoms on antidepressant response highlights the urgency to discover possible pharmacological targets for disease management. The generation of reliable data regarding the neurological, physical and social correlates impacting PSD is a crucial factor in the field of stroke recovery. Ultimately, a sound understanding of PSD etiology may lead to novel therapeutic approaches that can propagate into successful management of this stroke outcome.

1.2 Purpose of the Study and Objectives

PSD remains profoundly problematic due to its high prevalence, negative impact, and lack of effective treatments. Several hypotheses regarding the etiology of PSD have circulated since the early 1980s, beginning with Robinson’s lesion location theory\textsuperscript{37}. It was published that stroke victims suffering from left anterior lesions were more prone to PSD than those of right or posterior lesions. His hypothesis not only stimulated several debates, but also generated the formation of subsequent hypotheses by other groups exploring other biological and psychosocial components relevant to the disease. Despite such increasing interest in PSD research, no conclusive data have been collected to date. The primary objective of this study was to assess novel biological components that may contribute to the etiology PSD. In particular, concentration changes of specific inflammatory markers and their ability to modulate tryptophan (TRP) metabolism post-stroke was investigated as a possible cause of PSD. Tryptophan was of special interest since it acts as a precursor to both the mood modulatory neurotransmitter serotonin and neurotoxic kynurenine (KYN) metabolites.

KYN is formed by metabolism of TRP by indoleamine 2,3-dioxygenase (IDO); therefore, an increase in IDO activity as stimulated by increases in pro-inflammatory cytokines post-stroke is thought to initiate the development of PSD. Here, it is hypothesized that a decrease in serotonin synthesis accompanied by an accumulation of neurotoxic KYN
metabolites act as a possible mechanism of the disease. This study measured the KYN/TRP ratio as a marker of IDO activity in order to elucidate the hypothesis. Additionally, measurements of pro- and anti-inflammatory cytokines were taken and analyzed to examine the relationship between cytokines, IDO, and PSD. Finally, demographic and clinical factors were characterized between depressed and non-depressed stroke patients in order to elucidate any markers that may contribute to post-stroke inflammatory response mechanisms.

1.3 Statement of Research Hypotheses and Rationale for Hypotheses

1.3.1 Primary Hypothesis

**Hypothesis 1:** Increased IDO enzyme activity will be found in stroke patients experiencing depressive symptoms compared to non-depressed stroke patients as evidenced by an increased kynurenine/tryptophan (KYN/TRP) ratio.

**Rationale:** Initially, an increased KYN/TRP ratio was documented among depressed immunotherapy patients\(^3\), in post-partum depression\(^3\), and in major depression\(^4\). Most recently, our group demonstrated that an elevated KYN/TRP ratio was associated with depression among coronary artery disease patients, a group similar with respect to age and a high prevalence of cerebrovascular risk factors\(^4\). It is hypothesized that post-stroke stimulation of IDO activity increases the production of KYN from its TRP precursor. Since TRP also acts as a precursor to serotonin synthesis\(^4\), decreased levels of serotonin are left available for synapses between mood regulatory neurons. In addition, KYN can be metabolized into neurotoxic metabolites\(^4\) that may cause further damages to areas of the brain responsible for processing emotions. The effect of decreased serotonin synthesis accompanied by an escalation of neuronal injuries is hypothesized to play a major role in the etiology of PSD. Thus, it is of interest to measure IDO activity as a potential marker of disease progression. Since IDO has an extremely short half life, an indirect measurement of enzyme activity (KYN/TRP ratio) will be
utilized instead. The KYN/TRP ratio has been used as a sensitive estimate of IDO activity and cellular immunity both in vivo and in vitro since the late 1980s\textsuperscript{46, 47}.

### 1.3.2 Secondary Hypothesis

**Hypothesis 2:** Increased levels of pro-inflammatory cytokines (IL-1\(\beta\), IL-6, TNF-\(\alpha\), IFN-\(\gamma\)) and decreased levels of anti-inflammatory cytokines (IL-10) will be found in stroke patients experiencing depressive symptoms compared to non-depressed stroke patients.

**Rationale:** Early pre-clinical studies have documented several cases of behavioural disturbances in animals administered a variety of pro-inflammatory cytokines\textsuperscript{48, 49}. These behavioural changes were collectively termed “sickness behaviors” and are thought to mimic those of depressive symptoms observed in humans\textsuperscript{50, 51}. More recent experimental and epidemiological studies have reported higher levels of inflammatory markers within the nervous and circulatory systems of depressed people\textsuperscript{52-58}. Marked increases in peripheral pro-inflammatory cytokines have been documented in several post-stroke cases\textsuperscript{59-64}. By linking the two findings together, the development of depression in immunocompromised patients is thought to be cytokine-mediated. This theory has been supported by reported cases of depression after cytokine immunotherapy\textsuperscript{65-68} where pro- and anti-inflammatory cytokines are thought to have opposite effects. Although data regarding the role of pro- and anti-inflammatory cytokines remain controversial, it has been hypothesized that a balance between both inflammatory markers is crucial for maintaining mental fitness\textsuperscript{69-71}. According to the mechanism proposed for the primary hypothesis, elevations in pro-inflammatory cytokines and reductions in anti-inflammatory cytokines contribute to IDO activation and may contribute to PSD.
1.4 Review of the Literature

1.4.1 Post-stroke Depression

1.4.1.1 Prevalence

One systemic review of observational studies reported a 33% prevalence of PSD at any time after stroke\(^ {10}\). Although this number concurs with most PSD studies, it is difficult to define the true prevalence of PSD due to methodological (evaluation time, study setting, inclusion/exclusion criteria) and diagnostic (different cut-off scores in rating scales, non-consistent use of structured-clinical interviews, clinical findings only) variability present amongst studies. PSD may also be over-diagnosed due to similar somatic symptoms produced by other medical illnesses, or under-diagnosed particularly in patients with co-morbid cognitive impairment. The correct attribution of somatic symptoms is important since it is relevant to accurate scoring of depression severity scales, namely the Hamilton Depression Scale (HAM-D), Montgomery Asberg Depression Rating Scale (MADRS) and Beck Depression Inventory (BDI). Furthermore, the frequency of PSD may increase in prevalence over time since symptoms may even begin to appear one year post-stroke irrespective of the level of handicap\(^ {13, 72, 73}\). Conversely, some patients with early onset PSD (0-3 months post-stroke) are not necessarily affected at one year follow-up\(^ {74, 75}\). Ultimately, quantification of PSD prevalence proves to be challenging due to the dynamic nature of the disease in both its early and late stages.

1.4.1.2 Risk Factors

Although the prevalence of PSD remains unpredictable, the disorder has been documented to affect a high percentage of stroke victims (at least 30%). Thus, it is important to investigate predictive demographic factors early after stroke onset in order to determine those at highest risk for the disease. One study has shown that stroke severity, non-lacunar stroke
subtype and the melancholy index of the HAM-D (which includes depressed mood, feelings of guilt, work inhibition, psychic anxiety and general somatic symptoms) were significant risk factors for PSD at 3 months follow-up\textsuperscript{76}, and adjustment for stroke severity increased the predictive value of the melancholy index at the same follow-up. Other studies have demonstrated that prior personal or family history of major depression\textsuperscript{75}, poor social support\textsuperscript{74}, greater impairment in activities of daily living (ADLs)\textsuperscript{74, 77, 78}, cognitive impairment\textsuperscript{79} and one or more negative life events during the six months prior to stroke\textsuperscript{75} increases risk for PSD. Time needed for recovery may also play an important role as it has been documented that those who have not yet recovered from the disease at the one year mark have a high risk of developing chronic depression\textsuperscript{74}. Despite these separate findings a more recent systematic review reported that only the degree of physical disability, stroke severity and cognitive impairment are consistently associated with the disease\textsuperscript{80}.

Because stroke often occurs in discrete lesion locations, researchers have examined the neuroanatomical correlates of depression to identify regions that would put one at most risk of PSD. Currently, literature surrounding lesion location remains controversial. There is some evidence that PSD is accompanied by left anterior and left basal ganglia lesions and proximity to the frontal pole has been positively correlated with depressive symptoms\textsuperscript{37, 81-85}. However, these results have not been consistency replicated\textsuperscript{23, 86} and the reasons for these discrepancies are not known. Thus, ready identification of patients at risk for PSD remains complicated. Further studies are necessary to support, add to, or even contrast the current evidence available before a sound pattern of PSD risk factors can be elucidated.

1.4.1.3 Demographics

To date, little is understood on the demographic determinants related to PSD onset. Whether age increases the likelihood of developing PSD remains controversial. A couple of
studies support a correlative relationship between the two variables\textsuperscript{16, 87} while others report no association between them\textsuperscript{9, 19, 88, 89}. With gender, there have been reports that women are more likely to suffer from depression in the general population; however many studies have found minimal to no effect between gender and PSD\textsuperscript{16, 22, 87, 89, 90}. Despite these negative findings, a more recent systemic review evaluating sex differences in the prevalence of PSD reported the disease to be slightly more common among women than men after combining post-stroke cases ranging from less than two weeks to 15 years\textsuperscript{91}. The same factors explaining the greater prevalence of depression in women among cardiac patients may also be applicable to post-stroke cases, including elements of genetics, psychosocial inequities, issues related to recovery, differential support and access to rehabilitation\textsuperscript{92}. Interestingly, women\textsuperscript{93, 94} and stroke patients less than 59 years of age\textsuperscript{94} have been found to be more susceptible to post-stroke anxiety (PSA), a disease that often manifests with PSD.

More recently, racial and ethnic disparities have been examined as potential demographic correlates for PSD. In one study, white, non-Hispanic acute stroke patients from a national cohort of Department of Veterans Affairs were more likely to be diagnosed with PSD even after adjusting for sociodemographic and clinical characteristics\textsuperscript{95}. Another study examining the relationship between ethnicity and risk for post-stroke mortality reported that Non-Hispanic Black veterans with a history of stroke had a higher risk for mortality than did Non-Hispanic White veterans\textsuperscript{96}. Because PSD patients have been shown to have a higher mortality risk than non-depressed stroke patients\textsuperscript{27-31} racial and ethnic factors may be important predictors in the development of the disease. At this time, it remains unclear whether racial and ethnic backgrounds can be utilized as correlates for PSD.

Whether the stroke victim was an inpatient or an outpatient may be predictive of PSD. A systematic review reported that inpatients with left anterior lesions were significantly more
likely to have MDD than outpatients\textsuperscript{97}. Note however, that this finding was only applicable to the acute stroke period as no difference in mood was found between in- and outpatients during the chronic stroke period.

### 1.4.1.4 Clinical Diagnosis of PSD

It is well-known that both major and minor depression are possible consequences of stroke, though the true prevalence of each remains less certain. Major depression can be accurately diagnosed in those with stroke\textsuperscript{98, 99} using the Diagnostic and Statistical Manual Version of Mental Disorders, Fourth Edition (DSM-IV)\textsuperscript{100}. According to the manual, a patient is diagnosed with major depression if at least five of the following nine symptoms are met: depressed mood, anhedonia, appetite/weight change, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, feelings of worthlessness or guilt, diminished concentration, or recurrent thoughts of death. The five symptoms must include either depressed mood or anhedonia and must persist for at least two weeks prior to evaluation. In contrast, minor depression must only meet three of the previously mentioned nine symptoms with either depressed mood or anhedonia listed as one of the three symptoms. Although studies have documented major depression to occur in approximately 25\%\textsuperscript{89, 98} of all stroke patients, the occurrence of minor depression remains more elusive, with a reported prevalence ranging from 14-31\%\textsuperscript{89, 98}.

Currently, there is no consensus on how to define "early" and "late" onset PSD. However, Robinson demonstrated that 30\% of stroke survivors who are non-depressed at initial evaluation in an acute hospital setting will be diagnosed with depression 6 months later\textsuperscript{101}. Following that criterion, one study examined the individual characteristics of early and late-onset PSD, where early and late-onset were described as 3-6 months and 24 months post-stroke respectively. It was found that the frequency of melancholic, vegetative, and psychological
symptoms and morning depression were significantly greater in patients with early-onset post-stroke major or minor depression than in patients with late-onset major or minor depression. Furthermore, early-onset post-stroke major depression was associated with larger lesion volumes, and late-onset post-stroke major and minor depressions were associated with poorer social functioning. Another study reported that early-onset PSD is characterized by anxiety, loss of libido and feelings of guilt, whereas later-onset PSD is more frequently associated with diurnal variation of mood and social isolations. Still, the prognostic differences and clinical patterns between early-onset and late-onset PSD remain quite vague and are suspected to result from differences in the biological or psychological processes acquired from the stroke.

Recently, diagnosis of PSD has shifted the emphasis from expressed mood and feelings to the patient’s affect and behavior. This is especially true in cases where PSD is complicated by significant neurological and functional impairment. Dominant hemispheric strokes are often associated with aphasia making it difficult for patients to articulate thoughts and emotions of sadness, hopelessness and guilt. On the other hand, patients with non-dominant hemisphere stroke may still have intact language but be stricken with speech prosody, thus affecting their ability to comprehend the importance voice tone and attitude expression in their speech (e.g. the ability to imply sarcasm through tone of voice).

Patients with language impairments are not the only group of individuals who require special attention. Several somatic and vegetative symptoms that are important clinical features of PSD may be misattributed to a simple distaste for hospital environment. For example, weight loss due to post-stroke dysphagia may be ascribed to a disliking for hospital food. Insomnia may be noted as difficulty sleeping in a hospital setting rather than a symptom of depression. In all cases, the opposite effect may also be true where patients are misdiagnosed with depression due to misinterpretation of the aforementioned symptoms. Note, that vegetative symptoms are
significantly more common in depressed patients over the first two years post-stroke\textsuperscript{103} and that certain somatic symptoms, specifically loss of appetite, psychomotor retardation and fatigue have been found to be predictive of depression\textsuperscript{99}. Thus, it remains crucial for physicians be attentive to such symptoms despite their unspecific nature.

\subsection*{1.4.1.5 Hypertension and the Vascular Hypothesis of Depression}

Cerebral blood flow (CBF) and vasomotor reactivity are specific hemodynamic changes that play a major role in stroke. Once CBF falls below threshold, delivery of vital nutrients through the brain's microvasculature becomes compromised. This subsequently leads to detrimental effects on neuronal functioning. If further decline in regional CBF occurs, cellular mechanisms governing ionic equilibrium are also jeopardized\textsuperscript{105}. Furthermore, patients with stroke or transient ischemic attacks have reduced vasomotor reactivity, indicating that cerebral arterioles are unable to dilate in order to compensate for increased blood demand\textsuperscript{106}.

Hypertension is well-known to the public by its simplest definition--a condition characterized by consistently elevated arterial blood pressure. However, hypertension entails much more complicated hemodynamic changes than the previous definition describes. With respect to blood vessels, it is characterized by two main factors: (1) the structural remodeling of cerebral arteries via increasing wall thickness/lumen ratio\textsuperscript{107} and (2) impaired endothelium-mediated vasodilatation as a result of collagen build-up\textsuperscript{108}. Ultimately, the effects of decreased lumen size and decreased vessel distensibility may reduce both CBF and cerebrovascular reactivity. Abnormal changes in regional CBF have been documented in the past where hypertensive subjects had decreased CBF to the subcortical regions of the brain as well as to the limbic and paralimbic structures\textsuperscript{109}. Thus, not only may hypertension create a vulnerability state for the development of neurodegenerative disorders via oxidative stress mechanisms, but it may also be relevant to the development of depression since limbic structures of the brain are widely
known to regulate emotions and behaviour. Indeed, both of the aforementioned hemodynamic properties have previously been found to be impaired in subjects with depressive symptoms\textsuperscript{110, 111} where a reduction in blood flow velocity was especially prevalent in subjects suffering from a DSM-IV depressive disorder\textsuperscript{110}. Furthermore cerebrovascular reactivity (CVR) is significantly improved after antidepressant treatment at 21 months follow-up\textsuperscript{111}. Interestingly, CVR has also been found to be significantly reduced in a group of depressed patients free of vascular risk factors\textsuperscript{112}. This suggests that major depression may be the actual cause of CVR function rather than its consequence. A combination of results from both studies suggests that the link between hypertension and depression may be bidirectional with both leading to increased risk for stroke.

Because depressive syndromes have been shown to be comorbid with cerebrovascular lesions and cardiovascular risk factors, a vascular hypothesis to depression was introduced by Alexopoulos and colleagues in the late 1990s. Here, it was stated that “cerebrovascular disease may predispose, precipitate, or perpetuate some geriatric depressive symptoms”, and are uniquely attributed to cases of late-onset depression\textsuperscript{113}. Further research has described the symptomatology of the disease as distinct from non-vascular depression with more cognitive impairment, prominent psychomotor retardation, apathy, lassitude and pronounced disability\textsuperscript{114}. However, not all studies report a specific symptom profile of vascular depression\textsuperscript{115}. Nevertheless, the vascular depression hypothesis remains a topic of interest as positive associations between cardiovascular risk factors (i.e. hypertension\textsuperscript{114}, hyperlipidemia\textsuperscript{116}), cerebro-\textsuperscript{117} and cardiovascular diseases\textsuperscript{118}, diabetes\textsuperscript{119} and depression have been documented in the past.

In terms of size and location of the stroke lesion, Alexopoulos and his team postulated that the neurocircuitry responsible for processing emotions can be affected by not only one large
infarct but by several macrovascular or microvascular lesions. Here, neuroimaging data consisted of acute infarctions and white matter changes confined to three frontal-subcortical loops of the brain that were later described to be key features of vascular depression. In support of the multi-lesion hypothesis, one study found no relationship between focal vascular pathology and PSD in post-mortem elderly subjects\textsuperscript{31}. This finding suggests that the vascular burden from chronic accumulation of multiple lesions may be a crucial determinant to the etiology of PSD rather than a single large infarct from stroke\textsuperscript{120}. Two years prior, Brodaty and colleagues proposed the same neuroanatomical change of focus for PSD stating “depression after stroke may be related to cumulative vascular brain pathology rather than side and severity of single stroke”\textsuperscript{121}.

\textbf{1.4.1.6 Treatment of PSD}

Currently, antidepressants are prescribed to either prevent the occurrence of PSD or as treatment of a newly diagnosed case. In 2005, a Cochrane review was published examining the efficacies of both indications for therapy within a post-stroke population\textsuperscript{122}. The results, however, were discouraging as no evidence of improved mood or cognitive function was found in either the treatment or prevention group. Even at best, there were only some documented effects of reduced depression severity within the treatment group. These negative findings may reflect heterogeneity between studies, including differences in patient characteristics, methods of diagnosis, assessment tools of depression and cut-off scores used in those forms of assessments. Furthermore, patient recruitment varied from a few days to two years after stroke onset. This likely complicated the analysis since the balance of risks and benefits of pharmacotherapy may change over various stages of stroke recovery. Finally, the primary goals of therapy in many trials were unclear which made it difficult to define important outcomes such as remission and recovery. As a result of the methodological limitations encountered during the
review process, no successful recommendations regarding the applicability of antidepressants to post-stroke conditions were made.

In contrast to the Cochrane review, more recent data supports the use of antidepressants for the prevention of the disease. One RCT examining the SSRI escitalopram found the drug to be significantly superior to placebo in preventing the development of PSD\textsuperscript{123}. The same study also reported that problem-solving therapy, a manual-based intervention that originated from England for treating medical patients with depression\textsuperscript{124}, was significantly superior to placebo. Other classes of antidepressants have also been effective in preventing PSD, though the study designs were less rigorous. In one study, 5.7\% of patients receiving the tetracyclic acid (TeCA) mirtazapine developed PSD compared to 40\% of non-treated patients\textsuperscript{125}. While those proportions were significantly different, the study was not placebo controlled and participants were not blinded to treatment, which likely introduced a bias in favour of mirtazapine. As well, more than 90\% of people screened for recruitment were not enrolled as participants. Milnacipran, which can be classified as a serotonin and norepinephrine reuptake inhibitor (SNRI), was given open label to 11 patients and compared to historical controls and found to be associated with improved PSD in rehabilitative in-patients\textsuperscript{126}. Finally, a 2007 meta-analysis exploring prophylactic uses of antidepressants significantly favoured the treatment group outcome. The study examined 10 randomized clinical trials where a total of 703 non-depressed post-stroke patients were identified and reported a difference in PSD occurrence rate of about 17\% between treated and non-treated groups (PSD occurrence of 29.17\% and 12.54\% in non-treated and treated groups, respectively)\textsuperscript{127}. The authors also noted that the prophylactic effects of antidepressants were not related to duration of use. However, the previously quoted studies were the basis of that meta-analysis, thus study quality was a problem.
Additional publications suggest that antidepressants may reduce other post-stroke sequelae. Several studies have suggested their positive effect on abating symptoms of cognitive impairment that are often found co-morbid with PSD\textsuperscript{128-130}, and these improvements are likely to remain stable over the next 2 years in the absence of subsequent reinjury to the central nervous system\textsuperscript{131}. It has been suggested that post-stroke recovery from impaired activities of daily living (ADLs)\textsuperscript{132} and cognition\textsuperscript{133}, and reductions in aggression and irritability\textsuperscript{134} may also be enhanced by antidepressants if treatment were to begin within the first few months of stroke onset. Some physicians opt to prescribe antidepressants to stroke patients before a formal diagnosis of depression is made in order to prevent such disabilities. Although the prophylactic use of these drugs has been reported to significantly reduce the occurrence of PSD\textsuperscript{127}, it does not completely eradicate the disease and may manifest into different disease characteristics of PSD compared to patients without treatment. For example, one study randomly assigned non-depressed patients to receive nortriptyline, fluoxetine, or placebo for three months and examined the occurrence of depression thereafter\textsuperscript{135}. In patients treated with antidepressants, lesion volume and degree of social impairment were associated with late-onset PSD. In contrast, those who were on placebo developed PSD associated with the severity of ADL impairments. The differences in the clinical and pathological correlates between groups suggest that the pathophysiology of the disease changes with the use of antidepressants, and that these subtle differences may be important to post-stroke recovery.

Physicians are often reluctant to prescribe antidepressants to older stroke patients co-morbid for other medical illnesses due to the perceived risk of side effects (e.g. drug-drug interactions) leading to morbidity. Therefore, the selection of the antidepressant class with the lowest risk/benefit ratio is of great importance. Both selective serotonin reuptake inhibitors (SSRIs)\textsuperscript{136-138} and tricyclic antidepressants (TCAs)\textsuperscript{139} have been suggested to significantly
reduce depressive symptoms in some PSD patients. However, the use of TCAs in elderly populations with vascular disease remains a safety concern among the medical community. A systematic review comparing SSRIs and TCAs in patients vulnerable to stroke and cardiovascular disease found that those receiving TCAs were at significantly greater odds of developing non-serious cardiovascular adverse events (AEs) than those on SSRIs. These non-serious events include the following: heart palpitations, chest pain, angina, arrhythmia, hypertension and hypotension-syncope. Note, however, that serious cardiovascular AEs such as death, cerebrovascular disease, heart failure, TIA, stroke and MI were not associated with the use of TCA.

Interestingly, a couple of studies have documented potential benefits to SSRI use in cardiovascular disease patients. Most notably, congestive heart failure (CHF) patients on active aspirin medication yielded additional anti-platelet benefits during concomitant therapy with SSRIs. Prior data from the same research group found that the SSRI sertraline and its neurologically inactive metabolite N-desmethylsertraline (NDMS) exhibited potent, dose-dependent antiplatelet activity in both in vitro and ex vivo clinical scenarios in patients undergoing coronary stenting. These findings suggest that the use of SSRIs after ischemic stroke may translate into improvement of depressive symptoms and a decreased rate of mortality that would previously have been caused by unwanted cardiovascular AEs.

The findings above are likely the reason why SSRIs remain the most recommended treatment for PSD due to their believed favourable tolerability (e.g. less serious cardiovascular side effects, lack of anticholinergic effects). However, one must not discount the negative side effects of SSRIs, as they are known to elicit sexual dysfunction, weight gain, and sleep disturbances during long-term therapy. A recent study in post-menopausal women reported that those using SSRIs had a 45% increased relative risk of incident stroke and 32% increased
risk of death compared to those with no antidepressant use. When analyzed by stroke type, SSRI use was associated with incident hemorrhagic stroke and fatal stroke. The results are consistent with prior evidence suggesting SSRIs may increase the risk of abnormal bleeding due to their antiplatelet effects when combined with aspirin. Although the risk of death was similarly increased among TCA users, no significant differences in risk between SSRI and TCA users were found. However, it remains difficult to determine whether the non-significant results can be attributed to drug-type exposure alone or unaccounted differences in other cardiovascular risk factors. For example, depression has been an established risk factor for cardiovascular morbidity and mortality and has been associated with an increased risk of stroke. This raises the possibility that any “excess” risk to stroke may be caused by depression and not solely on drug use. Thus, it is necessary to assess the effect of different antidepressants specifically on the PSD population in order to clarify their side effects. The optimal length of treatment is unclear, though one research group recommends carrying on treatment for 4-6 months followed by slow withdrawal of the drug. Conversely, one meta-analysis reported that the prophylactic effects of antidepressants were not related to the duration of drug intake. Since there has been several documented cases of robust placebo responses during antidepressant trials, it would be necessary to examine the significance of placebo-effects before determining the best treatment time-course for PSD patients.

1.4.1.7 Co-morbid Depression and Cognitive Decline Post-stroke

Cognitive symptoms are hypothesized to be indicators of chronic depression in the elderly and have been found to persist in major depressive disorder (MDD) outpatients in remission. Although the appearance of cognitive deficits remains under scrutiny, several studies suggest that it may be immune-mediated. For example, higher levels of inflammation during baseline assessments have been shown to predict cognitive symptoms at follow-up in
depressed patients\textsuperscript{157}. Thus, inflammation may act as an initiator and contributor to cognitive symptoms of depression in the overall progression of the disease.

It has been reported that cognitive function and depressive symptoms are independently associated with risk of mortality within the elderly population\textsuperscript{158}. Here, the study participants were stratified into best, middle and worst groups based on scores collected from various depression and cognition scales. It was found that older participants in the worst cognitive function or worst depressive symptoms tertile at baseline had higher rates of mortality than those in the middle and best tertiles. Furthermore, for each grouping of cognitive function, greater depressive symptoms were associated with higher mortality rates, and for each grouping of depressive symptoms, worse cognitive function was associated with higher mortality rates. Thus, the combination of poor cognitive function and depressive symptoms appear to increase the likelihood of mortality in an additive fashion, suggesting that the risk for poor outcome is best explained by the cumulative effects of two or more risk factors.

With relation to post-stroke periods, one study related cognitive functioning to depression and anxiety whereby verbal learning and reduced cognitive speed were correlated with increased depression and anxiety scores\textsuperscript{159}. Furthermore, stroke patients with both depression and cognitive impairment have been shown to present with longer durations of depression than depressed patients without cognitive impairment\textsuperscript{160}. This finding was most pronounced during the initial evaluation period of the study and highly associated with left hemispheric lesions. Whether the acute post-stroke period and left hemispheric lesions are absolutely related to co-morbid depression and cognitive decline requires further research. However, it is apparent that cognition and mood post-stroke are linked and neither should be ignored during the rehabilitative process.

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1.4.2 Inflammatory Processes and Stroke

1.4.2.1 Time course of inflammatory molecules post-stroke

Experiments inducing focal ischemia in rats via medial cerebral artery occlusion (MCAO) have given insight to the inflammatory events that occur post-stroke. Circulating monocytes have been detected within the capillaries and venules after 4 to 6 hours of induced ischemia\textsuperscript{161}. The study also detected accumulation of polymorphonuclear neutrophils (PMNs) 12 hours after the same injury\textsuperscript{161}, though PMN infiltration may occur within 6 hours and with greater severity in reperfused tissues than in permanently occluded tissues\textsuperscript{162}. Mechanistically, it has been reported that leukocytes and PMNs exacerbate tissue damage via edema formation, physical obstruction through adherence to the vessels, and the release of oxygen radicals, pro-inflammatory cytokines, and cytolytic enzymes leading to cellular necrosis\textsuperscript{162-164}. In support of these findings, administration of inflammatory markers into rat models has been found to augment increases in brain water content and tended to correlate with neutrophilic infiltration into the parenchyma\textsuperscript{165}.

1.4.2.2 Cytokines

Cytokines are small, soluble glycoproteins (~15-25kd) that function locally or systemically to orchestrate immune responses. They are produced by a variety of activated cell types including endothelial cells, platelets, leukocytes, and fibroblasts\textsuperscript{166}. Additionally, autocrine, paracrine, or endocrine effects are possible, depending on the particular cytokine\textsuperscript{105}. Once released into the environment in response to an activating stimulus (e.g. stroke), they control the growth and differentiation of different cell types through specific membrane receptors\textsuperscript{105}. Ultimately, intracellular signaling pathways are activated and alter gene transcription patterns within the cell, one of which modulates the expression of their own specific target cell receptor.
In addition to being mediators of inflammation, cytokines also induce the expression of cell adhesion molecules on endothelial cells that propagates cerebral ischemia and contribute to tissue damage via alterations in levels of endogenous mediators such as tissue-plasminogen-activator (tPa)\textsuperscript{105}. One post-mortem study of elderly patients showed that intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) were increased in the cerebral endothelium in late life depression\textsuperscript{167}. Combining these two findings suggests a possible causal relationship between inflammatory cytokines and MDD.

1.4.2.3 Cytokines and Stroke: Pro- Versus Anti-inflammatory Cytokines

Cytokines are not stationary inflammatory markers and thus, are able to coordinate immune responses between several physiological systems in the body. Post-stroke plasma elevations of different cytokines\textsuperscript{168-172} (including IL-1β, IL-6, IL-8, and TNF-α) and their mRNA expression in mononuclear cells\textsuperscript{168-172} have been previously reported and are significantly correlated with brain infarct volume\textsuperscript{171, 173}, functional disability\textsuperscript{173}, risk of recurrent stroke\textsuperscript{172}, and less favourable prognosis\textsuperscript{171}. Thus, not only is the inflammatory response related to stroke severity, but clinical outcome as well. Two major categories of cytokines are the pro- and anti-inflammatory cytokines which play opposite roles in regulating stroke outcome.

Pro-inflammatory cytokines (IL-1, TNF-α, IL-6, IL-8 and IL-18) function to amplify immunological responses during reperfusion injury after acute cerebral ischemia. However, they may also induce the depressogenic effects in depressed patients\textsuperscript{174, 175}. For example, increased levels of IL-1β, TNF-α and IL-6 have been observed in the cerebrospinal fluid (CSF)\textsuperscript{176, 177} and plasma\textsuperscript{178-183} of patients suffering from mood disorders. In addition, inhibition of these cytokines and their respective signaling pathways can improve depressed mood\textsuperscript{184}. Furthermore, both acute and chronic infectious and non-infectious diseases that primarily affect immune regulation are often reported to be accompanied by depression\textsuperscript{185-188}. Note, however,
that the opposite is also true where immune dysregulation is found to be common in major depression disorder, suggesting that depression by nature may be an autoimmune disease. In contrast to pro-inflammatory cytokines, anti-inflammatory cytokines (IL-10, IL-4 and insulin-like growth factors (IGF)) are proposed to have beneficial effect to the injured brain. In one animal model, IL-10 administration to MCAO treated rats reduced infarct size compared to vehicle treated controls. The opposite is also true where low levels of IL-10 have been linked to larger infarct size and early neurological deterioration. Additionally, both IL-10 and IGF have been shown to block lipopolysaccharide (LPS) and TNF-α induced behavioural changes in rats. Although these results implicate neuroprotective properties of anti-inflammatory cytokines to depression, their exact roles as immune modulators are clouded by studies that have associated both increased and decreased IL-10 levels with poor outcome and neurological worsening. Still, the balance between pro- and anti-inflammatory cytokines must also be considered, highlighted by an elevated IL-6/IL-10 ratio in major depression.

Although the unfortunate prognosis of PSD has been well-documented, there is limited research available explaining why certain post-stroke fates occur and the underlying mechanisms involved in their development. With regards to mortality, the relationship between pro- and anti-inflammatory cytokines may provide clues as to why the rate of mortality is significantly higher in the PSD population compared to healthy aging individuals. Here, robust evidence has been gathered from experiments supporting the development of an “immunodepression syndrome”, as summarized by two recent publications. It is hypothesized that the fate of injured brain tissue is dependent on the intricate balance between pro- and anti-inflammatory cytokines where divergence from their naturally occurring concentrations is thought to induce an immune response. Such prominent changes in
inflammatory markers are believed to facilitate systemic infection, thus resulting in a higher risk for mortality post-stroke.

1.4.3 Inflammatory Processes and Depression

1.4.3.1 Sickness Behaviour

Sickness behaviour is a collective term describing a constellation of behavioural disturbances in animal models that mimic the depressive symptoms of humans. These characteristics are comprised of malaise, decreased motivation (fatigue, lethargy, adipsia and anorexia), decreased pleasure (anhedonia), psychomotor retardation, affective and cognitive changes, hyposomnia or hypersomnia, decreased physical and social activities, poor concentration and confusion. Current research suggests that inflammatory response mechanisms may play a role in such behavioural disturbances. For example, administration of the bacterial endotoxin LPS or inflammatory cytokines in animals produces some of the behavioural characteristics encompassed in sickness behaviour. Furthermore, the absence of pro-inflammatory genes may inhibit the appearance of these depressive characteristics. In support of this, one study found that mice deficient for the IL-6 gene are less likely to exhibit the depressiogenic effects induced by LPS administration compared to mice that do express the IL-6 cytokine.

1.4.3.2 Animal models of stroke

Behavioural correlates of depressive-like symptoms in animal models mainly include: 1) reduced preference for sucrose solution when both the sweetened liquid and water are made available and 2) reduced locomotor and exploratory activity. The former observation is an indicator of anhedonia and the latter suggests changes in incentive and motivation. A recent study demonstrated both these symptoms in a novel animal model of stroke where MCAO
treated rats exhibited decreased locomotor activity and reduced sucrose solution preference\textsuperscript{208}. Moreover, the observed behavioural abnormalities were ameliorated after the administration of citalopram. These results are congruent with another study that also reported reduced sucrose intake in MCAO-treated rats compared to control rats. However, the researchers took a different approach to restore sucrose consumption. Instead, they administered the IL-1 receptor antagonist (IL-1Ra) to MCAO-treated rats and found that it produced the same alleviation of behavioural disturbances as antidepressants in the previously mentioned study\textsuperscript{209}. Results from both findings not only confirm the presence of depressive symptoms post-stroke, but also suggest that the disease may be immune-mediated.

1.4.3.3 Clinical Cases of Immunotherapy or Immune Challenges

Clinically, cytokines have been shown to be effective in the treatment of various medical conditions including hepatitis C, leukemia, multiple sclerosis, Kaposi’s sarcoma, melanoma, myeloma, renal carcinoma and other forms of cancer. However, cytokine immunotherapy may also lead to pro-inflammatory molecule release causing depression\textsuperscript{210-217}, and may even exacerbate preexisting psychotic symptoms\textsuperscript{218}. For example, the therapeutic use of cytokines (e.g. IL-2, IFN-a) in the treatment of malignancies and chronic viral infections is frequently complicated by cognitive, emotional, and behavioural disturbances\textsuperscript{219}. Moreover, antidepressant therapy can prevent or attenuate cytokine induced depression\textsuperscript{220-223} and may even possess a cytokine-linked anti-inflammatory effects\textsuperscript{222, 224-228}. These findings have opened up new avenues for alternative treatments to depression, since pro-inflammatory cytokine inhibitors and anti-inflammatory drugs may be able to prevent or attenuate depressive symptoms in humans.

In cases of immune challenges, one study found that mild stimulation of the primary host defense in humans (using a safe and well-tolerated dose of endotoxin) produces negative effects on both emotional and cognitive function\textsuperscript{229}. Elevations in both anxiety and depression levels
have been reported in other human cases of immune challenges\textsuperscript{186, 230} with those of lower socioeconomic status most affected\textsuperscript{231}. All together, these findings suggest that pro-inflammatory cytokines may play a crucial role in the pathogenesis of mood disorders in humans, with implications for novel cytokine targeting therapies in neuropsychopharmalogical research.

1.4.3.4 Cytokines in Clinical Depression and Cognitive Decline

The involvement of cytokines in the etiology of psychiatric disorders has been extensively reviewed in the past by various authors\textsuperscript{174, 175, 184, 232, 233}. Under physiological conditions, cytokine concentrations remain low in the body but are up-regulated during various disease states, particularly those involving inflammation. With regards to depression, both experimental and epidemiological research has found that depressed people have higher levels of inflammatory markers\textsuperscript{52-58, 234}. For example, one study examined the levels of inflammatory cytokines in a community-based sample of already depressed elderly subjects and found that persons with depressed mood had higher median plasma levels of IL-6 and TNF-α\textsuperscript{57}. These results became more significant after adjustments for health and demographic variables, suggesting that depressed mood is either causing or caused by systemic inflammation.

Clinical studies have associated depression with increased levels of systemic cytokines and acute phase proteins\textsuperscript{52, 58, 235-238} although such results were not always reproducible\textsuperscript{239, 240}. Note, however, that the complex balance between pro- and anti-inflammatory cytokines may play a crucial role in the development of mood disturbances, highlighted by reports of elevated IFN-γ to IL-4\textsuperscript{70, 241} and IL-6 to IL-10\textsuperscript{69} ratios in major depression. This may explain why a few studies did not find a positive correlation between pro-inflammatory cytokines and depression since none of them explored the significance of this balance. In addition, one of the authors based their concentrations on lipopolysaccharide (LPS) induced peripheral blood mononuclear
cell (PBMC) cytokine production, which may be drastically different from the natural biological concentrations of interest.

Cytokines are believed to affect cognitive function via active transport into the CNS through afferent neurons that centrally activate cognitive responses, or upon local release by activated microglia causing neurodegeneration\textsuperscript{242}. As previously mentioned, the neuropsychiatric effects of cytokine therapy on cancer and HIV patients encompasses cognitive changes that may involve a decline in verbal memory, cognitive speed and executive function\textsuperscript{216, 243, 244}. Other forms of neurological disease associated with cognitive impairment have been well documented in the past. Most notably, the severity of dementia in Alzheimer’s disease patients is often accompanied with higher levels of circulating cytokines than controls, including IL-1\textbeta\textsuperscript{245-248}, IL-6\textsuperscript{247, 249, 250}, IL-18\textsuperscript{251}, and TNF-\alpha\textsuperscript{248}, although conflicting reports exist\textsuperscript{251, 252}. This is not a disease related phenomenon since healthy aging individuals who experience cognitive decline also exhibit higher levels of inflammatory markers\textsuperscript{253-257} independent of demographic status and social status, and may also be at higher risk for mortality\textsuperscript{258}. Although the mechanism of cytokine-induced cognitive changes has yet to be elucidated, it has been suggested to involve damages to the fronto-subcortical circuitry. For example, one study found that stroke lesions of the frontal lobe were associated with amusia (the absence of music perception), and that the disorder also entailed general cognitive deficits in working memory, learning, semantic fluency, executive functioning, and visuospatial coordination\textsuperscript{259}. Therefore, post-stroke elevations in cytokines may contribute to post-stroke cognitive deterioration alongside depression.

The precise mechanisms behind cytokines and their ability to induce radical changes in mood states remain unanswered. Generally, it is believed that increased peripheral cytokine levels enter the brain and alter neurotransmitter metabolism, neuroendocrine function, and neural plasticity mechanisms responsible for mood regulation\textsuperscript{184}. Additionally, cytokine-mediated
interactions between neurons and glial cells may contribute to cognitive impairment (especially in cases of memory and learning) via alterations in cholinergic and dopaminergic pathways\textsuperscript{242, 260}. The next step would be to determine the cytokine concentrations and their time of activation post-stroke to create a more detailed definition of their pathological outcome. Nevertheless, elevations in cytokine levels may be useful prognostic markers for PSD despite their detrimental effect to neuronal functioning.

1.4.4 Current Hypotheses on the Etiology of Post-Stroke Depression

The etiology of PSD is thought to be multifactorial involving both psychosocial vulnerability and biological mechanisms. In addition, several scientists believe that location of the infarct plays a crucial role in understanding the mechanisms behind the disease. Thus, three major hypotheses currently exist in the etiology of PSD: lesion location, psychosocial factors, and biological factors\textsuperscript{261}.

1.4.4.1 Lesion Location Hypothesis

Those who support the lesion location hypothesis believe that PSD is directly caused by focal damage to brain regions involved in the mood regulatory system. An early pioneer of the lesion location hypothesis is Robinson and his team who were the first to report that left-hemisphere lesions located to the vicinity of the frontal pole were more frequently associated with PSD than lesions elsewhere in the brain\textsuperscript{82}. Since then a couple of other studies have reported the same phenomenon\textsuperscript{74, 159, 262, 263}. These findings were encouraging to the lesion location hypothesis since they support theories regarding: 1) frontal structures in the regulation of emotional behavior and 2) lateralized differentiation in the organization of emotion, where the left side of the brain is more often activated than the right side during emotional processing\textsuperscript{264, 265}. In support of this, a MRI study found that left lesions of the frontal-subcortical
circuits (i.e. pallidum and caudate) predisposed stroke patients to depression with size of infarcts being larger in depressed patients\textsuperscript{12}. Furthermore, the severity of depression has been associated with left frontal lobe damage, with the exception of the basal ganglia\textsuperscript{266}. However, two more recent systematic reviews\textsuperscript{267, 268} and several other studies\textsuperscript{76, 89, 269-273} failed to confirm the association between left-sided strokes and depression, while one review stated such an association varied depending on whether patients were sampled as inpatients or from the community\textsuperscript{97}. Post-stroke cognitive impairment may also be related to lesion location as comorbid MDD and cognitive impairment has been associated with left-sided lesions\textsuperscript{274}.

To counter the unsuccessful replication of their findings, Robinson and colleagues carried out a meta-analysis\textsuperscript{275} and claimed to have found an interaction between lesion location and time of PSD onset. Here, left-hemisphere lesions would increase the risk of PSD especially in the first couple of months after stroke, whereas in the subacute to chronic course, psychosocial factors would play a more important role. In addition, it was suggested that the proximity of the left hemispheric lesion to the frontal pole was correlated with depression severity, but not in cases of right-hemisphere stroke\textsuperscript{276}. However, prior to such claims, Gainotti and his team had tested this same assumption and found that symptom profiles and the anatomical and clinical correlates of major PSD were no different in the acute and chronic stages of stroke\textsuperscript{270}. Currently, confirmation or rejection of Robinson and colleagues’ findings has yet to be elucidated. However, many researchers agree that the etiology of PSD does not and cannot rely on the lesion location theory alone.

1.4.4.2 Psychosocial Factors Hypothesis

The psychosocial hypothesis states that PSD is a psychological reaction to the physical devastation caused by stroke whereby disability is the major predictor to disease development. Several studies have supported this hypothesis\textsuperscript{22, 270, 277, 278} even after controlling for other stroke
characteristics including size and location of infarct\textsuperscript{85, 89, 279}. Lack of social companionship\textsuperscript{272, 273, 280} and absence of family support\textsuperscript{74} have also been associated with the development of PSD and are predictive of depression severity at six months post-stroke\textsuperscript{272}. Additionally, the development of functional disabilities as a result of the stroke may lead to greater depressive symptoms which would further complicate functional outcome\textsuperscript{20, 33, 281}. In support of this, improvement of depressive symptoms post-stroke has been also associated with better functional recovery\textsuperscript{88}.

Although the severity of functional disability has been shown to be a strong predictor of depression at 3 months post-stroke\textsuperscript{74, 85} the association disappears after around 12 to 24 months post-stroke\textsuperscript{74, 85, 279}. Instead, few social contacts outside the immediate family and diagnosis of the disorder at an in-hospital setting contributes most to depression at those times. Thus, disability may only be a risk factor for depression in the acute and subacute post-stroke period, where it does not determine the onset of depression per se, but interacts with it to limit the results of long-term rehabilitation\textsuperscript{280}. These suggestive findings have led to the hypothesis that PSD is at least, in part, a consequence of the direct biological consequence (e.g. immune response) of the stroke.

\textbf{1.4.4.3 Other Biological Factors}

It has been hypothesized that during acute brain infarction, there is decreased monoamine synthesis leading to decreased serotonin levels in the brain. In blood, serotonin is present in high concentrations in platelets and released into the plasma when platelets aggregate at the site of tissue damage. It is then subsequently catabolized by monoamine oxidase enzyme activity in the liver and lungs. In the CNS, serotonin is present in high concentrations in localized regions of the midbrain, serving as a neurotransmitter. The level of CSF or brain serotonin remains controversial—some studies associate MDD with reduced levels of CSF serotonin and increased levels of serotonin turnover\textsuperscript{282, 283} whereas other studies find no
differences in CSF concentrations of serotonin metabolite (5-hydroxyindoleacetic acid) between depressed and healthy subjects\textsuperscript{284, 285}.

It has been proposed that PSD is caused by ischemic brain lesions that directly disrupt neural circuits involved in mood regulation\textsuperscript{286}. In particular, it has been postulated that stroke lesion interrupts the biogenic amine containing axons ascending from the brainstem to the cerebral cortex, thereby leading to a decreased production of serotonin and norepinephrine in uninjured limbic structures of frontal and temporal lobes as well as basal ganglia\textsuperscript{287}. Thus, deficits in important mood regulatory neurotransmitters or failure of the body to up-regulate their respective receptors after stroke may trigger PSD. This hypothesis has been supported by studies demonstrating significantly lower CSF and plasma concentrations of serotonin\textsuperscript{288} and CSF concentrations of its metabolite, 5-hydroxy-indoleacetic acid\textsuperscript{289}, in PSD patients compared to non-depressed stroke survivors. Moreover, one study reported an inverse correlation between serotonin receptor binding in the left temporal cortex and HAM-D scores of depressed patients\textsuperscript{290}.

In further support of the biological hypothesis, one study found that the most commonly reported symptoms in a group of post-stroke patients were discouragement/hopelessness, self-criticalness, tiredness and feelings of being punished\textsuperscript{159}, all of which describe non-physical symptoms. Furthermore, a community-based post-stroke cohort was found to have an elevated risk for clinically significant depressive symptoms that was not mediated by functional disability\textsuperscript{35}. Lastly, one study reported an association between the presence of small silent strokes and late-onset depression, suggesting that patients who are unaware of their existing cerebral lesions develop depression independently of psychological mechanisms related to functional debilitation\textsuperscript{291}.  

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1.4.5 Neurological Consequences of Reduced Serotonin Synthesis and the Formation of Neurotoxic Metabolites

1.4.5.1 Serotonergic Hypothesis of Depression

The serotonergic hypothesis of depression has been in existence for over 40 years\textsuperscript{292} and proposes that diminished activity of 5-HT pathways is responsible for the pathophysiology of depression. The hypothesis was evidenced by countless observations of patient response to treatment with tricyclic antidepressants in which inhibition of 5-HT and noradrenaline reuptake was presumed to enhance 5-HT-mediated neuroendocrine responses. Moreover, the development of the more efficacious SSRIs suggested that depressive symptoms can sufficiently be attenuated as a result of increased synaptic 5-HT concentration\textsuperscript{293}. Today, 5-HT is recognized to influence many symptoms of depression, including mood, appetite and sleep patterns, physical activity, sexual interest and cognitive dysfunction.

Imaging investigations of the 5-HT receptors via ligand detection in conjunction with positron emission tomography (PET) or single photon emission computed tomography (SPECT) have provided consistent and convincing evidence that the binding density of 5-HT\textsubscript{1A}\textsuperscript{294, 295} and 5-HT\textsubscript{2}\textsuperscript{296} receptors is significantly decreased in MDD patients. The reduction in receptor binding density was particularly prominent in areas of the brain involving the amygdala\textsuperscript{297}, midbrain\textsuperscript{297, 298}, and brainstem\textsuperscript{299}. Age-related effects may also be of significant importance in which reduced receptor binding density occurs with increasing age\textsuperscript{298, 300, 301}. Similar results have been reported for the serotonin transporter (SERT), where drug-free unipolar depressed patients exhibited reduced SERT availability in the brain stem\textsuperscript{299}. However, these findings are not necessarily reproducible as no difference in SERT availability between depressed and healthy volunteers has also been documented\textsuperscript{302}. Similarly, no significant difference in 5-HT binding potential was found between recovered depressed patients and healthy controls in one study\textsuperscript{303} while another study documented widespread persistent reductions in 5-HT receptor
binding among patients who have already recovered from the disease. Taken together, these results suggest that reduced binding density may represent a genetic abnormality that confers vulnerability to recurrent major depression rather than an observation made only during the course of the disease\textsuperscript{303}.

In relation to post-stroke cases, the presence of an acute infarct is hypothesized to reduce monoamine synthesis (e.g. 5-HT) within the brain. In the past, PSD patients have been consistently documented to have lower CSF concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid compared non-PSD patients\textsuperscript{289}. More recently, one study measured the CSF levels of serotonin in PSD patients\textsuperscript{288} and compared these levels to plasma concentrations of serotonin. It was reported that serotonin concentrations in the CSF and plasma of PSD patients were considerably lower than non-depressed patients and that a greater number of PSD patients had lower than normal serotonin concentrations than the control group. Furthermore, a sound correlation was found between the plasma and CSF serotonin concentrations in both PSD and control patients, suggesting that plasma concentrations of serotonin may be useful in predicting the central concentrations in patients with stroke or depression.

1.4.5.2 Tryptophan and Central Serotonin Synthesis

Tryptophan is an essential amino acid that can only be obtained through external sources. Once absorbed by the body, tryptophan travels around the peripheral circulation in equilibrium between its albumin-bound and free form, the former comprising of 90% of the body’s tryptophan level\textsuperscript{42}. Tryptophan can only be transported across the BBB in its free form by the L-type amino acid transporter\textsuperscript{304} and once in the CNS, tryptophan acts as a precursor in various metabolic pathways. The end products of these pathways are comprised of various proteins, kynurenine, and serotonin\textsuperscript{42}. Note that brain TRP levels can be considered a reflection
of its plasma levels since TRP transport across the BBB directly affects the amount of metabolites synthesized.

Tryptophan hydroxylase (TPH) is located in the cytoplasm of neurons of the raphe nuclei and acts as the rate limiting enzyme of 5-HT synthesis. Using molecular oxygen and the cofactor tetrahydobiopterin, it catalyzes the hydroxylation of TRP into 5-hydroxytryptophan (5-HTP). A subsequent decarboxylase enzyme then rapidly converts 5-HTP into 5-HT using pyridoxal-5'-phosphate as co-factor\(^4\). Serotonin is then either: 1) bound by the carrier protein serotonin-binding protein (SBP) and released into the extracellular fluid (synapse) via calcium-dependent exocytosis; or 2) metabolized into 5-hydroxyindoleacetic acid (5-HIAA) by monoamine oxidase enzymes and excreted from the cell via energy dependent mechanisms. Following release into the synapse, 5-HT can be recycled back into the neuron through SERT reuptake located on the pre-synaptic membrane or metabolized into 5-HIAA in the post-synaptic neuron.

Several researchers have examined the role of TRP and 5-HT in depression and the clinical and neuropsychological consequences of abnormal 5-HT activity in the brain. For example, one of the most reliable pieces of evidences linking 5-HT abnormalities to depression was first documented in the mid-1980s, where total plasma tryptophan was significantly lower in depressed patients compared to controls\(^5\). More recently, researchers have constructed a technique to induce a brief tryptophan depleted state in healthy subjects in order to assess the mood effects of reducing central 5-HT function. This is simply accomplished by administering an amino acid mixture free of TRP; thus, lowering both plasma and brain TRP concentrations. Because the rate limiting step of 5-HT synthesis is determined by TPH activity, and because TPH is only 50% saturated with TRP under normal physiological conditions\(^6\), tryptophan depletion is predicted to produce a transient lowering of central 5-HT activity.
The return of depressive symptomatology as a result of TRP depletion has been documented in recovered depressed patients withdrawn from medication\textsuperscript{307, 308}. However, TRP depletion appears insufficient to cause clinically defined depressive symptoms in healthy volunteers\textsuperscript{308}. Although the difference in response to tryptophan depletion between the two populations has yet to be elucidated, it has been postulated that depression may be the result of persistent neurobiological changes that produce clinically significant mood abnormalities when affected individuals are exposed to low 5-HT activity\textsuperscript{309}. The types of neurobiological changes remain undefined but are hypothesized to involve altered or impaired innervations in neurocircuitry that consistently leads to negative biases in emotional perception. Whatever the mechanism, it can be said that tryptophan depletion studies generally support a casual relationship between decreased central 5-HT and depression, and that those with risk factors for depression or a family history of depression are most affected.

1.4.5.3 Central Tryptophan Availability

Knowledge underlying the specificities of tryptophan uptake into the serotonergic neurons remains scarce. In its most simple definition, it is generally agreed upon that a dynamic, regulatory barrier exists between plasma and intraneuronal tryptophan concentrations. Although it is known that the L-type amino acid transporter is responsible for tryptophan transport into the brain, two important factors surrounding this transport system must be considered in central tryptophan availability: 1) the ratio between free tryptophan to its albumin-bound form since only the free fraction crosses the BBB; and 2) the competitive nature of other large neutral amino acids (LNAA = tyrosine, valine, leucine, isoleucine and phenylalanine) for entry into the brain via the same transporter.

Several studies have reported lower tryptophan and tryptophan to LNAA ratio (TRP/LNAA) in MDD patients compared to healthy controls\textsuperscript{305, 310-312} or subjects with obsessive
compulsive disorder (OCD)\textsuperscript{311}. The ratio appears to be most correlated with depressed mood\textsuperscript{313}, retardation\textsuperscript{313}, and melancholic features\textsuperscript{314}. While some studies demonstrated reduction by using total tryptophan, others have found reductions in only free tryptophan. Depression severity has also been related to lower TRP/LNAA ratio among the depressed population\textsuperscript{315}, though the opposite finding has also been documented\textsuperscript{312}. TRP/LNAA analysis may also successfully predict treatment response to antidepressants, with a lower baseline TRP/LNAA ratio indicative of greater improvements in depressive symptoms\textsuperscript{316-318}. Additionally, one study reported that good responders exhibited a steady increase in TRP/LNAA ratio as the length of treatment proceeded for six weeks\textsuperscript{319}. All in all, there is substantial evidence to support the relationship between reduced central tryptophan availability and the presence of depression. Indeed, increased brain tryptophan availability can increase brain 5HT synthesis\textsuperscript{320}. However, these findings have yet to be tested among the PSD population.

1.4.5.4 Kynurenine Biosynthesis and Metabolism

Tryptophan is a ubiquitous molecule that is not only essential for protein synthesis but plays a crucial role in central 5-HT metabolism. Additionally, the amino acid is a precursor for the kynurenine pathway of metabolism in astrocytes, infiltrating macrophages, microglia and dendritic cells\textsuperscript{321, 322}. The end products include kynurenine and several of its metabolites, some of which are neurotoxic and others which are neuroprotectant. Under this pathway, the indole ring of tryptophan is first metabolized by the rate limiting enzymes tryptophan 2,3-dioxygenase (TDO), found highly concentrated in hepatic cells\textsuperscript{323}, or by indoleamine 2,3-dioxygenase (IDO), most prominently expressed by cells in the CNS and infiltrating macrophages\textsuperscript{321, 324}. It can then be inferred that TDO regulates homeostatic plasma tryptophan concentration in non-inflammatory conditions while the extra-hepatic IDO predominantly maintains brain tryptophan levels and is thus, of special interest in to PSD.
IDO is up-regulated in response to infection and tissue inflammation by certain cytokines and can be induced in vitro by other inflammatory molecules such as LPS and amyloid peptides. Once IDO metabolizes tryptophan into kynurenine (KYN) it proceeds along the pathway until nicotinamide adenosine dinucleotide (NAD) is achieved as the final product. Several neuroactive intermediates are generated during this process and comprising of: 1) 3-hydroxykynurenine and 3-hydroxyanthranilic acid, both free-radical generators; 2) quinolinic acid, the excitotoxin and selective N-methyl-D-aspartic acid (NMDA) receptor agonist; and 3) kynureninc acid (KA), a neuroprotective NMDA antagonist. A detailed diagram of TRP metabolism and its products are displayed in Figure 1. Since the kynurenine pathway produces both neurotoxic and neuroprotective metabolites, the ratio between these products are of great importance when correlating their concentrations to the pathological consequences of PSD. For example, the KA/KYN ratio has been show to be decreased in depressed hepatitis C patients undergoing IFN-α therapy and MDD patients.

Several pro-inflammatory cytokines possess the ability to induce IDO activity, including IFN-α, IFN-β, IFN-γ, IL-2, IL-6, IL-18, and TNF-α. Among this list, IFN-γ has consistently been reported to be the most potent regulator of the enzyme. Conversely, the anti-inflammatory cytokine IL-4, IL-10 and TGf-β act to inhibit IDO activity. Interestingly, one study found that IL-10 is able to suppress IFN-γ induced IDO expression in cells derived from the hypothalamic-pituitary-adrenal axis (HPA). It cannot be stressed enough that the balance between pro- and anti-inflammatory cytokines is crucial for homeostatic maintenance of the human body. Therefore, an excess of pro-versus anti-inflammatory cytokines have the potential to cause mood dysfunction in several neurological diseases if IDO activity exceeds its normal boundaries of TRP metabolism.
Figure 1. The KYN Pathway: TRP Metabolism and its Products
1.4.5.6 Linking Cytokines and Tryptophan Metabolism to the KYN/TRP ratio

A newer hypothesis to the etiology of depression involving the metabolic pathways of tryptophan was recently described\(^3\). Increased cytokine levels (such as in the case of stroke) upregulates IDO activity, an enzyme that is widely distributed in the intestinal tissues, lungs, placenta and brain\(^{291, 343, 346-349}\). Because it is in direct competition with TPH for the metabolism of tryptophan into 5-HT, heightened IDO activity as a result of elevated in cytokine levels may reduce tryptophan availability for 5-HT production. Consequently, central serotonin concentrations become sparse, leading to mood complications and behavioural disturbances in the affected individual. In support of this hypothesis, several studies have shown a significant decrease in serum TRP concentrations in patients treated with cytokines\(^{350-352}\). In addition, TRP levels have been found to be decreased in depressed patients\(^{314}\) while the administration of TRP produces an antidepressant effect\(^{340}\). Based on such findings, one is inclined to postulate that clinical conditions associated with increases in pro-inflammatory cytokines lead to activation of the IDO enzyme, subsequently resulting in decreased 5-HT synthesis and thus, increased risk of depression.

We postulate that the mechanism above may have a significant effect on the etiology of PSD. To reiterate, elevations in post-stroke cytokine levels shunts tryptophan metabolism from the serotonin pathway into the kynurenine pathway via upregulation of the IDO enzyme. Under the kynurenine pathway, tryptophan is catabolized into kynurenine; thus reducing the availability of tryptophan to be converted into the mood regulatory neurotransmitter 5-HT. Kynurenine is subsequently broken down into several neuroactive intermediates, including three potent neurotoxins; the hydrogen peroxide generators, 3-hydroxykynurenine and 3-hydroxyanthranilic acid\(^{223}\) and the NMDA agonist, quinolinic acid (QUIN)\(^{335}\). Indeed, one study reported IFN-\(\alpha\) immunotherapy treatment in hepatitis C patients significantly increased
peripheral blood KYN\textsuperscript{353}. This was accompanied by marked increases in CSF KYN and QUIN and correlated with the severity of depressive symptoms.

Several studies have examined the effects of the neurotoxic kynurenine metabolites. 3-hydroxykynurenine levels are elevated in Huntington’s disease patients\textsuperscript{354, 355} and has been shown to induce neuronal cell death in cortical and striatal cell cultures\textsuperscript{44}. Intracerebral injection of 3-hydroxyanthranilic acid can decrease choline acetyltransferase activity\textsuperscript{356}, thus, decreasing acetylcholine synthesis. QUIN is produced by infiltrating macrophages, microglia and dendritic cells under inflammatory conditions and acts as a potent agonist of the neuronal NMDA subtype of glutamate receptors\textsuperscript{355}. Moreover, several neurodegenerative conditions have been linked to QUIN-induced apoptosis, including Huntington’s disease\textsuperscript{357} and HIV-associated dementia\textsuperscript{358}. Most recently, it was suggested that KYN preferentially metabolized along the QUIN pathway at the subcortical level (amygdala/striatum) in mice models\textsuperscript{359}. This has its importance since a reduction in 5-HT receptor binding density has been associated with MDD and is most pronounced in the amygdala. Altogether, it is possible that these metabolites inflict further damage to the post-stroke brain by destroying essential neuronal pathways responsible for regulating mood and cognition.

Because measurement of IDO activity has not been thoroughly explored in stroke and is difficult to measure, the kynurenine to tryptophan ratio (KYN/TRP) has been proposed as a marker for its activity instead. The KYN/TRP is a reliable marker of IDO activity based on its reproducibility in past studies examining depression and related neurologic diseases. For example, enhanced TRP degradation and higher KYN/TRP ratios in Alzheimer's disease, Parkinson's disease, Huntington's disease, cancer, malaria, and rheumatoid arthritis have been associated with advanced stages of the diseases and more severe symptoms and fatal outcomes\textsuperscript{360-367}. Furthermore, it has been reported that the inflammatory marker neopterin, is correlated
with both KYN/TRP ratio and kynurenine concentrations and inversely correlated with tryptophan in cases of immune activation\textsuperscript{368, 369}. Elevations in the KYN/TRP ratio has not only been associated with the severity of well-known inflammatory diseases but has also been linked to cases of depression. Individuals undergoing cytokine therapy for hepatitis C and cancer developed depressive symptoms that were specifically linked to increases in plasma KYN and KYN/TRP ratio\textsuperscript{38, 337}. Similar increases in the KYN/TRP ratio have also been found in women with post-partum depression\textsuperscript{39, 370}. Most recently, our group demonstrated that a higher KYN/TRP ratio was significantly associated with greater depression scores in coronary artery disease (CAD) patients, a relatable population to the stroke population\textsuperscript{41}. Taken all together, these findings support the possibility of a biological contribution to PSD (alongside the current psychosocial models) through IDO activation, whereby upregulation of IDO activity stimulates the KYN pathway; thus depleting the amount of TRP available for 5-HT synthesis and increasing the levels of KYN neurotoxins. A summary of KYN/TRP levels in psychiatric, neurodegenerative, and inflammatory illnesses is displayed in Table 1.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
Illness & KYN/TRP Ratio & Neurotoxin Levels \\
\hline
Psychiatric & High & Elevated \\
Neurodegenerative & Moderate & Normal \\
Inflammatory & Low & Low \\
\hline
\end{tabular}
\caption{Summary of KYN/TRP Levels}
\end{table}

\subsection*{1.4.6 Rationale for this Study}

Currently, no definite relationship has been established between pro-inflammatory cytokine levels and PSD in humans. This study attempts to determine the relationship between such and expects to find a positive causal relationship between plasma pro-inflammatory cytokine concentrations and depression after stroke. In addition, this study also aims to examine the counterintuitive reports of elevated anti-inflammatory cytokine levels\textsuperscript{192, 191} in post-stroke patients. Instead, it is proposed that anti-inflammatory cytokines are involved in an innate protective mechanism initiated by the body to counterbalance the depressiogenic effects induced by pro-inflammatory cytokines.
Finally, the study will examine IDO activity as measured by the KYN/TRP ratio. Since TRP can only be obtained through diet, and since KYN\textsuperscript{371} and TRP compete with LNAAs for entry through the BBB, peripheral measurements of both molecules should reflect the concentrations circulating within the CSF. As summarized in the literature review, an elevated KYN/TRP ratio will act as a marker for both possible neurotoxicity and decreased central TRP availability. The additive affects of the neurotoxic KYN metabolites and reduced 5-HT synthesis is predicted to play a significant role in the development of PSD. Indeed, increased KYN/TRP ratio has recently been associated with depressive symptoms of other diseases (e.g. post-partum depression, CAD); however, this will be the first study to examine its relationship to PSD.
Table 1: Mean KYN/TRP Levels in Various Depression, Neurodegenerative Diseases, and Inflammatory Illnesses

<table>
<thead>
<tr>
<th>Author</th>
<th>Condition</th>
<th>KYN/TRP Controls*</th>
<th>KYN/TRP Patients*</th>
<th>p-value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kohl³⁹</td>
<td>Post-partum depression</td>
<td>29.4 ± 44.7 (n=86)</td>
<td>35.1 ± 47.5 (n=9)</td>
<td>0.067</td>
<td>KYN/TRP measured 4 days after birth; Edinburgh Postnatal Depression Scale (EPDS) score ≥ 12 positive for depression</td>
</tr>
<tr>
<td>Myint⁴⁰</td>
<td>Major depression disorder</td>
<td>25.0 ± 11.0 (n=58)</td>
<td>17.0 ± 14.0 (n=189)</td>
<td>0.036</td>
<td>DSM-IV used for diagnosis of MDD; fasting blood levels drawn</td>
</tr>
<tr>
<td>Forrest³⁶⁷</td>
<td>Huntington's disease</td>
<td>33.8 ± 8.3 (n=11)</td>
<td>45.0 ± 7.9 (n=40)</td>
<td>&lt;0.05</td>
<td>KYN/TRP significantly higher in only patients with the severest form of the disease (≥37 CAG triplet expansions on the gene)</td>
</tr>
<tr>
<td>Widner³⁶⁵</td>
<td>Alzheimer's disease</td>
<td>34.1 ± 9.91 (n=20)</td>
<td>46.1 ± 19.8 (n=21)</td>
<td>0.018</td>
<td>AD patients with cognitive score lower than the median (&lt;6; as measured by the Mini Mental State Examination (MMSE)) had significantly higher KYN/TRP (p=0.02) than those with MMSE≥6</td>
</tr>
<tr>
<td>Widner³⁶⁶</td>
<td>Parkinson's disease</td>
<td>36.1 ± 8.2 (n=11)</td>
<td>58.5 ± 34.2 (n=7)</td>
<td>&lt;0.05</td>
<td>Means displayed only compare controls to patients with the severest form of PD. Mean difference between controls and mild-moderate PD patients was non-significant</td>
</tr>
<tr>
<td>Bonaccorso³⁸</td>
<td>Hepatitis C virus (Before and after IFN-α therapy)</td>
<td>39.0 ± 14.0</td>
<td>61.0 ± 31.0</td>
<td>&lt;0.001</td>
<td>Patient group with IFN-α therapy, control group without IFN-α therapy. Treatment resulted in significantly higher depression scores at 4-6 months (p=0.03) as measured by the Hamilton Depression Scale (HAM-D)</td>
</tr>
</tbody>
</table>

*Reported as mean ± SD unless otherwise indicated
<table>
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<th>p-value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lögters³⁷²</td>
<td>Major trauma (with or without sepsis)</td>
<td>5.6 ± 0.4 (n=42)</td>
<td>21.9 ± 1.1 (n=17)</td>
<td>&lt;0.01</td>
<td>KYN/TRPx100 reported here. Plasma levels measured 6 days after trauma; patient group is with sepsis, control group without sepsis</td>
</tr>
<tr>
<td>Huengsberg³⁷³</td>
<td>HIV</td>
<td>34.9 (32.4, 36.9)Φ (n=72)</td>
<td>50.5 (44.9, 54.5)Φ (n=82)</td>
<td>&lt;0.01</td>
<td>Most patients were on treatment during the course of the study (53 patients took zidovudine (AZT); 23 took didanosine or zalcitabine)</td>
</tr>
<tr>
<td>Pertovaara³⁷⁴</td>
<td>Sjögren's syndrome</td>
<td>Females (n=139): 24.3 (21.0–28.9)*</td>
<td>Females (n=170): 34.0 (25.1–44.3)*</td>
<td>&lt;0.0001</td>
<td>Patients were not age-matched; mean years control = 45 ± 11 years vs. mean years patients = 60 ± 12 years</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Males (n=7): 27.0 (23.6–32.1)*</td>
<td></td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>Schroecksnadel³⁶³</td>
<td>Rheumatoid arthritis</td>
<td>26.9 ± 8.10 (n=49)</td>
<td>37.9 ± 42.4 (n=22)</td>
<td>N/A</td>
<td>KYN/TRP tended to be higher in men than women, strongly associated with age (p&lt;0.01), and significantly correlated with neopterin (p&lt;0.05)</td>
</tr>
<tr>
<td>Swardfager⁴¹</td>
<td>CAD (depressed vs. non-depressed)</td>
<td>38.5 ± 15.7 (n=24)</td>
<td>45.6 ± 20.0 (n=71)</td>
<td>0.055</td>
<td>All patients had CAD; control group represents those who were non-depressed, patient group represents those who were depressed</td>
</tr>
<tr>
<td>Wirleitner³⁷⁵</td>
<td>Coronary heart disease</td>
<td>28.1 ± 5.25 (n=35)</td>
<td>36.3 ± 13.0 (n=35)</td>
<td>&lt;0.01</td>
<td>KYN/TRP positively correlated with neopterin levels. Neopterin concentrations also positively correlated with older age</td>
</tr>
<tr>
<td>Zangerle³⁷⁶</td>
<td>HIV</td>
<td>30.7 ± 8.7 (n=40)</td>
<td>79.2 ± 60.3 (n=45)</td>
<td>&lt;0.001</td>
<td>KYN/TRP significantly decreased (p&lt;0.001) in patient group during anti-retroviral therapy (ART)</td>
</tr>
</tbody>
</table>

*Reported as mean ± SD unless otherwise indicated
ΦReported as median (95% confidence intervals)
*Reported as median (interquartile ranges)
<table>
<thead>
<tr>
<th>Author</th>
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<th>KYN/TRP Controls*</th>
<th>KYN/TRP Patients*</th>
<th>p-value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schroecksnadel³⁶⁹</td>
<td>Gynecological cancer</td>
<td>35.1 (19.2-59.0) <em>(n=19)</em></td>
<td>41.4 (not reported) <em>(n=20)</em></td>
<td>N/A</td>
<td>Patients with ovarian cancer had higher KYN/TRP trending on significance compared to controls</td>
</tr>
<tr>
<td>Suzuki³⁶⁰</td>
<td>Lung cancer</td>
<td>32.9 ± 9.10 <em>(n=45)</em></td>
<td>47.1 ± 21.3 <em>(n=123)</em></td>
<td>&lt;0.001</td>
<td>Higher KYN/TRP ratio was associated with advanced stages of the disease</td>
</tr>
<tr>
<td>Weinlich³⁷⁷</td>
<td>Malignant melanoma</td>
<td>26.9 ± 8.10 <em>(n=49)</em></td>
<td>46.3 ± 20.7 <em>(n=87)</em></td>
<td>&lt;0.001</td>
<td>KYN/TRP and neopterin levels were positively correlated. Patients with KYN/TRP above the median (=41.2 μmol/mmol) had a significantly decreased survival time compared to those above the median (p=0.0025)</td>
</tr>
</tbody>
</table>

*Reported as mean ± SD unless otherwise indicated
†Reported as median (interquartile ranges)
2. METHODS

2.1 Study Design

This was a cross-sectional observational study examining the role of several neurochemical factors in the etiology of PSD. Patients meeting the World Health Organization Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (WHO-MONICA) Project\textsuperscript{378} and National Institute of Neurological Disorders and Stroke (WHO-NINCDS)\textsuperscript{379} criteria for stroke with CT or MRI evidence of acute ischemic infarcts were invited to participate into the study. Based on the WHO-NINCDS guidelines, stroke was defined as “a sudden, nonconvulsive, focal neurologic deficit persisting for greater than 24 hours” and excluded cases of transient ischemic attacks (TIAs). Five different health and rehabilitation centres were utilized for recruitment: (1) Sunnybrook Health Sciences Centre, (2) Baycrest Rehabilitation Centre, (3) St. John’s Rehabilitation Centre, (4) York Central Hospital and (5) Toronto Rehabilitation Centre. Only patients who had an ischemic stroke within 12 weeks of the initial assessment who spoke and understood English were considered for enrolment into the study. If the patient fully agreed to the voluntary terms outlined for the study, a written informed consent was completed (Appendix A). The individual then remained an active participant until all consent terms were met unless the patient chose to terminate participation prematurely.

At baseline interview, patients were considered depressed if they met the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for depression, either major or minor. Once screening tools and standardized mood questionnaires were completed, all participants had blood samples drawn for cytokine, KYN, TRP, and LNAA analyses. In addition, general cognitive function and stroke severity was examined in all consenting participants.
2.2 Subjects

2.2.1 Inclusion and Exclusion Criteria

In all of the five listed recruitment sites, the study was approved by their respective Research Ethics Boards (REBs) as shown by the most recent approval letters in Appendixes B through F. The eligibility criteria for the study were carefully considered before approaching patients and are described below:

Inclusion Criteria:

- Age ≥18 years
- Speaks and understands English
- Clinical diagnosis of stroke according to WHO-MONICA\textsuperscript{378} and NINCDS\textsuperscript{379} criteria
- Evidence of acute infarction on either CT or MRI
- Maximum time since stroke onset <12 weeks
- Written, informed consent

Exclusion Criteria:

- Subarachnoid or intracranial hemorrhage
- Severe aphasia or dysarthria that would interfere with the assessor’s ability to understand patient response
- Imminently suicidal or, in the opinion of the affiliated clinician, has inadequate family monitoring for suicidality
- Significant acute medical illness, including:
  - Infection
  - Drug overdose
  - Alcohol abuse
  - Untreated hypothyroidism
- Significant acute neurologic illness, including:
  - Decreased Level of Consciousness (LOC)
  - Parkinson’s disease
  - Huntington’s disease
  - Multiple sclerosis
- Presence of a premorbid Axis I psychiatric diagnosis, including:
  - Major depressive disorder
  - Schizophrenia
  - Bipolar disorder
  - Dementia
- Concomitant use of psychotropics except for short acting benzodiazepines

- Uncontrolled diabetes
- Uncontrolled anemia
- Severely disturbed liver, kidney, or lung function
- Binswanger’s disease
- Hydrocephalus
- Subdural hematoma
- Progressive supranuclear paralysis
- Severe aphasia
2.2.2 Demographics and Medical History

Patient demographics including age, body mass index (BMI), level of education, employment status, living status, marital status, history of depression, and number of vascular risk factors (including hypertension, cigarette smoking, obesity, hyperlipidemia, and diabetes) were either collected through patient interviews or medical histories stored on hospital electronic databases. Current or past medical illnesses were determined through a review of patient history, physical examinations, and/or routine laboratory test results by a licensed practitioner as indicated in the patient charts. Finally, the time since stroke onset and concomitant medications were recorded based on information gathered by the attending physician upon admission.

2.3 Clinical Assessments

2.3.1 Depression Scales

Centre of Epidemiological Studies Depression Scale (CES-D)

The Centre for Epidemiological Studies Depression Scale (CES-D)\textsuperscript{380} is a 20-item self-rated questionnaire that was originally designed for use in community surveys as a means of determining one’s depression quotient. Today, it is clinically employed as a screening tool for depression and examines various aspects of the respondent’s perceived mood, anxiety and physical functioning within the past seven days. However, because certain somatic symptoms listed on the CES-D were originally intended to elicit symptoms of depression in otherwise healthy individuals, the appropriateness of the scale requires validation among the stroke population. Indeed, the scale has previously been validated in post-stroke patients using a structured psychiatric interview as an established criterion. Here, a cut-off score of 16 was found to be highly predictive of clinical depression, with a specificity of 90%, a sensitivity of
86%, and a positive predictive value of 80%\textsuperscript{381}. Thus, a score of 16 or greater was used as an indicator of depressive symptoms in this study but may not have necessarily reflected definite clinical depression. The CES-D has also been shown to exhibit high inter-observer reliability and concurrent construct validity with several depression measures in the geriatric stroke population (e.g. Geriatric Depression Scale (GSD), Zung Scale), as well as high discriminant validity with measures of other factors including social functioning, cognition, and disability\textsuperscript{382, 383}. As such, it has consistently been used as a screening instrument for depression among the post-stroke population, including the NINCDS Stroke Data Bank\textsuperscript{384} and other various PSD studies\textsuperscript{22, 385-387}.

**Structured Clinical Interview for the DSM-IV: Depression Module (SCID)**

In cases where patients scored 16 or greater on the CES-D, the attending psychiatrist was called to further assess their depressive symptoms to ensure that they were not evoked by the physical impairments suffered from stroke. The psychiatrist then confirmed a clinical diagnosis of depression according to the DSM-IV criteria for a Major Depressive Episode (MDE)\textsuperscript{388}, using the depression module of the Structured Clinical Interview for the DSM-IV (SCID). The use of the SCID has previously been validated among the PSD population\textsuperscript{98, 99}. Patients were considered to be suffering from major depression if at least five of the following nine modules were met in the past two weeks: depressed mood, anhedonia, changes in weight or appetite, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, feelings of worthlessness or guilt, difficulties in concentration and decision-making, or recurrent thoughts of death; with at least depressed mood or anhedonia present among their symptoms. Patients were considered to be suffering from minor depression if they met three of the nine modules, with at least depressed mood or anhedonia present among their symptoms. Those who scored 16 or higher on the CES-D but failed to meet a formal diagnosis of
depression were placed in a "depressive symptoms only” group. However, the clinically depressed group and the depressive symptoms only group were eventually combined for analytical purposes due to the limitations of a small sample size. Thus, the patient population used for analyses were not necessarily diagnosed with clinical depression. The CES-D has consistently been used as a screening instrument for depression among the post-stroke population, including the NINCDS Stroke Data Bank\(^{384}\) and other various PSD studies\(^{22, 385-387}\). Even a briefer version of the CES-D that only included half of the original questions was found to be a positive predictor of depression when compared to the DSM-IV criteria, with a sensitivity of 97\%, a specificity of 84\%, and a positive predictive value of 85\%\(^{389}\).

### 2.3.2 Cognition Scales

**Mini Mental State Examination (MMSE)**

In addition to depression measurements, all patients were evaluated for general cognitive function post-stroke. The Mini-Mental State Examination (MMSE)\(^{390}\) is the most is a popular screening tool for this purpose and was administered to all recruited participants. It consists of a brief, 30-point questionnaire for cognitive ability and evaluates various mental functions including orientation, registration, short-term memory, attention, calculation, and visuo-spatial skills. A patient who scored less than 24 was considered to be cognitively impaired and a patient who scored 24 or more was considered non-cognitively impaired. Previously, the MMSE has detected differences in concentration and memory function between depressed and non-depressed stroke patients\(^ {391}\), as well as worse global cognitive function in PSD patients with significant executive dysfunction\(^{392}\). The MMSE has also been used to test the efficacy of different antidepressants on reducing cognitive impairment in the PSD population\(^{128-130}\).
2.3.3 Stroke Severity and Functional Disability

National Institutes of Health Stroke Scale (NIHSS)

Since the study was interested in examining the biological correlates related to PSD, it was important to evaluate stroke severity and functional disability as possible confounding factors to the results. To fulfill this task, The National Institute of Health Stroke Scale (NIHSS) was administered to all stroke patients by the treating medical team or by a NIHSS certified research associate. The NIHSS is a systematic assessment tool designed to quantitatively evaluate the neurological deficits and degree of recovery of stroke patients. The scale examines the following outcomes: level of consciousness, extraocular movements, visual field defects, facial muscle function, extremity strength, sensory function, coordination (ataxia), language (aphasia), speech (dysarthria), and hemi-inattention (neglect). In this study, the level of stroke severity was scored as follows: 0 = no stroke; 1-4 = minor stroke; 5-15 = moderate stroke; 15-20 = moderate/severe stroke; 21-42 = severe stroke.

Previously, The NIHSS has demonstrated high intra- and inter-observer reliability to non-neurologists and showed that study coordinators can be rapidly and reliably trained via certification videos/DVDs to administer the NIHSS. Furthermore, these certification videos are reliable across multiple venues. NIHSS scores are also robust predictors of stroke outcome and their values at baseline correlate strongly with the Trail of Org 10172 in Acute Stroke Treatment (TOAST) stroke subtype diagnosis. In general, lacunar strokes are related to lower NIHSS scores and thus, forecast better patient outcome. NIHSS scores are also well correlated with infarct volumes, indicating strong concurrent validity of the scale.
2.3.4 Blood Draw

Blood was drawn from inpatients by IV technicians at the time of their routine clinical blood draws. Although blood orders were usually carried out in the morning (approximately 9:00 a.m.), there was no guarantee of a standardized collection time or that the desired blood fasting levels were obtained. Serum levels of various pro- and anti-inflammatory cytokines (IL-1β, IL-6, IL-10, IL-18, TNF-a, IFN-g) and kynurenine were analyzed, as well as plasma levels of tryptophan and LNAA. Blood samples used for cytokine and kynurenine analysis were collected in SST gel vacutainer tubes, while TRP and LNAA were collected in EDTA vacutainer tubes.

Immediately after collection, blood samples were centrifuged at 1000g for 10 minutes. Plasma or serum samples were transferred into labeled cryovials after centrifugation and stored at -70°C until assayed. Because only the free fraction of plasma TRP reflects its concentration in the brain, TRP samples required an additional ultrafiltration process to separate its free form from its protein-bound form. Here, 1.0 cc of plasma was transferred into an ultrafiltration device (Centrifree 4104®, Millipore) and centrifuged at 1000g for an additional 15 minutes. Mechanistically, the device retained all protein-bound TRP while allowing for the smaller free TRP to pass through the 30,000 kDa membrane into ultrafiltrate reservoir cups. The cups containing the free TRP were then capped and stored with the other samples at −70°C until ready for batched analysis.

2.4 Laboratory Analyses

2.4.1 Cytokines Assay

This study focuses on peripheral cytokine measurements. Several cytokines, including IL-1α, IL-1β, IL-6, and TNF-α, are capable of crossing the blood brain barrier.
Although it cannot be positively confirmed that plasma cytokine levels are a direct reflection of CSF levels, peripheral and central cytokine concentrations have been correlated in the past\textsuperscript{400}. Finally, reproducibility of several past studies have indicated plasma cytokines to be reliable markers of depression\textsuperscript{175, 401} and stroke outcome\textsuperscript{171, 191, 192, 400, 402}.

Cytokine measurements of IL-6, IFN-\(\gamma\), and I-10 were performed by Dr. Angela Panoskaltsis-Mortari who heads the Cytokine Reference Laboratory at the University of Minnesota. The technique employed for all three cytokines was multiplex immunobead-based assay using the Human Fluorokine Multianalyte Profiling (MAP) Base Kit A (R&D Systems, Minneapolis, MN). This bead-based assay only requires a small sample size, allows the user to define specific molecules of interest, and is known to be highly efficient and flexible for the simultaneous detection of process-related molecules via Luminex xMAP\textsuperscript{R} technology. Each sample has been evaluated for cytokine sensitivity, precision, recovery, sample linearity, and specificity by the company panel. A brief mechanistic summary of the technique is provided as follows from the company website:

"Detection is achieved through a bead-based antibody-antigen sandwich method. Briefly, samples are incubated with color-coded beads that are pre-coated with analyte-specific capture antibodies for the molecule of interest. Expression levels are determined following incubation with a biotinylated detection antibody and streptavidin-conjugated phycoerythrin (PE). Using a Luminex\textsuperscript{R} analyzer, independent lasers determine the color of each bead and the magnitude of the PE-derived signal, which is directly proportional to the levels of bound analyte."

The procedure is similar to that described by Djoba Siaway et al\textsuperscript{403}, who evaluated and compared several commercial bead-based luminex cytokine assays. The group reported that R&D Systems Fluorokine-MAP assays were one of the most accurate for the measurement of cytokine concentrations in whole blood culture supernatant and achieved good recovery ranges and reproducibility for most cytokines. According to the data provided
by R&D Systems, assay sensitivities were 1.11 pg/mL for IL-6, 0.3 pg/mL for IL-10, and 1.27 pg/mL for IFN-γ. A more detail description of assay sensitivities as described as the minimum detectable dose is shown in Table 2 below:

**Table 2: Cytokine Assay Sensitivities**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Number of Assays</th>
<th>MDD range</th>
<th>MDD mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>43</td>
<td>0.10 - 1.11 pg/mL</td>
<td>0.36 pg/mL</td>
</tr>
<tr>
<td>IL-10</td>
<td>43</td>
<td>0.07 - 0.30 pg/mL</td>
<td>0.13 pg/mL</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>42</td>
<td>0.09 - 1.27 pg/mL</td>
<td>0.31 pg/mL</td>
</tr>
</tbody>
</table>

**2.4.2 KYN Assay**

The KYN assay was performed by the Pharmacy Quality Control and Research Group at Sunnybrook Health Sciences Centre. The technology utilized was reverse phase high performance liquid chromatography (HPLC) at UV detection of 258nm. The exact steps taken for the KYN assay were provided by Scott Walker in a personal communication. Briefly, the method for KYN involves protein precipitation with an equal volume of 3% perchloric acid. The sample was centrifuged for 10 minutes and 0.1 mL of the supernate was injected directly into the high liquid chromatographic system using an autoinjector (715 Ultra WISP, Waters Canada). The mobile phase consists of 9% acetonitrile in 0.05 M potassium phosphate mono basic, which was pumped through a reverse phase 5 µm ODS column, 250 mm x 4.6 mm (Symmetry; Waters Corp) at 1.0 mL/min using a chromatographic pump (P4000; ThermoSeparation Systems, San Jose CA). KYN was detected using an ultraviolet detector (UV 6000; ThermoSeparation Systems, San Jose CA) at a wavelength of 258nm. Chromatograms were recorded on a computer using the ChromQuest software (ThermoQuest, San Jose CA).

The standard curve was constructed by creating 9 standards from a common serum stock sample. To approximately 10 mL of serum, additional KYN was added to the KYN present in the sample to create standards with 0, 0.125, 0.250, 0.3125, 0.50, 0.75, 1.0, 1.5 and 2 mg/L of additional KYN added. Analysis of these standards in duplicate resulted in linear
relationship between the KYN peak area and concentration with a slope and intercept (due to the endogenous kynurenine in the common serum stock). Unknown concentrations of KYN in plasma were determined by dividing the KYN peak area in the unknown sample by the slope. Replicate error, as measured by the coefficient of variation (CV(%) = 100 x standard deviation/mean), based on duplicate analysis of standards between 0 mg/L of kynurenine added to 2 mg/L added ranged from 1.54% to 0.5% with deviations from the known or expected concentrations averaging less than 7%.

2.4.3 TRP and LNAA Assay

The TRP and LNAA assays were performed by Dr. Simon Young in the Department of Psychiatry at McGill University who used the same procedure as described by Anderson et al. Details of the TRP and LNAA assays were provided by Simon Young via personal communication:

“Plasma was deproteinized by adding one part of 1 M perchloric acid to three parts of plasma, mixing and centrifuging. Tryptophan in plasma was measured by high-performance liquid chromatography (HPLC) on a Waters Bondapack C18 reverse phase column (Phenomenex, Torrance, California) with fluorometric detection (Anderson and Purdy, 1979). The other large neutral amino acids (LNAA) (tyrosine, phenylalanine, leucine, isoleucine, valine) were measured on a Beckman System Gold amino acid analyzer using precolumn derivatization with o-phthalaldehyde and gradient reverse phase HPLC with fluorometric detection. Aminoadipic acid was used as an internal standard.”

2.5 CT Scan Analysis

The majority of patients admitted to Sunnybrook for acute stroke had CT scans performed within the first 24 hours of admission. Lesion characteristics were determined from CT scans obtained without a contrast agent on a General Electric LightSpeed VCT series scanner (General Electric Healthcare, Waukesha, WI). Ischemic lesions were manually traced
on these images using Medical Image Processing, Analysis, and Visualization (MIPAV; National Institutes of Health, Bethesda, MD). Lesion area was measured with the use of a digitizing tablet (Sigma Scan, version 3.0, Jandel Scientific) from tracings of the infarct on each slice of the CT scan on which the lesion appeared. The area of the lesion on each slice was multiplied by the slice thickness (1 mm$^3$) and summed to obtain the total lesion volume. The criteria for anterior lesion was that its anterior most border must extend in a rostral manner past the 40% anterior-posterior (A-P) distance. The criterion for posterior lesion was that its posterior border must extend in a caudal manner past the 60% A-P distance. Lesions that fell between the 40% and 60% A-P distance were classified as intermediate. Lesions that eclipsed both the 40% and 60% A-P distances was classified as extending. Infarct side (left vs. right) was determined by examining which side the lesion laid to the longitudinal fissure on the CT scans.

2.6 Statistical Analyses

The Statistical Package for the Social Sciences (SPSS), version 17.0 was used for all statistical analyses.

2.6.1 Preliminary Data Transformations

NIHSS scores are supposed to be collected upon patient admission and entered into the chart. However, 23 scores were missing from the charts. The NIHSS score was imputed at the group mean for both the control and depression group (since the stroke scale was not performed on all patients) in order to keep a consistent sample size for analyses. NIHSS scores were collected based on forms that were filled out by the attending medical staff upon patient admission. Thus, about half of all scores were missing as some of the NIHSS forms were not contained within the patient charts. In total, 23 NIHSS scores were imputed—18
from the control group and 5 from the depressed group. In the case of the MMSE, a percent score was calculated for all patients who could not complete the entire examination due to physical impairments from stroke (e.g. motor injuries would void the individual form drawing and writing activities), where the correct number of responses was divided by the largest possible score that can be obtained during assessment. The percent was then multiplied by 30 (the standard MMSE total score) to maintain an invariable range of scores and the method has been validated among the elderly population\textsuperscript{405, 406}. Additionally clinical measurements of cytokine assays were sometimes returned with undetectable levels or below the lowest level of detectability of the assay. When this occurred, the lowest level of detectability for each cytokine (1.11 pg/mL for IL-6, 0.30 pg/mL for IL-10, and 1.27 pg/mL for IFN-γ), was used as the imputed value.

Although this method would generate cytokine values that deviate from the true mean, the main goal of this study was not to capture low concentrations of pro-inflammatory cytokines but their elevated levels. Thus, the approach should not affect our ultimate intent of finding clinically significant elevated cytokine concentrations. However, data pertaining to anti-inflammatory cytokines may be affected since our hypothesis predicts the presence of decreased concentrations. This limitation will be explored further in the discussion.

2.6.2 Planned Analyses

Clinically depressed patients and those who only exhibited depressive symptoms (CES-D≥16) were combined into one group and collectively labeled the "depressed group". As mentioned previously, the number of patients in the study with major versus minor depression made subanalysis by depression subtype unfeasible. Thus, throughout this paper, the term "depressed group" will be used to represent the combined depressed and depressive symptoms group. Those who did not exhibit any clinically significant signs of depressive
symptoms (CESD≤15) were labeled the "non-depressed group". Complete demographic data were collected during recruitment and compared between the two groups using independent samples t-tests for continuous data (e.g. age, BMI) and chi-square or Fisher's exact tests for categorical data (e.g. marital status, employment status, living situation). Variables that were significantly different between groups were included as covariates in subsequent analyses.

2.6.2.1 Primary Analysis

Hypothesis 1: Increased idoleamine 2,3-dioxygenase (IDO) enzyme activity will be found in stroke patients experiencing depressive symptoms compared to non-depressed stroke patients as evidenced by an increased kynurenine/tryptophan (KYN/TRP) ratio.

Demographic and clinical characteristics were compared between depressed and non-depressed groups using independent samples t-test for continuous variables and Pearson's chi-square analysis for categorical variables. ANCOVA was used to compare differences in mean KYN/TRP ratio between groups with any between-group demographic or clinical differences entered as covariates. Depression was also analyzed as a continuous variable by looking at the relationship between CES-D scores and KYN/TRP using a partial correlation.

2.6.2.2 Secondary Analyses

Hypothesis 2: Increased levels of pro-inflammatory cytokines (IL-6, IFN-γ) and decreased levels of anti-inflammatory cytokines (IL-10) will be found in stroke patients experiencing depressive symptoms compared to non-depressed stroke patients.

Skewness and kurtosis were examined for each cytokine variable. If the data presented itself as unskewed, ANCOVA was used to compare differences in mean cytokine levels between depressed and non-depressed patients, with the appropriate demographic variables acting as covariates as determined during preliminary analysis. If the data presented itself as being skewed, a non-parametric Mann-Whitney test was employed instead to determine the
mean cytokine difference between patient groups. Additionally, Spearman's rank rho were performed between all cytokines and the KYN/TRP in order to determine the relationship between the two variables.

2.7 Sample Size Calculations

This is the first study to evaluate the relationship between KYN/TRP ratio and its predictive value of depression post-stroke. Thus, estimates of effect size were based on previously published data examining the relationship between KYN/TRP ratio and depression in CAD patients\(^{41}\). Since the primary analysis of this study was based on ANCOVA statistics, sample size calculations for the multivariate data were not possible as no supportive data was available for this method\(^{407}\). Thus, all effect estimates were based on the procedure for independent samples t-tests. Based on the CAD study, mean KYN/TRP ratios and the weighted standard deviation were used in the equation described as follows. Effect size (d) were calculated according to the equation \(d = (m_A - m_B) / \sigma\); where \(m_A\) = the population mean for group A (MDD patients), \(m_B\) = the population mean for group B (non-depressed controls), and \(\sigma\) = the population standard deviation. Required sample size was calculated according to the equation \(n = 2 \left[ \frac{(z_{\alpha} + z_{\beta}) \sigma}{\Delta} \right]^2\) or \(n = 2 \left[ \frac{(z_{\alpha} + z_{\beta})}{d} \right]^2\) where \(\Delta = (m_A - m_B)\), the difference between the two population means, \(z_{\alpha}\) = the z-value that corresponds to the accepted \(\alpha\) level (probability of type I error), and \(z_{\beta}\) = the z-value that corresponds to the accepted \(\beta\) level (probability of type II error). For \(\alpha = 0.05\), \(z_{\alpha} = 1.96\) and for \(\beta = 0.20\), \(z_{\beta} = 0.84\). It was determined that for \(\alpha = 0.05\), 88 patients per group would be necessary to obtain an observed power of 0.80.
2.8 Control of Confounders

Both depression and stroke have been individually and collectively linked to several clinical and cardiovascular risk factors in the past research studies. For this reason, potential confounders were compared between depressed and non-depressed patients. Based on the statistical analyses, variables found to be significantly different between groups were considered as covariates in the primary analyses. Only hypertension and levels of LNAA were found statistically significant among all measured demographic and clinical factors. As previously mentioned, hypertension has previously been found in subjects with depressive symptoms\textsuperscript{110} and thus may be pertinent to the development of PSD.

3. RESULTS

Patient recruitment began at the inpatient stroke unit at Sunnybrook Health Sciences Centre in July 2007. A list of possible study participants was provided weekdays by a nurse responsible for keeping track of admitted stroke patients. This list of potential research subjects is generated as part of the Heart and Stroke Foundation Centre for Stroke Recovery research initiative. Potential recruits were subsequently approached by the study coordinator to ask if they were willing to participate in a research study. The coordinator described the premise of the study, what was required of the patient if he or she chose to participate, and the risks and benefits of those requirements. However, because the research is complex in its variability, the ability for each individual to understand the research also varies. Thus, the capacity for consent was verified by independent physicians for all patients as part of standard care. As a further precaution, the assessor had the patients repeat the purpose and details of the study to ensure that they were fully aware of their responsibilities with participation. Finally, the study requires consenting to questionnaires and a blood sample, which are not
complicated tasks to understand in themselves. A flowchart of patient recruitment progress can be viewed in Figure 2 along with a table of ineligibility in Table 3.

**Figure 2. Patient Recruitment**

- **Ischemic Stroke Patients**
  - **n=489**

- **Minimum Length of Stay ≥5 days**
  - **n=346**

- **Screened**
  - **n=303**

- **Eligible**
  - **n=125**

- **Not Eligible**
  - **n=178**

- **Recruited**
  - **n=61**

- **Completed**
  - **n=54**

- **Non-Depressed**
  - **n=38**

- **Depressive Symptoms**
  - **n=16**
Table 3: Participant Ineligibility

<table>
<thead>
<tr>
<th>Reason for Ineligibility</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of Depression</td>
<td>34</td>
</tr>
<tr>
<td>Non-English Speaking</td>
<td>29</td>
</tr>
<tr>
<td>Severe Aphasia/Dysarthria</td>
<td>19</td>
</tr>
<tr>
<td>Decreased LOC</td>
<td>15</td>
</tr>
<tr>
<td>Neurological Disorder</td>
<td></td>
</tr>
<tr>
<td>Dementia</td>
<td>18</td>
</tr>
<tr>
<td>Parkinson’s Disease</td>
<td>5</td>
</tr>
<tr>
<td>Other</td>
<td>9</td>
</tr>
<tr>
<td>Medical Illness</td>
<td></td>
</tr>
<tr>
<td>Infection</td>
<td>13</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>3</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
</tr>
<tr>
<td>Other</td>
<td>27</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>178</strong></td>
</tr>
</tbody>
</table>

At the time of this paper, 61 patients have agreed to participate in the study with recruitment ongoing. All individuals who were selected as participants met the eligibility criteria, signed a written informed consent and provided a blood sample for biomarker analyses. However, due to loss of follow-up, only 54 (38 non-depressed, 16 depressive symptoms) were used for analyses. Based on these numbers, the study group consists of 30% of stroke patients experiencing significant depressive symptoms, which is an agreement with previously published results regarding the prevalence of PSD\(^10\).

3.1 Demographic and Clinical Characteristics

All pertinent demographic and clinical characteristics are reported in Table 4 via independent t-test and chi-square analyses. It is uncertain how long a patient was depressed because the diagnosis of depression was made by our team. However, patients with a prior history of depression or who were on anti-depressants at the time of approach were excluded.
from the study. Therefore, it is unlikely that the depression evaluated by our team was a result of a previous history of depression rather than the events prompted by stroke.

**Table 4: Participant Demographics and Assessment Scores by Depression Group**

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Non-depressed (n=38)</th>
<th>Depressive Symptoms (n=16)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>52.6%</td>
<td>50.0%</td>
<td>0.860</td>
</tr>
<tr>
<td>Age</td>
<td>71.3 ± 17.7</td>
<td>69.4 ± 14.3</td>
<td>0.674</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.0 ± 4.8</td>
<td>26.1 ± 5.4</td>
<td>0.228</td>
</tr>
<tr>
<td>Time between date of stroke and assessment (days)</td>
<td>30.2 ± 42.0</td>
<td>25.6 ± 36.8</td>
<td>0.713</td>
</tr>
<tr>
<td>Marital status (% married)</td>
<td>39.5%</td>
<td>68.8%</td>
<td>0.233</td>
</tr>
<tr>
<td>Living situation (% living with others)</td>
<td>68.4%</td>
<td>75.0%</td>
<td>0.751</td>
</tr>
<tr>
<td>Employment (% retired)</td>
<td>76.3%</td>
<td>75.0%</td>
<td>0.286</td>
</tr>
<tr>
<td>Education (% with Bachelor’s degree or greater)</td>
<td>36.8%</td>
<td>18.8%</td>
<td>0.514</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Assessment Scores</th>
<th>Non-depressed (n=38)</th>
<th>Depressive Symptoms (n=16)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CES-D score</td>
<td>6.2 ± 5.0</td>
<td>26.8 ± 10.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MMSE imputed score</td>
<td>26.6 ± 4.3</td>
<td>25.55 ± 4.7</td>
<td>0.090</td>
</tr>
<tr>
<td>NIHSS score</td>
<td>7.6 ± 5.6</td>
<td>11.4 ± 5.4</td>
<td>0.097</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Blood Markers</th>
<th>Non-depressed (n=38)</th>
<th>Depressive Symptoms (n=16)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LNAA (µg/mL)</td>
<td>578.8 ± 147.0</td>
<td>538.6 ± 141.2</td>
<td>0.022</td>
</tr>
<tr>
<td>KYN (µmol/L)</td>
<td>2.8 ± 1.6</td>
<td>3.3 ± 1.6</td>
<td>0.582</td>
</tr>
<tr>
<td>TRP (µg/mL)</td>
<td>8.8 ± 2.5</td>
<td>9.3 ± 3.7</td>
<td>0.120</td>
</tr>
</tbody>
</table>

For continuous variables, data is represented as mean ± SD

No significant differences were found between patient groups except for hypertension (p=0.043). Although PSD is often found comorbid with cognitive impairments, the current patient population did not display such a relationship as no significant difference was found between mean MMSE scores (non-depressed group=26.6±4.3, depressed group=25.6±4.7, F<sub>2,49</sub>=2.53, p=0.090). Plasma markers were examined using ANCOVA analysis with hypertension acting as the covariate. Mean LNAA concentrations were found to be significantly lower in the depressed patient group (F<sub>2,51</sub>=4.10, p=0.022), while other plasma markers including TRP and KYN were found to be non-significant.
Lesion characteristics of stroke patients with and without depression can be viewed in Table 5. The mean stroke lesion volume among all stroke patients was 21.89 ± 50.8 cm$^3$ but was not significantly different among non-depressed and depressed stroke patients (non-depressed=23.6 ± 58.3, depressed=18.3 ± 31.8, $p=0.763$). The majority of stroke patients (38.5%) had lesions located in the intermediate region (between anterior and posterior lesions). The rest of the patient population had lesions in the following locations: 35.9% of in the anterior circulation, 23.1% in the posterior circulation, and 2.6% extended from the anterior circulation to the posterior circulation. There was no significant difference in lesion locations between groups ($\chi^2=3.74$, $p=0.291$). Among all patients, 54.9% had right-sided lesions, 43.1% had left-sided lesions, and 2.0% had lesions spanning both the left and right sides. Finally, there was no significant difference in infarct side between depressed and non-depressed patients ($\chi^2=0.49$, $p=0.781$).

**Table 5: Stroke Lesion Characteristics by Depression Group**

<table>
<thead>
<tr>
<th></th>
<th>Non-depressed (n=27)</th>
<th>Depressive Symptoms (n=13)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volume (cm$^3$)</strong></td>
<td>23.6 ± 58.3</td>
<td>18.3 ± 31.8</td>
<td>0.763</td>
</tr>
<tr>
<td><strong>Location</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• %Anterior</td>
<td>34.6%</td>
<td>38.5%</td>
<td>0.291</td>
</tr>
<tr>
<td>• %Posterior</td>
<td>30.8%</td>
<td>7.7%</td>
<td></td>
</tr>
<tr>
<td>• %Intermediate</td>
<td>30.8%</td>
<td>53.8%</td>
<td></td>
</tr>
<tr>
<td>• %Extending</td>
<td>3.8%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td><strong>Laterality (% Right)</strong></td>
<td>59.3%</td>
<td>61.5%</td>
<td>0.781</td>
</tr>
</tbody>
</table>

For continuous variables, data is represented as mean ± SD

Almost all patients had at least one cardiovascular risk factor based on the entire study population, including hypertension (71.4%), hypercholesterolemia (37.0%), diabetes (18.5%) or cigarette smoking (18.5%). All recorded cardiovascular risk factors were found to be higher in the depressed patient population as displayed in Table 5; however, only the proportion of hypertensive patients in the depressed groups was significantly greater than the non-depressed group (93.8% vs. 65.8%, $\chi^2=4.48$, $p=0.04$).
Based on different medical histories, all patients were treated with a variety of medications including anti-hypertensives (57.4%), aspirin (66.7%), beta-blockers (37.0%), calcium channel blockers (29.6%) and diuretics (16.7%). The proportion of patients taking a certain medications did not differ between patient groups. Although the regular use of psychotropics was counted as an exclusion criterion during recruitment, short acting benzodiazepines (e.g. lorazepam) were permitted in this study to sedate or to aid patients in their sleep. Medications consumed by both patient groups by proportion are displayed in Table 6 via chi-square analysis.

Table 6: Cerebrovascular Risk Factors and Concomitant Medication Use by Depression Group

<table>
<thead>
<tr>
<th>Cerebrovascular Risk Factors</th>
<th>Non-depressed (n=38)</th>
<th>Depressive symptoms (n=16)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>65.8%</td>
<td>93.8%</td>
<td>0.043</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>34.2%</td>
<td>43.8%</td>
<td>0.507</td>
</tr>
<tr>
<td>Obesity (BMI ≥ 30)</td>
<td>31.6%</td>
<td>25.0%</td>
<td>0.629</td>
</tr>
<tr>
<td>Diabetes</td>
<td>15.8%</td>
<td>25.0%</td>
<td>0.426</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>18.4%</td>
<td>18.8%</td>
<td>0.977</td>
</tr>
<tr>
<td>Total number of CVRFs</td>
<td>1.7 ± 1.2</td>
<td>2.1 ± 1.1</td>
<td>0.198</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concomitant Medications</th>
<th>Non-depressed (n=38)</th>
<th>Depressive symptoms (n=16)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>68.4%</td>
<td>62.5%</td>
<td>0.673</td>
</tr>
<tr>
<td>β-blockers</td>
<td>36.8%</td>
<td>37.5%</td>
<td>0.964</td>
</tr>
<tr>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; channel blockers</td>
<td>23.7%</td>
<td>43.8%</td>
<td>0.140</td>
</tr>
<tr>
<td>Diuretics</td>
<td>15.8%</td>
<td>18.8%</td>
<td>0.790</td>
</tr>
<tr>
<td>Insulin</td>
<td>15.8%</td>
<td>25.0%</td>
<td>0.426</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>13.2%</td>
<td>6.3%</td>
<td>0.461</td>
</tr>
</tbody>
</table>

For continuous variables, data is represented as mean ± SD

3.2 Assay Results

A total of 54 plasma samples (38 non-depressed, 16 depressive symptoms) were collected and sent for analysis. Plasma markers of interest were KYN, TRP, LNAA and the cytokines IL-6, IL-10, and IFN-γ. However, several cytokine samples were below the lower limit of the assay’s detectability. As previously mentioned, these limits were 1.11 pg/mL for
IL-6, 0.30 pg/mL for IL-10, and 1.27 pg/mL for IFN-γ. Thus, imputed scores were also used for cytokine statistical analyses, where the lowest level of detectability was used as the imputed values.

3.3 Biological Correlates of Post-stroke Depression

3.3.1 Hypothesis 1: KYN/TRP ratio as a marker of depression

As displayed in bar graph form in Figure 3, ANCOVA analysis revealed a significant difference in mean KYN/TRP ratio between depressed and non-depressed patient groups with hypertension and LNAA concentrations as covariates (non-depressed group=69.3±36.90, depressed group=78.3±42.03, F_{3,50}=4.61, p=0.006) and an observed power of 86.4%.

Figure 3. Mean KYN/TRP ratios in stroke patients with and without depressive symptoms

A box-plot displaying further differences in the range, median, and interquartile ranges of the KYN/TRP ratios can be viewed in Figure 4. However, partial correlation analysis revealed a non-significant relationship between CES-D scores and KYN/TRP while
controlling for hypertension and disparities in LNAA concentrations ($r_{50}=-0.06$, $p=0.67$). A scatter plot outlining the relationship between CES-D scores and KYN/TRP ratio is displayed in Figure 5.

**Figure 4. Box-plot of unadjusted KYN/TRP ratios in stroke patients with and without depression**

![Box-plot of unadjusted KYN/TRP ratios](image)

**Figure 5. Correlation between CES-D total scores and KYN/TRP ratio**

![Scatter plot](image)

The same ANCOVA analysis was conducted after separating the combined depression group into “depressed” and “depressive symptoms only” groups such that a total of three
groups existed. The results remained significant between groups ($F_{4,49}=4.027$, $p=0.007$), where those in the depressive symptoms category had a much higher KYN/TRP that the other two groups. These results suggest that KYN/TRP may act as a red-flag for those most prone to depressive symptoms post-stroke and possibly developing clinical depression thereafter. No comment can be made about the depressed group due to the small sample size.

Other exploratory analysis found a significant correlation between KYN/TRP levels and LNAA concentrations using bivariate correlation analysis ($r^2=0.21$, $p<0.001$). A scatter-plot of the relationship can be viewed in Figure 6.

**Figure 6. Correlation between LNAA concentrations and KYN/TRP ratio**

![Correlation between LNAA concentrations and KYN/TRP ratio](image)

The presence of hypertension between depressed and non-depressed patients was explored further since chi-square analysis found a significantly greater number of hypertensive patients in the depressed than the non-depressed population via chi-square analysis (93.8% vs. 65.8%, $\chi^2=4.48$, $p=0.03$). Unfortunately, the production of a correlative relationship between the two variables was not possible since systolic pressure over diastolic pressure values were not collected during recruitment. To observe the reverse relationship where groups were
separated by the presence of hypertension rather than depression, ANOVA analysis revealed a significant difference in mean CES-D scores between hypertensive and non-hypertensive patients (non-hypertensive=7.0 ± 5.7, hypertensive=14.2 ± 12.9, $F_{1,52}=3.9$, $p=0.05$). A graph of this relationship can be viewed below in Figure 7.

**Figure 7. Mean CES-D Scores by Hypertension Group**

Further exploratory analysis was conducted to determine the relationship between hypertension and the KYN/TRP, the KYN/TRP values were first separated into two groups, “low” vs. “high” (based on the median), proceeded by chi-square analysis with hypertension acting as the other variable. However, results were non-significant (percent non-hypertensive above the median KYN/TRP ratio=50% vs. percent hypertensive above the median KYN/TRP ratio=73%, $\chi^2=2.36$, $p=0.12$). When ANOVA was conducted using the same variables, the results were no different in significance between hypertension and mean KYN/TRP ratio (mean KYN/TRP non-hypertensive=59.5±41.7, hypertensive=76.3±36.6, $F_{1,52}=2.3$, $p=0.16$).

Although the relationship between hypertension and KYN/TRP was not significant, hypertension acted as a significant co-variate in our analysis of KYN/TRP and depression.
group. Thus, the effect of anti-hypertensive medication on the results of this primary hypothesis was also examined. Hypertension was diagnosed based on reviewing the past medical history in the patient chart. Out of all 54 patients, 40 were hypertensive and 31 patients were on anti-hypertensive medication. The distribution of hypertensive/non-hypertensive patients on anti-hypertensive medication is listed in Table 7 as follows:

**Table 7: Distribution of Hypertensive Patients on Anti-Hypertensive Medication**

<table>
<thead>
<tr>
<th></th>
<th>Hypertensive</th>
<th>Non-hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>On anti-hypertensives</td>
<td>27</td>
<td>4</td>
</tr>
<tr>
<td>Not on anti-hypertensives</td>
<td>13</td>
<td>10</td>
</tr>
</tbody>
</table>

Those who did not have a history of hypertension but were currently placed on anti-hypertensives as an inpatient may have acquired hypertensive characteristics because of complications from stroke. Likewise, those who had a history of hypertension but were not give anti-hypertensives as an inpatients may have been taken off the medication due to medical complications as seen fit by the attending doctor. When both hypertension and the use of anti-hypertensive were controlled for in analysis of the primary hypothesis, The KYN/TRP ratio remained significantly higher in the depressed group (F_{4,49}=4.0, p=0.007). Thus, the presence or absence of anti-hypertensive medication did not significantly alter our results.

### 3.3.2 Hypothesis 2: Inflammatory markers of depression

Because preliminary analysis found the cytokine data to be highly skewed, a Mann-Whitney test was employed to compare the mean cytokine levels between depressed and non-depressed stroke patients with hypertension acting as the covariate. All three measured cytokines (IL-6, IL-10, IFN-γ) revealed non-significant results (p=0.32, p=0.19, p=0.21) respectively. When the imputed values were used for analysis, results remained non-significant (p=0.627, p=0.216, p=0.297) respectively. However, about half or more of the data
required imputation creating a skewed set of data that would be hard to detect significance even with non-paramatric tests. Nevertheless, the values remained numerically in the direction of expectation, where pro-inflammatory cytokine levels were greater in depressed group and the anti-inflammatory cytokine levels were lower in the depressed group. A more detailed summary of the results can be viewed in Table 8.

**Table 8: Measured and Imputed Cytokine Plasma Levels by Depression Group**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Non-depressed</th>
<th>Depressed</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unadjusted values</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>30</td>
<td>7.9 ± 5.0</td>
<td>9.3 ± 3.7</td>
<td>0.329</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>13</td>
<td>1.9 ± 1.5</td>
<td>1.0 ± 0.4</td>
<td>0.192</td>
</tr>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>27</td>
<td>5.1 ± 3.7</td>
<td>5.3 ± 1.7</td>
<td>0.205</td>
</tr>
<tr>
<td><strong>Imputed values</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>54</td>
<td>4.9 ± 5.0</td>
<td>5.7 ± 5.0</td>
<td>0.627</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>54</td>
<td>0.59 ± 0.86</td>
<td>0.56 ± 0.42</td>
<td>0.216</td>
</tr>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>54</td>
<td>3.1 ± 3.2</td>
<td>3.5 ± 2.4</td>
<td>0.297</td>
</tr>
</tbody>
</table>

*Based on Mann-Whitney statistics
Data is represented as mean values ± SD

Due to the high number of samples lost as a result of undetectability, chi-square analysis was conducted to compare if there was a significant difference in percentage of cytokine detectability between groups depression group. This was not the case as displayed in the table below. Thus, detectability of cytokines should not have affected our results and was not treated as a covariate during any analyses.

**Table 9: Cytokine Assay Detectability by Depression Group**

<table>
<thead>
<tr>
<th></th>
<th>Non-depressed</th>
<th>Depressed</th>
<th>χ²</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>56.8%</td>
<td>56.3%</td>
<td>0.001</td>
<td>0.973</td>
</tr>
<tr>
<td>IL-10</td>
<td>18.4%</td>
<td>37.5%</td>
<td>2.242</td>
<td>0.134</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>47.4%</td>
<td>56.3%</td>
<td>0.355</td>
<td>0.551</td>
</tr>
</tbody>
</table>

Spearman’s rank rho analysis revealed a relationship trending toward significance between measured unadjusted IL-6 concentrations and KYN/TRP ratio (rho=0.33, p=0.08) and is exhibited in scatter-plot form in Figure 8. However no other cytokine resulted in a similar relationship with the KYN/TRP ratio. In addition, non-significant correlations were found
between all cytokines and total CES-D score when both the raw and imputed data were used for analysis.

**Figure 8. Correlation between unadjusted IL-6 concentrations and KYN/TRP ratio**

Finally, the magnitude of variability imposed by combining the depression groups was observed. When the combined depression group was separated into “depressed” and “depressive symptoms only” such that a total of three groups existed rather than two, the results did not significantly differ from the originally reported results after running the same analysis. The time of blood collection with respect to stroke onset was also evaluated for its variability. Only 10 of the total 54 patients had blood drawn within greater than 1 month post-stroke, 2 of which were in the depressed group. However, our preliminary analysis showed that time since stroke and enrollment into the study did not significantly differ between patient groups, thus, the inclusion of patients >3 months post-stroke should not significantly affect our results. Nevertheless, when the 10 patients were removed from the subject population and the cytokine analysis were rerun, they did not significantly differ from our initially reported results.
4. DISCUSSION

This study is the first to support the role of IDO activation in the etiology of PSD via the KYN/TRP ratio marker. Mean KYN/TRP ratio was significantly higher in depressed than non-depressed stroke patients. However, the severity of depressive symptoms, as measured by total CES-D score, did not significantly correlate with KYN/TRP ratio or plasma cytokine levels. Additionally, cytokine levels did not significantly differ between patient groups, but a correlation trending on significance was found between KYN/TRP and IL-6 plasma levels. Finally, the percent of hypertensive patients was significantly higher in depressed stroke patients, a finding supported by a higher mean CES-D score among hypertensive patients than non-hypertensive patients. No significant difference in mean KYN/TRP was found between hypertensive and non-hypertensive patients.

4.1 The Role of IDO Activation in Post-stroke Depression

Several studies examining neurological illnesses associated with unstable levels of inflammatory markers have reported elevations in KYN/TRP ratio, including in Alzheimer’s disease, Parkinson’s disease, HIV infection, rheumatoid arthritis, coronary heart disease and healthy aging. Additionally, an increase KYN/TRP ratio has also been associated with states of depression including women with post-partum depression and depressed hepatitis C and cancer immunotherapy patients. However, despite several speculations of a causal relationship between activation of the KYN pathway and the development of PSD by various research groups, no association has been made to date. This study is the first to report a significant difference in mean KYN/TRP ratio between depressed and non-depressed stroke patients with a high observed power of 86.4%. Although this result
was relatively surprising since the sample size calculation predicted that 88 patients per group was required to fulfill an observed power of 80%, we were still able to demonstrate significantly elevated KYN/TRP ratios among depressed patients.

The results presented in this study agree with our hypothesis that IDO is activated upon inflammatory burden post-stroke. Because the IDO enzyme has an extremely short half-life, direct measurement of its plasma concentrations was not feasible. Thus, the ratio of its primary metabolite KYN to TRP was used as a marker of its activity instead. An increase in IDO activity may reduce 5-HT production since the enzyme is in direct competition with TPH for the metabolism of TRP into 5-HT. Consequently, central 5-HT levels are decreased below the level for normal mood and behavioural regulations. For example, TRP levels have been found to be decreased in depressed patients\textsuperscript{314} while the administration of TRP produces an antidepressant effect\textsuperscript{340}. Thus, depletion of TRP as a result of increased IDO activity may explain our finding that the depressed patient population had a significantly higher KYN/TRP than non-depressed subjects.

Increases in KYN levels may also affect mood since it can be further metabolized into several neurotoxic metabolites including three potent neurotoxins; the hydrogen peroxide generators, 3-hydroxykynurenine and 3-hydroxyanthranilic acid\textsuperscript{223} and the NMDA agonist, quinolinic acid (QUIN)\textsuperscript{335}. Because these intermediates are neuroactive, central elevations in their concentrations may destroy essential neurons responsible for controlling mood and behaviour within the limbic structures of the brain. For example, 3-hydroxykynurenine levels have been found to be elevated in other neurological disorders such as Huntington’s disease patients\textsuperscript{354, 355} and has been shown to induce neuronal cell death in cortical and striatal cell cultures\textsuperscript{44}. Intracerebral injection of 3-hydroxyanthranilic acid can decrease choline acetyltransferase activity\textsuperscript{225} thus, decreasing acetylcholine synthesis. Finally, several
neurodegenerative conditions have been linked to QUIN-induced apoptosis, including Huntington’s disease and HIV-associated dementia. Furthermore, laboratory studies have demonstrated the production of QUIN to be immune mediated (in this case, stroke) and acts as a potent agonist of the neuronal NMDA subtype of glutamate receptors. It is thus no surprise that a significantly elevated KYN/TRP ratio was detected in the depressed patient population as a combination of both decreased serotonin synthesis and increase neurotoxic KYN metabolites can result in the clinical manifestations of PSD. Also note that stroke severity was not significantly different between groups, suggesting that the described biological pathway may be partially responsible for PSD rather than the sole influence of post-stroke physical impairments.

An effort was made to control the potential demographic confounders collected during the recruitment period. The presence of both hypertension and low LNAA concentrations significantly differed between patient groups and were thus used as covariates in the analyses. Properties of hypertension including decreased lumen size and decreased vessel distensibility have previously been found to be prominent in patients with depressive symptoms and those who also suffer from reduced blood flow velocity were diagnosed with ad DSM-IV depressive disorder. LNAA directly competes with TRP for transport into the brain and because the direction of TRP metabolism affects synthesis of 5-HT and KYN, significantly different levels of LNAA would be expected from depressed patients.

In this study, plasma levels of LNAA were significantly lower in depressed patients while TRP levels (although not significant) were numerically higher. This counters past studies that have reported lower TRP and TRP/LNAA ratio in MDD patients compared to healthy controls. Our contrasting results may include one of the following three explanations, a combination of all, or other factors outside our control. First, it is common for
clinical studies to measure plasma TRP in the morning after overnight fast since TRP can only be obtained exclusively through dietary sources. Although our study attempted to ensure all blood samples were collected in the morning (approximately 8 a.m.) during the IV technologist’s regular rounds, patients were known to have breakfast as early as 7 a.m. in some cases, while blood draw times were delayed to approximately 11 a.m. in other cases. Thus, variability in collection time and diet may have contributed to the unexpected increase in TRP availability among stroke patients. Secondly, the amount of dietary tryptophan and LNAAs (e.g. tyrosine) consumed may differ between patient groups depending on their willingness to eat. Since one of the most defining symptoms of depression includes the loss of appetite, variations in TRP and LNAA consumption between depressed and not depressed patients may have confounded our results. Finally, peripheral levels of TRP, KYN, and LNAA may not parallel with its central availability. It would be necessary to devise experiments looking at both the central and peripheral availability of these molecules in order to validate if an increased TRP/LNAA ratio or its opposite is representative of the observed depressive symptoms in PSD patients.

Interestingly, a significant positive correlation could not be found between total CES-D scores and the KYN/TRP. Therefore, it would be necessary to test this hypothesis on a greater sample population of depressed patients as our study was limited to a small group of subjects. This was mostly due to a decrease in sample availability in order to prevent the confounding effect of plasma analyses from two different laboratories. Furthermore, because research regarding the clinical and neuropsychiatric correlates of KYN/TRP are still in its primitive phase, other demographic or clinical factors that have not yet been documented may be confounding the analysis. For example, it has been documented that post-stroke major and minor depressions have different prognoses. The prognosis of MDD has been shown to be
more favourable than that of minor depression, with resolution of depressive symptoms in 75% of the MDD patient population compared to only 30% of the minor depression cohort\textsuperscript{411}. In addition, those with minor eventually developed major depression. By increasing sample size, we would be able to segregate post-stroke MDD and minor depression groups along with other demographic variables that each cohort may entail. Determination of these factors may lead to more significant results when controlling for them during partial correlations.

Whether elevations of the KYN/TRP ratio is a phenomenon directly related to PSD and other neurological illness or a marker that can be employed in otherwise healthy individuals has yet to be elucidated. It would be necessary to conduct the same study by also enrolling healthy, non-stroke depressed and non-depressed individuals. Although the relationship between depression and inflammation has been well-documented in the past, there is still uncertainty whether the former induced the latter or vice versa. Through the inclusion of healthy individuals in a longitudinal study design one may be able to decipher if the relationship between the two variables is uni- or bidirectional depending on the individuals neurological state and also assess the role of the KYN/TRP ratio in both subgroups. Moreover, the addition of follow-up visits would allow for the time-course evaluation of the KYN/TRP ratio and changes in inflammatory states of each participant. Even so, current animal models suggest that IDO is most active following cerebral ischemia\textsuperscript{412,413} and may be necessary to induce sickness behaviours\textsuperscript{208}, suggesting that basal cytokine levels are rarely sufficient enough to alter mood states via IDO induction.

4.2 The Role of Cytokines in Post-stroke Depression

Since the inflammatory pathways are triggered under post-stroke conditions and are
the primary inducers of IDO activity, elevated pro-inflammatory cytokines (IL-6, IFN-γ) and diminished anti-inflammatory cytokines (IL-10) were expected among the depressed patient population. Although the numerical mean values equate to the anticipated direction of movement, none of the measured levels significantly differed between patient groups. However, a positive correlation trending on significance was found between IL-6 levels and KYN/TRP ratio as demonstrated in Figure 5, supporting the role of pro-inflammatory cytokines in IDO activation.

It is possible that a significant difference in cytokine levels between groups could not be detected due to a broad separation between time of study assessment and time when inflammation is most pronounced post-stroke. As displayed in Table 3 the average time between stroke and recruitment averaged 25 to 30 days in the depressed and non-depressed groups respectively, with some patients reaching almost 3 months post-stroke before the initial evaluation. However, animal studies have detected circulating monocytes within the capillaries and venules 4 to 6 hours after induced ischemia, as well as an accumulation of PMNs 12 hours after the same injury\textsuperscript{161}. At this time inflammatory molecules initiate their activity and exacerbate tissue damage via edema formation, release of oxygen radicals, cytokines, and cytolytic enzymes leading to cellular necrosis\textsuperscript{162-164}. Because our cytokine concentration measurements occurred as late as 3 months post-stroke for both depressed and non-depressed patients, it was difficult to determine an accurate reflection of cytokine level differences between patient groups. Furthermore, cytokines have previously been reported to increase the risk of recurrent stroke\textsuperscript{172}. Since a few of our participants were not first-time stroke patients, elevated cytokine levels may have already been present prior to their most recent ischemic attack regardless of their mood state. This would also confound our ability to precisely define significant differences in mean cytokine levels between depressed and non-
depressed subjects. However, because only four of our patients were previously diagnosed with stroke, it is unlikely that recurrent stroke played a major role in cytokine variability in our study. Nevertheless, previous history of stroke was treated as a covariate in further analysis and did not significantly change the results. Once sample size is increased and thereby, also increasing the likelihood of recruiting patients with previous stroke, the same statistics should be repeated while controlling for this variability. Taking everything into account, the optimal recruitment time would be 5 days to 1 month post-stroke such that undesired early spikes in cytokine concentrations or late undetectable differences between groups can be eliminated.

The time of blood collection relative to stroke onset may have also hindered cytokine detectability in this study which decreased the power of our analyses. Currently, the majority of the data regarding the time course of cytokines post-stroke are available for IL-6. While it has been suggested that its activity may remain elevated up to one year post-stroke\(^4\), another study has reported peak IL-6 response to occur 4 days post-stroke\(^6\). The latter study also found the detection of IL-6 to be significantly higher only up to 1 month post-stroke in stroke patients compared to healthy controls. Although this numerical value was still 127% of controls after 3 months, the difference was no longer statistically significant. Similarly, the cytokine IL-10 has also been reported to peak in response 3 days after stroke\(^4\). Thus, assaying plasma samples collected from our patients 5 days to 3 months post-stroke may have altered detection levels, as some may have had their cytokine concentrations documented at its peak response while others may have had their concentrations recorded during the tail-end of response. Based on past research, it would be necessary to narrow cytokine analysis to only those patients recruited up to 1 month post-stroke. However, because our analysis was limited by low detectability, patients recruited up to 3 months post-stroke were included to increase
yield. As recruitment continues and assay procedures become stabilized, our ability to assess stroke survivors only up to 1 month post-ischemic attack will increase in power. However, it is important to note that, only 10 of the total 54 patients had blood drawn within greater than 1 month post-stroke, 2 of which were in the depressed group. Furthermore, our preliminary analysis showed that time since stroke did not significantly differ between patient groups (Table 3). Thus, the inclusion of patients greater than 3 months post-stroke should not have significantly affected our results. As suspected, when the 10 patients were removed from the subject population and the cytokine analyses were rerun, they did not significantly differ from our initially reported results.

Blood collection time relative to morning or night periods may also have an effect on our study. Fasting blood levels were collected from patients in the mornings (around 9:00 am) based on the IV technician's regular rounds in the wards. However, this was not always the case, as the IV technician may have made rounds with ±2 hours of the study's 9:00 am standard. This is a limiting factor to our study since cytokines have been found to be regulated in accordance with the circadian rhythm. Such diurnal rhythms are believed to follow consistent and remarkable patterns as demonstrated across a wide range of experiments. These studies reported production of pro-inflammatory cytokines (IL-2, IL-6, IL-12, TNF-α, IFN-γ) to be maximal during nocturnal sleep, peaking between 1–4 a.m.\textsuperscript{416-419}. Although these findings suggest it would be most appropriate to draw blood from patients at night, a change in procedure remains implausible since laboratory workers are not present at that time to collect and centrifuge the blood for further analyses. Thus, our ability to detect levels of pro-inflammatory cytokines may have been hindered by their natural fluctuations in accordance with circadian rhythms. It should be noted nonetheless that all samples were taken in the morning, and none were taken in the evening, or between 1 and 4 am. This should minimize
the impact of sample time variability. Finally, identifiers of possible mediators that enhance or suppress actions of nocturnal sleep on pro- and anti-inflammatory cytokines include high levels of growth hormone and prolactin and low levels of cortisol and catecholamines, respectively\textsuperscript{420-423}. Thus, further research should explore how these molecules vary with changing levels of cytokines and the possible role they may play in PSD progression.

The importance of pro- versus anti-inflammatory response during post-stroke conditions was previously discussed. Two recent publications have described what is believed to be an “immunodepression syndrome”, where the fate of the injured brain tissue is dependent on the intricate balance between pro- and anti-inflammatory cytokines\textsuperscript{196, 197}. Moreover, divergence from their naturally occurring concentrations is thought to induce an immune response. A couple of studies have since then reported increased pro- to anti-inflammatory cytokine ratios in major depression, including the IL-6/IL-10\textsuperscript{69} and the IFN-\textgreek{g}/IL-4 ratio\textsuperscript{70, 71}. Unfortunately, low cytokine detectability hampered our ability to evaluate the “immunodepression syndrome” theory in our study as only a few patients had well detected pro- and anti-inflammatory cytokines to generate such a ratio. This was a major drawback since a balance between pro- and anti-inflammatory cytokine stimulation may be particularly important with regards to IDO activation. In fact, cytokine ratios may even hold a predictive value in the development of PSD when tied to the KYN hypothesis. Hopefully our finding that IL-6 concentrations are trending towards significance with the KYN/TRP ratio will generate greater interest in this field of study.

In summary, the numeric value of both raw and imputed cytokine levels were directionally in line to our prediction, but not in full agreement with past studies reporting significant differences in cytokine levels between depressed and non-depressed healthy individuals\textsuperscript{52, 58, 235-238}. This was surprising since our hypothesis anticipated stroke to
significantly elevate inflammatory marker concentrations such that those suffering from the highest level of inflammation would begin to manifest depressive symptoms. Thus, it would be worthwhile to replicate our assays to improve the reliability of our findings. Furthermore, measurements of LPS stimulated cytokine levels have been conducted in previous studies and may mimic altered states of immune activation representative of post-stroke periods\textsuperscript{424}. Evaluation of unstimulated and stimulated cytokine levels may also be necessary to further elucidate the relationship between inflammation, stroke and depression.

4.3 The Role of Hypertension in Post-stroke Depression

Our study reports a greater percent of hypertensive patients among the depressed population. With respect to blood vessels, hypertension is characterized by two main factors: (1) the structural remodeling of cerebral arteries via increasing wall thickness/lumen ratio\textsuperscript{107} and (2) impaired endothelium-mediated vasodilatation as a result of collagen build-up\textsuperscript{108}. Ultimately, the effects of decreased lumen size and decreased vessel distensibility may reduce both CBF and cerebrovascular reactivity. CBF have been found to be decreased in hypertensive subjects compared to healthy controls\textsuperscript{109}. The limbic and paralimbic structures of the brain, which are known to house the major areas responsible for emotional processing, were found most altered due to this decrease. Both our prediction and results agree with these concepts and findings and match other clinical studies that report impaired blood flow velocity in subjects suffering from a DSM-IV depressive disorder\textsuperscript{110}.

However, similarly to the relationship between depression and inflammation, no conclusive statements can be made about the order of their appearance. Although research surrounding this topic remains scarce, one study has reported CVR to be significantly reduced in a group of depressed patients free of vascular risk factors\textsuperscript{112}. This suggests that major
depression may be the actual cause CVR malfunction rather than its consequence. Although there is no definitive evidence linking depression to the development of hypertension, a couple of studies have found that depression can impair the management and prognosis of hypertension\textsuperscript{425,426}. Since our study focused solely on stroke patients, it would be necessary to include otherwise healthy depressed and non-depressed groups into the study to further evaluate the possible relationship between depression and hypertension and whether this phenomenon is only applicable to stroke conditions. It would also be necessary to include raw blood pressure values to be able to analyze the data from a numerical standpoint.

Because depression was found to be significantly related to hypertension and KYN/TRP, an analysis was conducted to compare the levels of KYN/TRP between hypertensive and non-hypertensive patients. Although the numeric value was higher in hypertensive compared to non-hypertensive patients, the results were non-significant (p=0.12 for chi-square analysis, p=0.16 for ANOVA analysis). Currently, this is the first study to examine the relationship between KYN/TRP and hypertension. Furthermore, no other study has previously examined the triple relationship between stroke, hypertension and the KYN/TRP. As our results indicate a significantly higher KYN/TRP ratio in depressed subjects while controlling for hypertension, the next step would be combine the depressed and hypertensive variables to generate four patient groups (non-depressed and non-hypertensive, non-depressed and hypertensive, depressed and non-hypertensive, and depressed and hypertensive) and evaluate their association to KYN/TRP ratio. Due to the small sample size of depressed patients in our study, we were unable to carry out the analyses ourselves as only two subjects qualified for the depressed and non-hypertensive group. Thus, active recruitment would allow for a greater number of individuals in order to evaluate the proposed relationships.
In support of the triple relationship between depression, KYN/TRP ratio and hypertension, previous studies have produced results that make it worthwhile to explore this concept further. Most recently, it was reported that kynurenine formation by IDO-expressing endothelial cells contribute to arterial vessel relaxation and regulation of blood pressure in systemic inflammation. Although the effects and significance of this finding have yet to be elucidated, the regulation of vascular tone by IDO may substantiate a link between hypertension and PSD. However, the results described from that study appear to favour an opposite outcome (hypotension) than the one hypothesized by this study (hypertension). Conversely, animal models may favour an outcome more in line the predictions of this study.

As previously mentioned, KA is a NMDA antagonist that counters the neurotoxic effects of the QUIN metabolite. It is synthesized in the brain by the enzyme kynurenine aminotransferase-1 (KAT-1) which concentrations have been reported to be significantly reduced in spontaneously hypertensive rats (SHR) compared to normotensive Wistar Kyoto rats (WKY). The same research group further reported that all examined strains of SHR possessed a missense mutation in the KAT-1 gene but not in any of the WKY outbred strains. These results suggest that a mutation in the KAT-1 gene leading to deficient amounts of KA production may be associated with increased effects of the neurotoxic KYN metabolites in hypertensive subjects. In keeping with stroke, further investigations searching for a similar KAT-1 mutation in the human genome may relate hypertension to an elevated KYN/TRP ratio among the PSD population.

The KA levels are also affected by the availability of the kynureninase enzyme. As depicted in Figure 1, kynureninase is responsible for converting KYN into anthranilic acid which is subsequently metabolized into 3-hydroxyanthranilic acid. It has been shown that inhibition of kynureninase as achieved by the intraperitoneal administration of m-
benzoylalanine results in large accumulation of KA in the brain. Furthermore, one animal study documented reduced basal blood pressure after KA injection into the rostral ventrolateral medulla (RVLM) in SHR but not in normotensive WKY. Since the RVLM is pertinent to the tonic and reflexive regulation of arterial blood pressure, kynureninase is speculated to play a role in blood pressure regulation. Along this line of logic, those with a genetic predisposition to increased kynureninase activity will experience higher concentrations of the neurotoxic KYN metabolites under states of inflammatory stress (e.g. stroke) than those without the genetic predisposition, and that this observation will be most pronounced in hypertensive patients.

Finally, a reduction of TRP and simultaneous increase of KYN and QUIN concentrations have been observed in patients with chronic renal insufficiency and correlated with the severity of the disease. Similar results are exhibited in renal insufficient rats where QUIN and L-kynurenine levels in serum, brain, and CSF are increased parallel to the severity of renal incompetence. Since the kidney is well-known for its homeostatic control of blood volume via renin-angiotensin-aldosterone system and anti-diuretic hormone (ADH) release from the adrenal cortex and posterior pituitary, damage to the organ may lead to abnormal blood pressures (e.g. hypertension). Indeed, rat models with induced uremia (a kidney disease where urea and other waste products normally excreted into the urine are retained in the blood) display significantly decreased TRP plasma levels and augmented concentrations of the KYN metabolites 3-hydorxykynurenine and QUIN. This suggests that an elevated KYN/TRP ratio may be responsible for the severity of uremic symptoms such as neuropathy, and increases one's susceptibility to infections, anemia and hypertension. Thus, future PSD studies may also want to look into kidney function and how it may be related to KYN/TRP and hypertension.
Several other risk factors relating depression to hypertension have been documented in the past that were not included in this study. For example, a combination of depression and anxiety symptoms as measured by the General Well-Being Schedule and DSM-IV diagnostic criteria, have been associated with elevated risk of incident hypertension in a couple of studies. One of these studies also reported that race and gender may also play a role since the effect of anxiety was more pronounced in black women compared to white women or men. Certain aspects of depressive symptomatology may also be related to hypertension. In a 4-year follow-up prospective study, hopelessness was associated with increased incidence of hypertension in initially normotensive men. Here, men reporting high levels of hopelessness at baseline were 3 times more likely to become hypertensive than men who were not hopeless. Finally, it is widely accepted that blood pressure and susceptibility to hypertension are influenced by genetic factors. Furthermore, twin, adoption and family studies have linked genetics to the etiology of mood disorders. A combination of the two findings suggests that a shared genetic vulnerability may play a role in the relationship between depression and hypertension. Indeed, one study found that individuals with positive family histories of hypertension exhibit more depressive-like behavior when exposed to mental stress tasks. Results from this study also suggest that a combination of genetic and psychological factors (i.e. stressors) may be involved with cardiovascular hyperreactivity. Thus, inclusion of all the aforementioned risk factors (anxiety, race, specific aspects of depressive symptomatology, genetics, and psychological well-being) should be executed in future studies in order to achieve a well-rounded understanding of hypertension and its effect on mood disorders.

Finally, pre-clinical studies suggest that a mutation in the KAT-1 gene leading to deficient amounts of KA production may be associated with increased effects of the
neurotoxic KYN metabolites in hypertensive subjects. Thus, future studies may choose to measure the KA/KYN ratio as an indicator of KAT-1 activity, where individuals with high blood pressure would be expected to have a lower KA/KYN ratio than normotensive subjects. This finding would also suggest the presence of higher KYN levels in hypertensive subjects such that an elevated KYN/TRP should be observed. Recently, the KA/KYN ratio has been speculated to be a biomarker for neuroprotection as one study reported reduced levels to be correlated with depression severity in hepatitis C immunotherapy patients. Using this logic, a lower KA/KYN ratio may be indicative of PSD severity, with hypertensive PSD patients displaying the lowest KA/KYN ratios.

4.4 Limitations

This study was primarily limited by the small sample size, particularly with respect to the depressed group. Initial sample size calculations predicted statistical significance to be achieved at 88 patients per group. However, this study only represents a post-stroke population of 38 non-depressed and 16 depressed patients. Although results of the primary hypothesis regarding differences in KYN/TRP ratios was significant under ANCOVA analysis with a high observed power of 86.4%, the results from the secondary hypothesis examining differences in cytokine levels were not significant. Furthermore, when the imputed values were used for analysis, results remained non-significant. However, about half or more of the samples required imputation creating a very skewed set of data that would be hard to detect for significance even with the Mann-Whitney Test. Thus, sample size of the depressed patient population should be increased to alleviate the impact of this limitation.

The secondary limitation to this study was the high level of undetectable cytokine samples. To control for this loss, we opted to create imputed values for the missing data by
substituting them with the lower limit of the assay’s detectability. Although this method generated cytokine values that deviated from their true means, the main goal of this study was not to capture low concentrations of pro-inflammatory cytokines but their elevated levels. Thus, the approach should not affect our ultimate intent of finding clinically significant elevated cytokine concentrations. This is supported by our analysis examining differences in undetectability of cytokines between patient groups which did not yield significant results.

From a more conservative perspective, it is likely that the technique overestimated all data since it was not possible to generate discrete values for cytokine levels below the outlined assay sensitivities. Even so, we felt that this method was the most applicable to our data and provided us with the most conservative estimates. Unfortunately, incorporating the imputed values to our analysis did not yield any significant results since such a large majority of our patient population had cytokine levels below the detection limit of the assay. For example, mean levels of pro-inflammatory cytokines could in truth, be significantly higher in the depressed group even though the individual values may be lower than the assay sensitivity. The reverse is also true where imputation would over-estimate plasma pro-inflammatory cytokine levels in the non-depressed group if they were indeed lower as predicted in hypothesis 2. Data pertaining to anti-inflammatory cytokines were much more affected since our hypothesis predicts the presence of decreased concentrations. Imputation of a depressed patient's anti-inflammatory cytokine level would overestimate the true lowest level of interest. Taken all together, imputation would still make it very difficult to accurately define differences in cytokine concentrations between patient groups.

Due to the small sample size, we were unable to categorize patients according to the DSM diagnosis of major depression. Thus, patients who were clinically depressed and displayed milder forms of depressive symptoms were combined and analyzed as one group.
Although differences in mean KYN/TRP ratio was significant under ANCOVA analysis, it is possible that separation between clinically depressed and those who only exhibit depressive symptoms (such that three groups would be employed in the analyses) would have yielded different results. For example, patients who only display depressive symptoms may have lowered the actual mean KYN/TRP ratio and pro-inflammatory cytokines among the clinically depressed patient population. Correlative analysis also revealed that a possible, positive correlation may exist between patients with the severest cases of depressive symptoms and KYN/TRP ratio. Separation of the current "depressed group" into its major and minor components would aid in this investigation. Furthermore, many studies examining the biological correlates mentioned in this study categorize patients explicitly based on the DSM diagnosis criteria. On-going recruitment would increase the sample size such that a more in depth analysis can be carried out between non-depressed, depressive symptoms only, and depressed patients. Moreover comparisons with other studies would carry greater legitimacy by eliminating heterogeneity between different methods of study design.

In summary, the major limitations of this study were sample size, low levels of cytokine detectability, and the shortcoming of having to combine milder depressive symptoms patients with clinically depressed patients into one group due to small sample size.

4.5 Recommendations for Future Research

The primary recommendation would be to increase the sample size of the study population with greater emphasis on recruitment of depressed patients. The addition of more patients would increase the observed power to detect differences in mean cytokine levels between patient groups. Furthermore, it would no longer be a necessity to create a heterogeneous group consisting of those with milder forms of depressive symptoms with those...
who are clinically depressed. This would allow for analysis of the individual depression groups according to the DSM diagnosis criteria as well as more appreciable comparisons with other study populations by decreasing heterogeneity between studies. Since it has been previously reported that cytokine levels are associated with recurrent stroke, it would be useful to longitudinally track the depressive outcomes of these patients and assess their relationship to cytokine concentrations and the KYN/TRP ratio. Indeed, the study is still actively recruiting patients and has included two follow-up visits at 6 and 12 weeks post-baseline assessment to track the time-course of these biological correlates, as well as demographic influences on depression severity. Recently, low level of education, low income, severity of stroke, worse functional outcome, and self-reported problems with ADLs have been shown to be predictive of depression symptoms at 3 months post-stroke\textsuperscript{440}. Moreover, low social support at three months and loneliness and low satisfaction with social network at six months has been reported to be predictive of post-stroke psychological distress\textsuperscript{441}.

It would be interesting to examine the triple relationship between depression, KYN/TRP ratio and hypertension in greater depth as our study indicates that 93.8% of the depressed patient population was also hypertensive. Several demographic risk factors relating depression to hypertension that have been documented in the past were not included in this study, including race and level of anxiety. In addition, the relationship between hypertension and KYN/TRP ratio was close to trending on significance. We hope that by collecting patient demographics and clinical history in greater depth, we will be able to control for these factors during analyses and generate significant results between hypertension, depression and KYN/TRP ratio. It would also be interesting to include a psychophysiological aspect to the study by submitting patients to certain mental tasks (or stressors) while measuring their cardiovascular response. Previously, it has been found that both men and women with a
positive family history of hypertension exhibit higher tonic levels of blood pressure and heart rate during such stressors. The next step would be linking the relationship between hypertension and stress to depression, the KYN/TRP ratio, and other biological correlates of mood disorders. Also, it would be worthwhile to investigate whether this observation applies to otherwise healthy but hypertensive and depressed individuals, or if it has a greater effect on those suffering from neurological conditions such as stroke, since stroke itself is can also be considered a stressor.

Genetics of depression may play a role in the etiology of PSD based on findings from past studies. Polymorphisms of the SERT have been repeatedly associated with several neurological illnesses. Coded by the SLC64A gene in the SERTLPR, the presence or absence of 43 kb yields a long (l) variant or short (s) variant of the gene. Current research suggests a significant association between the s genotype and PSD among all ethnicities. It has also been demonstrated that individuals with the SERTLPR s/s genotype have a 3-fold higher odds of PSD compared with l/l type carriers. Additionally, another SLC64A polymorphism known as the intron 2 VNTR (STin2 VNTR) has been implicated in the etiology of PSD. STin2 VNTR is located in intron 2 and consists of a variable number (usually 9, 10, or 12) of nearly identical 17-bp segments, with the 9-repeat allele (STin2.9) conferring increased odds of major depression and bipolar disorder. In fact, one research group reported that stroke patients with the STin2 9/a2 or 12/12 genotype had 4-fold higher odds of PSD compared with STin2 10/10 genotypes.

There are also several known mammalian 5-HT receptor genes, including 5-HT1A/B/D/E/F, 5-HT2A/B/C, 5-HT3A/B/C/D/E, 5-HT4, 5-HT5A/B, 5-HT6, 5-HT7. Moreover, some of these receptor genes may encode additional receptor variants. Within this group, polymorphisms in the 5-HT1A gene have been the most investigated for its role in psychiatric
disorders and treatment responses. The 5-HT1A receptor is encoded by an intronless gene located on human chromosome 5q11.2–q13 and it spans about 1200 bp and was first cloned by was first cloned by Fargin and Albert⁴⁴⁷, ⁴⁴⁸. Both chronically stressed animal models and subjects with MDD have been reported to have a significantly decreased dorsolateral prefrontal cortex and hippocampal expression of 5-HT₁A mRNA⁴⁴⁹, ⁴⁵⁰. Conversely, enriched expression of the gene carrying the G allele of the C(-1019)G polymorphism has been associated with higher risk of depressive episodes and greater resistance to antidepressant therapy⁴⁵¹-⁴⁵⁷. Moreover, the polymorphism may be sex-linked as one study reported significantly higher binding potential of the receptor in females compared to males, especially in regions of the dorsal raphe, amygdala, anterior cingulate, cingulate body, and the medial and orbital pre-frontal cortices⁴⁵⁴. Thus, further exploration into the 5-HT₁a polymorphism and its role in PSD should be implemented in future studies.

Finally polymorphisms within cytokine coding genes may also be related to PSD. Several studies have reported that polymorphisms in IL-1a, IL-1Ra and TNF-a genes⁴⁵⁸-⁴⁶¹ and polymorphisms within the promoter region of the IL-6 gene are associated with increased risk of stroke⁴⁶², ⁴⁶³. However, whether these polymorphisms are linked to both increased risk of stroke and the development of depression thereafter requires further research. Thus, it would be worthwhile to study both SERT and cytokine genetic components in our study population and examine their roles in the etiology of PSD.

4.6 Clinical Implications

Detection of elevated KYN/TRP ratio early post-stroke would aid clinicians in determining which patients are most prone to PSD. In these cases, immediate preventative measures can be taken to inhibit disease manifestation. Since the prevalence of depression
post-stroke is documented to be as high as 30%, some clinicians opt to prescribe antidepressants to their patients immediately following stroke even before a formal diagnosis of the disease is made. However, research regarding prompt pharmaceutical interventions post-stroke is scarce.

Previously, a Cochrane systemic review was conducted examining methods for preventing depression post-stroke\textsuperscript{464}. The group investigated randomized controlled trials comparing pharmaceutical agents with placebo or psychotherapy against standard care as means of intervention. After reviewing 10 pharmaceutical trials and 4 psychotherapy trials, the authors concluded that there was no clear effect of pharmacological therapy on the prevention of depression. Conversely, there was a significant improvement in mood for psychotherapy patients. However, although the prevention of depression was evident, the treatment effects were reported to be small. Overall, the general consensus is that more reliable data is required before recommendations can be made about either technique. Additionally, the results from our study indicating a significantly higher mean KYN/TRP among those displaying depressive symptoms should also be explored alongside future pharmacotherapy studies, and may provide an improved selection criterion for patients who should undergo antidepressant treatment immediately following stroke.

The ability to predict treatment response has always been of great interest to clinicians since it would allow them to determine the best available medical intervention for their patients on an individual basis. One method currently being explored for the individualization of pharmacotherapy is the use of biomarkers as a predictor to treatment response. According to the Biomarkers Definitions Working Group, a biomarker can be described as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention”\textsuperscript{465}.”
For example, results from earlier research proposed that SSRIs would be most beneficial to those who exhibit low TRP/LNAA ratios, while TCAs would generate a better response in patients with a high TRP/LNAA ratio\textsuperscript{466}. This hypothesis has since then been refuted by other studies that reported no significant difference between antidepressant response and TRP/LNAA ratio\textsuperscript{467, 468}. Thus, a rational biomarker that is predictive of antidepressant response specific to PSD patients has yet to be identified.

The KYN/TRP ratio has previously been hypothesized to be a potential biomarker for stroke volume, where an elevated KYN/TRP ratio may be indicative of a larger infarct size\textsuperscript{469}. Results from our study suggest that the KYN/TRP ratio may be utilized as a probable biomarker to predict antidepressant response in PSD. For example, a higher KYN/TRP ratio would indicate elevated levels of the neurotoxic KYN metabolites and lower TRP availability for 5-HT synthesis. If this is the case, the 5-HT levels may already be so depleted that inhibiting 5-HT uptake from the synapse via SSRI treatment will prove to be ineffective in preventing depression. In addition, activation of the NMDA receptors and oxidative stress mechanisms via QUIN and 3-hydroxykynurenine production may degenerate the SERT-containing neurons at the site of SSRI action. Because our results already indicate a significant difference in KYN/TRP ratio between depressed and non-depressed patients, the next step would be to examine whether this biomarker is a suitable predictor of antidepressant response in a RCT study.

As outlined in the review of literature, activation of the KYN pathway via pro-inflammatory cytokines contribute to post-stroke glutamate excitotoxicity and subsequent calcium accumulation through the production of QUIN. Since QUIN is an endogenous NMDA receptor agonist, it may be advantageous to treat PSD patients exhibiting high KYN/TRP ratio with drugs that inhibit the NMDA receptor. In fact, one study reported that
the NMDA antagonist memantine was able to block oligodendrocyte NMDA receptors at clinically significant therapeutic concentrations after induced ischemia, as well as improve the recovery of action potentials in myelinated axons\textsuperscript{470}. Clinically, memantine has been demonstrated to significantly improve aphasia severity in chronic post-stroke aphasic patients, with its beneficial effects persisting during long-term follow-ups. However, the best outcomes were achieved when the drug was combined with constraint-induced aphasia therapy (CIAT), a form of therapy that forces the patient to communicate verbally without using gestures, non-word sounds or writing\textsuperscript{471}. Thus, the same concept may be applied to PSD, where a therapeutic regimen combining a NMDA receptor antagonist with an antidepressant and psychotherapy may aid in preventing disease manifestation. Furthermore, if response to antidepressant alone is hindered by the neurodegeneration of the SERT containing neurons due to KYN toxicity, an add-on of a NMDA receptor antagonist may facilitate recovery.

It is important to note that activation of the KYN pathway not only produces neurotoxic metabolites, but also the neuroprotective kynurenic acid (KA). KA acts as a NMDA antagonist and opposes the negative effects of QUIN. However, studies examining the measure of its action compared to other KYN metabolites have been limited. The KA/KYN ratio has been proposed to be a measure of neuroprotection since reduced levels of this ratio has been associated with MDD patients\textsuperscript{40}, and correlated with depression severity in hepatitis C patients undergoing immunotherapy\textsuperscript{337}. Although these findings suggest potential treatment opportunities with KA to help restore mood balances in post-stroke patients, it does not readily cross the BBB\textsuperscript{371} and required large doses to be effective in preclinical studies\textsuperscript{472}.

A more plausible route would be to inhibit the kynureninase and kynurenine-3-monooxygenase (KMO) enzymes responsible for converting KYN into its neurotoxic metabolites, such that KYN would be shunted into the kynurenine aminotransferase pathway
to form KA. Nicotinylalanine was the first compound that was reported to act in this manner; it significantly increased the brain content of KA while also preventing the induction of seizures\textsuperscript{473, 474}. The more recently developed KMO inhibitors, m-nitrobenzoyl-alanine (mNBA) and 3,4-dimethoxy[-N-4-(nitrophenyl)thiazol-2yl]-benzenesulfonamide (Ro 61-8048) have also been reported to increase brain KA synthesis\textsuperscript{475, 476} as well as significantly reduce infarct volume in MCAO treated rats\textsuperscript{477}, reduce post-ischemic neuronal death in gerbil hippocampal slice cultures\textsuperscript{476}, and decrease extracellular basal ganglia concentrations of glutamate that would have otherwise accumulated from overproduction of QUIN\textsuperscript{475}. Finally, KMO is also inhibited by the compound PNU156561 (formerly FCE28833A), which has been documented to increase levels of KYN by tenfold and KA by 80-fold in rat hippocampal dialysates for 24 hours after a single dose\textsuperscript{478}.

Most recently, orally active hydroxyamidine small molecule inhibitors (INCB023843 or INCB024360) have been shown to suppress IDO activity in the plasma of mice and dogs\textsuperscript{479}. For example, they were reported to decrease plasma KYN concentrations \textit{in vivo} in wild-type mice to those seen in \textit{Ido}-deficient mice, suggesting these compounds can completely block IDO function. The same study also documented decreased tumour size in tumour-bearing mice after application of the inhibitor. Although the results are promising, the study only measured KYN concentrations as a marker of IDO activity rather than the more appropriate KYN/TRP ratio. Thus, it would be worthwhile to replicate the study using the KYN/TRP as marker for IDO activity before making premature implications on the efficacy of the molecules.

To conclude, the KYN/TRP ratio was increased in those with PSD. The potential role of this ratio as a biomarker for response to antidepressant treatments should be explored. Furthermore, sound knowledge of the role of KYN pathway activation in PSD can lead to the
development of novel pharmacotherapies (e.g. kynureninase and KMO inhibitors, hydroxyamidine inhibitors) to prevent or prohibit disease manifestation and other debilitating stroke outcomes. As research continues to reveal the mechanisms involved in PSD etiology and safe mediums of inhibiting its development, individualization of pharmacotherapies for the disease is a reasonable goal for the future.

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APPENDIX A: Post-stroke Depression Patient Consent Form

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Stroke and Depression: The Role of Cytokine - Serotonin Interactions
Patient Information and Consent

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Dr. J. Ween Baycrest
Dr. W. Goldstein York Central Hospital
Dr. M. Waldman St. John’s Rehab

1. Information for subject:
You are being asked to participate in a study conducted at Sunnybrook Health Sciences Centre under the supervision of the above investigators. Participation is voluntary and will involve the following:

2. Description and purpose of the trial:
The purpose of this study is to evaluate determinants of the development of depressive and cognitive (memory and thinking) symptoms after a stroke. Both symptoms are common post-stroke, and may be related to levels of serotonin (an important brain chemical involved in regulating mood and thinking). We are interested in assessing the relationship between different types of cytokines (naturally produced inflammatory chemicals) and serotonin, and the role they play in any depressive or cognitive symptoms that you may or may not have. In addition, we are also interested in the impact of cytokines and other chemicals related to
serotonin production on the size of the hippocampus (an important brain structure involved in regulating mood and thinking).

3. Study Details:

If you agree to participate in this study, you will be asked to undergo an assessment with a trained research assistant. The process of assessment will involve the following:

a) Assessments:
The study coordinator will first meet with you and review your medical chart in order to assess your eligibility for the study. If you are eligible to participate, the study coordinator will then interview you using standard questionnaires that assess your mood, cognition and physical functioning. Certain details (e.g. medical history, demographic characteristics, current medications and details of your stroke) will be copied from your medical chart. Any CT and MRI scans conducted clinically during your hospital stay will also be analyzed to determine the characteristics of your stroke. Lastly, if you report experiencing significant depressive symptoms, the study physician will meet with you for further assessment and treatment, if necessary. This study will not interfere with your selection of treatment choice. However, information will be collected regarding your response to treatment through the questionnaires described above.

b) Blood Draw:
A sample of blood will be drawn in order to measure levels of certain signaling molecules related to the serotonin and inflammatory systems (called cytokines, kynurenines and tryptophan). A total of 31 mL (2 tablespoons) of blood will be drawn.

c) Cheek Swab:
A sample of skin cells from the inside of your cheek will be taken using a sterile cotton-tipped swab. The DNA inside these cells will be used for us to determine which forms of certain genes (“polymorphisms”) you have that are related to the cytokine, kynurenine and serotonin pathways. Your sample will be identified only by a unique number and will be destroyed once the genetic tests are complete.

4. Benefits:
You will not benefit directly from participation in this study.

5. Risks:
When your blood is drawn, there may be some discomfort and/or bruising, however these are expected to be very mild. The mouth swab is simple and painless.

6. Alternative Treatments:
You are eligible to receive treatment for your stroke and any depressive symptoms you may have even if you choose not to participate in this study. Participation in this study will not affect your treatment in any way.
7. Costs:
You will be given a $20.00 honorarium each time you visit Sunnybrook for the purposes of this study. If you participate in the MRI substudy, you will be given a $40.00 honorarium for your third visit. You will incur no costs as a result of participation in this study.

8. Participation/Termination:
Your participation in this study is voluntary. Thus, if you do not wish to take part in this study or wish to withdraw at any time after commencing the study, your care will not be affected in any way.
You may be withdrawn from the study, at any point, if the investigator of this study considers it to be in your best interest. You may withdraw your consent at any point during the study.

9. Confidentiality:
Your identity in this study will be treated as confidential. Certain Sunnybrook research staff, the Sunnybrook Research Ethics Board, and other agencies as required by law, may need to review your medical chart. We will have access to your medical chart for information on: blood pressure, heart rate, prescribed drugs, depressive symptoms and health status for 1 year. On all data collected for this study, you will be identified only by a unique number. If you disclose the intention to harm yourself or others, this information may not be kept confidential, as required by law.

10. Contacts:
If you or your substitute decision maker have any questions about this study or for more information, you may contact the Study Co-ordinators: Philip Francis or Amy Wong (416-480-6100 x3185), Dr. Krista L. Lanctôt (416-480-6100 x2241) or Dr. Nathan Herrmann (416-480-6100 x6133).

If you have any questions about your rights as a research subject, you may contact Dr. Philip Hébert, the Chair of the Sunnybrook Research Ethics Board, at 416-480-4276.
Consent to Participate in this Study:

I, (patient’s name) ______________________________________ have read the above information and fully understand the nature and the purpose of the study in which I have been asked to take part. The explanation I have been given has mentioned both the possible risks and benefits of the study. I understand that I will be free to withdraw from the study at any time without affecting my subsequent treatment by my doctor in any way. I voluntarily consent to participate in this study.

_________________________________
Name of Patient (typed or printed)

_________________________________  _________________
Signature of Patient                  Date

_________________________________
Name of Investigator (typed or printed)

_________________________________  _________________
Signature of the Investigator         Date

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APPENDIX B: Sunnybrook REB Approval Letter

RENEWED ETHICS APPROVAL

RESEARCH ETHICS BOARD
SUNNYBROOK
HEALTH SCIENCES CENTRE
C819, 2075 Bayview Avenue
Toronto, Ontario
M4N 3M5

Must be completed annually for all on-going studies
Please return to room C819

Project Title:
Post-Stroke Depression: The Role of Cytokine-Serotonin Interactions in Treatment Response

Project Identification Number: 380-2004

Original Approval Date: December 8, 2004

Principal Investigator: Dr. Krista Lanctôt

Full Address Including Room Number: Sunnybrook Health Sciences Centre
2075 Bayview Ave., Room FG05
Toronto, ON M4N 3M5

Full Board Review Required: Yes ☐ No ☑

If industry sponsored, please note there is a $500 re-approval fee. Invoicing information including contact name and full mailing address is required for each renewal.
Progress of Study (provide a brief summary of patient accrual):

A total of 81 ischemic stroke patients have been recruited to date. Of these, 76 (53 non-depressed, 12 depressive symptoms, 11 depressed) have completed all required study procedures. 3 withdrew consent during the baseline assessment due to fatigue and 2 completed all assessments but were discharged prior to blood collection. Of the 11 depressed patients, 3 were treated with citalopram.

Of the 81 patients recruited, 78 were recruited from Sunnybrook, 1 from Baycrest and 2 from Toronto Rehab.

Amendments to the study (must be submitted for approval):

1. Submitted May 13, 2005; Approved June 1, 2005
2. Submitted January 24, 2006; Approved February 28, 2006

In addition, this protocol has been submitted to, and approved by, the Baycrest and Toronto Rehabilitation Institute REBs. It is currently under review by the St. John’s Rehab REB.

Changes in scientific knowledge that could impact on the study and action taken:

None.

Unexpected or adverse events and action taken:

No adverse events were experienced in the 3 patients treated with citalopram.

Protocol violations and actions taken:

None

Expected date of completion:

December 2010 (date has been extended due to the addition of new cognitive measures).
Sunnybrook Health Sciences Centre
Research Ethics Board
Renewed Ethics Approval

Please sign below:

My signature certifies the following information is correct and I will not use any procedures, which have not been approved by the Board.

Signature of Principal Investigator

Date

The above study is ethically acceptable and has received renewed ethics approval. This study may continue at Sunnybrook Health Sciences Centre.

Chair, Research Ethics Board

Date of Review

Date of Full Board Review (if required):

Cap 6-07
APPENDIX C: York Central REB Approval Letter

York Central Hospital
10 Trench Street
Richmond Hill, ON
Canada L4C 4Z3
Phone 905-883-1212
Fax 905-883-2455
www.yorkcentral.on.ca

November 30, 2009

Krisia Lanctôt
Sunnybrook Health Sciences Center
2075 Bayview Ave - Room FG05
Toronto, ON M4N 3M5
Tel: 416-480-6100 x3165

CC: Robert Mitchell, robert.mitchell@sri.utoronto.ca

Study Title: The Role of Cytokine-Serotonin Interactions in Post-Stroke Depression
- YCH Secondary Research Application - Renewal (Dated: September 3, 2009)
- Renewal information (Dated: September 3, 2009)
- Informed Consent Form (Dated: September 1, 2009)

Dear Dr. Lanctôt:

The York Central Hospital (YCH) Research Ethics Board (REB) acknowledges receipt of and approves all the documents noted above in regards to the study titled: The Role of Cytokine-Serotonin Interactions in Post-Stroke Depression; and grants approval of the study for one year which is to be conducted in accordance with all submitted and approved documentation.

If during the course of the project, there are any confidentiality concerns, changes in the approved protocol or subject information or new information that must be considered with respect to the project, these should be brought to the attention of the REB. The YCH REB acknowledges that the data will be transported electronically and/or physically off site. In the event of a privacy breach, you are responsible for reporting the breach to the YCH REB. As the submitting investigator, you are responsible for the ethical conduct of this study.

Should this project extend beyond one year, you are responsible for maintaining ongoing ethical approval with consideration to the date noted on this letter. The REB must also be notified of the completion or termination of this study and a final report provided. If you have any questions regarding this letter, please contact the REB Research Coordinator, Abel Cheng at 905-883-1212 ext 7569.

Sincerely,

[Signature]

Abel Cheng, BSc, MEd (o), CCRP
Research Coordinator
Research Ethics Board (REB)
York Central Hospital
10 Trench St, Richmond Hill ON L4C 4Z3
Email: acheng@yorkcentral.on.ca
APPENDIX D: Baycrest REB Approval Letter

Notification of REB Continued Approval

Date: May 14, 2010

To: Lanctot, K., Ween, J., Herrmann, N., Black, S., Sahlas, D., Gladstone, D.

Re: The Role of Cytokine-Serotonin Interactions in Post-Stroke Depression (REB# 06-62)

REB Review Type: Annual
REB Initial Approval Date: February 13, 2007
REB Expiry Date: February 13, 2011
Consent Form(s) Currently Approved for Use: ICF (Version #4, September 1, 2009)

The above-named study has received continued approval from the Baycrest Research Ethics Board (REB) until the expiry date noted above. If the study is expected to continue beyond the expiry date, you are responsible for ensuring the study receives re-approval. The REB must also be notified of the completion or termination of this study and a final report provided.

If, during the course of the research, there are any serious adverse events, confidentiality concerns, changes in the approved project, or any new information that must be considered with respect to the project, these should be brought to the immediate attention of the REB. In the event of a privacy breach, you are responsible for reporting the breach to the Baycrest REB and the Baycrest Privacy Office (in accordance with Ontario health privacy legislation – Personal Health Information Protection Act, 2004). Additionally, the Baycrest REB requires reports of inappropriate/unauthorized use of the information. As the Principal Investigator, you are responsible for the ethical conduct of this study.

The Baycrest Research Ethics Board operates in compliance with the Tri-Council Policy Statement, ICH/GCP Guidelines, the Ontario Personal Health Information Protection Act (2004), and Part C, Division 5 of the Food and Drug Regulations of Health Canada.

Sincerely,

Angela Troyer, Ph.D.
Chair, Baycrest Research Ethics Board
APPENDIX E: St. John's REB Approval Letter

TO Dr. Krista L. Lanctôt  
Dr. Murray Waldman  
DATE: September 16, 2009

FROM Manuel Gomez, MD

RE The Role of Cytokine-Serotonin Interactions in Post-Stroke Depression

Project Identification Number: 2008-10  
Approval Date: September 16, 2009

The Research Ethics Board (REB) of St. John’s Rehab Hospital has conducted a review of the research protocol referenced above on the above captioned date, and has received renewed ethics approval for the involvement of human subjects as specified in the protocol and the information sheet/consent form.

Should your study continue for more than one year you must request a renewal on or before one year from this approval date. Please advice the Board of the progress of your research annually and/or any adverse reactions or deviations which may occur in the future.

The above Project Identification Number has been assigned to your project. Please use this number on all future correspondence.

Thank you for keeping the Board informed.

[Signature]
Manuel Gomez, MD, MSc.  
Chair, Research Ethics Board

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APPENDIX F: Toronto Research Institute REB Approval Letter

August 11th, 2010

Dr. Krista Lancot
Sunnybrook Health Sciences Centre
2075 Bayview Avenue
North York, ON
M4N 3M5

Dr. Abe Snaiderman
Toronto Rehab Site Investigator
Toronto Rehab Institute
550 University Avenue
Toronto, Ontario
M5G 2A2

Dear Drs. Lancot and Snaiderman:

RE: TRI REB # 07-034
The Role of Cytokine Interactions in Post-Stroke Depression

The above-named study has received continued approval from the Toronto Rehab Research Ethics Board until the expiry date noted below. If the study is expected to continue beyond the expiry date, you are responsible for ensuring the study receives re-approval. The REB must also be notified of the completion or termination of this study and a final report provided.

If, during the course of the research, there are any serious adverse events, changes in the approved protocol or consent form or any new information that must be considered with respect to the study, these should be brought to the immediate attention of the Board.

Sincerely,

[Signature]

[ ] Paul Oh MD, MSc, FRCPC
Chair, Research Ethics Board
Toronto Rehabilitation Institute

[ ] Ann Heesters BEd, BA, MA, PhD(ABD)
Vice Chair, Research Ethics Board
Toronto Rehabilitation Institute

August 14, 2007
Date of Initial REB Approval

August 14, 2011
Expiry Date of REB Approval

TRI REB conforms with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans and Ontario Privacy Legislation PHIPA
APPENDIX G: Study Roles

Study Concept and Design: Drs. Krista Lanctôt and Nathan Herrmann

Data Acquisition: Amy Wong

- Consisted of participant recruitment, completion of all study assessments, collection of medical histories through chart review, venipuncture and database management

Cytokine Assays: Dr. Angela Panoskaltsis-Mortari, University of Minnesota

Kynurenine Assay: Mr. Scott Walker, Sunnybrook Health Sciences Centre

Tryptophan Assay: Dr. Simon Young, McGill University

CT Analysis: Dr. Richard Aviv, Sunnybrook Health Sciences Centre

Data Analysis and Interpretation: Amy Wong, Drs. Krista Lanctôt, Nathan Herrmann

Funding: A Heart and Stroke Foundation grant to: Drs. Krista Lanctôt (PI), Nathan Herrmann (Co-PI), Sandra Black, Demetrios Sahlas, David Gladstone

Study Supervision: Drs. Krista Lanctôt, Nathan Herrmann
APPENDIX H: Presentations and Publications

Presentations


Wong A. Cytokine and serotonin interactions in post-stroke depression. Presented at Brain Sciences Rounds. Department of Neurology, Sunnybrook Health Sciences Centre Toronto, Canada, November 2009.


Other Publications


