The Inflammatory and Neuroanatomical Factors Involved in Post-stroke Depression

By

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ABSTRACT

This cross-sectional study examined neurobiologic correlates of depression in ischemic stroke patients. Depression severity was measured with a standardized scale (Center for Epidemiologic Studies Depression Scale; CES-D). Eighty-two patients (53.1% male, mean (± SD) age 71.9 ± 14.2 years, mean (± SD) National Institutes of Health Stroke Scale (NIHSS) score 4.6±4.7, mean (± SD) CES-D score 12.6 ± 10.8) were recruited. A linear regression controlling for age and stroke severity (NIHSS) determined that the kynurenine to tryptophan ratio (β= -0.105, p=0.369) was not significantly associated with CES-D (primary hypothesis) (overall model R²=0.069, F3,73=1.805, p=0.154). Secondary analyses suggested one instance of cytokines favouring inflammatory states in mild depressive symptomatology; IFN-Ɣ/IL-10 (OR, 2.17; 95% CI, 1.02-4.64, p=0.045). For the most part however, inclusion of cytokines and neuroimaging correlates such as atrophy, lesion location and white matter changes were non-significant. Longitudinal studies are necessary to identify the possible neurobiologic correlates of depressive symptoms post-stroke.
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Section 1: Introduction

1.1 Statement of the Problem

Stroke is one of the most common causes of death in industrialized countries.\textsuperscript{1} For those who survive, it can negatively impact physical and neuropsychiatric domains leading to impairments in these areas. While apathy, anxiety and cognitive impairment are common post-stroke sequelae,\textsuperscript{2} the onset of depression after stroke is one of the most frequent neuropsychiatric outcomes.\textsuperscript{3} Depression in particular can significantly affect the stroke patient, the family and staff involved in their care, and the healthcare system at large.\textsuperscript{3} Regarding negative consequences for the patient, post-stroke depression (PSD) has been linked to increased mortality\textsuperscript{4}, reduced functional\textsuperscript{5} and rehabilitative\textsuperscript{6} success, and poorer quality of life.\textsuperscript{3, 7} Diminished quality of life and increased depressive symptoms are also commonly experienced by caregivers of PSD patients\textsuperscript{8}. With respect to the impact of PSD on the health care system, it has been correlated with an increased duration of inpatient stay and increased number of outpatient clinic visits.\textsuperscript{3, 9}

PSD affects approximately one third of all stroke survivors\textsuperscript{10} and its prevalence surpasses the 30-day (4.9\%) and lifetime (17.1\%) prevalence of depression found by the U.S. National Comorbidity Survey.\textsuperscript{3, 11} Studies propose that the prevalence of PSD reaches a maximum at roughly 3-6 months, diminishes by 50\% at 1 year, and then it may remain elevated at approximately 20\% for 2 years and beyond.\textsuperscript{3, 12, 13} This last finding is troublesome because it demonstrates that PSD can be a chronic, non-remitting disease in many stroke survivors. Compounding this is the issue of antidepressant treatment efficacy. Current therapies may reduce depressive symptom severity, however they may not lead to the remission of symptoms.\textsuperscript{14, 15} Given that functional disability and cognitive impairment are strongly associated with PSD,\textsuperscript{16} these deficits may not improve with the use of current antidepressant therapies.\textsuperscript{3, 14} Taking these concerns together, it is evident that more research must focus on the mechanisms involved in the onset of PSD, in order to develop efficacious treatment strategies for this emotionally debilitating disease.
1.2 Purpose of the Study and Objectives

It is evident that PSD is a serious health concern to the patient, their caregivers, and the entire health system at large. Because of its high prevalence among stroke survivors and the negative consequences it can incur, researchers have devoted much energy into developing and testing hypotheses on the etiology of this disease. Over the years, the biological and psychosocial mechanisms of PSD have been examined, forming multiple lines of reasoning and debate over which domain contributes more to the development of PSD.12 However, the general consensus among experts in the field today is that these two viewpoints are not contradictory, rather, they may interact to instigate the development of depressive symptoms.12

The overarching objective of this study is to investigate the neurobiological correlates of PSD through the assessment of current contemporary hypotheses, namely the inflammatory and neuroanatomical factors which may have a role in its development. Principally, this study aims to uncover the relationship between acute inflammatory serum biomarkers and post-stroke depressive symptom severity in a cohort of ischemic stroke patients. In doing so, the kynurenine (KYN) to tryptophan (TRP) ratio (K/T) will be assessed for its association with depressive symptoms. The K/T ratio is considered to be an indicator of indolamine 2,3-dioxygenase (IDO) activity,17,18 which is an enzyme that catalyzes the conversion of TRP into KYN. Tryptophan is a precursor for the neurotransmitter serotonin,19,20 and so acute depletion of this molecule by way of increased KYN production may lead to the development of depressive symptoms post-stroke. Furthermore, the metabolism of KYN increases the amount of neurotoxic21 KYN metabolites, which may also have a detrimental effect on mood regulating centers of the brain. Furthermore, IDO activation can be influenced by both elevations in pro-inflammatory cytokines22-28 and reductions in anti-inflammatory cytokines.29,30 Pro- versus anti-inflammatory cytokine imbalance has been studied with respect to major depression31-35 and post-stroke outcomes36,37 in the past with positive results. Therefore as a secondary aim I will examine the relationship between pro-
inflammatory and anti-inflammatory cytokines and post-stroke depressive symptoms, as a possible intermediary link between an elevated K/T ratio and depressive symptoms.

Lesion volume, \(^{38-43}\) white matter change (WMC), \(^{38-48}\) and atrophy \(^{39, 40, 45, 49}\) are other biological correlates that have been studied in the context of PSD with mixed findings, however MDD has been consistently linked with neurodegeneration and neuroprogression. \(^{50-60}\) A comprehensive approach to investigating atrophy, lesion volume and WMC may help to clarify their role in the context of PSD, as these measurements are not mutually exclusive. Therefore, I additionally sought to explore the relationship between neuroimaging findings and post-stroke depressive symptom severity, as these might represent chronic factors involved in the development of PSD. Specifically, hippocampal thickness, whole brain atrophy, WMC and lesion volume may be important independent correlates of depressive symptom severity post-ischemic stroke. Finally, the fact that (1) depression has been associated with neurodegeneration, \(^{50-60}\) (2) KYN metabolites are elevated in many neurodegenerative diseases and stroke, \(^{61-66}\) and (3) KYN metabolites have also been implicated in the process of neurodegeneration, \(^{21, 50, 67-77}\) suggest that an elevated K/T ratio may be more relevant in contributing to the development of PSD in the presence of neurodegeneration, than either variable alone. Thus, I will also assess the role of an interaction between the K/T ratio and any significant radiological findings from the previous exploratory analysis, in contributing to depressive symptoms in this population.

In summary, the key objectives of this study are to explore the inflammatory and neuroanatomical contributions and their potential interactions, to the development of PSD.
1.3 Statement of Research Hypotheses and Rationale for Hypotheses

**Hypothesis 1:** Serum levels of the K/T ratio, as a measure of IDO activity, will positively correlate with depressive symptom severity post-ischemic stroke.

**Rational:** Pro-inflammatory cytokines are released during the inflammatory response following infarction, and elevations in cytokines stimulate the synthesis of the enzyme IDO. In turn, this increases the production of KYN from TRP, thereby increasing the K/T ratio. Changes in the levels of KYN and its metabolites have been associated with neuropsychiatric outcomes such as bipolar mania, schizophrenia, and importantly, major depression. Furthermore, our group observed a significant positive correlation between the K/T ratio and depression scores in coronary artery disease (CAD) patients, a disease which shares common grounds with stroke. TRP is the precursor for the neurotransmitter serotonin, and a depletion in serotonin has been associated with major depressive disorder (MDD). Additionally, acute tryptophan depletion paradigms have caused transient depressive symptoms in recovered MDD patients. Once IDO catalyzes the conversion of TRP to KYN, KYN can be further degraded into excitotoxic metabolites such as quinolinic acid (QUIN) which has been demonstrated to play a role in neurodegenerative processes. Furthermore, this neurotoxic metabolite may affect the emotion centres of the brain; a recent study discovered that the brains of deceased patients with MDD and bipolar disorder had increased QUIN immunoreactivity in the prefrontal cortex and hippocampus. Thus, taking these findings together, depressive symptoms experienced post-stroke may be the result of either acute tryptophan depletion or the production of excitotoxic KYN metabolites that may contribute to the onset and maintenance of depression, respectively. Additionally, changes in the K/T ratio have been correlated with depression in non-stroke patients, therefore it is possible that elevations in the K/T ratio may be associated with PSD as well.
**Hypothesis 2:** Elevated pro-inflammatory cytokines IFN-Ɣ, TNF-α, IL-6, IL-18, and IL-1β and a reduction in the anti-inflammatory cytokine IL-10, as evidenced by an elevated immunologic ratio between these pro-inflammatory cytokines and IL-10, will correlate with depressive symptom severity post-ischemic stroke.

**Rational:** IDO activation can be stimulated by both elevations in pro-inflammatory cytokines and reductions in anti-inflammatory cytokines. Therefore it is plausible that a change in the levels of these cytokines post-stroke may be associated with increased IDO activation. These particular cytokines were chosen because they have been shown to be disrupted post-stroke, or because they have been implicated in stimulating IDO, and represent both helper T cell type 1 (IFN-Ɣ, TNF-α, IL-1β, IL-18) and type 2 (IL-6 and IL-10) cytokines. Further, after immune challenge with IFN-α, levels of CSF KYN and QUIN in hepatitis C virus (HCV) infected patients increased significantly and were significantly correlated with depression scores. Pro- versus anti-inflammatory cytokine imbalance has been studied with respect to major depression and post-stroke outcomes in the past. With respect to major depression, it was observed that the IL-6/IL-10 ratio was significantly elevated in major depressive disorder (MDD) patients compared to controls, and multiple studies have observed a reduction in these immunologic ratios upon antidepressant treatment. Researchers have also found that patients with PSD had significantly higher serum ratios of TNF-α/IL-10 and IL-6/IL-10 than controls, and reduced serum IL-6 and elevated serum IL-10 were significantly associated with lower degree of patient disability. Additionally, IL-4 and IL-10 polymorphisms, which related to lower anti-inflammatory cytokine production were significantly associated with PSD. This suggests that immune imbalance may be more indicative of PSD symptoms than studying pro- and anti-inflammatory markers in isolation and therefore is the impetus for the secondary hypothesis of this study.
1.4 Review of the Literature

1.4.1 Prevalence

One of the primary causes of death in Canada is due to stroke.\textsuperscript{108} The overall incidence of stroke in Canada is 14.4 per 10,000, however, for patients 80 years and older this number rises to approximately 132 per 10,000. The length of stay for patients in hospital is approximately 3 weeks per indexed episode, and 18.2% of patients die in the hospital within 28 days.\textsuperscript{108}

While this mortality rate is high, there has also been much progress in the area of post-stroke interventional and rehabilitative services that are contributing to an increased number of stroke survivors. These patients must deal with many post-stroke functional, cognitive and emotional impairments.\textsuperscript{3} The onset of depression after stroke is one of the most frequent neuropsychiatric outcomes and has been linked to increased mortality rate\textsuperscript{4}, reduced functional\textsuperscript{5} and rehabilitative\textsuperscript{6} success, and poorer quality of life in stroke survivors.\textsuperscript{3, 7} In the acute phase after a stroke (approximately 1 month) the prevalence of depressive disorders and depressive symptoms ranges from 5% to 63%.\textsuperscript{109} Furthermore, one study estimated overall pooled prevalence of depression post-stroke was approximated at 33% for the follow-up period between 2 weeks and 5 years post-stroke.\textsuperscript{10} A recent systematic review and meta-analysis by Ayerbe \textit{et al.} assessed fifty studies published between 1983 and 2011 and found the pooled prevalence of depression to be 29% (95% CI 25–32) and can remain at this level for up to 10 years post-stroke.\textsuperscript{110} In addition, they found that depression within one month post-stroke had a prevalence of 28% (95% CI 23–34), depression at one to six months post-stroke had a 31% (95% CI 24–39) prevalence, at six months to one year the prevalence increased to 33% (95% CI 23–43), and at more than one year the prevalence remained elevated at 25% (95% CI 19–32).\textsuperscript{110} This surpasses the 30-day (4.9%) and lifetime (17.1%) prevalence of depression found by the U.S. National Comorbidity Survey.\textsuperscript{3, 11} Variations in the methods of selecting the study population, i.e. inclusion/exclusion criteria, assessment time points, and lack of an operational definition of PSD in order to determine appropriate diagnoses, are all factors involved in the lack of consensus on the prevalence of PSD.\textsuperscript{3, 5, 111, 112} In terms of the study population, there is a large
disparity between the degree of disability in inpatient versus outpatient units, which is problematic because depression has been correlated with functional impairment in the past. Thus, studies exclusively assessing inpatients or outpatients may misjudge the overall prevalence in these populations. With respect to the timing of assessments, studies observing patients in the acute (weeks) phase post-stroke may calculate higher frequencies PSD than studies with patients in the more chronic (months to years) phases post-stroke. As mentioned earlier, studies suggest that the prevalence of PSD reaches a maximum at roughly 3-6 months, diminishes by 50% at 1 year, and then it may remain elevated at approximately 20% for 2 years and beyond. The lack of consensus on the prevalence of these post-stroke sequelae is troublesome, despite this, it is evident that depression after a stroke is a frequent and debilitating disease that warrants further investigation.

1.4.2 Clinical Correlates and Phenomenology of PSD

The reliable diagnosis of PSD is a difficult task for clinicians, since stroke usually leads to the development of functional disabilities and neurological deficits. In some cases the stroke, as opposed to the underlying presence of depression, may be credited for these impairments. For instance, physical disabilities such as paralysis may render the patient unable to participate in certain daily activities. However, the patient may also be experiencing anhedonia, which is the inability to enjoy activities that were once considered pleasurable by the individual. Dysphagia may interfere with the ability to assess disturbances in appetite and fluctuations in weight which may also manifest as somatic symptoms of depression. Additionally, aphasic patients who are experiencing depressive symptoms may be unable to express these feeling properly or entirely.

Compounding the issue of diagnosis is the even larger problem of how to define PSD. An operational definition of this disorder is difficult due to the presence of other neuropsychiatric disorders that may accompany it. These conditions may include anxiety, apathy, and pseudobulbar affect (emotional incontinence), which have the potential to occur within the context of depressive symptoms or exclusive of them. For instance, one study observed that apathy, defined as diminished motivation,
occurred independently in 19.8% of post-stroke patients and occurred concurrently with depression in 20.6% of patients. Despite the overlap between depression and other post-stroke neuropsychiatric sequelae, the DSM-IV-TR classifies post-stroke major depression as “mood disorder due to stroke with major-depressive-like episode” (American Psychiatric Association 2000), while post-stroke minor depression is associated with “research criteria” in DSM-IV. To receive the diagnosis of major depression, patients must display at least five out of these nine symptoms: 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. Additionally, these symptoms must be present for two weeks at minimum and depressed mood or anhedonia must constitute one of the five symptoms present. To receive the diagnosis of minor depression, patients must exhibit two to four of these nine symptoms and once again the presence of depressed mood or anhedonia is required. Spalletta and colleagues studied 200 first-ever stroke patients within 3 months post-stroke and observed that 25% of the cohort had major PSD and 31% had minor PSD. In the past, minor PSD has been linked to increased mortality risk and diminished ability to take part in activities of daily living. Therefore, physicians should take care in diagnosing both forms of this disorder, since the consequences of either could be devastating.

Researchers have attempted to further characterize PSD in terms of early- and late-onset of symptoms. A study assessing 142 patients over a period of 2 years post-stroke found that symptoms such as anxiety, loss of libido, guilt and irritability were only more frequent in the depressed group before 6 months. In a 3-year longitudinal study assessing 80 patients, researchers observed a 25% prevalence of major PSD acutely and a 31% prevalence at 3 months. In addition, 60% of the patients diagnosed with ‘early depression’ (zero to three months) experienced remission of depressive symptoms at one-year. It was also determined that patients who were diagnosed with early depression and still had symptoms at one-year were more likely to experience chronic depression. This finding is in agreement with studies by Wade et al. and Robinson et al. who have demonstrated that as many as half of their participants characterized as having early- or late-onset depression, were still depressed at one-year follow-up.
However, Whyte and Mulsant suggest that depression developing acutely post-stroke is more likely to spontaneously remit.\textsuperscript{12}

In terms of the psychosocial correlates of PSD, functional impairment,\textsuperscript{5, 13, 122, 125-128} stroke severity,\textsuperscript{129} personal history of depression,\textsuperscript{130, 131} female gender,\textsuperscript{47, 128, 129, 132} social isolation,\textsuperscript{130, 132} neuroticism\textsuperscript{133} and cognitive impairment\textsuperscript{134, 135} have been found to moderate the risk of PSD in the past. A meta-analysis by Hackett and Anderson, however, determined that only one half of the aforementioned factors were significantly associated with PSD across various studies.\textsuperscript{16} These factors include functional impairment, stroke severity, and cognitive impairment.\textsuperscript{16} Another recent meta-analysis by Ayerbe \textit{et al.} found that disability after stroke, personal history of depression pre-stroke, cognitive impairment, stroke severity, lack of social support, and anxiety were all important predictors of PSD.\textsuperscript{110} This study also observed that poorer quality of life, mortality and disability are significant independent outcomes of depression.\textsuperscript{110} Moreover, the presence of cognitive impairment in the context of PSD has been associated with diminished recovery of depressive symptoms,\textsuperscript{136} and deficits in the areas of memory, visual perception and language have also been linked with worse long-term PSD outcomes.\textsuperscript{41, 137, 3}

Numerous studies have also attempted to correlate lesion characteristics with PSD. Lesions in left anterior and left basal ganglia regions have been associated with the development of PSD and the severity of depressive symptoms have been correlated with the proximity of the lesion to the frontal pole.\textsuperscript{125, 138-142} The time course of this relationship may also be important; studies have shown that it is strongest at 6 months post-stroke,\textsuperscript{139} however other researchers have failed to reproduce these findings.\textsuperscript{143, 144} Although there is much debate in this field over the true impact of lesion location of PSD, the general concept behind this research is that certain brain circuits involved in mood regulation can be altered by a stroke, leading to the onset of depression.\textsuperscript{3, 45} This concept will be discussed in further detail elsewhere in this thesis.
1.4.3 Treatments for Post-stroke Depression

Many pharmacological treatments for PSD have been tested over the past three decades. These treatments include antidepressant therapies and psychostimulants. Regarding antidepressant medications, many open-label and placebo controlled studies have determined that currently accepted treatments for depression, such as selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), and tricyclic antidepressants (TCAs) are effective at lessening the symptom severity of depressive symptoms post-stroke. However, by DSM-IV standards, multiple meta-analyses have found that the effects of these treatments are not significant enough to demonstrate that depressive symptoms remit completely. Although, a randomized trial that found nortriptyline was more efficacious at reducing depressive symptoms than either fluoxetine or placebo, also determined that both fluoxetine and nortriptyline taken for 3 months resulted in improvement of executive functioning at 2 years. This is a significant finding since some researchers believed that smaller reductions in depressive symptoms by antidepressants may leave patients dealing with impairments in cognition and function. Researchers have also assessed prophylactic treatment of depressive symptoms post-stroke. Yet the results of these placebo-controlled prophylactic studies are not in unanimous agreement with one another. Some studies’ findings support the use of antidepressants in patients without evidence of depressive symptoms, while others show no significant benefit of proactive treatment in the acute post-stroke phase. In addition, multiple meta-analyses have examined the utility of prophylactic treatment for PSD, however these too have produced mixed findings. For instance, one meta-analysis by Chen et al. observed a significant difference in the incidence of PSD in antidepressant treated patients versus non-treated patients. That study was then challenged by a Cochrane review that failed to find strong evidence in support of antidepressant prophylaxis of PSD. This review attributes its lack of findings to the methodological heterogeneity of studies, such as differing periods between stroke and timing of study enrollment, wide range of medications administered and length of trial periods, and finally, a mix of
depression scales or classifications utilized across studies may have prevented this group from accurately investigating treatment prophylaxis in PSD.\textsuperscript{169, 170}

Concerns regarding the safety of TCAs and SSRIs for use in elderly patients with vascular disease has prompted much investigation on their negative effects. A systematic review assessing the use of SSRIs and TCAs in patients with vascular disease, for example, stroke and cardiac disease, observed that TCAs were not significantly associated with increased frequency of ‘serious’ cardiovascular adverse events, such as death due to heart failure, stroke or myocardial infarct.\textsuperscript{171} Furthermore, SSRIs were significantly less likely to cause ‘non-serious’ cardiovascular AEs compared to TCAs.\textsuperscript{171} Conversely, in a retrospective, clustered secondary data analysis from the National Veterans Health Administration long-term care nursing homes (\(n=6\,577\)), it was found that antidepressant medications use (OR = 1.39, \(P < .0001\)) was associated with the odds of falling by nursing home residents.\textsuperscript{172} Furthermore, another study found that there is an increased risk of stroke in women who use SSRIs (OR=1.45, 95\% CI 1.08-1.97).\textsuperscript{173} With these conflicting findings, it may be important to explore non-pharmaceutical interventions for treatment of PSD, such as psychoeducation,\textsuperscript{174} care coordination\textsuperscript{175} and nursing home visits,\textsuperscript{176} which have been demonstrated to reduce depressive symptoms and improve quality of life in stroke patients.\textsuperscript{3}

1.4.4 Ischemic and Inflammatory Responses in Stroke

Both \textit{in vitro} and \textit{in vivo} models of cerebral ischemia have shaped our understanding of the pathophysiology of stroke.\textsuperscript{177, 178} A stroke occurs when cerebral blood flow is momentarily cut-off, depriving brain tissue of crucial oxygen and glucose that is required for normal brain function. When this occurs, it can lead to irreversible brain injuries.\textsuperscript{178} Abrupt cessation of blood flow to a specific area of the brain followed by reperfusion results in a chain of inflammatory events at the cellular and molecular level, which in turn lead to ischemic damage. First and foremost, ischemia disrupts the activity of neurons and triggers their cell death, however it effects the glia and vascular cells as well.\textsuperscript{177} Occlusion of the middle cerebral artery (MCAO) results in the formation of an ischemic territory, which is the total area effected by the stroke and includes the ischemic core and the ischemic penumbra.\textsuperscript{178} The ischemic core is
affected quickly and incurs the most damage, since it is the area where blood flow is most limited. The core of the ischemic territory becomes necrotic mainly due to lack of sufficient energy supply for adenosine triphosphate (ATP) production. ATP is necessary for the neuron to maintain a gradient of charges across its membrane; when this does not occur, sodium and calcium ions build up in the cytoplasm, leading to the influx of water, organelle dysfunction, cell membrane deterioration, and its eventual demise (necrotic cell death). The ischemic penumbra, the area surrounding the ischemic core, is hypoxic, meaning it has limited but sufficient blood supply from collateral vasculature to prevent cells in this region from undergoing instantaneous cell death. In this way, the ischemic penumbra represents a zone of cells at risk of becoming necrotic, however its fate is determined by a number of different factors. Because blood supply in this region is adequate to maintain the production of ATP, cells in the ischemic penumbra do not die immediately, however they are under stress because blood flow is not at an optimum level. This undue strain increases the susceptibility to certain factors that may disrupt their equilibrium and instigate necrotic cell death. One of these critical factors is excessive production of glutamate in extracellular spaces, leading to NMDA receptor activation and a cascade of calcium dependent events. Eventually proteases and free-radical producing enzymes are activated, and this may instigate necrotic cell death or apoptosis in the ischemic penumbra. However, the magnitude of damage in this region depends on the severity of the infarct and the energy status of the neurons involved. (reviewed in)

Hypoxic and ischemic neurons set off the inflammatory response through the release of ‘danger signals’ that trigger immune cells. Studies involving animals as well as humans, have demonstrated that “innate” immune cells, for instance, macrophages and neutrophils, as well as “adaptive” immune cells such as T-lymphocytes, invade the ischemic territory. The release of “danger signals” from injured neurons, microglia and astrocytes (local immune cells of the brain) cause innate and adaptive immune cells to release cytokines. Danger signals may include components released from within the cell after it has undergone necrosis, and these signals are collectively referred to as danger-associated molecular patterns (DAMPS). DAMPS can then associate with innate immune cells such as microglia
and macrophages, by binding to toll-like receptors (TLRs) expressed on the surface of these cells. This interaction can prime the inflammatory response and instigate a series of events which feedback to amplify the production of cytokines, causing further cell injury and anti-inflammatory cascades.

Therefore, it is evident that a stroke can lead to damage at both gross and cellular levels. The next section will discuss the role and association of pro- and anti-inflammatory cytokines in acute ischemic stroke.

1.4.4.1 Cytokine Elevations Post-stroke

As illustrated above, cytokines released by innate and adaptive immune cells can wreak havoc on neurons, microglia and astrocytes which feedback to amplify the immune response and cause further destruction. After an ischemic stroke, a number of different pro-inflammatory cytokines are released. Cerebrospinal fluid (CSF) and plasma of post-stroke patients has been demonstrated to contain raised amounts of the pro-inflammatory cytokines IL-1β, TNF-α, IL-6 and IL-8. Researchers have found IL-6 to correlate with larger stroke volume, stroke severity and functional impairment. TNF-α has also been correlated with stroke severity and neurological outcome.

Moreover, another study found that IL-6 and TNF-α were associated with risk of recurrent ischemic stroke, and IL-6 has been demonstrated to remain elevated at 3 months and 1 year post stroke. Anti-inflammatory cytokines such as IL-10 may have the opposite effect post-stroke. IL-10 production has been demonstrated to act in a neuroprotective manner post-ischemic stroke. Briefly, animal and in vitro models have demonstrated that IL-10 can significantly reduce infarct size and enhance neuronal survival. Furthermore, it has been found that lower IL-10 is associated with clinical worsening and larger infarct volume.

Insight from ‘immunomodulation’ therapeutic strategies post-stroke, such as ‘ischemic tolerance’, have highlighted the importance of a balance between pro- and anti-inflammatory mediators in influencing the fate of neurons. Ischemic tolerance is an outcome that occurs when a sublethal insult prevents an organ from sustaining a successive lethal insult. For instance, a transient, non-injurious ischemic event in the brain will prevent the brain from incurring harm from a successive
injurious ischemic event.\textsuperscript{178, 179, 194} The success of ischemic tolerance relies on a balance between immunosuppression, i.e. through interferon-β (IFN-β) mechanisms,\textsuperscript{195} and pro-inflammation,\textsuperscript{196-199} demonstrating that the equilibrium between pro- and anti-inflammatory cytokines has the potential to have a positive or detrimental impact on the post-stroke brain, depending on their relative involvement in the ischemic event. Yet, past research suggests that a skewed pro- to anti-inflammatory response is also associated with depression. Myint \textit{et al.} observed significant elevations in plasma IFN-Ɣ/IL-4 in depressed subjects and upon antidepressant treatment, the level of IFN-Ɣ/IL-4 ratio decreased significantly.\textsuperscript{200} Considering the importance of equilibrium between inflammatory mediators in ischemia post-stroke and MDD, it seems plausible that this inflammatory balance is an important factor in the development of PSD as well. The next part of this review will discuss this balance as it relates to sickness behaviour.

1.4.5 Sickness behaviour

In mammals, sickness behaviour is a term used to describe a cluster of behavioural symptoms that ensue due to acute infections or wounded tissue.\textsuperscript{57} These symptoms may include malaise, reduced pain tolerance, fever, diminished energy, avoidance of social situations, lassitude, anorexia, poor concentration and anxiety.\textsuperscript{201, 202} Pro-inflammatory cytokines have been intensely investigated in relation to sickness behaviour, and much evidence points towards the involvement of TNF-α, IL-1β,\textsuperscript{203-207} and IL-6.\textsuperscript{202, 208, 209} Specifically, mice or rats injected with IL-1β or TNF-α centrally or systemically exhibit a wide range symptoms indicative of sickness which is temporal and dose-dependent.\textsuperscript{202} These pro-inflammatory cytokines also reduce the expression of clock gene transcripts that are important for the maintenance of diurnal rhythms.\textsuperscript{210, 211} The role of IL-6 in cytokine-induced sickness behaviour is different than IL-1β and TNF-α, in that its administration does not induce any behavioural symptoms,\textsuperscript{202} although lipopolysaccharide (LPS) induced sickness behaviour is diminished in mice that produce less IL-6.\textsuperscript{208} Therefore, IL-6 is believed to play a role in sickness behaviour by inducing the expression of other cytokines in the brain.\textsuperscript{212}
The severity and extent of sickness behaviour is controlled by anti-inflammatory cytokines. It is hypothesized that they exert their effects by blocking pro-inflammatory cytokine expression and by hindering cross-talk and communication with inflammatory pathways. For example, LPS induced sickness behaviour is dampened by central IL-10 injection. Furthermore, fever brought on by LPS administration is more severe and drawn out in mice that produce less IL-10. These findings emphasize the importance of a balance between pro- and anti-inflammatory cytokines in regulating sickness behaviour. An aged brain also demonstrates the importance of this balancing act; older mice have elevated expression of pro-inflammatory cytokines and reduced expression of anti-inflammatory cytokines, for instance IL-6 and IL-10 and their sickness response to LPS injection is more pronounced and intense than in young mice. (Reviewed in)

1.4.6 Inflammatory Markers and Depression

The behaviours displayed by depressed individuals are quite similar to those exhibited in sickness behaviour. These include sadness, fatigue, anorexia/weight loss, sleep disturbance, reduced pain tolerance, anhedonia and difficulty concentrating. In patients who receive immunotherapy in the form of IFN-α or IL-2, approximately 33% experience symptoms of depression. Additionally, researchers assessing otherwise healthy MDD patients have consistently demonstrated that inflammation is exaggerated in these individuals. For instance, patients with depression have been found to have elevated acute phase proteins, chemokines, adhesion molecules and pro-inflammatory cytokines in their serum and cerebrospinal fluid (CSF). In particular, cytokines and acute phase proteins associated with depression include IL-6, CRP, TNF-α, IL-1β, α-1-acid glycoprotein, α-1-antichymotrypsin and haptoglobin, and human macrophage chemoattractant protein-1, soluble intracellular adhesion molecule-1 and E-selectin are some of the adhesion molecules and chemokines reported to be elevated in depression. Furthermore, multiple studies have observed a reduction in ratios between pro- and anti-inflammatory cytokines with antidepressant treatment and genetic variations in IL-1β and TNF-α alleles have been demonstrated to be associated with antidepressant
treatment resistant depression and elevated risk of developing depression. Patients with depressive symptoms have significant elevations in inflammatory molecules as well. Specifically, Suarez et al. observed that higher Beck Depression Inventory (BDI) scores were correlated with elevated TNF-alpha and IL-8 production.

Moreover, patients burdened with diseases such as cancer, CVD and viral infections exhibit depressive symptoms which are also associated with inflammation. In particular, multiple studies have found that inflammation after a stroke is associated with PSD. Researchers have found an association between PSD and serum TNF-α and IL-6 as well as serum IL-18 post-stroke. Immunologic ratios of TNF-α/IL-10 and IL-6/IL-10 were also demonstrated to be significantly elevated in patients with PSD compared to controls, and a study assessing IL-4 and IL-10 polymorphisms in patients with PSD, which related to lower anti-inflammatory cytokine production, found that these alleles were significantly associated with depression in these individuals. Conversely, some studies have failed to find a relationship between the post-stroke cytokine production and depressive symptoms. One such study by Jimenez et al. failed to find a relationship between the cytokines IL-1β, TNF-α, IL-6, as well as C-reactive protein, although they did find that serum leptin levels were independently associated with PSD and that patients with MDD had significant elevations in leptin levels at discharge and one month post-stroke. Leptin is an adipocyte hormone that has an important role in regulating appetite and energy equilibrium, however recent research has also focused on its role in neuronal plasticity and protection. Furthermore, leptin has also been studied for its potential to act as a biomarker for vascular risk factor in stroke and heart attacks, and higher serum levels have been associated with depression in the past. Although these researchers failed to find a relationship between cytokines and PSD, their finding on the relationship between leptin and PSD may represent a new avenue for depression biomarkers post-stroke. Another study involving IL-18 in acute stroke found that serum IL-18 was significantly elevated in alexithymic (emotionally unaware) patients but not in depressed patients. In addition, a study by Ormstad et al. investigating the relationship between 13 cytokines, depression and post-stroke fatigue, determined that depression was not associated with any of the
cytokines studied. However, post-stroke fatigue was significantly positively associated with acute serum levels of IL-1β 6 months post-stroke and acute serum levels of IL-ra and IL-9 were negatively correlated with post-stroke fatigue at 12 months post-stroke. Interestingly, IL-ra, an endogenous antagonist to IL-1β, is involved in neuronal protection mechanisms and has been shown in animal models to minimize sickness behavioural displays. This suggests that some of the depressive symptoms observed post-stroke may be more related to a sickness response than a true depression response.

Although sickness behavior and depression are both associated with elevations in inflammatory markers and mirror each other in terms of the behavioural symptoms, their differing durations suggest that their etiologies are unique. Sickness behaviour is a motivational and adaptive response during infection which will resolve once the body eliminates the pathogen, however depression does not follow the same course. As mentioned earlier, about a third of patients receiving IFN-α or IL-2 immunotherapy experience symptoms of depression and investigation into the cause of this helped researchers paint a picture of two forms of cytokine-induced depressive symptoms. One of which are the early-onset somatic disturbances of treatment that most, if not all patients experience and these consist of lethargy, decreased food intake, increased sensitivity to pain and disturbances in sleeping. The other form of cytokine-induced depressive symptoms are psychological in nature, late-onset, and are exhibited in as many as 50% of these patients. These symptoms involve mild disturbances in cognition, and a depressed, anxious or irritable mood. Interestingly, patients receiving immunotherapy that were treated prophylactically with an SSRI demonstrated improvement in the aforementioned mood disturbances without any changes in somatic depressive symptoms. Furthermore, patients’ depression rating scores prior to treatment initiation, determined by the Montgomery–Asberg Depression Rating Scale (MADRS), were significantly associated with depressive symptoms at the end of treatment, and patients who experienced the psychological symptoms of depression after immunotherapy also had significantly elevated plasma levels of cortisol and adrenocorticotropic hormone (ACTH) which are indicative of a heightened pituitary-adrenal response. Dantzer et al. suggest that these findings
indicate both physiological and psychological mechanisms in the susceptibility to developing depression from cytokine immunotherapy. He also adds that “It is possible that depression represents a maladaptive version of cytokine-induced sickness, which could occur when activation of the innate immune response is exacerbated in intensity and/or duration...” This may also be the case in the development of PSD, since stroke is associated with marked elevations in pro-inflammatory cytokines. The next section of this literature review will outline some of the biological mechanisms involving inflammation-associated depression; hopefully shedding light on factors that incur ‘increased vulnerability’ to this form of depression.

1.4.7 IDO-mediated Mechanisms of Depression

1.4.7.1 Kynurenine Pathway and its Metabolites under Normal and Inflammatory Conditions

IDO is an enzyme involved in the conversion of TRP to KYN. Under normal physiologic conditions the enzyme tryptophan 2,3-dioxygenase (TDO) is mostly responsible for this process, which takes place in the liver. However, inflammatory processes trigger a shift in enzyme activity to IDO. This shift in metabolism occurs mainly due to elevations in pro-inflammatory cytokines during an immune response which induce IDO. IDO gene transcription is most potently activated by IFN-Ɣ, however other pro-inflammatory cytokines such as IFN-α, IFN-β, IL-2, IL-6, IL-18, and TNF-α have also been demonstrated to induce IDO activity. Anti-inflammatory cytokines, such as IL-4 and IL-10 can inhibit IFN-Ɣ-stimulated IDO activation. IDO is present in the lungs, kidneys, spleen, blood and brain, although the majority of IDO catalytic activity takes place in lymphoid tissues and blood. Glucocorticoids released by the adrenal glands during stress can also upregulate TDO activity in the liver, causing further increases in KYN.

Under normal physiologic conditions, KYN metabolised from TRP contributes to energy stores through a pathway that leads to ATP production. As seen in Figure 1 the steps involved in this pathway are as follows: in the liver, an enzyme called kynurenine-3-monooxygenase (KMO) converts KYN into 3-hydroxykynurenine (OHK), OHK is further metabolized by an enzyme called kynureninase to 3-
hydroxyanthranilic acid (HAA), HAA can then be completely oxidized to make ATP or catabolised by 3-hydroxyanthranilic acid oxygenase into quinolinic acid (QUIN) which will eventually be converted to nicotinamide adenine dinucleotide (NAD) by quinolinate phosphorybosltransferase.\textsuperscript{278} ATP is the main product of HAA under normal conditions.\textsuperscript{278} KYN also has the potential to undergo conversion to kynurenic acid (KYNA) by an enzyme called kynurenine aminotransferase.\textsuperscript{275, 278}

**Figure 1.** TRP and KYN Metabolism Pathway (Adapted from Mandi and Vecsei\textsuperscript{105} and Myint et al.\textsuperscript{275})
As mentioned above, the majority of TRP metabolism normally occurs in the liver, however a small amount can take place elsewhere, such as the brain. In the brain, the main site of TRP metabolism is within astrocytes and microglia. Most KYNA production occurs in astrocytes, while QUIN formation takes place in macrophages and microglia. QUIN is then taken up by astrocytes and mainly used to produce NAD and enable glycogen storage in the brain.

Under inflammatory conditions, IFN-Ɣ, IFN-α, IFN-β, IL-2, IL-6, IL-18, and TNF-α induce IDO activity. Pro-inflammatory cytokines can also stimulate the activity of KMO leading to increased production of OHK and therefore causing QUIN levels to increase. Anti-inflammatory cytokines, such as IL-4 and IL-10 are capable of significantly reducing QUIN production stimulated by TNF-α and IFN-Ɣ in human monocyte-derived macrophages. Until inflammation is reduced or completely subsides, QUIN will continue to be produced. Picolinic acid, a metabolite of the complete oxidation of HAA, was demonstrated to significantly increase IFN-Ɣ-dependent expression of TNF-α mRNA, therefore the KYN pathway itself may have a role perpetuating QUIN production as well.

During the inflammatory response, IDO activation may also play a pivotal role in immune regulation and suppression. Its activation may suppress the immune response by reducing the proliferative capacity and inducing apoptosis of T lymphocytes. In general, this may be carried out through decreased TRP availability, increased production of oxidative and cytotoxic KYN metabolites, and lastly by indirectly causing naïve cluster of differentiation (CD) 4 T cells to differentiate into regulatory T cells (Treg cells) through the production of transforming growth factor (TGF-β). Recent evidence has suggested that this last mode of action is carried out via the aryl hydrocarbon receptor (AHR). It has been demonstrated that KYN can bind to the AHR, leading to IDO induction and the generation of (forkhead box P3) Foxp3 Tregs; TGF-β may also amplify the interaction between KYN and AHR by upregulating AHR expression. The general result of IDO activation is a dampened helper T cell type 1 (Th1) response, which shifts the immune response to helper T cell type 2 (Th2) activation, while also stimulating the production of Tregs. Th1 and Th2 cells produce distinct antagonistic cytokines that aim to repress each others’ immune response and to perpetuate the production
of their own cytokines.\textsuperscript{105, 296} Th1 cytokines include IFN-\(\gamma\), TNF-\(\alpha\), IL-1, IL-18 and while Th2 cytokines include IL-6, IL-4, and IL-10.\textsuperscript{105} Consequently, Tregs have a repressive effect on both the Th1 and Th2 responses, driving the immune system towards equilibrium.\textsuperscript{105, 297}

Aside from the effects of KYN on immunosuppression, inflammation-mediated IDO activation may affect neurotransmitter levels and synaptic transmission in the brain. As mentioned previously, tryptophan is an essential amino acid that acts as a precursor for serotonin synthesis.\textsuperscript{298} Therefore, elevated IDO activation may reduce the amount of tryptophan available for serotonin synthesis\textsuperscript{275} and influence the rate of serotonin degradation by IDO’s ability to convert serotonin into formyl-5-hydroxykynuramine.\textsuperscript{299} These two effects of IDO activation may compromise the synaptic transmission of serotonin.\textsuperscript{275} Furthermore, QUIN has been demonstrated to act as an N-methyl-d-aspartate (NMDA) receptor agonist\textsuperscript{300} and animal models have demonstrated its excitotoxic and neurodegenerative capacities.\textsuperscript{75, 301, 302} KYNA, on the other hand, at higher than normal concentrations acts as an antagonist to multiple ionotropic excitatory amino acid receptors.\textsuperscript{21} At physiological concentrations, KYNA acts as a competitive antagonist of the NMDA receptor\textsuperscript{303} and researchers have found that neurotoxicity brought on by QUIN is diminished significantly by KYNA.\textsuperscript{301} KYNA has also been found to exhibit antagonistic properties at the \(\alpha7\)-nicotinic acetylcholine receptor (\(\alpha7\)nAchR) at physiologic concentrations.\textsuperscript{304} However, Kynurenine amino-transferase, the enzyme responsible for the conversion of KYN into KYNA, has not been demonstrated to be upregulated by cytokines. Therefore, inflammation may create an imbalance between QUIN and KYNA, potentially increasing neurons’ susceptibility to excitotoxic neurodegeneration. Taken together, these findings suggest that inflammation-induced IDO activation may have a negative impact on many mechanisms of synaptic transmission. The following segment of this literature review will highlight on the relationship between KYN, its metabolites, and depression, as well as touch upon certain mechanisms in which toxic KYN metabolites may lead to neurodegeneration and therefore hypothetically, depression.
1.4.7.2 Kynurenine and Psychiatric Disorders

Changes in the levels of KYN and its metabolites have been associated with major depression, bipolar mania, and schizophrenia. With respect to depression, Myint et al. found the ratio of K/T, KYNA and KYNA/KYN in depressed patients was significantly different than controls. Specifically, the K/T ratio was significantly elevated in depressed patients, and KYNA, as well as KYNA/KYN levels were significantly reduced in depressed patients. Another study by Steiner et al. discovered significantly elevated QUIN immunohistochemistry staining in the subgenual anterior cingulated cortex and anterior midcingulate cortex in depressed patients compared to controls. KYN was also shown to be elevated in MDD patients who attempted suicide compared to controls. Post-partum depression has also been associated with elevations in the K/T ratio. Additionally, in adolescents with melancholic depression, researchers using magnetic resonance spectroscopic imaging observed that levels of KYN and the HAA/KYN ratio were positively associated with increased cell turnover, as measured by choline levels in the right caudate and left putamen, respectively. Choline is a brain metabolite associated with neuronal membrane breakdown that has been associated with MDD in the past. With respect to PSD, no study to our knowledge has examined the relationship between the K/T ratio or KYN metabolites and depression. The only closely related study was conducted by our group in coronary artery disease (CAD), and we found a significant association between depression scores and the K/T ratio. CAD also shares common risk factors with stroke and a study assessing independent predictors of CAD, using cardiac computed tomography (CTA), found that acute ischemic stroke is independently associated with increased risk and more severe CAD in contrast to patients with “acute chest pain at low-to-intermediate risk for acute coronary syndrome.” Moreover, the latest results from studies measuring the levels of TRP, KYN and its metabolites in relation to depression have demonstrated that disruptions in the KYN pathway may be more relevant to the symptoms of somatization. This has been defined in the past as a “multisomatoform disorder characterized by medically unexplained, functional or psychosomatic symptoms” and within the general population it
has a prevalence of 4-7%.\textsuperscript{314} Maes and colleagues demonstrated this by conducting a study evaluating KYN metabolism in depressed patients, patients with comorbid depression and somatization, patients with just somatization, and controls.\textsuperscript{305} They found that plasma TRP was lower in patients with somatization than depression and the KYN/KYNA ratio as well as the K/T ratio were elevated significantly in somatization compared to depression.\textsuperscript{305} Furthermore, reduced TRP and increased KYN/KYNA and K/T were associated with somatic symptom severity.\textsuperscript{57, 305, 313} Their group attributes these results to the findings that: a reduction in serotonergic activity increases pain,\textsuperscript{315} KYN can also stimulate pain and peripheral neuropathy,\textsuperscript{316} and KYNA can prevent these symptoms\textsuperscript{317} potentially by blocking the nociceptive NMDA receptor or by stimulating the activity of the anti-nociceptive G-protein coupled receptor.\textsuperscript{313, 318}

In studies involving cytokine immunotherapy, the relationship among inflammation, cytokines, and kynurenine metabolites has been well established. One study observed significant increases on the MADRS score in hepatitis C patients treated with 24 weeks of IFN-\(\alpha\) immunotherapy.\textsuperscript{319} They also found increases in the K/T and KYN/KYNA ratio, which was significantly associated with total MADRS scores over time.\textsuperscript{319} Another study by Raison \textit{et al.} found that hepatitis C patients treated for 12 weeks with IFN-\(\alpha\) had significantly elevated peripheral (blood) and central (CSF) concentrations of KYN.\textsuperscript{106} Elevations in CSF KYN were correlated with marked elevations in CSF QUIN and CSF KYNA.\textsuperscript{106} Furthermore, CSF QUIN and CSF KYN were associated with CSF IFN-\(\alpha\) and depressive symptom scores on the MADRS.\textsuperscript{106} CSF QUIN and KYN were also associated with TNF-\(\alpha\) receptor 2 and MCP-1.\textsuperscript{106} Of note, the concentration of TRP in the CNS depends on the concentration of large neutral amino acids (LNAA) (tyrosine, phenylalanine, leucine, isoleucine, valine) which compete with TRP to be transported across the blood brain barrier (BBB), and is also reliant on the central demand for TRP.\textsuperscript{275, 298} Furthermore, approximately 60\% of KYN in the brain is contributed from the systemic circulation\textsuperscript{320} and which is transported across the BBB by the large amino acid transporter.\textsuperscript{19} KYNA and QUIN, however, enter the CNS through passive diffusion, this implies that central KYN metabolism contributes to the
The majority of CSF QUIN and KYNA. The results of this study by Raison et al. suggest that depression caused by IFN-α therapy is associated with KYN metabolism as well as central inflammation.

1.4.7.3 Kynurenine Metabolites and Neurodegeneration

KYN metabolites have been associated with neurodegenerative diseases in the past. For instance, researchers discovered 3-4 fold increases in the levels of OHK and QUIN in the neostriatum and cortex of Huntington’s disease patients, and QUIN has also been linked to AIDS-associated dementia. OHK was also demonstrated to cause striatal neuron toxicity through the formation of reactive oxygen species. Elevations in the K/T ratio have been significantly correlated with the progression, severity and mortality of multiple neurodegenerative diseases, such as Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, as well as stroke. The development of depression has also been hypothesized to have ties with mechanisms of neuroprogression. Neuroprogression is a term given to the process of gross and cellular neurodegeneration which has been hypothesized to have a link to the cause and/or consequence of worsening depression and cognitive decline. In one case register study the rate of dementia increased by 13% with every depressive episode leading to hospital admission. Furthermore, the number of depressive episodes and length of each episode has been associated with diminished brain volumes. For instance, an increased number of depressive episodes is linked with decreased hippocampal, basal ganglia, orbitofrontal and subgenual volumes, and the extent of the episode has been associated with diminished cerebral grey matter volume. In a meta-analysis by McKinnon et al. it was determined that compared to controls, MDD patients had reduced hippocampal volumes, and patients with ‘moderate’ (2–9 years) and chronic (>10 years) illness duration had smaller hippocampal volumes as well. Another meta-analysis by Videbech et al. observed an 8% decrease in right hippocampal volume and 10% decrease in left hippocampal volume in MDD and right hippocampal volume was negatively correlated with the number of depressive episodes. In a study comparing first episode, never-treated depressed patients to multiple episode depressed patients, researchers observed both patient types to have cognitive
deficits in recollection memory, which they attributed to hippocampal dysfunction.\textsuperscript{60} Furthermore, only patients with recurrent episodes had decreased hippocampal volumes and statistical analysis demonstrated that length of illness is significantly correlated with hippocampal volume reduction.\textsuperscript{57, 60} Taken together, these studies suggest that the process of neuroprogression may have a role in the etiology of depression.

There is some research that implicates inflammation and oxidative stress in the development of neuroprogressive depression as well.\textsuperscript{50, 67, 68, 57} In particular, inflammation-induced IDO and KMO activation and the production of KYN metabolites have been hypothesized to play a role in these neurodegenerative processes,\textsuperscript{50, 67, 68} through pro-oxidative mechanisms,\textsuperscript{69-72} disruption in energy and metabolism,\textsuperscript{73, 74} and through glutamate excitotoxicity.\textsuperscript{21, 75, 76} This last mode of action is carried out by the previously mentioned KYN metabolite, QUIN, which has been demonstrated to act as an NMDA receptor agonist\textsuperscript{300} and animal models have demonstrated its excitotoxic and neurodegenerative capacities.\textsuperscript{21, 75, 301, 302} Interestingly, inflammation in the systemic circulation can induce the expression of NMDA receptors, potentially further increasing the excitotoxic effects of QUIN.\textsuperscript{313} Furthermore, KYNA production occurs in astrocytes, while QUIN formation takes place in macrophages and microglia,\textsuperscript{281-283} and QUIN is then taken up by astrocytes\textsuperscript{281} and mainly used to produce NAD and enable glycogen storage in the brain.\textsuperscript{275, 284, 285} However, at high concentrations QUIN inhibits the uptake of glutamate by astrocytes, which may lead to increased synaptic concentrations of glutamate and eventually cause increased glutamatergic transmission.\textsuperscript{324} Interestingly, observations in MDD patients have demonstrated decreased density of astrocytes in brain regions associated with depression.\textsuperscript{76, 310} Researchers have demonstrated that the neurodegenerative capabilities of QUIN can also occur through mechanisms other than glutamatergic excitotoxicity.\textsuperscript{76} One study found that QUIN exposure to hippocampal nerve cells \textit{in vitro} caused extensive degeneration, acute swelling and damage to postsynaptic elements.\textsuperscript{77} Another \textit{in vitro} study measuring lipid peroxidation in rat brain homogenate, found QUIN increased lipid peroxidation in a concentration-dependent manner.\textsuperscript{72} Whether or not these mechanisms represent acute or chronic factors the etiology of PSD has yet to be determined. However, one study involving cardiac arrest patients found marked reductions in hippocampal volume compared to controls 21 days after cardiac arrest.\textsuperscript{325} The
researchers of this study postulated that this reduction in volume signifies the hippocampus’ increased susceptibility to global brain ischemia.\textsuperscript{325} This may imply that oxidative and neurotoxic factors can have a relatively acute effect on brain volume and neurodegeneration. However, because of the cross-sectional nature of this study one cannot determine whether the hypoxic (or oxidative/excitotoxicity/etc.) consequences are acute or chronic.

KYN and its metabolites have also been studied in relation to stroke. Darlington et al. observed an elevated K/T ratio post-stroke, found that KYNA levels were significantly elevated in patients who died within 3 weeks of stroke, and also demonstrated that the HAA/AA ratio was significantly correlated with lesion volume.\textsuperscript{66} As mentioned above, at physiological concentrations, KYNA acts as a competitive antagonist of the NMDA receptor\textsuperscript{303} and has also been found to exhibit antagonistic properties at the $\alpha$7nAchR at physiologic concentrations.\textsuperscript{304} One explanation the researchers give for the positive association between KYNA levels and mortality is that excessive KYNA production may be an adaptive physiological mechanism in response to higher than normal glutamatergic transmission that occurs due to ischemia.\textsuperscript{66} In turn, elevations in this glutamate antagonist might cause glutamate hypofunctioning and leave already compromised post-stroke patient vulnerable to death.\textsuperscript{66} Another research group assessing the relationship between KYN metabolites and stroke outcomes found that the K/T ratio correlated with stroke severity and infarct volume.\textsuperscript{326} Furthermore, worse stroke outcome at 3 months was associated with a higher K/T ratio.\textsuperscript{326} In patients with traumatic brain injury researchers observed increased conversion of TRP to KYN compared to controls, and decreased production of KYNA.\textsuperscript{327} The multiple findings that depression, as well as KYN metabolites have been associated with neurodegeneration and stroke, and KYN metabolites are associated with neuroprogression, suggests that the combined effect of an elevated K/T ratio and neurodegeneration may be more relevant in contributing to the development of PSD, than either variable alone. The subsequent section of the introduction will explore the relationship between tryptophan, serotonin, and depression, as these have also been implicated in the development of MDD, and may also have a role in the etiology of PSD.
1.4.8 Serotonin Hypothesis of Depression

Nearly 4 decades ago the serotonin hypothesis of depression was created, postulating that a reduction in serotonergic transmission was an important mediator in the onset of depressive symptoms. This idea came from the finding that synaptic reuptake of serotonin and noradrenaline was blocked by TCAs; researchers believed that this mechanism of improved serotonergic transmission was the key to improvement of symptoms in depressed individuals, and the advent of SSRIs in the pharmaceutical world gave further credence to this hypothesis.

A combination of positron emission tomography (PET), single photon emission tomography (SPET) and ligand imaging techniques have been used in recent years in an attempt to uncover the link between serotonin and depression. Findings from those studies demonstrate that 5-H1A receptors have diminished bindings in regions such as the frontal, temporal, and limbic cortex, as well as the raphe nuclei in unmedicated depressed patients, although these results have not been consistently replicated. Moreover, reduced 5-H1A binding in recovered depressed patients signifies that this may not represent acute depression and binding at this receptor in the context of panic disorder is also reduced raising the concern of how unique these findings are to depression relative to other mood disorders. Furthermore, a study investigating 5-HT2A receptor binding observed significantly higher binding potential in the frontal cortex, parietal cortex, and occipital cortex of recovered depressed individuals. Conversely, other studies have demonstrated reduced, as well and no change in binding density at the 5-HT2A receptor. With regards to the serotonin transporter (SERT), a study by Malison et al. found significant decreases in binding potential at SERT in the brainstem of unipolar MDD patients, but yet again, another group of researchers failed to replicate these findings; with no change in SERT binding in the midbrain between MDD patients and controls (reviewed in)

In PSD, there is evidence that the levels of serotonin metabolites in CSF are also altered. A study observing the differences in CSF 5-HIAA between depressed and non-depressed stroke patients, as well as controls, found that 5-HIAA is significantly reduced in PSD patients compared to the other two patient
Another group of researchers investigated the differences in plasma and CSF serotonin concentrations in PSD patients compared to non-depressed stroke patients. They observed overall plasma and CSF serotonin concentrations in the PSD group to be lower than in the control group, as well as a 90% reduction in plasma serotonin in the PSD group compared to a 13.3% reduction in the control group, and also observed an 80% reduction in CSF serotonin in the PSD group and whilst the control group had a mere 6.7% reduction in CSF serotonin. Additionally, a study by Mayberg et al. observed increased 5-HT2 binding in PSD patients compared to stroke patients without depressive symptomatology, and this was significantly associated with symptom severity as well.

Evidence from animal and in vitro studies suggest that certain modes of serotonin neurotransmission may be important in the development of immune-mediated depression. For instance, researchers have demonstrated that serotonin turnover and tryptophan uptake (the precursor to serotonin synthesis) are stimulated by LPS or pro-inflammatory cytokine administration. Additionally, through p38 mitogen activated kinase (MAPK) mechanisms, it was demonstrated that IL-1β and TNF-α are both able to acutely regulate the neuronal serotonin transporter (SERT). These researchers consequently demonstrated that LPS administration in mice caused increased SERT activation and behavioural despair, which did not occur in mice deficient in the interleukin-1 receptor (IL-1R). Furthermore, SERT knockout mice administered with LPS did not perform worse in behavioural models of despair. In a separate study, researchers observed a 72% decrease in the expression of the 5-HTR1A serotonin receptor when hepatoblastoma, myelocyte-derived and T cell leukemia-derived cell lines were treated with IFN-α, this effect was diminished with either cessation of IFN-α treatment or administration of a TCA (desipramine) or a serotonin reuptake inhibitor (fluoxetine). Recent findings in humans corroborate these results; patients treated with IFN-α demonstrated significant increases in p38 MAPK activation, this activation was significantly more pronounced in patients that developed depression, as assessed by the MADRS. These results suggest a possible role of serotonin synaptic transmission in the development of cytokine-associated depressive symptoms and may be associated PSD in the acute stages.
post-stroke. However, whether dysfunctional serotonergic transmission is a mediator in the development of PSD is still a topic of debate.

1.4.8.1 Tryptophan and Depression

Tryptophan (TRP) is an essential amino acid that is used by the body to produce serotonin, kynurenine, melatonin and the trace amine tryptamine. In the systemic circulation TRP exists in two forms: albumin-bound and free, although at basal states the majority (90%) is bound to albumin, which cannot be transported across the blood-brain barrier (BBB). Thus, TRP transport across the BBB depends on the amount of albumin-bound and unbound TRP, as well as the relative amount of TRP compared to the concentration of large neutral amino acids (LNAA), which compete for the binding site on the L-type amino acid transporter at the BBB. Once TRP crosses the BBB, it is metabolized into serotonin in the raphe nuclei, a small cluster of neurons in the brainstem. The rate-limiting enzyme involved in the conversion of TRP to serotonin is tryptophan hydroxylase (TPH) (reviewed in).

‘Tryptophan depletion’ has been used by many researchers to study the effects of reduced serotonin activity on depressed mood. This method came from the finding by Cowen et al. that total plasma tryptophan was significantly reduced in patients with depression compared to controls, and it involves giving patients an amino acid mixture that is deficient in TRP. Reductions in TRP will reduce the amount of TRP catalyzed by TPH, and therefore transiently reduce serotonin synthesis. This method demonstrated that patients who had a depressive episode in the past and subsequently recovered, were sensitive to the effects of tryptophan depletion, causing temporary relapse in depressive symptoms in these individuals. Conversely, the depressive effects of tryptophan depletion in healthy volunteers without risk factors for developing depression were not significant. Cowen et al. suggest these findings indicate that patients who have vulnerability factors to depression and have experienced depression in the past, are more likely to develop depression after diminished serotonin activity. They further suggest that depression may permanently alter the neurobiology of the serotonergic system so that when
previously depressed individuals are subjected to tryptophan depletion, they display a depressed response. (reviewed in 336)

Studies by Maes et al. and Hoes et al. measuring the excretion of xanthurenic acid (XA), a metabolite of the KYN pathway, in depressed patients, were the first to postulate a relationship between IDO activity, TRP depletion, and depression.362, 363 Their results demonstrated that a ‘shunt’ in KYN metabolism by way of IDO activation, reduced TRP levels in depressed patients.57, 362, 363 This same mechanism is plausible in the context of PSD, where stroke is characterized by marked elevations in cytokines,99-104 leading to IDO activation,28, 274 an increase in the conversion of TRP to KYN, an elevated K/T ratio, and a subsequent reduction in the amount of TRP available for serotonin synthesis. Studies involving cytokine immunotherapy corroborate this theory, finding significant reductions in serum TRP upon cytokine administration.364, 365 Furthermore, Maccay et al. demonstrated that at baseline and after TRP depletion, patients with traumatic brain injury had increased conversion of TRP to KYN compared to controls, and decreased production of KYNA.327 In a study assessing plasma levels of TRP in rehabilitative stroke patients, it was found that patients on antidepressants had plasma tryptophan concentrations approximately 70-80% of the mean value measured in patients not using antidepressants.366 The results of those studies suggest that a reduction in TRP following a stroke may be associated with PSD, however research is scarce on the topic. The following portion of this review will examine the relationship between neuroimaging outcomes such as atrophy, white matter lesions, and acute infarct volume with PSD, as this field has put much effort into determining this post-stroke sequelae’s neuroanatomical roots.

1.4.9 Neuroanatomical Correlates of Post-Stroke Depression

Few studies have assessed the relative effects of acute infarcts, WMC, and atrophy together in relation to PSD.39, 40, 45, 48 The first study to do so, by Vataja et al., hypothesized that an intricate relationship between these neuroanatomical findings contribute to PSD in a similar way to their interaction within fronto-subcortical pathways in the development of post-stroke dementia.40, 367 These
researchers failed to find associations between both WMC or atrophy and PSD, while subsequent studies assessing all three outcomes have found associations with WMC and atrophy in isolation, without any additive effects. Similar to these aforementioned groups, this thesis aims to take a comprehensive approach to assessing neuroanatomy as it relates to PSD, however an overview of the literature on previously established relationships between each radiologic finding and PSD is necessary in order to justify this approach.

### 1.4.9.1 Lesion Location

The Lesion Location Hypothesis was first proposed in the late 1970s by Robinson and colleagues, and it was based on the idea that discrete lesions in the aminergic mood and emotion centres of the brain can cause depression in otherwise healthy individuals post-stroke. The first CT study by Robinson et al. investigating this theory found that the distance of the lesion to the frontal pole was inversely correlated with depressive symptom severity in left hemispheric strokes. A subsequent study by this group published a year later demonstrated similar results, fueling the notion that left hemisphere frontal strokes are more highly associated with PSD than any other region. This theory then developed into the idea that there were left anterior lesion preferences in PSD patients, from the finding that stroke patients with single lesions in the left anterior quadrant of the brain had elevated depression scores compared to any other quadrant (left posterior, right anterior, right posterior); that group was also more likely to develop MDD than the others. The justification put forward for those findings was that monoaminergic (noradrenergic and serotonergic) neural circuits involved in the regulation of mood travel from the brainstem to the frontal cortex and travel in an anterior to posterior fashion, stretching from deep to more shallow layers of the cortex. If fibers from this circuit were damaged by a lesion closer to the frontal pole, a more pronounced decrease in these neurotransmitters would ensue, causing depressed mood. However, the findings of many other studies were not in agreement with the left anterior lesion preference of PSD. The work of Starkstein et al. added onto previous findings by demonstrating that the contribution of lesions within the basal ganglia and thalamus were equally as important as cortical
strokes in their association to PSD. These observations were rationalized by the fact that the basal ganglia is involved in five frontal-subcortical loops involved in motor control, cognitive function and mood. Three of which are associated with depression, and encompass the orbitofrontal cortex (OFC), dorsolateral prefrontal cortex (DLPFC) and anterior cingulate (ACC) regions (reviewed in ). Furthermore, Herrmann et al. studied the contribution of basal ganglia lesions in PSD and found that MDD patients had greater involvement of the basal ganglia in lesions than non-depressed stroke patients. Other researchers attempted to correlate depression with lesion locations characterized by specific brain regions. For instance, Singh et al. found that depressive symptoms were most significantly predicted by inferior frontal lesions, while Kim and Choi-Kwon demonstrated that the prevalence of PSD was higher in the frontal-temporal region than parieto-occipital regions. The findings from three meta-analyses complicated the state of the literature even further, with one meta-analysis by Singh et al. finding that methodological disparities were too vast to combine data from studies. While Carson et al. found no significant preference of lesion laterality in PSD and Bhogal et al. observed a difference in lesion laterality preference between in patient and community stroke survivors. Hospital patients with strokes in the left hemisphere had a higher likelihood of developing depression than hospital patients with strokes of the right hemisphere, while the opposite was found for out patients. Additionally, some evidence suggests a possible contribution of timing of onset of depressive symptoms to lesion laterality, with a meta-analysis by Robinson et al. finding that in the acute stage post-stroke (approximately 6 months) depression severity and distance of the lesion to the frontal pole in left-sided strokes are inversely correlated. At this time, mixed findings in this area of PSD research demonstrate that more work must be done to clarify the association between depression and lesion location. (reviewed in)

Few studies have explored the effect of lesion volume on depression post-stroke. Two studies by Tang et al. failed to find a significant difference in lesion volume between depressed and non-depressed stroke patients. However, in a study by Vataja et al. it was observed that depressed patients had significantly larger lesions in both the right and left caudate and pallidum. Furthermore, Nys and colleagues found a significant difference in lesion volume between patients with moderate to severe
depressive symptoms, mild depressive symptom, and no depressive symptoms; patients with more severe symptoms had significantly larger lesions than patients with mild or no symptoms. The findings of Terroni et al. support these results with their observation that lesion volumes in the amygdala, ventral anterior cingulated cortex, subgenual cortex, hippocampal subiculum, and dorsal anterior cingulated cortex are significantly larger in patients with first major depressive episode post-stroke than non-depressed patients. Moreover, another study by Zhang et al. demonstrated that depressed patients had significantly larger infarct volumes on either the left or right side, however there was only a trending difference in total infarct volume between depressed and non-depressed groups.

1.4.9.2 White Matter Changes, PSD and the Vascular Depression Hypothesis

Age related white matter change (WMC) is another marker of neurodegeneration which may be associated with the development of PSD. It is a disease of the small vessels and represents incomplete ischemia mainly related to occlusive venous collagenosis. The walls of deep cerebral vessels undergo hardening and thickening, due to vascular risk factors such as hypertension, and cause reduced blood flow to this region, leaving the white matter vulnerable to cerebral ischemia. The damage that ensues can cause dysfunction of axonal transmission and may result in a wide array of clinical impairments, from rapid disruption of motor control to more gradual, slight changes in cognition, emotion, sensory or motor function. In relation to emotion, a hypothesis was constructed by Alexopoulos et al. to describe the unique cases of depression among elderly individuals. These cases were unique because patients with ‘vascular depression’ have more cognitive impairment, particularly in fluency and naming, more psychomotor retardation, poor insight, and less irritability and guilt than depressed patients without vascular disease. In their seminal paper on vascular depression, Alexopoulos et al. stated: “Cerebrovascular disease can predispose, precipitate, or perpetuate some geriatric depressive symptoms.” Alexopoulos et al. classifies this as being clinically separate from non-vascular depression due to the fact that there is increased prevalence of depression amongst patients with concomitant vascular disease. Furthermore, there is lower prevalence of family history of mood disorders compared
to early-onset depression in the elderly, \(^{398}\) and lastly, WMCs in the context of depression are most evident in the aforementioned frontal-subcortical loops, \(^{399-401}\) involved in mood regulation (DLPFC, ACC, and OFC). \(^{379}\) With respect to late life depression, one meta-analysis found that the occurrence and severity of white matter change is more pronounce in late life depression, with odds above 4 for white matter lesions in late versus early onset depression. \(^{402}\) More recent studies using both CT \(^{403}\) and MRI \(^{404}\) have found strong relationships between late-life depression and WMC. In particular, a study by Olesen et al. found that in the elderly, more severe WMC is an independent correlate of MDD over 5 years. \(^{403}\) While results from a 3-year follow up study indicate a causal relationship between WMC and late-life depression, with the finding that the development of depression during the third year of study was significantly correlated with the progression of WMC and was also a significant predictor of depression in year 3. \(^{404}\)

Researchers have also sought to examine the relationship between WMC and depression post-stroke, particularly with respect to the interaction between acute infarcts and the progressive, chronic infarcts that are representative of WMC. The first study to assess this relationship in the realm of PSD research was by Vataja et al. who examined the connection between whole brain atrophy, WMC, and stroke lesion characteristics. \(^{40, 44}\) However, they observed that white matter lesions and whole brain atrophy were not significantly different across groups with and without depression, the only significant radiological findings were the aforementioned volume differences in the caudate, pallidum, \(^{40}\) and putamen. \(^{45}\) Many other studies failed to find a relationship between white matter lesions and PSD. \(^{39, 41-43, 46, 47}\) Although, this cohort was then investigated by Pohjasvaara et al. using the BDI to rate depressive symptom severity as opposed to the previous studies, \(^{48}\) which used DSM-III-R and International Classification of diseases (ICD)-10 criteria to dichotomise clinically depressed versus non-depressed patients. \(^{40, 45}\) It was found that depressive symptoms were more severe amongst patients with subcortical ischemic vascular disease, i.e. periventricular and deep white matter lesions. \(^{48}\) Another study by Tang et al. demonstrated that compared to non-depressed patients, patients with depression had a greater chance of having severe deep white matter lesions or frontal lobe infarct, and in a multivariate analysis severe
white matter change held as an independent predictor of depression post-stroke. Those results demonstrate the importance of considering white matter lesions in the presence of acute infarcts when assessing their association with depression, as this thesis plans to consider as well.

1.4.9.3 Atrophy and Depression

As previously mentioned, researchers have extensively studied the complex relationship between depression and atrophy. There have been many findings which indicate the role of neuroprogression in MDD. For instance, the number of depressive episodes and length of each episode has been associated with diminished brain volumes. In particular, an increased number of depressive episodes are linked with decreased hippocampal, basal ganglia, orbitofrontal and subgenual volumes, and the extent of the episode has been associated with diminished cerebral grey matter volume. In a meta-analysis by McKinnon et al. it was determined that compared to controls, MDD patients had reduced hippocampal volumes, and patients with ‘moderate’ (2–9 years) and chronic (>10 years) illness duration had smaller hippocampal volumes as well. Another meta-analysis by Videbech et al. observed an 8% decrease in right hippocampal volume and 10% decrease in left hippocampal volume in MDD and right hippocampal volume was negatively correlated with the number of depressive episodes. In a study comparing first episode, never-treated depressed patients to multiple episode depressed patients, researchers observed both patient types to have cognitive deficits in recollection memory, which they attributed to hippocampal dysfunction. Furthermore, only patients with recurrent episodes had decreased hippocampal volumes and statistical analysis demonstrated that length of illness is significantly correlated with hippocampal volume reduction. With respect to PSD, existing atrophy in the context of an acute infarct may perhaps exacerbate or instigate a neuroprogressive pathway. The results of Starkstein et al. indicate that this may be the case, with their finding that the lateral and third ventricles in depressed patients were significantly larger than controls. Although, Vataja et al. did not find any link between depression and atrophy analyzed by cortical (frontal, parietal, and occipital lobes) and central (temporal, frontal, and occipital horns) and medial temporal lobe (entorhinal cortex and hippocampus) regions. More
recently, Tang et al. investigated the relationship between cortical atrophy and PSD, and a multivariate analysis determined that severe frontal lobe atrophy independently predicted the presence of depression.\textsuperscript{39} Another study by Fu et al. assessing the relationship between depressive symptoms, WMC and atrophy, observed that left inferior frontal gyrus atrophy significantly predicted depressive symptom severity.\textsuperscript{49} These positive findings suggest that location of atrophy, as opposed to lesions may be more highly associated with PSD. The results of these studies further emphasize the importance of considering multiple neuro-radiological measurements in the effort to determine the mechanisms of PSD, as a comprehensive approach to studying these domains implies that they are not mutually exclusive.

In summary, this study aims to assess the relationship between IDO activation, as measured by the K/T ratio, and depressive symptom severity. Alterations in the levels of KYN and its metabolites have been associated with major depression\textsuperscript{88-92, 305} and the K/T ratio has been demonstrated to correlate with stroke severity, lesion volume, and poor outcomes.\textsuperscript{326} It is presumed that acute elevations in the K/T ratio post-stroke will influence mood initially through a reduction in available TRP for serotonin synthesis, and more gradually through the production of excitotoxic and neurodegenerative KYN metabolites. In addition, previous reports have demonstrated pro-inflammatory cytokine elevations in the context of MDD\textsuperscript{215-225, 228, 230-233} as well as PSD.\textsuperscript{36, 243} However, researchers have failed to find a relationship between PSD and post-stroke pro-inflammatory cytokine elevations.\textsuperscript{244, 254-255} As outlined in section 1.4.6, the balance between pro- and anti-inflammatory cytokines may be a better marker for depression than either type of these cytokines in isolation, as evidence by many positive findings in both MDD\textsuperscript{31-35} and PSD.\textsuperscript{36, 37} This study will attempt to clarify the role between changes in pro- and anti-inflammatory cytokine production in relation to post-stroke depressive symptom severity, as an upstream link in the pathway of IDO-mediated depression. Lastly, few studies have assessed the relative effects of acute infarcts, WMC, and atrophy together as they relate to PSD.\textsuperscript{39, 40, 45, 48} Those have produced mixed results, with one study finding WMC\textsuperscript{48} and atrophy\textsuperscript{39} to be significantly associated with PSD. Thus, failure to account for pre-existing or additional brain pathology may explain the lack of consistency in previous findings. This thesis aims to explore a comprehensive approach in assessing the association between
neuroanatomy and post-stroke depressive symptoms, and thus will assess the relationship between depressive symptom severity and each neuroimaging measurement. In particular, this study will measure whole brain atrophy, hippocampal atrophy, lesion volume, and WMC. Lastly, the combined effect of an elevated K/T ratio and neurodegeneration may be more relevant in predicting the presence of PSD than either variable alone and therefore, this study will also attempt to explore this notion as its final exploratory aim.

Section 2: Materials and Methods

*Methods were conducted, as has been done by our Group in the past406-408

2.1 Study Design

This clinical study was cross-sectional and correlational in nature. It examined the neuroanatomical and inflammatory correlates of severity of post-stroke depressive symptoms. Accordingly, ischemic stroke patients were recruited, all having met World Health Organization MONICA Project (1988) and National Institute of Neurological Disorders and Stroke (WHO-NINDS)409 criteria for stroke with visual CT-based evidence of an acute brain infarct. All patients were recruited from either an in-patient unit or the out-patient Stroke Prevention Clinic (SPC) at Sunnybrook Health Sciences Centre, which is an acute care hospital with regionalized stroke service. They were assessed in terms of mood, cognition, and stroke severity. Demographic information and medical history were obtained through hospital chart reviews and patient interviews. This study has received Research Ethics Board approval by Sunnybrook Health Sciences Centre (Appendix 1). All patients provided written, informed consent and each received a photocopy of the signed consent form (Appendix 2).
2.2 Inclusion Criteria and Exclusion Criteria

Inclusion criteria:

- Age: ≥ 18 years old
- Gender: male or female
- Language: speaks and understands English
- Clinical diagnosis of stroke according to the WHO-NINDS criteria
- Acute cerebral infarction with visual CT-based evidence

Exclusion Criteria:

- Subarachnoid or intracranial hemorrhage
- Severe aphasia or dysarthria that would preclude neuropsychiatric testing
- Imminently suicidal or, in the opinion of the affiliated clinician, has inadequate family monitoring for suicidality
- Impaired level of consciousness that would preclude from neuropsychiatric testing
- Significant acute medical illness likely to affect neuropsychiatric symptoms, including:
  - Infection
  - Drug or alcohol abuse
  - Uncontrolled diabetes
  - Uncontrolled anemia
  - Severe disturbance in liver, kidney, or lung function
  - Untreated hypothyroidism

- Significant acute neurological illness likely to affect neuropsychiatric symptoms, including:
  - Decreased Level of Consciousness (LOC)
  - Active brain tumour
  - Parkinson’s disease
  - Huntington’s disease
  - Multiple sclerosis
  - Binswanger’s disease
  - Hydrocephalus
  - Subdural hematoma
  - Progressive supranuclear palsy
  - Severe aphasia

- Presence of a previous Axis I psychiatric diagnosis, such as mood disorder, an anxiety disorder or schizophrenia
  - Major Depressive Disorder (history of)
  - Schizophrenia
  - Bipolar disorder
  - Dementia

- Concomitant use of psychotropic medication, except short-acting benzodiazepines for sedation (e.g. lorazepam)
- Current use of antidepressant pharmacotherapy
2.3 Demographics, Medical History and Clinical Assessments

2.3.1 Demographic and Medical History

The method for obtaining demographic information and clinical history was either through chart review or patient interviews. Age, gender, time since stroke, marital status, current and expected living situation, current and expected employment status and educational history were documented. Past and current medical histories included thorough accounts of all surgical history, cardiovascular risk factors, concomitant medications, and psychiatric history.

2.3.2 Clinical Assessments

2.3.2.1 Stroke Severity Scale

The National Institutes of Health Stroke Scale (NIHSS)\textsuperscript{410} was used to measure stroke severity, which was carried out by clinicians at the time of patient admission, attained through chart review, or retrieved from the chart utilizing a standardized method as has been done before.\textsuperscript{411} Stroke severity is an established risk factor for depression and an important outcome associated with physical disability.\textsuperscript{129} The NIHSS was used by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) Stroke Data Bank.\textsuperscript{412} Stroke severity, measured using the NIHSS, was demonstrated to significantly correlate with stroke outcomes.\textsuperscript{413} In terms of functional impairment, as assessed by the Barthel Index and the modified Rankin Scale, the NIHSS was found to significantly associate with this negative post-stroke outcome.\textsuperscript{414}

2.3.2.2 Depressive Symptom Severity Scale

Depressive symptoms were rated using the Centre for Epidemiological Studies Depression (CES-D) scale,\textsuperscript{415} which is a patient self-report scale. It was also utilized by the NINCDS Stroke Data Bank,\textsuperscript{412} and it was formerly validated, using the structured clinical interview in Stroke Data Bank patients, to be reliable with previously accepted diagnostic criteria. When the CES-D was evaluated against a number of different
depression rating scales in aging stroke patients, it was established to retain good external and concurrent validity.\textsuperscript{416} Previous studies have utilized this scale in the context of PSD.\textsuperscript{144,417} Using a cut-off of $\geq 16$ in the post-stroke population was found to be extremely indicative of depression (sensitivity 86\%, specificity 90\%, positive predictive value 80\%).\textsuperscript{418} In older, non-stroke populations, this same cut-off score was sensitive enough to detect physical, cognitive, and psychosocial impairments with had clinical relevance,\textsuperscript{419,420} as well as relevant and significant depressive symptoms.\textsuperscript{419,420}

\textbf{2.3.2.3 Global Cognition Scale}

Upon the patient giving written informed consent, the Mini-Mental State Examination (MMSE)\textsuperscript{421} was carried out. This scale was chosen to screen global cognition due to the fact that in acute care settings it is extensively utilized, it is also a means of rapid assessment of cognition and has been validated in this setting.\textsuperscript{422} The MMSE has previously been demonstrated to associate well with the CAMCOG (cognitive and self-contained part of the Cambridge Examination for Mental Disorders of the Elderly) in a stroke rehabilitation setting.\textsuperscript{423} Due to the fact that this study is exploratory and the possibility of low admissibility of a more lengthy assessment in an acute stroke setting, this concise, although validated and clinically relevant cognitive scale was chosen.

\textbf{2.4 Blood Sampling}

For inpatients, blood withdrawal was carried out by IV technicians upon their scheduled clinical blood draws, and outpatients met with the SPC study coordinator for their blood to be drawn. Because of the variability in blood collection time, it was not possible to have an absolute standardized collection time, however every effort was made to conduct blood draws in the morning (approximately 7:00 a.m.). Serum concentrations of various pro- and anti-inflammatory cytokines (TNF-$\alpha$, IL-6, IL-10, IFN-$\gamma$, IL-1$\beta$ and IL-18), and kynurenine, and plasma concentrations of tryptophan and LNAA were assessed. Blood samples utilized for cytokine and kynurenine analysis were collected in SST gel vacutainer tubes, and TRP and LNAA were collected in EDTA vacutainer tubes.
After a maximum of 2 hours post-collection, the samples of blood were centrifuged at 1000g for 10 minutes. Plasma or serum sample were relocated into labeled cryovials following centrifugation and kept at -70°C until analyzed. An extra ultrafiltration step was necessary for TRP samples to divide the free form from protein-bound form. Here, 1.0 ccof plasma was moved into an ultra filtration device (centrifree 4104®, Millipore) and centrifuged at 1000g for an extra 15 minutes. The device maintained all protein-bound TRP while enabling the smaller free TRP to move across the 30,000 kDa membrane into ultrafiltrate reservoir cups. The cups holding free TRP were then capped and held with the other samples at -70°C until ready for batched analysis.

2.5 Serum and Plasma Analyses

2.5.1 Kynurenine Assay

KYN concentrations were calculated by the Pharmacy Quality Control and Research Group at Sunnybrook Health Sciences Centre with high-performance liquid chromatography (HPLC) at UV detection of 258 nm, as described elsewhere. Personal communication with Scott Walker was carried out for a comprehensive protocol of the KYN assay. Briefly, an equal volume of 3% perchloric acid was used for protein precipitation. After centrifugation for 10 minutes, a 0.1 mL aliquot of the supernate was injected directly into the high liquid chromatograph system using an autoinjector (715 Ultra WISP, Waters Canada). The mobile phase consisted of 9% acetonitrile in 0.05 M potassium phosphate mono basic, pumped through a reverse phase 5 μm ODS column, 250 mm × 4.6 mm (Symmetry; Waters Corporation, Milford, Massachusetts, USA). KYN was detected using an ultraviolet detector (UV 6000; ThermoSeperation 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 obtained by making 9 standards from a common serum stock sample. Additional KYN was added to roughly 10 mL of serum, to yield standards with 0, 0.125, 0.250, 0.3125, 0.50, 0.750, 1.0, 1.5, and 2 mg/L of extra KYN added. Examination of these standards in duplicate produced a linear relationship between the KYN peak area and concentration with a slope and intercept (because of
the endogenous kynurenine in the common serum stock). The obtain unknown concentrations of KYN in plasma the KYN peak area in the unknown sample was divided by the slope. Replicate error, as assessed 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%.

2.5.2 Cytokine Assay

Cytokine measurements of TNF-α, IL-6, IL-10, IFN-γ, IL-1β and IL-18 were carried out by Dr. Angela Panoskaltsis-Mortari who runs the Cytokine Reference Laboratory at the University of Minnesota. A multiplex immunobead-based assay using the Human Fluorokine Multianalyte Profiling (MAP) Base Kit A (R&D Systems, Minneapolis, MN) was utilized to assay all cytokines. All cytokine samples from this study were assessed for sensitivity, precision, recovery, sample linearity, and specificity by the company panel. The company website gives an overview of the methods:

“Detection is achieved through bead-based antibody-antigen sandwich method. Briefly, samples are incubated with colour-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® analyzer, independent lasers determine the colour of each bead and the magnitude of the PE-derived signal, which is directly proportional to the levels of bound analyte.”

Assay sensitivities were 0.2 pg/ml for IL-10, 1.0 pg/ml for IFN-γ, 0.09 pg/ml for TNF-α, 0.057 pg/ml for IL-1β, 0.1 pg/ml for IL-6, and 12.5 pg/ml for IL-18, which are comparable to the sensitivities of used by other studies observing stroke and depressed populations by means of standard ELISA assays. However, researchers have noted that multiplex technology can distinguish a superior range of values compared to ELISA or Western blotting.
2.5.3 Tryptophan and LNAA Assay

Dr. Simon Young, who resides at the Department of Psychiatry at McGill University, kindly assessed concentrations of TRP and LNAA, as explained elsewhere in detail by Anderson et al. Through personal communication with Simon Young, a thorough protocol of the TRP and LNAA assays was obtained and is presented here:

“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 Bondpack C18 reverse phase column (Phenomenex, Torrance, CA) 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 pre-column derivatization with o-phthalaldehyde and gradient reverse phase HPLC with fluorometric detection. Aminoadipic acid was used as an internal standard.”

2.6 Neuroimaging

As a component of routine hospital procedures, patients had two to three CT scans carried out. This study assessed the final CT scan so as to account for the complete infarct and analyze all ‘tissue at risk’. The final scan needed to be performed at a minimum of 24 hours after the stroke. All CT scans were collected without a contrast agent on a General Electric LightSpeed VCT series scanner (General Electric Healthcare, Waukesha, WI). The CT images were skull stripped with the Image Edit function of Analyze 11.0 (Mayo Clinic, Rochester, MN). The de-skulled brains were transformed using the segment function of Statistical Parametric Mapping 5 (APM5; Wellcome Department of Imaging Neuroscience, University College London, London, England). This function was employed to make transformation matrices that outlined the process required to align de-skulled brains with a standard template previously aligned with Montreal Neurological Institute (MNI) space. The Normalise: Write function of SPM5 utilized the transformation matrices to normalize the de-skulled brains to this MNI space template. The normalized de-skulled brains
were assessed to make sure that they were aligned well. The scans that did not align properly with MNI space were not used and therefore these patients were removed from the study.

2.6.1 Lesion Volume and location Measurement

Areas of infarcted tissue were traced on these images using Analyze version 11.0 (Mayo Clinic, Rochester, MN) [Figure 2]. Lesion area was obtained by tracing the infarct slice-by-slice on the CT scan, then the area of the lesion on each slice was multiplied by slice thickness (1mm$^3$) and summed to get the total lesion volume. This method has been demonstrated to have high reproducibility in acute ischemic stroke.\textsuperscript{429}
Figure 2. Axial slice showing a patient with a large right hemispheric infarct (radiological perspective: flipped)
Lesion location was determined using the methods constructed by Robinson et al.\textsuperscript{138} to test their lesion location hypothesis of PSD.\textsuperscript{406} This included determining an anterior-posterior (AP) ratio by assessing the distance of the most anterior border of the lesion to the frontal pole and then dividing this value by the total length of the AP ratio in the same slice. Depending on the AP ratio, lesions were grouped into four distinct types: ‘Anterior’ lesions are located <40\% from the frontal pole, ‘Posterior’ lesions are situated >60\% from the frontal pole, ‘Extending’ lesions are <40\% and >60\% from the frontal pole, and ‘Intermediate’ lesions are >40\% but <60\% from the frontal pole.\textsuperscript{406}

\textbf{2.6.2 White Matter Change Analysis}

WMCs were carried out with a CT and MRI-validated visual rating scale, the Age-Related White Matter Changes (ARWMC) scale [\textbf{Figure 3}].\textsuperscript{430} This scale has many advantages to the extensively used Fazekas scale,\textsuperscript{431} including a more in depth assessment of brain regions. The ARWMC scale goes beyond the Fazekas scale in terms of this because it analyzes the brain as 10 discrete areas, as opposed to ‘deep’ and ‘periventricular’. The ARWMC regions are as follows: bilateral frontal lobes, bilateral temporal lobes, bilateral parieto-occipital lobes, bilateral basal ganglia, and bilateral infratentorial areas. The scoring system is as follows: 0 (no lesions), 1 (focal lesion), 2 (beginning of confluence lesions), 3 (diffuse involvement of the entire region). However, the basal ganglia are scored by to another system: 0 (no lesions), 1 (1 focal lesion), 2 (>1 focal lesion), 3 (confluent lesions).
Figure 3. Axial slice showing a patient with grade 2 white matter changes in bilateral frontal and parieto-occipital regions.

2.6.3 Measurement of Hippocampal Thickness

Hippocampal thickness was measured using previously described methods by Gao et al. 2003. Briefly, as seen in Figure 4, the width of the medial temporal lobe (MTL) was used as a marker for hippocampal thickness. Using Analyze version 11.0 (Mayo Clinic, Rochester, MN), the MTL was oriented at an angle of approximately 30° caudal to the Anterior Commissure – Posterior Commissure (AC-PC)
plane. The intercollicular sulcus (ICS) was used as a landmark to obtain the best longitudinal view of the hippocampus and parahippocampal gyrus, and the MTL width was measured midway and at its thinnest portion between the anterior–posterior borders of the midbrain. Since the scans were co-registered with a template that was calibrated to be 24% larger than normal, the measurement obtained for hippocampal thickness was then multiplied by 0.76 to obtain the true thickness of the hippocampus. Measurements for right and left hippocampal thickness were averaged to create one final value per patient.

Figure 4. Axial slice showing the measurement of left hippocampal width (radiological perspective) by aligning the hippocampus 30° caudal to the AC-PC plane.
2.6.4 Whole Brain Atrophy Measurement

Whole brain atrophy as assessed by total ventricular volume measurement. The left and right lateral ventricles as well as the third ventricle were manually traced using Analyze version 11.0 (Mayo Clinic, Rochester, MN) [Figure 5]. The area of the ventricles on each slice was then multiplied by slice thickness (1mm$^3$) and summed to get the total volume of left and right lateral ventricles as well as the third ventricle. Total ventricular volume was then normalized by dividing this value by the total intracranial volume (TIV). This produces a ratio value of total ventricular volume: total intracranial volume in order to account for varying brain volumes across subjects.
Figure 5. Axial slice showing a manual tracing of the left and right lateral ventricle, as well as the third ventricle.

Aside from lesion location analysis, which was carried out by Phillip Francis, all neuroimaging conducted was overseen and verified by an experienced neuroradiologist, Dr. Fu-Qiang Gao, to ensure
preservation of correct methodology. Briefly, training was done by Dr. Gao using a training set of 20 random images. Once those were found to be in agreement with Dr Gao’s tracings, scores, or measurements, the remainder were done initially by the candidate and then checked by Dr. Gao.

2.7 Planned Analyses

2.7.1 Initial Descriptive Statistics

The Statistical Package for the Social Sciences (SPSS), version 20.0 was used for all statistical analyses. Initially, all demographic variables were assessed in terms of their correlation with the CES-D. Any variables found to be significantly associated with depression scores were then used as covariates in latter analyses. These variable comprised of the following: age (years), gender (F/M), time between stroke and assessment (days), vascular risk factors; cigarette smoking (Y/N), hypertension (Y/N), obesity (Y/N), elevated cholesterol (Y/N), and diabetes (Y/N), education level (ordinal), living situation (alone or with others), marital status (ordinal), employment status (ordinal), lesion location (ordinal), lesion laterality (right/left), stroke severity (NIHSS score), and global cognition (MMSE) score. All of these variables were analyzed for a significant correlation with depression scores. Continuous variables were analyzed with spearman’s rank correlation coefficient (\(\rho\)) (Non-parametric). Pearson’s product-moment correlation coefficient (\(r\)), was not employed because CES-D patient scores failed the Kolmogorov-Smirnov test for normality \((p>0.05)\). Gender, living situation, cigarette smoking, hypertension, diabetes, elevated cholesterol, and obesity were examined using an independent-samples Student’s \(t\)-test, which compared the mean CES-D score between the sexes, living situations, and individual vascular risk factor groups. The education level, employment status, and marital status were investigated using a one-way analysis of variance (ANOVA), which compared the mean CES-D score between the multiple groups.

For serum concentrations of IFN-\(\gamma\), TNF-\(\alpha\), IL-6, IL-10, IL-18, and IL-1\(\beta\) that were below the limit of detectability, missing values were imputed at the above mentioned values, however there were no undetectable sample for IL-6 or IL-18. Because of the fact that undetectable samples were imputed at the lower limit of detectability, n the absolute values of kurtosis and skewness were more than 2 standard
deviation (SD) of the error. It then became imperative to log-transform the cytokine dataset in order to retain a normally distributed dataset, as has been done by our group and many other researchers have dealt with this issue in similar ways in the past. Furthermore, this method should not interfere with our objective of examining the role of increased proinflammatory cytokine concentrations in PSD, except in the case of IL-10, an anti-inflammatory cytokine, where the goal was to detect decreased concentrations in PSD patients. This drawback will be discussed in further detail within the discussion. For the calculation of immunologic ratios, they were determined with raw values and log-transformed afterwards. Possible intercorrelations between individual cytokines were also assessed.

2.7.2 Primary Analyses

**Hypothesis 1:** It is hypothesized that serum levels of the K/T ratio, as a measure of IDO activity, will positively correlate with depressive symptom severity post-ischemic stroke.

To test the primary hypothesis, a simple linear regression was performed to assess the relationship between CES-D total score and the K/T ratio. All important covariates from the initial descriptive analysis were included in the model. Furthermore, K/T ratio was recently found to be significantly elevated in somatization compared to depression and also associated with somatic symptom severity. In a study involving IL-2 and/or interferon-α cancer therapy, symptoms characterized by the researchers as ‘neurovegetative’, including lack of sleep, poor appetite, and decreased energy, were significantly more pronounced in patients with mild depressive symptoms than patients within the ‘absent’ depressive symptom range, as measured by the MADRS. As a result, the relationship between K/T ratio and CES-D may not be linear. Therefore, an ordinal regression was also performed to determine if the K/T ratio significantly predict CES-D scores as tertiles. By dividing our study population into tertiles based on CES-D total scores, this will reveal if the K/T ratio is associated with none, mild, or moderate depressive symptoms. For all tests, multicollinearity was assessed; tolerance statistics were calculated and examined. However for an ordinal regression, the amount of covariates allowed in the model is determined by taking the smallest (n) outcome category and dividing it by 10. Out of the three CES-D tertiles, the smallest outcome category has
an n value of 26, thus, the model can only hold 2 covariates. Therefore, a decision was made to include NIHSS scores as the sole covariate for all subsequent ordinal regressions, since stroke severity has been consistently identified as an important independent predictor of PSD in the past.\textsuperscript{16, 110}

2.7.3 Secondary Analyses

**Hypothesis 2:** Elevated pro-inflammatory cytokines IFN-\(\gamma\), TNF-\(\alpha\), IL-6, IL-18, and IL-1\(\beta\) and a reduction in the anti-inflammatory cytokine IL-10, as evidenced by an elevated immunologic ratio between these pro-inflammatory cytokines and IL-10, will correlate with depressive symptom severity post-ischemic stroke.

To test my secondary hypothesis, separate simple linear regressions were performed to assess the relationship between CES-D total score and all individual cytokines as well as their immunologic ratios with IL-10. Since patients with depressive symptoms not meeting DSM-IV outlines for MDD have significant elevations in inflammatory molecules,\textsuperscript{214, 236} a set of ordinal regressions were conducted to determine which cytokines or cytokine ratios may significantly predict CES-D scores as an ordinal variable. By dividing our study population into tertiles based on CES-D total scores, this will reveal if elevated cytokines or an elevated pro- to anti-inflammatory cytokine ratio is associated with none, mild, or moderate depressive symptoms. For all tests, significant clinical characteristics were included as covariates and tolerance statistics were calculated and examined.

2.7.4 Exploratory Analyses

Lesion volume,\textsuperscript{38-43} WMC,\textsuperscript{38-48} and atrophy\textsuperscript{39, 40, 45, 49} have been studied in the context of PSD with mixed findings, however MDD has been consistently linked with neurodegeneration and neuroprogression.\textsuperscript{50-60} A comprehensive approach to investigating atrophy, lesion volume and WMC may help to clarify their role in the context of PSD, as these measurements are not mutually exclusive. Thus, I sought to explore the relationship between neuroimaging findings and post-stroke depressive symptom severity. Specifically, hippocampal thickness, whole brain atrophy, WMC and lesion volume may be important independent correlates of depressive symptom severity post-ischemic stroke. In order to assess which neuroimaging findings were significantly associated with CES-D score, average hippocampal thickness, whole brain
atrophy, the global ARWMC score for WMC, and lesion volume were entered into a backward stepwise linear regression and CES-D score was entered as the dependent variable. CES-D scores were compared across acute lesion locations to determine if they were significantly different across groups, if so, these locations would be included as a covariate in this analysis. The removal criterion for backwards regression model was set to \((P \leq 0.1)\). A separate backward stepwise linear regression was conducted to determine the significance of white matter lesion location in relation to PSD. As determined by a paired samples t-test, there was no significant difference between right and left ARWMC scores, thus, each bilateral region score (frontal lobes, temporal lobes, parieto-occipital lobes, bilateral basal ganglia, and infratentorial) were summed to create 5 ARWMC scores as opposed to 10 bilateral scores. These 5 regional scores were entered into the regression model with CES-D score as the dependent variable. For all tests, significant clinical characteristics were included as covariates and tolerance statistics were calculated and examined.

The fact that (1) depression has been associated with neurodegeneration, \(^{50-60}\) (2) KYN metabolites are elevated in many neurodegenerative diseases and stroke, \(^{61-66}\) and (3) have also been implicated in the process of neuroprogression, \(^{21, 50, 67-77}\) suggests that an elevated K/T ratio in the presence of neurodegeneration may be more relevant in contributing to the development of PSD, than either variable alone. In order to explore the possible joint contributions of an elevated K/T ratio and neurological pathology in post-stroke depressive symptom severity, a simple linear regression was conducted taking into account any significant neuroimaging correlates from the previous analysis, in conjunction with the K/T ratio. A custom model was created taking into consideration the K/T ratio and neuroimaging correlates separately, alongside interaction terms between these independent variables. Significant clinical characteristics were also included and tolerance statistics were calculated and examined.

2.7.5 Sample Size Calculation

The K/T ratio has never been assessed in relation to depressive symptom severity post-ischemic stroke. Therefore, the effect size \((f^2)\) was approximated according to an earlier study published by our group involving CAD patients assessed for the association between the K/T ratio and depression. \(^{92}\) For a multiple linear regression the coefficient of determination \((R^2)\), based on the CAD study, was used to calculate \(f^2\),
where $f^2 = \frac{R^2}{1 - R^2}$. Subsequently, $f^2$ was used to determine the required sample size ($n$), based on the $\alpha$ level (probability of type I error) set to 0.05 ($\alpha = 0.05$), and the $\beta$ level (probability of type II error) set to 0.2 ($\beta = 0.2$). Thus, it was determined that with $\alpha = 0.05$, an estimated $f^2 = 0.1433$, and 3 independent predictors in the multiple linear regression model, the required sample size is 80, to obtain an observed power of 0.80.

### 2.8 Controlling for Confounding Variables

Many clinical and vascular risk factors have been found to be associated with stroke and depression. Therefore, confounding variables were tested for their correlation with depressive symptom severity and any confounders determined to be significantly associated with depressive symptom severity were included in subsequent *a priori* and exploratory analyses as important covariates. It was determined that NIHSS scores and age were significantly correlated with depression scores. In one meta-analysis stroke severity was found to be significantly associated with PSD across various studies.\(^{16}\) Another recent meta-analysis by Ayerbe *et al.* also found stroke severity to be an important predictor of PSD.\(^{110}\) Furthermore, pro- and anti-inflammatory cytokines,\(^ {228, 436, 437}\) as well as the K/T ratio\(^ {438, 439}\) have been found to positively correlate with age. Patients with a history of depression and those who are currently using antidepressant pharmacotherapy were excluded from this study since antidepressant use has been demonstrated to alter the ratios of pro- to anti-inflammatory cytokines, increasing the production of IL-10,\(^ {32-35}\) and a history of depression is an established risk factor for MDD, though the mechanisms behind its development may vary from the etiology of PSD.

*Methods were conducted, as has been done by our Group in the past\(^ {406-408}\)
Section 3: Results

3.1 Demographic and Clinical Characteristics

Eighty two patients were recruited to participate in this study. Table 1 displays the results of independent samples t-tests, ANOVAs and Spearman’s correlations between CES-D scores and clinical and demographic characteristics.

Table 1. Clinical demographic characteristics (n=82)

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Mean ± SD or %</th>
<th>Range</th>
<th>*R , t, or F Value</th>
<th>*p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>71.9 ± 14.2 years</td>
<td>33-98</td>
<td>0.254</td>
<td>0.022*</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>53.1%</td>
<td></td>
<td>-0.558</td>
<td>0.578</td>
</tr>
<tr>
<td>Living situation (% alone)</td>
<td>32.1%</td>
<td></td>
<td>1.100</td>
<td>0.275</td>
</tr>
<tr>
<td>Employment Status (% retired)</td>
<td>73.8%</td>
<td></td>
<td>1.409</td>
<td>0.247</td>
</tr>
<tr>
<td>Marital Status (% married)</td>
<td>51.9%</td>
<td></td>
<td>-0.271</td>
<td>0.787</td>
</tr>
<tr>
<td>Education level (%&gt;high school)</td>
<td>50.6%</td>
<td></td>
<td>1.528</td>
<td>0.192</td>
</tr>
<tr>
<td>Time since stroke, days</td>
<td>25 ± 35</td>
<td>4-155</td>
<td>0.105</td>
<td>0.353</td>
</tr>
</tbody>
</table>

Clinical Assessments

<table>
<thead>
<tr>
<th>Clinical Assessments</th>
<th>Mean ± SD or %</th>
<th>Range</th>
<th>*R , t, or F Value</th>
<th>*p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CESD</td>
<td>12.6 ± 10.8</td>
<td>0-47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIHSS</td>
<td>4.6±4.7</td>
<td>0-19</td>
<td>0.231</td>
<td>0.042*</td>
</tr>
<tr>
<td>MMSE</td>
<td>25.8 ± 4.2</td>
<td>11-30</td>
<td>0.034</td>
<td>0.762</td>
</tr>
</tbody>
</table>
Mean ± SD or % | Range | *R, t, or F Value | *p Value
---|---|---|---
**Plasma and Serum Markers**
KYN ($\mu$ mol/L) | 2.98 ± 1.57 | 0.59-8.46 | -0.075 | 0.503
TRP ($\mu$ g/mL) | 2.87±0.68 | 2.08-5.05 | 0.012 | 0.935
LNAA ($\mu$ g/mL) | 564.55 ± 145.08 | 273.68-977.14 | -0.145 | 0.314

*r, t, or F values and their corresponding *p-values reflect the results of bivariate Spearman’s correlations with CES-D scores for continuous variables, independent samples t-test between CES-D score and categorical variables, and ANOVAs between CES-D scores and ordinal variables.

These descriptive statistics revealed that NIHSS score ($\rho=0.231, p=0.042$) and age ($\rho=0.254, p=0.022$) were significantly associated with CES-D score, thus, these were included as covariates in all subsequent analyses. As displayed in Table 2, there were no significant differences in depression scores between any of the vascular risk factors (VRFs) assessed. However there a trend ($t=-1.680, p=0.097$) for depression scores to be higher in the hypertensive group. Of note, hypertension (81.5%) and hyperlipidemia (66.3%) were especially common in this patient population, and 93.8% of this patient sample had at least one VRF.

**Table 2.** Vascular risk factors (VRFs) (n=82)

<table>
<thead>
<tr>
<th>Vascular Risk Factors</th>
<th>Mean ± SD or %</th>
<th>Range</th>
<th>* t Value</th>
<th>*p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>81.5%</td>
<td>-1.680</td>
<td>0.097</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>20.3%</td>
<td>0.722</td>
<td>0.473</td>
<td></td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>66.3%</td>
<td>-0.212</td>
<td>0.833</td>
<td></td>
</tr>
<tr>
<td>Obesity (BMI&gt;=30)</td>
<td>20.0%</td>
<td>0.143</td>
<td>0.887</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>21.5%</td>
<td>1.564</td>
<td>0.122</td>
<td></td>
</tr>
<tr>
<td>Total number of VRFs</td>
<td>2.09 ± 1.109</td>
<td>0-5</td>
<td>1.076</td>
<td>0.380</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index. *t values and their corresponding *p-values reflect the results of independent samples t-test between CES-D score and categorical variables.
As shown in Table 3, there were no significant differences in depression scores between lesion locations (F\(_{3,41}=0.622, p=0.605\)) or lesion laterality (F\(_{1,56}=0.371, p=0.545\)). An equal proportion (28.6%) of patients has stroke in the anterior and posterior regions, while 24.5% had extending lesions and 18.4% had intermediate lesions. Approximately two thirds (64.7%) had right-sided lesions, while 35.3% had left-sided lesions.

Table 3. Lesion location (n=45) and laterality (n=58)

<table>
<thead>
<tr>
<th>Lesion Location</th>
<th>%</th>
<th>* F Value</th>
<th>*p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior</td>
<td>28.6%</td>
<td>0.622</td>
<td>0.605</td>
</tr>
<tr>
<td>Intermediate</td>
<td>18.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior</td>
<td>28.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extending</td>
<td>24.5%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Laterality</th>
<th>%</th>
<th>* F Value</th>
<th>*p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>64.7%</td>
<td>0.371</td>
<td>0.545</td>
</tr>
<tr>
<td>Left</td>
<td>35.3%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*F values and their corresponding *p-values reflect the results of ANOVAs between CES-D scores and ordinal variables.
As displayed in Table 4, independent samples t-tests revealed no significant differences in mean CES-D scores between groups of patients using and not using certain medications.

**Table 4.** Concomitant medication use

<table>
<thead>
<tr>
<th>Medication</th>
<th>% of patients using medication</th>
<th>*t</th>
<th>*p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>62.5%</td>
<td>-0.021</td>
<td>0.98</td>
</tr>
<tr>
<td>Anti-hypertensives†</td>
<td>35.5%</td>
<td>-1.65</td>
<td>0.103</td>
</tr>
<tr>
<td>Insulin</td>
<td>16.3%</td>
<td>1.69</td>
<td>0.096</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>17.5%</td>
<td>-0.059</td>
<td>0.95</td>
</tr>
</tbody>
</table>

†Anti-hypertensives include: Ca\(^{2+}\) Channel Blockers, \(\beta\)-blockers, and diuretics. *t values and their corresponding *p-values reflect the results of independent samples t-test between CES-D score and categorical variables.

### 3.2 Assay Results

Plasma samples from 82 patients were gathered and sent out for testing of IL-6, IL-18, TNF-\(\alpha\), IFN-\(\gamma\), IL-1\(\beta\), and IL-10. There were 82 samples sufficient to analyze for IL-6, IL-10, and IFN-\(\gamma\), 62 samples for IL-18, 59 samples for TNF-\(\alpha\), and 54 samples for IL-1\(\beta\). Samples of cytokines that were below the assay’s limit of detectability were imputed at the lower limit of detectability for each cytokine. The aforementioned sensitivities were 0.2 pg/ml for IL-10, 1.0 pg/ml for IFN-\(\gamma\), 0.09 pg/ml for TNF-\(\alpha\), 0.057 pg/ml for IL-1\(\beta\), 0.1 pg/ml for IL-6, and 12.5 pg/ml for IL-18, however there were no undetectable samples for IL-6 or IL-18. Imputed cytokine levels were included in all statistical analyses.

### 3.3 Primary Analyses

A simple linear regression was conducted to determine the relationship between the K/T ratio and depression scores as a continuous variable. After controlling for age and NIHSS scores, the overall model was not significant \((R^2=0.069, F_{3,73}=1.805, p=0.15)\), with neither the K/T ratio \((\beta=-0.105, p=0.37)\), nor the NIHSS score \((\beta=0.190, p=0.10)\), nor age \((\beta=0.143, p=0.21)\) significant independent predictors of CES-D score. A scatter plot of the relationship between serum K/T and CES-D scores is shown in **Figure 6**.
Next, an ordinal regression was performed to determine if the K/T ratio significantly predicts CES-D tertiles. The lowest CES-D tertile scores ranged from 0-6, the middle tertile scores ranged from 7-15, and the highest tertile scores ranged from 16-47. For an ordinal regression, the number of covariates allowed in the model is determined by taking the smallest (n) outcome category and dividing it by 10. Out of the three CES-D tertiles, the smallest outcome category has an n value of 26, thus, the model can only consider 2 covariates. The NIHSS scores was chosen as the sole covariate for all subsequent ordinal regressions, since stroke severity has been consistently identified as an important independent predictor of PSD in the past. The ordinal regression model was conducted and demonstrated that neither the K/T ratio (odds ratio [OR], 0.996; 95% confidence interval [CI], 0.985-1.007, p=0.51) or NIHSS score (OR, 1.077; 95% CI, 0.987-1.177, p=0.095) were significant independent predictors of CES-D tertile. Figure 7 displays a bar graph of mean serum K/T ratio across CES-D tertiles.
**Figure 7.** Bar graph of mean serum K/T levels across stroke patients in low, middle, and high CES-D tertiles

![Bar graph of mean serum K/T levels across stroke patients in low, middle, and high CES-D tertiles](image)

### 3.4 Secondary Analyses

Simple linear regressions were used to determine the relationship between each cytokine and depression scores, as well as the IL-10 immunologic ratio and depression scores. After controlling for age and NIHSS scores, none of the overall models were significant, nor were the cytokines or immunologic ratios significant predictors. The results of each overall linear regression model and individual cytokines as predictors are summarized in **Table 5**. A sample (IFN-Ɣ and IFN-Ɣ/IL-10) of scatter plots showing the relationship between plasma cytokines and CES-D scores is shown in **Figure 8** and **9**.
Table 5. Final linear regression models for each cytokine as it predicts CES-D score

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Model</th>
<th>$R^2$</th>
<th>$^*F$</th>
<th>Df</th>
<th>$^\dagger_\beta$</th>
<th>$^a$p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>Overall</td>
<td>0.063</td>
<td>1.61</td>
<td>3,72</td>
<td>-0.086</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>IL-6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-18</td>
<td>Overall</td>
<td>0.044</td>
<td>0.826</td>
<td>3,54</td>
<td>0.122</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>IL-18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>Overall</td>
<td>0.010</td>
<td>0.17</td>
<td>3,52</td>
<td>0.004</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>TNF-α</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-Ɣ</td>
<td>Overall</td>
<td>0.072</td>
<td>1.85</td>
<td>3,71</td>
<td>0.142</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>IFN-Ɣ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1 β</td>
<td>Overall</td>
<td>0.007</td>
<td>0.12</td>
<td>3,49</td>
<td>-0.043</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>IL-1 β</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>Overall</td>
<td>0.058</td>
<td>1.47</td>
<td>3,72</td>
<td>-0.038</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>IL-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6/IL-10</td>
<td>Overall</td>
<td>0.056</td>
<td>1.44</td>
<td>3,72</td>
<td>-0.006</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>IL-6/IL-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-18/IL-10</td>
<td>Overall</td>
<td>0.043</td>
<td>0.80</td>
<td>3,54</td>
<td>0.118</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>IL-18/IL-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α/IL-10</td>
<td>Overall</td>
<td>0.043</td>
<td>0.77</td>
<td>3,52</td>
<td>0.188</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>TNF-α/IL-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-Ɣ/IL-10</td>
<td>Overall</td>
<td>0.080</td>
<td>2.05</td>
<td>3,71</td>
<td>0.167</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>IFN-Ɣ/IL-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1 β/IL-10</td>
<td>Overall</td>
<td>0.038</td>
<td>0.65</td>
<td>3,49</td>
<td>0.185</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>IL-1 β/IL-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*F  values and their corresponding $^a$p-values reflect the results of the overall linear regression model for each cytokine, $^\dagger_\beta$ values and their corresponding $^a$p-values reflect the results of the individual cytokine as a predictor in the regression.
Next, a set of ordinal regression were performed to determine which cytokines or cytokine ratios may significantly predict CES-D tertiles. Results of ordinal regressions for each cytokine and cytokine ratio are displayed in Table 6. Briefly, the regression models revealed that most of the cytokines or cytokine ratios were not significant independent predictors of CES-D scores as tertiles. The only cytokine ratio found to be a significant predictor of mild depressive symptoms, i.e. the middle
CES-D tertile, was IFN-Ɣ/IL-10 (OR, 2.17; 95% CI, 1.02-4.64, p=0.045). There was a strong trend for TNF-α/IL-10 (OR, 1.88; 95% CI, 0.98-3.60, p=0.056) to be a significant predictor of the highest CES-D tertiles. Figure 10 presents a bar graph displaying plasma IFN-Ɣ/IL-10 across CES-D tertiles.

Table 6. Final ordinal regression model for each cytokine and cytokine ratio as it predicts CES-D tertiles

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Predictor</th>
<th>*OR</th>
<th>95% CI</th>
<th>9p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>IL-6</td>
<td>0.86</td>
<td>0.30-2.45</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>NIHSS</td>
<td>1.07</td>
<td>0.99-1.18</td>
<td>0.098</td>
</tr>
<tr>
<td>IL-18</td>
<td>IL-18</td>
<td>3.37</td>
<td>0.48-23.90</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>NIHSS</td>
<td>1.03</td>
<td>0.93-1.14</td>
<td>0.56</td>
</tr>
<tr>
<td>TNF-α</td>
<td>TNF-α</td>
<td>1.17</td>
<td>0.58-2.34</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>NIHSS</td>
<td>1.03</td>
<td>0.92-1.14</td>
<td>0.65</td>
</tr>
<tr>
<td>IFN-Ɣ</td>
<td>IFN-Ɣ</td>
<td>1.98</td>
<td>0.72-5.48</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>NIHSS</td>
<td>1.07</td>
<td>0.98-1.17</td>
<td>0.11</td>
</tr>
<tr>
<td>IL-1β</td>
<td>IL-1β</td>
<td>0.58</td>
<td>0.20-1.74</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>NIHSS</td>
<td>1.01</td>
<td>0.90-1.12</td>
<td>0.34</td>
</tr>
<tr>
<td>IL-10</td>
<td>IL-10</td>
<td>0.65</td>
<td>0.33-1.30</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>NIHSS</td>
<td>1.08</td>
<td>0.99-1.18</td>
<td>0.10</td>
</tr>
<tr>
<td>IL-6/IL-10</td>
<td>IL-6/IL-10</td>
<td>1.29</td>
<td>0.72-2.31</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>NIHSS</td>
<td>1.07</td>
<td>0.98-1.17</td>
<td>0.11</td>
</tr>
<tr>
<td>IL-18/IL-10</td>
<td>IL-18/IL-10</td>
<td>1.97</td>
<td>0.90-4.32</td>
<td>0.091</td>
</tr>
<tr>
<td></td>
<td>NIHSS</td>
<td>1.02</td>
<td>0.92-1.13</td>
<td>0.77</td>
</tr>
<tr>
<td>TNF-α/IL-10</td>
<td>TNF-α/IL-10</td>
<td>1.88</td>
<td>0.98-3.60</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>NIHSS</td>
<td>1.03</td>
<td>0.93-1.15</td>
<td>0.59</td>
</tr>
<tr>
<td>Cytokine</td>
<td>Predictor</td>
<td>*OR</td>
<td>95% CI</td>
<td>*p-Value</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------</td>
<td>-----</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>IFN-Ɣ/IL-10</td>
<td>IFN-Ɣ/IL-10</td>
<td>2.17</td>
<td>1.02-4.64</td>
<td>0.045**</td>
</tr>
<tr>
<td></td>
<td>NIHSS</td>
<td>1.07</td>
<td>0.98-1.17</td>
<td>0.12</td>
</tr>
<tr>
<td>IL-1β/IL-10</td>
<td>IL-1β/IL-10</td>
<td>1.70</td>
<td>0.70-4.11</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>NIHSS</td>
<td>1.01</td>
<td>0.90-1.12</td>
<td>0.91</td>
</tr>
</tbody>
</table>

*OR values and their corresponding *p-values reflect the results of individual cytokines and the NIHSS score as predictors in each ordinal regression model.

**Figure 10.** Bar graph of mean plasma IFN-Ɣ/IL-10 levels across stroke patients in low, middle, and high CES-D tertiles

3.5 Exploratory Analyses

In order to explore the relationship between neuroimaging findings and CES-D score, average hippocampal thickness, whole brain atrophy, the global ARWMC score for WMC, and lesion volume were entered into a backward stepwise linear regression and CES-D score was entered as the dependent variable. NIHSS score and age were included as covariates. The initial descriptive statistics did not reveal a significant difference in CES-D scores across lesion location or laterality, therefore this was not entered into the model as a covariate. The removal criterion for backwards regression model was set to (P≤0.1). The regression analysis performed 7 iterations, and all predictors were removed from the final
model. Global ARWMC score was excluded first (adjusted $R^2 = 0.001$, $\beta = -0.15$, $p=0.92$), followed by age (adjusted $R^2 = 0.020$, $\beta = 0.101$, $p=0.51$), lesion volume (adjusted $R^2 = 0.030$, $\beta = -0.127$, $p=0.35$), NIHSS score (adjusted $R^2 = 0.032$, $\beta = 0.145$, $p=0.29$), whole brain atrophy (adjusted $R^2 = 0.029$, $\beta = 0.203$, $p=0.18$), and lastly, average hippocampal thickness (adjusted $R^2 = 0.015$, $\beta = 0.179$, $p=0.18$).

Figure 11-14 display scatter plots indicating the relationship between CES-D scores and the neuroimaging outcomes.

**Figure 11.** Correlation between average hippocampal thickness and CES-D scores.
**Figure 12.** Correlation between total ventricular volume / total intracranial volume, as a measure of whole brain atrophy, and CES-D scores

![Figure 12](image1)

**Figure 13.** Correlation between global ARWMC scores and CES-D scores.

![Figure 13](image2)
A separate backward stepwise linear regression was conducted to determine the significance of white matter lesion location in relation to PSD. Because there was no significant differences in the mean ARWMC scores for right and left regions, (Table 7) each bilateral region score (frontal lobes, temporal lobes, parieto-occipital lobes, bilateral basal ganglia, and infratentorial) were summed to create 5 ARWMC scores as opposed to 10 bilateral scores. These 5 regional scores were entered into the regression model with CES-D score as the dependent variable. Controlling for NIHSS and age, the regression analysis performed 8 iterations, and all predictors were removed from the final model. Total frontal ARWMC score was excluded first (adjusted $R^2 = -0.021$, $\beta = -0.061$, $p=0.69$), followed by total temporal ARWMC score (adjusted $R^2 = -0.007$, $\beta = 0.059$, $p=0.640$), age (adjusted $R^2 = 0.006$, $\beta = 0.088$, $p=0.50$), total basal ganglia ARWMC score (adjusted $R^2 = 0.015$, $\beta = -0.138$, $p=0.30$), total infratentorial ARWMC score (adjusted $R^2 = 0.013$, $\beta = -0.127$, $p=0.30$), total parieto-occipital ARWMC score (adjusted $R^2 = 0.012$, $\beta = 0.119$, $p=0.34$), and lastly, NIHSS score (adjusted $R^2 = 0.013$, $\beta = 0.167$, $p=0.18$). Table 8 displays the results of Spearman’s correlations between each bilateral ARWMC score and CES-D scores.
Table 7. Results of paired samples t-tests for right and left regional ARWMC scores

<table>
<thead>
<tr>
<th>Right and Left ARWMC Regions</th>
<th>Mean ± SD</th>
<th>*t</th>
<th>dF</th>
<th>a p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Frontal</td>
<td>1.8±1.1</td>
<td>-0.60</td>
<td>66</td>
<td>0.55</td>
</tr>
<tr>
<td>Left Frontal</td>
<td>1.9±1.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right PO</td>
<td>1.2±1.4</td>
<td>0.77</td>
<td>66</td>
<td>0.45</td>
</tr>
<tr>
<td>Left PO</td>
<td>1.1±1.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right Temporal</td>
<td>0.4±0.9</td>
<td>0.90</td>
<td>66</td>
<td>0.37</td>
</tr>
<tr>
<td>Left Temporal</td>
<td>0.5±1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right Basal Ganglia</td>
<td>1.1±1.1</td>
<td>-0.68</td>
<td>66</td>
<td>0.50</td>
</tr>
<tr>
<td>Left Basal Ganglia</td>
<td>1.0±1.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right Infratentorial</td>
<td>0.3±0.8</td>
<td>-0.67</td>
<td>66</td>
<td>0.50</td>
</tr>
<tr>
<td>Left Infratentorial</td>
<td>0.2±0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*t values and their corresponding *p-values reflect the results of paired samples t-tests between the means of right and left regional ARWMC scores.
Table 8. Spearman’s correlations between bilateral ARWMC scores and CES-D scores.

<table>
<thead>
<tr>
<th>ARWMC Region</th>
<th>*R</th>
<th>*p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left Frontal</td>
<td>-0.053</td>
<td>0.67</td>
</tr>
<tr>
<td>Right Frontal</td>
<td>0.171</td>
<td>0.17</td>
</tr>
<tr>
<td>Left Parieto-occipital</td>
<td>0.087</td>
<td>0.49</td>
</tr>
<tr>
<td>Right Parieto-occipital</td>
<td>0.182</td>
<td>0.14</td>
</tr>
<tr>
<td>Left Temporal</td>
<td>-0.021</td>
<td>0.86</td>
</tr>
<tr>
<td>Right Temporal</td>
<td>-0.032</td>
<td>0.80</td>
</tr>
<tr>
<td>Left Basal ganglia</td>
<td>-0.095</td>
<td>0.45</td>
</tr>
<tr>
<td>Right Basal ganglia</td>
<td>0.063</td>
<td>0.61</td>
</tr>
<tr>
<td>Left Infratentorial</td>
<td>-0.114</td>
<td>0.36</td>
</tr>
<tr>
<td>Right Infratentorial</td>
<td>-0.008</td>
<td>0.95</td>
</tr>
</tbody>
</table>

*r values and their corresponding *p-values reflect the results of bivariate Spearman’s correlations with CES-D scores.

Because no significant neuroimaging predictors emerged from these regression models, the final exploratory analysis of assessing the interaction between elevated K/T ratio and neurological pathology in post-stroke depression severity was not completed.

4. Discussion

Serum K/T ratio did not correlate with depressive symptom severity and it was not significantly different across CES-D tertiles, indicating none, mild or moderate-severe depressive symptom severity. Similarly, secondary analyses revealed no significant correlations between any plasma cytokines or IL-10 immunologic ratios and depressive symptoms severity, except plasma IFN-Ɣ/IL-10 was found to be a significant predictor of mild depressive symptoms. Additionally, exploratory analyses demonstrated that none of the neuroimaging measurements or regional ARWMC scores were associated with depressive
symptoms. Finally, stroke severity, as measured by the NIHSS, and age were the only variables significantly associated with depressive symptoms in this study.

4.1 The KYN Pathway’s Influence on Post-stroke Depressive Symptoms

This study aimed to assess the relationship between depressive symptoms and the K/T ratio in ischemic stroke patients. After controlling for all important covariates, including age and NIHSS scores, the relationship between CES-D scores and K/T ratio was not significant. Our patient sample was divided into tertiles based on the severity of depressive symptoms, to determine if an elevated K/T ratio was associated with none, mild or severe depressive symptoms. However, the K/T ratio was not significantly different across CES-D groups. Changes in the levels of KYN and its metabolites have been associated with neuropsychiatric outcomes such as MDD, bipolar mania, and schizophrenia. With respect to MDD, researchers have observed both positive and negative findings. Regarding negative findings, these researchers did not find any difference in the levels of KYN, TRP, or xanthurenic acid (XA), another KYN metabolite produced by KMO, between depressed and non-depressed patients. Furthermore, two important meta-analyses by Hackett and Anderson and Ayerbe et al. found that functional disability and stroke severity were amongst the most significant correlates of PSD. While the studies assessed in those analyses did not include immunological correlates, in the context of PSD it is possible that immunological contributions may only play a minor role in the development of depression, and these are masked by the overwhelmingly strong relationship between PSD, stroke severity, and functional disability. Our finding that NIHSS scores significantly correlated with CES-D scores adds credence to this hypothesis. However, researchers today believe the etiology of PSD stems from the joint effects of biological and psychosocial mechanisms, and the CES-D may also not be the most appropriate scale, however this will be explored in depth in the limitations section below. In addition, in the context of coronary artery disease (CAD), we previously reported a significant positive association between the K/T ratio and degree of depressive symptoms (measured by the CES-D). The mean K/T ratio in our CAD
population was much lower (25.35±10.73μmol/L) than that of the present population (70.43±38.35μmol/L), in agreement with means reported in other CAD \textsuperscript{445} and stroke\textsuperscript{66,323} populations. The results in stroke suggest IDO activation is above and beyond the levels associated with CES-D scores in CAD. Furthermore, elevations in the K/T ratio have been significantly correlated with the progression, severity and mortality of multiple neurological and inflammation-associated diseases, such as Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, traumatic brain injury, cancer, malaria, HIV, rheumatoid arthritis, as well as stroke.\textsuperscript{61-63, 66, 326, 327, 446-450} With respect to stroke, our group previously found the K/T ratio to significantly correlate with post-stroke cognitive impairment (PSCI).\textsuperscript{323} Although units and measurement techniques varied between these studies, it seems as though the levels of the of the K/T ratio were substantially elevated in most of these groups\textsuperscript{61, 62, 449, 450} compared to our PSCI\textsuperscript{323} and PSD populations. This may imply that in stroke patients, extreme elevations in the K/T ratio make it difficult to decipher the relationship between a high K/T ratio and depression against the back-drop of other post-stroke sequelae,\textsuperscript{408} since the K/T ratio and KYN metabolites have been shown to strongly correlate with mortality\textsuperscript{66} and worse outcome post-stroke.\textsuperscript{326}

Furthermore, descriptive statistics revealed that insulin use was a near significant predictor of CES-D groups, and thus a post-hoc analysis was conducted controlling for insulin use as a covariate in the a priori hypothesis. It is interesting that insulin use was nearly significantly different between CES-D groups, given the established relationship between diabetes and depression.\textsuperscript{451} Furthermore, a study by Ormstad and colleagues identified serum glucose levels to be a significant predictor of post-stroke fatigue at 6 months post-stroke.\textsuperscript{256} However, this post-hoc analysis still revealed no significant relationship and it would be worthwhile for future research to attempt to characterize the relationship between insulin use, glucose levels and post-stroke depressive symptomatology further.

As discussed in the methods section 2.8.4, the necessary sample size calculated with an \(\alpha\)-level of 0.05, the power set to 80\%, and effect size of 0.1433 determined from our CAD population,\textsuperscript{92} was 80 participants. This is 2 patients less than the amount of patients included in our a priori hypothesis
(n=82), however this sample size did not generate a significant relationship between the K/T ratio and depression. One reason for this could be because that this study may not have had the power to detect the primary relationship; a post-hoc statistical power calculation revealed that the observed power to detect a statistically significant correlation between the K/T ratio and depression scores was approximately 51.0%, which is well below the initial sample size calculation accounting for a power of 80%.

Furthermore, as outlined in section 1.4.8.1, new research suggests that IDO activation may actually have a positive role in curbing the inflammatory milieu associated with stroke, and thus, dampening the relationship between cytokines, KYN, and PSD. Animal models of cerebral ischemia have demonstrated the neuroprotective effects of KYN, and IDO activation may suppress the immune response by reducing the proliferative capacity and inducing apoptosis of T lymphocytes. In general, this may be carried out through decreased TRP availability, increased production of oxidative and cytotoxic KYN metabolites, and lastly by indirectly causing naïve cluster of differentiation (CD) 4 + T cells to differentiate into Treg cells through the production of TGF-β. This last mode of action may be carried out via the AHR. It has been demonstrated that KYN can bind to the AHR, leading to IDO induction and the generation of Foxp3 Tregs; TGF-β may also amplify the interaction between KYN and AHR by upregulating AHR expression. The general result of IDO activation is a dampened Th1 response, which shifts the immune response to Th2 activation, while also stimulating the production of Tregs. Consequently, Tregs have a repressive effect on the Th1 and Th2 responses, driving the immune system towards equilibrium. Taking these novel findings into consideration, it seems plausible that the immunosuppressive effects of KYN metabolites may account for the lack of a relationship between inflammation-associated IDO activation and PSD in this study. This may be tested in animal studies in the future by looking at the immunosuppressive effects of kynurenine, quinolinic acid and 3-hydroxykynurenine and analyzing responses of animals to behavioural paradigms of depression.
The level of free TRP was also not significantly associated with CES-D scores. As mentioned in section 1.4.9.1, TRP is the precursor for the neurotransmitter serotonin, and a depletion in serotonin has been associated with major depressive disorder (MDD) in the past. Additionally, acute TRP depletion paradigms have caused transient depressive symptoms in recovered MDD patients. However, these symptoms were only present in patients with a personal history of depression who had recovered, while this study excluded patients with a history of depression due to the fact that PSD is hypothesized to have a different etiology than depression in the non-stroke population. Therefore, although it was hypothesized that acute changes in mood post-stroke are due to TRP depletion as a result of IDO activation, it seems more likely that disruptions in mood are the result of cytokine imbalance and may be more closely related to sickness behaviour. A discussed in section 1.4.6, one researcher discovered that post-stroke fatigue, not depression, was found to significantly correlate with pro-inflammatory cytokines. Furthermore, sickness behaviour is a motivational and adaptive response during infection which will resolve once the body eliminates the pathogen, while depression does not follow the same course. Only about a third of patients receiving IFN-α or IL-2 immunotherapy experience symptoms of depression, prompting researchers to characterize two forms of cytokine-induced depressive symptoms. One of which are the early-onset somatic disturbances of treatment that most, if not all patients experience, while the other form of cytokine-induced depressive symptoms are psychological in nature, late-onset, and are exhibited in as many as 50% of these patients. Furthermore, SSRI prophylaxis in these patients improved psychological mood disturbances without influencing the somatic depressive symptoms. Dantzer et al. suggests that “It is possible that depression represents a maladaptive version of cytokine-induced sickness, which could occur when activation of the innate immune response is exacerbated in intensity and/or duration...” These findings shed light on the results of the current study and may imply that acute depressive symptoms observed post-stroke are more closely related to early onset sickness behaviour than a true depression response. While the chronic mood disturbances after stroke, similar to the ‘late-onset,
psychological symptoms of immunotherapy, may be a result of increases in neurotoxic KYN metabolites wreaking havoc on emotion-regulating centres of the brain.

4.2 Influence Cytokines on Post-stroke Depressive Symptoms

As another possible mechanism, or as an upstream link in the pathway of IDO-mediated depression, the levels of pro- and anti-inflammatory cytokines were assessed in relation to PSD. This study failed to find a relationship between elevations in pro-inflammatory cytokines or reduction in IL-10 and CES-D scores in isolation. This is in contrast to previous reports of pro-inflammatory cytokine elevations in the context of MDD as well as PSD. However, certain researchers have failed to find a relationship between PSD and post-stroke pro-inflammatory cytokine elevations. As outlined in section 1.4.6, the balance between pro- and anti-inflammatory cytokines may be more highly associated with depression than either type of these cytokines in isolation, as evidence by many positive findings in both MDD and PSD. In this study, the only cytokine ratio found to be a significant correlate of CES-D tertiles was IFN-Ɣ/IL-10, however there was a strong trend for TNF-α/IL-10 to be a significant correlate of CES-D tertiles as well. This finding may be related to the aforementioned influence of IDO activation on immunosuppression. Since Tregs (possibly stimulated by KYN) are capable of producing IL-10 and IL-10 production in general, as well as through Tregs production has been demonstrated to act in a neuroprotective manner post-ischemic stroke. Briefly, animal and in vitro models have demonstrated that IL-10 can significantly reduce infarct size and enhance neuronal survival. Therefore, the immunologic ratio between pro-inflammatory cytokines and IL-10 may broadly signify immune imbalance between Th1 and Th2 immune responses, which would be detrimental to the post-stroke brain. Yet, past research suggests that a skewed Th1/Th2 response is also associated with depression. Myint et al. observed significant elevations in plasma IFN-Ɣ/IL-4 in depressed subjects and upon antidepressant treatment, the level of TGF-β1 increased and IFN-Ɣ/IL-4 ratio decreased significantly. In addition, the ordinal regression between IFN-Ɣ/IL-10 and CES-D tertiles revealed that an elevation in this immunologic
ratio was a significant predictor of the middle CES-D tertile, while TNF-α/IL-10 was trending to be a significant predictor of the highest CES-D tertile. In relation to PSD, the CES-D is a widely used and validated measure for quick screening of the presence and severity of depressive symptoms. In patients who have suffered from a stroke, a cut-off score of 16 or greater has a sensitivity ranging from 86-100% and specificity of 73-90%. In the present study, CES-D total scores were divided into tertiles; the lowest tertile ranged from 0-6, the middle tertile ranged from 7-15, and the highest tertile ranged from 16-47. Therefore, in this patient sample the highest tertile begins at the same cut-off previously found to have high sensitivity and moderate specificity in the context of PSD. Perhaps if more samples for TNF-α were assayed, the relationship between TNF-α/IL-10 and the highest CES-D tertile would become significant. This may be a limitation of the study that will be discussed in further detail below. Although a score between 7-15 was not previously validated for the detection of PSD, considering the CES-D as a depressive symptom severity continuum, it may indicate that a score between 7-15 represents mild depressive symptom severity. Depressive symptom severity, as opposed to categorizing patients into depressed and non-depressed groups, has also been correlated with elevations in inflammatory molecules by many researchers. For instance, Suarez et al. observed that higher BDI scores were correlated with elevated TNF-alpha and IL-8 production, indicating that a linear relationship exists between immune imbalance and post-stroke depressive symptom severity, as opposed to solely categorical associations. The results of this exploratory analysis are interesting, however they should be interpreted cautiously, since few groups have assessed the relationship between mild depressive symptoms and immune imbalance and more research is necessary in order to determine the significance of these findings in the context of PSD.

**4.3 Influence of WMC, Atrophy and Lesion Volume on Post-stroke Depressive Symptoms**

As mentioned in section 1.5, few studies have assessed the interaction between acute infarcts, WMC, and atrophy in relation to PSD, and all have failed to find joint associations between these three radiological finding and PSD. This thesis also failed to find a relationship between all of the neurological measurements assessed, including average hippocampal thickness, whole brain atrophy,
global ARWMC score for WMC, and lesion volume, and CES-D scores. There was also no association between any of the regional ARWMC scores and depressive symptom severity. Furthermore, because none of the aforementioned neuroimaging measurements were correlated with depressive symptom severity, the final exploratory analysis incorporating neuroanatomy with the K/T ratio was not pursued.

As outlined in section 1.5.1, lesion location and its association with PSD has been a highly debated topic. This study failed to find a relationship between lesion location (classified as anterior, intermediate, posterior and extending, as well as laterality) and depression scores. This is in agreement with multiple studies failing to find a relationship between left anterior lesions and PSD. Research by Robinson et al. assessing the timing of depressive symptom development is also not in agreement with the findings of this study, since they found that in the acute stage post-stroke (approximately 6 months) depression severity and distance of the lesion to the frontal pole in left-sided strokes are inversely correlated. However, in this study timing of assessment post-stroke ranged from 4-155 days; well within the ‘acute stage’ by the Robinson et al. classification and not significantly correlated with CES-D scores. The work of Starkstein et al. and Herrmann et al. demonstrated that lesions within the basal ganglia and thalamus were also associated with depression post-stroke. Unfortunately the method of assessing lesion location for this thesis did not dichotomize basal ganglia versus cortical strokes, as it was adopted from the Robinson et al. hypothesis of classifying lesions based on the 4 quadrants of the brain. This may be a limitation of the study considering the positive associations these authors found between basal ganglia lesions and PSD, however the mixed and inconclusive findings of meta-analyses indicate that more research still needs to be done to elucidate the relationship between all classifications of lesion location and PSD.

As previously discussed, most research supports the involvement of lesion volume in PSD, however, like this thesis, other researchers have failed to find a relationship. The variation in findings may be due to methodological differences between this thesis and the other studies that have assessed lesion volume in the past. This thesis, as described in the methods section, measured lesion volume by manually tracing lesion area for each slice on the CT scan, multiplying the area on each slice
by slice thickness (1mm³), and then summing to obtain the total lesion volume. This particular method has been found to have very good reproducibility in acute ischemic stroke. Studies have used many different methods, including similar methods to the one used here, however they varied by neuroimaging technique, as one study assessed on DWI, while another assessed on both CT and MRI images. Furthermore, a study by Vataja et al. estimated lesion volume by grouping lesion diameters into 4 categories and radii were then used in the equation for volume of a ball. Although, considering the differences in measurement techniques between studies, the mean ± SD (21.11±47.02 cm³) lesion volume of this study was quite similar to the volumes measured by these previous researchers. This may indicate that the difference in methodology between studies is most likely not the reason for variable findings between groups, and that more research should be conducted in the field to determine the importance of lesion volume in the development of PSD.

This thesis did not find a relationship between WMC and post-stroke depressive symptoms. Both the global and regional ARWMC scores did not significantly correlate with CES-D scores. As previously discussed in section 1.5.2, neurodegeneration in the form of age-related WMC is highly associated with depression among the elderly. However, in relation to PSD the evidence is less consistent; with multiple studies failing to find a relationship with WMC. Only two studies have found a relationship between WMC and PSD, one of which by Pohjasvaara et al. used a depression symptom severity scale (BDI) to measure depressive symptoms, like the present study. Pohjasvaara et al. used the Fazekas scale to measure WMC, while this study employed the ARWMC scale, which may account for the difference in findings. Yet, the Fazekas scale was observed to have reduced inter-rater reliability in assessing less severe white matter disease and is only suitable for MRI, while the ARWMC scale was developed to target these reliability issues and is also designed for use in both CT and MRI. These variations in findings and methodology make it difficult to decipher the true relationship, if any, between PSD and WMC. Although a clear association between late-life depression and WMC exists, depression in the context of stroke may not have its origins in pre-existing WMC.
Additionally, this thesis found that whole brain atrophy was also not significantly correlated with depression scores. Frontal lobe atrophy has been found to significantly correlate with late-life depression, and was proposed by one study as a risk factor for PSD. Section 1.4.8.3 covers the relationship established between depression and atrophy in the non-stroke population and how the neuroprogression hypothesis of depression plays into these findings. However, in section 1.5.3 it is made evident that atrophy in relation to PSD may be more complex. This thesis hypothesized that atrophy in the context of an acute infarct will be associated with depressive symptoms following a stroke, however because of the cross-sectional nature of the study one cannot assume that atrophy pre-existed the stroke lesion. This thesis had two measures of atrophy; hippocampal and whole brain. Three studies assessing atrophy and PSD demonstrated a relationship between atrophy and PSD, while two studies failed to find a relationship. All three of these positive studies used different methods to assess atrophy; one measured atrophy by visual rating, another measured the ventricle to brain ratio, and the third measured atrophy using a novel quantitative hybrid warping method. In contrast, both of the negative studies used a visual rating scale to assess atrophy. Furthermore, this study assessed whole brain atrophy by manually tracing the ventricles to obtain total ventricular volume and then normalizing these values by dividing this value by the total intracranial volume (TIV) to produce a ratio value of total ventricular volume: total intracranial volume. A limitation of this thesis is the variation in methodology between it, and the aforementioned positive and negative studies, making it difficult to compare findings between groups. Another limitation is that fact that this thesis measured hippocampal thickness on CT as opposed to hippocampal volume, which is only possible on MRI. This may also account for the negative findings; which will be discussed in the limitations section below. The reasoning behind including hippocampal thickness as a neuroimaging outcome was because of the findings in animal studies that demonstrate kynurenine metabolites (mainly quinolinic acid) to be especially toxic to the hippocampus, causing targeted neurodegeneration in this area specifically. Furthermore, administration of kynurenic acid or blockade of the enzyme that indirectly leads to quinolinic acid production (kynurenine-3-monooxygenase), reduces QUIN-induced neurotoxicity.
The hippocampus is also especially vulnerable to hypoxia, one study involving cardiac arrest patients found marked reductions in hippocampal volume compared to controls 21 days after cardiac arrest.  The researchers of this study postulated that this reduction in volume signifies the hippocampus’ increased susceptibility to global brain ischemia. This may imply that oxidative and neurotoxic factors can have a relatively acute effect on brain volume and neurodegeneration. However, because of the cross-sectional nature of this study one cannot determine whether the hypoxic (or oxidative/excitotoxicity/etc.) consequences are acute or chronic. Finally, a recent meta-analysis found that smaller amygdala volume in stroke patients was significantly associated with depression, however whole brain atrophy was not. This suggests that PSD research in the area of neurimaging should focus more attention on measuring discrete regional differences in brain volume, as this method may be better at determining the role of atrophy in PSD.

As previously mentioned in the discussion, physical disability, as measured by stroke severity or functional impairment, has been found by two important meta-analyses to be one of the most significant correlates of PSD. As suggested for the relationship between K/T and PSD, the same may be true for other biological measurements in relation to PSD, such as the neurimaging outcomes measured in this thesis. Thus, it is likely that they too, only play a minor role in the development of depression and these are overwhelmed by the strong relationship between PSD, stroke severity, and functional disability. Our finding that NIHSS scores significantly correlated with CES-D scores adds credibility to this notion. Whyte and Mulsant believe the etiology of PSD stems from the joint effects of biological and psychosocial mechanisms, however this thesis only found strong correlations between the NIHSS and CES-D. Many of the PSD studies mentioned in section 1.5 assessed either functional impairment or stroke severity alongside neurimaging findings. The results of these studies demonstrated that physical disability, as measured by either one of these psychosocial outcomes, is significantly correlated with depression. Furthermore, two of these studies found that after controlling for these outcomes, the effect of the particular neurimaging measurement on depression was negated.
stroke severity or functional impairment are strong enough to overrule any small neurobiological contributions in the development of PSD.44

As mentioned earlier, because none of the aforementioned neuroimaging measurements were correlated with depressive symptom severity, the final exploratory analysis assessing the joint effects of neuroanatomy and the K/T ratio on PSD was not pursued. Although we were not able to explore this aim, it is still worthwhile to briefly discuss the motivation behind it. The idea behind this exploratory analysis was fueled by the fact that depression (both PSD and MDD) have been found to significantly correlate with neurodegeneration in the form of atrophy, WMC, and acute stroke lesion volume. In addition, altered IDO activity has been significantly correlated with the progression, severity and mortality multiple neurodegenerative diseases, such as Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, as well as stroke and traumatic brain injury. KYN and its metabolites have also been demonstrated to cause neurodegeneration in animal and in vitro models. In particular, inflammation-induced IDO and KMO activation and the production of KYN metabolites have been hypothesized to play a role in neuroprogression, through pro-oxidative mechanisms, disruption in energy and metabolism, and through glutamate excitotoxicity. Neuroprogression is a term given to the process of gross and cellular neurodegeneration which has been hypothesized to have a link to the cause and/or consequence of worsening depression and cognitive decline. It then follows that an elevated K/T ratio in the presence of neurodegeneration may be more relevant in contributing to the development of PSD than either variable alone, as the aforementioned evidence suggests that these biological outcomes are not mutually exclusive. However, the results of this thesis do not support this notion, since there was no significant correlation between the K/T ratio, most of the serum cytokines, or any of the neuroimaging outcomes and depression scores. This is in agreement with two recent studies published by Ormstad et al. who assessed the correlations between 12 acute serum cytokine levels and depression, as well as infarct volume and laterality. They found that serum inflammatory markers, lesion volume and laterality were not associated with
depression, nor were the various measured cytokines significantly correlated with lesion volume or laterality.

4.4 Limitations

The first limitation of this thesis is the large variation in time of assessment post-stroke, which ranged from 4-155 days. This was not found to be a significant correlate of CES-D scores in the initial descriptive analysis, and recent evidence suggests that IDO activity remains elevated at least one year following severe brain injury. However, a study by Noonan et al. demonstrated that significantly elevated C-reactive protein (CRP) and white cell count levels at 18 months post-stroke were not associated with depression scores. Thus, in the present study, the broad range of time between date of stroke and assessment may have eliminated or dampened the association between the K/T ratio, as well as cytokine concentrations and depression scores, respectively. Yet, as seen in Appendix 4, bivariate Spearman’s correlations revealed no significant associations between time post-stroke and any of the serum biomarkers.

Secondly, the gold standard for the diagnosis of post-stroke depression is the Structural Clinical Interview Diagnostic (SCID) and Statistical Manual (DSM-IV) criteria. Thus it could be argued that using the CES-D to screen for the presence of depressive symptoms does not capture or identify a true depressive episode with clinical relevance. However, as mentioned previously, in patients who have suffered from a stroke, a cut-off score of 16 or greater has a sensitivity ranging from 86-100% and specificity of 73-90%. In the present thesis, 31.7% of patients had a score of ≥16, which is quite similar to a very recent meta-analysis by Ayerbe et al. that found the pooled prevalence of depression observed at any period to be approximately 29%, and 33% between 1 and 6 months post stroke. Furthermore, proper administration of SCID depends on sound clinical objectivity. In a study such as this one, the more researchers involved in administering a diagnostic test, the more likely the reliability of scores will be decreased. Conversely, the CES-D is a self-report screening scale, and researchers have suggested that depression screens may prevent patients from accurately lining up the emotions
they feel with descriptions of emotions in the assessment.\textsuperscript{464} Furthermore, there is scarce research on how adults perceive depression and how they interpret this paradigm into context with their personal emotions,\textsuperscript{465, 466} although some findings demonstrate that patients have trouble deciphering between “reactions to adversity” and depressed mood.\textsuperscript{464} However the main goal of this thesis was to characterize the relationship between the K/T ratio and depressive symptom severity, as it was hypothesized that their relationship was linear. Furthermore, K/T ratio was recently found to be significantly elevated in somatization compared to depression and also associated with somatic symptom severity.\textsuperscript{57, 305, 313} In a study involving IL-2 and/or interferon-\(\alpha\) cancer therapy, symptoms characterized by the researchers as ‘neurovegetative’, including lack of sleep, poor appetite, and decreased energy, were significantly more pronounced in patients with mild depressive symptoms than patients within the “absent” depressive symptom range, as measured by the MADRS.\textsuperscript{435} Thus, a depression scale was necessary in order to determine if mild, moderate, or severe depressive symptoms were associated with an elevated K/T ratio; something that a categorical diagnosis of depressed versus non-depressed would not have been able to detect as well.

Additionally, peripheral concentrations of cytokines and plasma KYN concentrations may not be representative of central concentrations. Firstly, although KYN can cross the blood—brain barrier,\textsuperscript{19, 467} peripheral concentrations of KYN may be much lower than central concentrations and thus, may fail to show strong correlations with depression. A study by Raison \textit{et al.} involving IFN-\(\alpha\) treatment observed large differences between peripheral and central KYN.\textsuperscript{106} However, this study also found significantly increased peripheral blood KYN was highly correlated with cerebral spinal fluid (CSF) KYN levels, and these levels were significantly associated with CSF levels of KYN metabolites.\textsuperscript{106} Furthermore, it was demonstrated in bivariate correlations that CSF KYN and CSF QUIN significantly correlated with depression scores, yet in a multiple linear regression taking into account CSF TRP, KYN, QUIN, KYNA, IFN-\(\alpha\), IL-6, sIL-6R, sTNFR2, MCP-1 and history of depression, the only KYN metabolite that emerged as a significant predictor of depression scores was CSF QUIN.\textsuperscript{106} Although CSF KYN was not \textit{directly} significantly associated with depression scores, CSF QUIN was found to
correlate with peripheral KYN concentrations\textsuperscript{106} and it is hypothesized that K/T ratio is correlated with depression scores in part due to toxic KYN metabolites, such as QUIN, wrecking havoc on emotional neuronal circuits of the brain. In terms of peripheral levels of cytokines and their representation of central concentrations, the same holds true. Elevations in cytokines have been consistently associated with depression in the non-stroke population\textsuperscript{215-225, 228, 230-233} and many studies have found them to be elevated in the context of PSD,\textsuperscript{16, 243} as well as stroke outcomes such as disability\textsuperscript{37} and fatigue.\textsuperscript{256} Associations between peripheral and central concentrations of cytokines have been documented,\textsuperscript{99} and cytokines have been demonstrated to cross the BBB, and they can also cross back from the CSF into peripheral circulation (reviewed in\textsuperscript{468}), however they should not be considered one in the same.\textsuperscript{469, 470} Thus the results of this study were also limited by the fact that we did not measure intrathecal production of inflammatory markers, as these may be more representative of concentrations in the brain.

On the same note, imputation of cytokine values at the lower limit of detectability may limit the utility of this study. Assay sensitivities were 0.2 pg/ml for IL-10, 1.0 pg/ml for IFN-\textgamma, 0.09 pg/ml for TNF-\textalpha, 0.057 pg/ml for IL-1\beta, 0.1 pg/ml for IL-6, and 12.5 pg/ml for IL-18, which are comparable to the sensitivities of used by other studies observing stroke and depressed populations by means of standard ELISA assays,\textsuperscript{103, 426, 427} however there were no undetectable samples for IL-6 or IL-18. While, imputing at the lower limit of detectability may create data that stray from the true mean, the key aim of this study was to examine increased levels of cytokines, as it was hypothesized that pro-inflammatory cytokines will be increased in depressed patients of this population.\textsuperscript{407} As such, we did not think this method would interfere with our objective of examining the role of increased cytokine production. Nonetheless, IL-10, an anti-inflammatory cytokine, was imputed at the lower limit of detectability, when our goal was to detect decreased concentrations in PSD patients.\textsuperscript{407} For IL-10, since values may be even lower than the limit of detectability, imputing them would artificially inflate concentrations. Imputation of values may warp the linear relationship between CES-D scores and cytokine levels and cause the researcher to see less of a correlation between CES-D scores and IL-10,\textsuperscript{407} as well as for the
IL-10 immunologic ratios. A significant relationship was observed between IFN-Ɣ/IL-10 and the middle CES-D tertile, however had there been less imputations for IL-10, the trend between TNF-α/IL-10 and CES-D tertiles may have become significant. Appendix 5 displays the percentage of values imputed for each cytokine and Appendix 6 shows the result of Chi-squared tests between depressed and non-depressed groups (CES-D≥16 and <16) and imputed versus non-imputed cytokines, demonstrating that there are no significant differences in imputation of any cytokines between groups.

Lastly, the use of CT neuroimaging for analyzing the radiological correlates of PSD may be considered another limitation of this thesis. In terms of WMC scoring, the ARWMC scale was shown to identify an increased number of small white matter lesions on MRI, while bigger infarcts were identified equivalently or better by CT. MRI is better at detecting the subtleties of WMC than CT and is less susceptible to artefacts such as bone hardening, especially near the medial temporal lobe. However, CT is more apt at distinguishing clinically significant lesions, is equivalent to MRI in measuring atrophy, and possibly more appropriate for use in geriatric populations because it is not as easily affected by motion artefacts. Furthermore, CT is the most extensively utilized neuroimaging device in the world and thus, this study may be more clinically applicable in the context of stroke.

4.5 Recommendations

The most important recommendation for future studies assessing the relationship between peripheral inflammatory markers and PSD, would be to track these markers and depressive symptom severity longitudinally. Although the K/T ratio was not significantly correlated with depressive symptom severity in this study, it would be interesting to see if an initial increase in the K/T ratio confers greater vulnerability to depression at 6, 12, 18 months and beyond. The aforementioned observation that the K/T ratio is initially much higher in post-stroke populations than in other neurological diseases adds legitimacy to this recommendation. Furthermore, studies propose that the prevalence of PSD reaches a maximum at roughly 3-6 months, diminishes by 50% at 1 year, and then it may remain elevated at approximately 20% for 2 years and beyond. This last finding is
troublesome because it demonstrates that PSD can be a chronic, non-remitting disease in many stroke survivors. It was hypothesized that one of the ways in which an increased K/T ratio contributes to PSD is through an increase in neurotoxic KYN metabolites which may cause neurodegeneration of the post-stroke brain. If this is the case, it seems more likely that the effects of these toxic KYN metabolites would produce a delayed effect, and may possibly contribute to the chronic, non-remitting cases of PSD.

Following on the recommendation for longitudinal studies, it would also be important for future studies to incorporate measures of functional outcome, as this would help decipher if the psychosocial impacts on depression stand compared to the biological contributions of inflammation. This may also help uncover the complex interaction between psychosocial stressors, inflammatory markers and depression. A recent meta-analysis revealed elevated levels of pro-inflammatory cytokines were associated with these stressors and elevated levels of IL-6, in particular, have been associated with childhood adversity as well. In the present study a significant correlation was demonstrated between NIHSS scores and the CES-D. Although disability, as measured by the NIHSS, has ties with functional outcome, the NIHSS is a measure of stroke severity, which strictly speaking is not a psychosocial outcome. Future studies might assess activities of daily living, using the Barthel Index, or the modified Rankin Scale as these scales typify the psychosocial outcome of functional dependence and will help to further characterize its place in the development of depression post-stroke.

In MDD, genetic variations in IL-1β and TNF-α alleles have been demonstrated to be associated with antidepressant treatment resistant depression and elevated risk of developing depression. While in PSD, IL-4 and IL-10 polymorphisms related to lower anti-inflammatory cytokine production were found to be significantly associated with depression. It would be worthwhile for future studies to assess genetic variations in pro- and anti-inflammatory cytokine alleles as they relate not only PSD, but also to increased activation of IDO as measured by the K/T ratio. This
may help to reveal populations that are more vulnerable to the effects of inflammation-associated IDO activation. Furthermore, a neurotrophin called brain derived neurotrophic factor (BDNF) is a factor that has been implicated in adult neuronal growth and researchers have demonstrated its impaired production in the context of depression, and in chronic or acute stress. In addition, reductions in peripheral BDNF concentrations have been associated with diminished hippocampal volumes in antidepressant-naive first-episode MDD, while a polymorphism in the BDNF allele called BDNFVal<sub>66</sub>Met, associated with a reduction in its release, was also found to be associated with reduced hippocampal volume (reviewed in). In relation to PSD, a very recent study by Kim et al. found that BDNF methylation status was positively correlated with the presence of PSD at baseline and follow-up, and the BDNFVal<sub>66</sub>Met polymorphism was associated with depression at baseline. Furthermore, a recent meta-analysis found a significant reduction of BDNF levels in PSD. Future studies may find it meaningful to analyze the association between serum levels of BDNF and hippocampal atrophy in depressed stroke patients.

Finally, future studies should assess the relationship between PSD and the potent inflammatory cytokine, IL-17. This is a cytokine produced by γδT cells and Th17 cells, that has been implicated in the process of post-stroke neurodegeneration (reviewed in). IL-10 may play a role in curbing detrimental IL-17 expression via intracellular suppressors of cytokine signalling (SOCS) proteins, which link up with cytokine receptor complexes and inhibit intracellular cascades. Furthermore, knockdown of one particular SOCS protein (SOCS3) lead to larger infarct volumes and worse neurological outcomes. As mentioned above, IDO may act to suppress immune responses via KYN binding to the AHR in dendritic cells and causing the generation of Tregs. Tregs are also capable of producing IL-10 and thus KYN binding to the AHR may shift cytokine production to IL-22 (IL-10 superfamily cytokine) production, as opposed to IL-17 production. In this way, KYN may actually have a positive effect on preventing neurodegeneration caused by IL-17. It would be interesting to measure the levels of IL-17 in patients with PSD, and even more intriguing would be to assess the ratios
of IL-17/IL-10 and IL-17/KYN in the context of PSD. These immunologic ratios may be more representative of neurodegeneration than the ones assessed in this thesis.

4.6 Conclusion

In conclusion, this thesis sought to investigate the neurobiological mechanisms of PSD through the assessment of current contemporary hypotheses, namely the inflammatory and neuroanatomical factors which may have a role in its development. Principally, this study aimed to uncover the relationship between acute inflammatory serum biomarkers and post-stroke depressive symptom severity. The K/T ratio was not significantly associated with depression scores. Similarly, while IFN-γ/IL-10 ratio was found to be a significant predictor of mild depressive symptoms, this was found in exploratory analyses, and no relationship was found with other ratios. As such, the finding lacked consistency. Furthermore, this thesis explored the relationship between neuroimaging findings and post-stroke depressive symptom severity, as it was hypothesized that they represent chronic factors involved in the development of PSD. Yet, none of the neuroimaging measurements of this study were associated with depression. The fact that stroke severity was significantly correlated with depression may indicate that psychosocial mechanisms override the effects of neurobiology in the etiology of PSD. With respect to immunologic ratios, longitudinal studies incorporating a measure of functional dependence are necessary to further characterize the relationship between psychosocial factors and immune imbalance within the realm of PSD research.
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List of Publications and Abstracts

Papers in Preparation
Bensimon K, Herrmann N, Ranepura N, Lanctôt KL. Elevated IFN-γ, TNF-α, IL-18 and a reduction in the anti-inflammatory cytokine IL-10 is associated with post-stroke depression.

Bensimon K, Herrmann N, Gao FQ Ranepura N, Lanctôt KL. The neuroanatomical correlates of post-stroke depression.

Published Papers

Published Abstracts


Presentations


The Renewal Form is an application for continuing ethics approval and must be submitted for review and approval prior to the study’s expiry date. Ethics approval expires each subsequent year from the day REB approval was initially granted unless otherwise indicated by the Sunnybrook REB. Failure to submit this form prior to the expiry date signifies that the study does not have REB approval and all research activities must be suspended. Conducting research without REB approval may result in a notice of non-compliance involving corrective action, up to and including, termination of the research study.

Principal Investigator (PI): Dr. Krista Lanctot

REB Project Identification Number (PIN): 380-2004

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

1. Date of initial Sunnybrook REB approval (dd/mmm/yyyy).
   08-Dec-2004

2. Type of REB review requested. (Final decision rests with the REB Chair.)
   ☑ Delegated Review   ☐ Full Board Review

3. Is this an Industry-Sponsored/Supported study?
   ☐ YES (If YES, complete the table below.) ☑ NO (If NO, proceed to question 4.)

   Invoicing Information for Industry-Sponsored/Supported Studies
   A fee of $500 Cdn is invoiced for all Industry-Sponsored/Supported Studies applying for continuing ethics approval.

   Invoice to the Following Company:
   Contact Name:                      E-mail:                      
   Telephone:                        
   Street Address:                   Suite:                      
   City:                             Province/State:               
   Country:                         Postal/Zip Code:               

4. Is this study open for enrollment at Sunnybrook? ☑ YES ☐ NO
   If YES, attach a copy of the current Informed Consent Form(s).
If NO, provide reasoning:

We have recruited the number of participants that we had planned to recruit. We are in the process of completing the database.

5. How many participants at Sunnybrook:

<table>
<thead>
<tr>
<th>Were planned for enrollment</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Were consented</td>
<td>107</td>
</tr>
<tr>
<td>Were enrolled</td>
<td>107</td>
</tr>
<tr>
<td>Are currently receiving study treatment/intervention</td>
<td>n/a</td>
</tr>
<tr>
<td>Completed study treatment/intervention &amp; are currently on follow-up</td>
<td>n/a</td>
</tr>
<tr>
<td>Completed study treatment/intervention &amp; follow-up</td>
<td>n/a</td>
</tr>
<tr>
<td>Withdrew consent</td>
<td>3</td>
</tr>
<tr>
<td>Were planned for inclusion in a chart review (retrospective or prospective)</td>
<td>n/a</td>
</tr>
<tr>
<td>Were included in a chart review (retrospective or prospective)</td>
<td>n/a</td>
</tr>
</tbody>
</table>

6. Have all Serious Adverse Events (SAEs) experienced by a Sunnybrook participant been reported to the REB?

☐ YES ☐ NO, will submit immediately ✑ NO SAEs have occurred

7. In the opinion of the PI, is there a concern or trend in the SAEs that have occurred with Sunnybrook participants?

☐ YES ☐ NO ✑ NO SAEs have occurred

If YES, provide details and action taken.

8. Have all significant protocol deviations/violations been reported to the REB?

☐ YES ☐ NO, will submit immediately ✑ NO significant deviations/violations to report

9. Since the last REB approval, is there any new ethical or scientific information outside of a protocol amendment that would be relevant to the continuing review of this study?

☐ YES ✑ NO

If YES, provide details.

10. Since the last REB approval, is there any change in the conflict of interest information provided to the REB for any of the investigators, study staff or members of their immediate family? ☐ YES ✑ NO

If YES, provide details.
11. Person completing this form.

<table>
<thead>
<tr>
<th>Title: Miss</th>
<th>First Name: Russanthy</th>
<th>Last Name: Velummailum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dept/Div: Neuropsychopharmacology</td>
<td>Institution: Sunnybrook Research Institute</td>
<td></td>
</tr>
<tr>
<td>Full Address: 2075 Bayview Avenue, Toronto, ON M4N 3M5</td>
<td>Room Number: FG-05</td>
<td></td>
</tr>
<tr>
<td>Telephone: 416-480-6100</td>
<td>Extension: 3185</td>
<td></td>
</tr>
<tr>
<td>E-mail: <a href="mailto:rvelumm@sri.utoronto.ca">rvelumm@sri.utoronto.ca</a></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

12. Statement of Principal Investigator (PI).

I assume full responsibility for the scientific and ethical conduct of this study and agree to conduct this study in compliance with the Tri-Council Policy Statement: Ethical Conduct for Research Involving Human Subjects (TCPS), Personal Health Information Protection Act (PHIPA) and any other relevant regulations or guidelines. I certify that all researchers and personnel involved in this study at this institution are appropriately qualified and trained to fulfill their role in this study.

[Signature]
Signature of Principal Investigator

[Date]
Date (dd.mmm.yyyy)

Research Ethics Office Use Only

The Sunnybrook REB has reviewed the information provided and confirms that this study has obtained ethics approval by way of:

☐ Delegated Review

☐ Full Board Review → Date of Full Board meeting: 

This study is only approved for the following period:

March 04/2012 to March 04/2013

Philip C. Hebert MD, PhD, FCFP
Chair, Research Ethics Board
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:
Participation in this study will involve three separate visits. The first visit will occur soon (less than 4 weeks) after you experienced a stroke, and the second and third visits will be scheduled 6- and 12-weeks after the first. Each visit will last approximately 1½ hours, but can be broken up into several shorter (20-30 minute) assessments if needed. Each visit 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 (1st Visit Only):
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.

d) MRI (Optional, 3rd Visit Only):
MRI scanning is a method of making pictures of the brain using magnetic waves. This gives us information about the structure of the brain, including the hippocampus. The scan will take less than one hour. The scan will involve lying still with a simple device placed around your head/neck. The MRI machine looks like a long narrow tube. Even though the tube is open, some people feel confined in small places. If this bothers you, please notify us. You may end the scan at any time by telling the MRI staff, who will be able to see you, hear you, and communicate with you at all times. When MRI pictures are taken, it is normal for the MRI machine to make noises (banging and clicking). You will be asked to wear earplugs or headphones for your comfort during the exam. The scans performed in this study are not optimized to find clinical abnormalities. However, on occasion, a member of the research team may notice a finding on a scan that seems abnormal, in which case we will consult a neurologist as to whether the abnormality merits further investigation. If this is done, a member of the research team will contact you. The decision as to whether to proceed with further clinical examination or treatment will be yours; with your permission, we would forward your results to your family physician for further follow-up.

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.
If you consent to the MRI—During the MRI procedure, you may be bothered by feelings of confinement (claustrophobia), and by clicking and banging noises made by the magnet. Because MRI uses strong magnetic fields, you may not participate in MRI scans if you have a pacemaker, an implanted defibrillator or certain other implanted electronic or metallic devices. An interview will be conducted prior to your MRI to advise the MRI staff if you have had brain surgery for a cerebral aneurysm, or if you have implanted medical or metallic devices, shrapnel, or other metal, such as metal in your eye. There is a small chance that we may observe something abnormal on your MRI. If this is the case, we will inform you, which may cause you anxiety, and suggest the need for further tests.

If you consent to the contrast medicine during MRI—The Omniscan injection is very safe. Rarely, the contrast medicine may leak out of the vein during the injection. If this does occur, there will be some swelling and tenderness, but this does not harm the tissue and the Omniscan is cleared naturally over the next day. Although uncommon, the Omniscan injection may cause a feeling of coolness in the arm or mild nausea in about 1 in 200 patients. Allergic reactions can occur, but they are extremely rare. These can include spasm of the airways, low blood pressure and asthma and only happen in about one in 10,000 cases. Severe reactions have been reported to occur at a rate of about one in 32,500.

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.
Stroke and Depression Study

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.

Consent to MRI Substudy

By placing my initials in the “no” box, I am stating that I do not consent to the MRI substudy.

By placing my initials in the “yes” box, I am stating that I do consent to the MRI substudy. However, I may still choose at any time not to complete the MRI scan, and still participate in the main study.

Consent to Contrast Injection

By placing my initials in the “no” box, I am stating that I do not consent to receiving the contrast medicine injection. However, if I placed my initials in the “yes” box above, I may still complete the MRI scan without the contrast medicine injection.

By placing my initials in the “yes” box, I am agreeing to receive the contrast medicine injection. However, I may choose at any time not to receive the contrast medicine, but still complete the MRI scan and/or participate in the main study.

_________________________________
Name of Patient (typed or printed)

_________________________________ _____________________
Signature of Patient    Date

_________________________________
Name of Investigator (typed or printed)

_________________________________ _____________________
Signature of the Investigator   Date
APPENDIX 3: Study Roles

**Study Concept and Design:** Drs. Krista Lanctot, Nathan Herrmann

**Data Acquisition:** Amy Wong, Lana Rothenburg

**Database Management:** Kira Bensimon, Nipuni Ranepura, Russanthy Velummailum

**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

**Lesion volume, Whole Brain Atrophy, Hippocampal Thickness, ARWMC scoring:** Kira Bensimon

**Lesion Location and Laterality:** Philip Lennox Francis

**Data Analysis and Interpretation:** Kira Bensimon, Drs. Krista Lanctot, Nathan Herrmann

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

**Study Supervision:** Drs. Krista Lanctot, Nathan Herrmann
Appendix 4: Bivariate correlations between timing of assessment post-stroke and serum biomarkers

<table>
<thead>
<tr>
<th>Serum Biomarker</th>
<th>Raw Mean±SD</th>
<th>*R</th>
<th>*p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>K/T</td>
<td>70.4±38.4</td>
<td>0.158</td>
<td>0.158</td>
</tr>
<tr>
<td>IL-6</td>
<td>6.6±9.1</td>
<td>-0.144</td>
<td>0.202</td>
</tr>
<tr>
<td>IL-18</td>
<td>287.6±170.1</td>
<td>0.001</td>
<td>0.993</td>
</tr>
<tr>
<td>TNF-α</td>
<td>2.5±3.3</td>
<td>0.039</td>
<td>0.774</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>0.8±0.7</td>
<td>-0.071</td>
<td>0.533</td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.4±0.5</td>
<td>0.121</td>
<td>0.389</td>
</tr>
<tr>
<td>IL-10</td>
<td>3.2±15.8</td>
<td>-0.068</td>
<td>0.547</td>
</tr>
<tr>
<td>IL-6/IL-10</td>
<td>26.3±76.1</td>
<td>-0.021</td>
<td>0.855</td>
</tr>
<tr>
<td>IL-18/IL-10</td>
<td>1054.9±1062.8</td>
<td>0.028</td>
<td>0.833</td>
</tr>
<tr>
<td>TNF-α /IL-10</td>
<td>8.4±10.7</td>
<td>-0.001</td>
<td>0.995</td>
</tr>
<tr>
<td>IFN-γ/IL-10</td>
<td>2.7±3.7</td>
<td>0.061</td>
<td>0.592</td>
</tr>
<tr>
<td>IL-1β /IL-10</td>
<td>1.3±1.5</td>
<td>-0.002</td>
<td>0.990</td>
</tr>
</tbody>
</table>

*\( r \) values and their corresponding *\( p \)-values reflect the results of bivariate Spearman’s correlations with CES-D scores, units for cytokines are expressed in pg/ml and the K/T ratio is expressed in umol/L.
### Appendix 5: Percent imputation of each cytokine

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Percent Imputed</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>0%</td>
</tr>
<tr>
<td>IL-18</td>
<td>0%</td>
</tr>
<tr>
<td>TNF-α</td>
<td>27.1%</td>
</tr>
<tr>
<td>IFN-Ɣ</td>
<td>23.8%</td>
</tr>
<tr>
<td>IL-1 β</td>
<td>5.6%</td>
</tr>
<tr>
<td>IL-10</td>
<td>58.0%</td>
</tr>
</tbody>
</table>

### Appendix 6: Percent of cytokines imputed in each cytokine group and Pearson’s Chi-squared test between imputed and non-imputed cytokines and CES-D groups

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>CES-D ≥ 16</th>
<th>CES-D &lt; 16</th>
<th>*Χ²</th>
<th>*p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>None imputed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-18</td>
<td>None imputed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>11.8%</td>
<td>33.3%</td>
<td>2.848</td>
<td>0.091</td>
</tr>
<tr>
<td>IFN-Ɣ</td>
<td>24.0%</td>
<td>23.6%</td>
<td>0.001</td>
<td>0.972</td>
</tr>
<tr>
<td>IL-1 β</td>
<td>6.7%</td>
<td>5.1%</td>
<td>0.049</td>
<td>0.825</td>
</tr>
<tr>
<td>IL-10</td>
<td>57.7%</td>
<td>58.2%</td>
<td>0.002</td>
<td>0.967</td>
</tr>
</tbody>
</table>

*Χ² values and their corresponding *p-values reflect the results of Pearson’s Chi-squared tests between imputed and non-imputed cytokines and CES-D groups.