ORIGINAL RESEARCH

Kynurenine and depressive symptoms in a poststroke population

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Background and purpose: Depression is a commonly occurring and persistent sequel of stroke affecting approximately 29% of patients. An immunological hypothesis has been put forward, and synthesis of kynurenine from tryptophan has been proposed to link inflammatory activity with neurotoxicity and neurotransmitter dysfunction. This study assessed the relationship between peripheral blood kynurenine and poststroke depressive symptoms.

Patients and methods: This was a multisite cross-sectional observational cohort study of patients with ischemic stroke. Depressive symptoms were assessed using the Center for Epidemiological Studies Depression (CES-D) scale and divided into high, medium, and low depressive symptom tertiles. Concentrations of kynurenine and tryptophan were assayed from fasting serum samples, and the kynurenine/tryptophan ratio was compared between tertiles. Serum cytokine concentrations were assayed in a subgroup of patients, and the ratio of proinflammatory (IL-6, IL-18, IFNγ, TNF, IL-1β) to anti-inflammatory (IL-10) cytokines compared.

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Results: In these patients (n=86, 52.3% male, mean age 71.7±14.2 years), there were no differences in kynurenine/tryptophan ratios between CES-D scale tertiles ($F_{2,76}$=0.04, P=0.96) controlling for relevant covariates. For cytokines (n=53), serum IL-1β concentrations ($F_{2,52}$=3.55, P=0.037) and serum ratios of IL-18/IL-10 ($F_{2,52}$=3.30, P=0.046), IFNγ/IL-10 ($F_{2,52}$=4.02, P=0.025), and IL-1β/IL-10 ($F_{2,52}$=4.34, P=0.019) were elevated in the middle CES-D tertile. Post hoc analyses suggested that serum ratios of IL-18/IL-10 (ρ=0.28, P=0.04), and IL-1β/IL-10 (ρ=0.43, P=0.001), as well as IL-1β (ρ=0.29, P=0.04), were significantly associated with fatigue.

Conclusion: Peripheral kynurenine/tryptophan ratios were not associated with depressive symptoms in a poststroke population. However, in exploratory analyses a proinflammatory bias was identified specifically in patients with mild depressive symptoms and associated with poststroke fatigue, suggesting an avenue for future research.

Keywords: kynurenine, tryptophan, cytokine, inflammation, stroke, depression

Introduction

Poststroke depression (PSD) affects approximately a third of all stroke survivors.[1] The prevalence of PSD reaches a maximum at roughly 3–6 months, diminishes by 50% at 1 year, and may remain elevated for 2 years and beyond.[2,3] As such, PSD can be chronic and nonremitting. Up to 35% of patients do not respond to currently available antidepressants, with high nonremission rates, and residual symptoms commonly persist.[4] A better understanding of the mechanisms associated with PSD is needed to direct the search for additional or adjunctive therapies.

A relationship between elevations in peripheral proinflammatory cytokines and depression has been suggested in many cross-sectional studies.[5] Many cytokines, particularly IFNγ, IL-6, IL-1β, and TNF, upregulate the indoleamine 2,3-dioxygenase

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enzyme in multiple central and peripheral cell types, which catalyzes the rate-limiting step in the synthesis of kynurenine (KYN) from tryptophan (TRP), thereby increasing their ratio (K/T) in blood.[6,7] Changes in both the K/T ratio and concentrations of KYN and its metabolites have been demonstrated cross-sectionally in stroke.[8] These changes have also been associated with major depressive disorder (MDD)[9–12] and depressive symptoms in patients with cardiovascular disease.[13] The further metabolism of KYN into excitotoxic metabolites, which are elevated centrally in MDD postmortem, has been suggested to contribute to neurodegeneration in ischemia and depressive disorders.[12,14]

A peripheral inflammatory response is observed poststroke, due to a complex cascade of cytokine signals released from both innate and adaptive immune cells, including macrophages, neutrophils, and T-helper cells.[15,16] Clinically, inflammatory status can be surveyed by considering relative concentrations of pro- and anti-inflammatory cytokines, eg, the anti-inflammatory cytokine IL-10 is produced by regulatory T cells, which drop precipitously poststroke.[16] IL-10 concentrations are often increased in tandem with proinflammatory cytokine concentrations in healthy individuals, but this relationship is lost in MDD,[17] where pro- versus anti-inflammatory cytokine imbalances have been found.[17–20] Pro- to anti-inflammatory cytokine imbalances have also been associated with poorer outcomes poststroke, in agreement with the neuroprotective properties of regulatory T cells and IL-10 in animal ischemia models.[21–25] Therefore, in the present study, the ratios of proinflammatory cytokines with IL-10 were explored in addition to cytokine concentrations.

While psychosocial, genetic, and physical disability correlates of PSD have been identified, relationships between inflammatory markers and PSD have not been consistently observed.[26–28] Although some studies have described relationships between cytokines and fatigue or sickness-like behavior,[26,28,29] rather than severe PSD per se, few studies have taken into account that different contributing factors may underlie severe and mild PSD strata. The present study undertook analyses without assuming linear severity relationships. Therefore, the present study examined relationships between the K/T ratio and symptoms of depression in patients poststroke and explored relationships between ratios of pro- to anti-inflammatory cytokines with depressive symptoms. The study also examined possible relationships between cytokines and specific symptoms to account for qualitative differences between patients in depressive symptoms poststroke. We hypothesized that the K/T ratio (primary) and concentrations of other inflammatory markers would differ between subjects with mild, moderate, and severe depressive symptoms in a poststroke population.

Patients and methods
Participants
This cross-sectional observational study recruited participants admitted to acute care regional stroke centers in Toronto, ON, Canada. Consecutive patients meeting National Institute of Neurological and Communicative Disorders and Stroke[30] and World Health Organization MONICA (multinational MONItoring of trends and determinants in CArdiovascular disease)[31] criteria for stroke were invited to participate in this study. Patients were recruited from Sunnybrook Health Sciences Centre (Toronto, ON, Canada) and three additional regional stroke centers (Toronto Rehabilitation Institute, St John's Rehabilitation Centre, and Baycrest Hospital). All patients were referred to the primary study site at Sunnybrook for uniform assessment by the same study personnel. The study was approved by local research ethics boards from each institution, and all participants provided written informed consent. All study procedures were followed in accordance with institutional research ethics boards.

Acute ischemic infarcts were verified from computed tomography or magnetic resonance imaging reports performed at admission for all patients. Inclusion criteria also required participants to speak and understand English. Exclusion criteria were: prestroke diagnosis of dementia or significant cognitive impairment (Mini-Mental State Examination [MMSE] score ≤24), primary hemorrhagic stroke, decreased level of consciousness, severe aphasia or dysarthria, significant acute medical or neurological illness other than stroke, and the presence of a premorbid axis I psychiatric disorder other than depression. Histories of major depression or stroke were permitted and controlled for in statistical analyses as needed.

Assessments
All neuropsychiatric testing was done within 24 hours of the blood draw. Severity of depressive symptoms was assessed using the Center for Epidemiological Studies Depression (CES-D) scale.[32] The CES-D scale is a self-report instrument that was used in the National Institutes of Neurological Communicative Disorders Stroke Data Bank,[33] and previously validated in stroke patients using a structured psychiatric interview. The CES-D scale has been shown to have high concurrent validity with other depression measures in stroke patients (both observer-reported and self-reported),[34] and has been used specifically as a measure of PSD.[34–37]

CES-D scale scores have also been dichotomized: a score ≥16 was highly predictive of clinical depression (sensitivity 86%, specificity 90%, positive predictive value 80%),[38] and a score of 16 or greater has been considered a valid and reliable indicator of PSD.[39] The CES-D scale was administered to the participant in the presence of trained research personnel, with assistance if necessary. The MMSE[40] was selected as a cognitive screening instrument to exclude significant cognitive dysfunction (MMSE score ≤24), because it is brief, widely used, and validated in acute care settings.[41] Stroke severity was assessed using the National Institutes of Health Stroke Scale (NIHSS),[42] either completed by clinicians upon patient admission or extracted from the medical chart using a standardized method.[43] For patients with an available clinical computed tomography scan, stroke lesions were traced and lesion locations and volumes recorded. Patient demographics, risk factors, comorbidities, and concurrent medications were obtained from hospital charts and by patient interview.

Blood sampling and serum analyses

Fasting blood was collected via venipuncture in SST™ Vacutainer® (BD, Franklin Lakes, NJ, USA) tubes at 7.30 am ±30 minutes on the morning after the clinical assessments were conducted. Blood samples were centrifuged at 1,000 g for 10 minutes at 4°C, and serum was separated and stored at −80°C until it was assayed (Sunnybrook Health Sciences Centre). TRP and KYN concentrations were determined by high-performance liquid chromatography (HPLC), as described previously.[44,45] TRP was measured by isocratic reverse-phase HPLC without derivatization or fluorescence detection.

For KYN, an equal volume of 3% perchloric acid was used for protein precipitation. After centrifugation, the concentration of L-KYN in the supernatant was measured by HPLC with ultraviolet detection at 258 nm. The mobile phase consisted of 9% acetonitrile in 0.05 M potassium phosphate monobasic, pumped through a reverse-phase 5 µm octadecyl column of 250×4.6 mm (Symmetry®; Waters, Milford, MA, USA). For those participants who provided enough sample, analyses of serum cytokines IL-6, IL-10, IFNγ, TNF, IL-1β and IL-18 were performed using a multiplex suspension bead-array immunoassay (Luminex, Austin, TX, USA). Cytokine concentrations were calculated from the median fluorescence intensities using the Luminex analyzer based on standard curve data obtained using the serially diluted cytokine standards provided in kits (BioSource, Burlington, ON, USA). Assay sensitivities were 0.2 pg/mL for IL-10, 1.0 pg/mL for IFNγ, 0.09 pg/mL for TNF, 0.057 pg/mL for IL-1β, 0.1 pg/mL for IL-6, and 12.5 pg/mL for IL-18.

Biochemical assays were performed blinded to all clinical information.

Statistical analysis

For initial descriptive analyses, relationships between CES-D scale total tertiles and serum concentration of KYN, TRP, K/T ratio, lesion volumes, and clinical and demographic covariates were compared. To test the primary hypothesis, a univariate analysis of covariance (ANCOVA) model was performed to assess differences in the K/T ratio between low, intermediate, and highest CES-D scale tertiles, where all important covariates from initial descriptive analyses were included. NIHSS score, age, sex, and history of depression were chosen as covariates a priori, since these were identified previously as independent predictors of PSD[2,46] and antidepressant use due to associations with depressive symptoms and immune markers.[19,20,47] Age in particular has been found to be positively correlated with the K/T ratio,[48] pro- and anti-inflammatory cytokines, and depression scores.[2] CES-D scale tertiles were chosen to best model the hypothesis that elevated K/T might manifest as mild depressive symptoms.

Since serum cytokine concentrations below the limits of detectability indicate low serum concentrations, values returned below detectable limits were imputed at the lower limit of detectability for that analyte: 0.2 pmol/L for IL-10, 1.00 pmol/L for IFNγ, 0.09 pmol/L for TNF, and 0.06 pmol/L for IL-1β. Serum assays and stroke-lesion volumes were log-transformed to maintain a normal distribution. For calculating proinflammatory cytokine to IL-10 ratios, they were calculated with raw values and log-transformed afterwards. Associations between immune imbalance and PSD were investigated using individual cytokine concentrations and the ratios of pro- to anti-inflammatory cytokines as exploratory outcomes in all patients for whom these data were available. ANCOVA models were performed to determine which serum cytokines or cytokine ratios were significantly elevated across CES-D scale tertiles. Tukey's post hoc test was used to identify difference between specific tertiles.

To explore which depressive symptoms were associated with the K/T ratio, or serum cytokines or their ratios, bivariate Spearman's correlations were conducted between each CES-D scale item and the K/T ratio, as well as the significant serum cytokine ratios from previous analyses. Possible heterogeneity due to histories of depression or antidepressant use were also explored in post hoc analyses. All statistical analyses were performed using SPSS software (version 20; IBM, Armonk, NY, USA). Error bars in all figures represent standard deviation.

Sample size

A sample size of at least 84, divided into three groups, provides a power of 80% to detect the minimum effect size that was observed previously in a cardiovascular population[13] with a two-sided α-value of 0.05.

Results

Patient characteristics

A total of 382 post-ischemic stroke patients were screened for the study, of whom 138 patients were carefully selected who met inclusion criteria and did not meet any criterion for exclusion. A total of 86 patients (mean age 71.7±14.2 years, 47.7% female) who agreed to participate and for whom sufficient serum samples were obtained were included in this analysis. Demographics and clinical characteristics of each tertile are presented in Table 1. CES-D scale scores ranged from 0 to 48, with a mean ± standard deviation CES-D scale score of 13.5±11.5. Based on 33rd and 66th percentile CES-D scale scores, CES-D scale tertile ranges were 0–6, 7–16, and 17–48, respectively. Of all demographic and clinical characteristics, only smoking (current) and hypertension differed between CES-D scale tertiles. Concurrent medications did not

differ significantly between CES-D scale tertiles. As continuous variables, only NIHSS score (r=0.25, P=0.02) and age (r=0.23, P=0.04) were associated with CES-D scale scores.

K/T ratio and depressive symptoms

KYN, TRP, and K/T ratio assay results are reported in Table 2. The K/T ratio did not differ between CES-D scale tertiles ($F_{2,76}$=0.04, P=0.96; see Figure 1) in a model controlling for NIHSS score ($F_{1,76}$=1.12, P=0.28), age ($F_{1,76}$=4.84, P=0.03), sex ($F_{1,76}$=0.15, P=0.70), history of depression ($F_{1,76}$=3.19, P=0.08), antidepressant use ($F_{1,76}$=0.22, P=0.64), hypertension ($F_{1,76}$=1.39, P=0.24), and smoking ($F_{1,76}$=4.54, P=0.04). Neither KYN ($F_{2,76}$=0.054, P=0.96) nor TRP ($F_{2,76}$=0.44, P=0.64) concentrations individually differed between CES-D scale tertiles; however, TRP was significantly lower in patients with a history of depression ($F_{2,76}$=5.43, P=0.022) and in older subjects ($F_{1,76}$=6.48, P=0.013).

Cytokine concentrations and ratios

Cytokines were assayed in the 53 patients who provided sufficient serum for analysis, and results are reported in Table 2. This subset of patients did not differ from patients

Table 1 Clinical demographic characteristics by depression tertiles (n=86)

	Mean ± SD or % (low)* n=30	Mean ± SD or % (medium)* n=28	Mean ± SD or % (high)* n=28	χ^2 or F**	P-value[†]
Age (years)	67.9±14.3	73.0±13.1	74.6±14.7	1.81	0.17
Sex (male)	56.7	53.6	46.4	0.63	0.73
History of depression	3.3	3.6	14.3	3.42	0.18
Antidepressant use	0	7.1	14.3	4.56	0.10
Living alone	20.0	42.9	28.6	3.64	0.16
Level of education > high school	55.1	40.7	40.7	10.89	0.37
Marital status (married)	56.7	42.9	53.6	12.47	0.13
Employment status (retired)	62.1	82.1	78.6	6.77	0.34
Days since stroke	25.4±35.1	22.9±37.3	20.1±28.6	0.18	0.84
Lesion volume (mm³) (n=71)	31,107±61,158	17,700±39,967	15,502±14,894	0.86	0.43
CES-D scale	2.7±2.2	11.9±2.7	26.6±9.8		
NIHSS	4.2±5.2	5.3±3.9	6.3±5.3	1.29	0.28
MMSE	26.3±4.2	27.2±2.3	26.3±4.4	0.51	0.60
Hypertension	73.3	75.0	96.4	6.22	0.045[‡]
Antihypertensive medication use	53.3	53.6	75.0	4.04	0.395
Diabetes	26.7	17.9	28.6	1.00	0.61
Hyperlipidemia	66.7	53.6	82.1	5.22	0.07
Cholesterol-lowering medication use	56.7	53.6	78.6	4.86	0.31
Obesity (BMI ≥30)	20.0	25.0	17.9	0.46	0.80
Smoking	33.3	3.6	17.9	8.49	0.014[‡]
Sum of risk factors[§]	2.2±1.4	1.8±1.0	2.4±0.9	15.08	0.129

Notes: *Lowest CES-D scale tertile (0–6), middle tertile (7–16), highest tertile (17–47); **χ^2 or F-values and corresponding [†]P-values reflect results of Pearson's χ^2 tests with CES-D scale tertiles for categorical variables and ANOVAs between CES-D scale tertiles and continuous variables, respectively; [‡]P-values represent significant ($P<0.05$) associations between the CES-D scale and clinical/demographic variables; [§]sum of risk factors represents the sum of potential risk factors, including hypertension, diabetes, hyperlipidemia, obesity, or smoking.
Abbreviations: SD, standard deviation; BMI, body mass index; CES-D, Center for Epidemiological Studies Depression; NIHSS, National Institutes of Health Stroke Scale; MMSE, Mini-Mental State Examination; ANOVA, analysis of variance.

Table 2 Immunological assay results*

Immunological markers	Mean ± SD or median (IQR)
KYN (μmol/L)	3.04±1.64
TRP (μmol/L)	43.88±11.82
K/T (μmol/mmol)	73.32±43.22
IL-6 (pg/L)	3.90 (2.29–7.82)
IL-10 (pg/L)	0.20 (0.20–2.07)
IL-18 (pg/L)	251.40 (163.20–348.30)
IL-1β (pg/L)	0.19 (0.12–0.40)
TNF (pg/L)	1.91 (0.09–3.28)
IFNγ (pg/L)	0.61 (0.23–1.00)

Notes: *n=86 for KYN, TRP, and K/T ratios; n=53 for cytokine assays.
Abbreviations: SD, standard deviation; IQR, interquartile range; KYN, kynurenine; TRP, tryptophan; K/T, KYN/TRP.

Figure 1 Mean serum kynurenine/tryptophan (K/T) ratios in low (0–6), medium (7–16), and high (17–47) Center for Epidemiological Studies Depression (CES-D) scale tertiles (n=86).

who did not provide sufficient serum with respect to age, sex, depression history, hypertension, or other comorbidities (all $P>0.05$). Of these immunological markers, all KYN, TRP, K/T ratio, IL-6, and IL-18 levels were within detectable range. Imputations were made for 21% of IL-1β, 27% of TNF, 60% of IL-10, and 5.1% of IFNγ samples. Comparison of serum concentrations of IL-6, IL-10, IL-18, IFNγ, TNF, and IL-1β, and their ratios with IL-10 across CES-D scale tertiles are shown in Table 3. Of the individual cytokines, only IL-1β concentrations differed significantly between CES-D scale tertiles. Ratios of IL-18/IL-10, IFNγ/IL-10 and IL-1β/IL-10 were also significantly different between CES-D scale tertiles. Post hoc tests revealed that serum IL-18/IL-10 ($P=0.046$), IFNγ/IL-10 ($P=0.025$), and IL-1β/IL-10 ($P=0.019$) (Figure 2) were elevated in the middle CES-D scale tertile compared to the first CES-D scale tertile.

Post hoc analyses

Post hoc analyses of specific depressive symptoms suggested that serum ratios of IL-18/IL-10 ($\rho=0.28$, $P=0.04$) and IL-1β/IL-10 ($\rho=0.43$, $P=0.001$), as well as IL-1β ($\rho=0.29$, $P=0.04$) were significantly associated with CES-D scale item 7 ("everything was an effort"). However, this association was not found with K/T ratios ($\rho=0.044$, $P=0.69$). The IFNγ/IL-10 ratio was associated with CES-D scale items 11 ("restless sleeping", $\rho=0.35$, $P=0.01$) and 17 ("crying spells", $\rho=0.29$, $P=0.04$). The IL-18/IL-10 ratio was also associated with CES-D scale item 17 ($\rho=0.29$, $P=0.04$). IL-1β was associated with CES-D scale item 15 ("people were unfriendly", $\rho=0.30$, $P=0.03$).

In antidepressant free-patients (n=80), K/T ratios did not differ between CES-D scale tertiles ($F_{2,79}=1.49$, $P=0.19$). In those with cytokine assays (n=47), ratios of IL-1β/IL-10 ($F_{2,46}=4.87$, $P=0.01$), IFNγ/IL-10 ($F_{2,46}=3.83$, $P=0.03$) and IL-1β concentrations ($F_{2,46}=4.41$, $P=0.02$) remained different
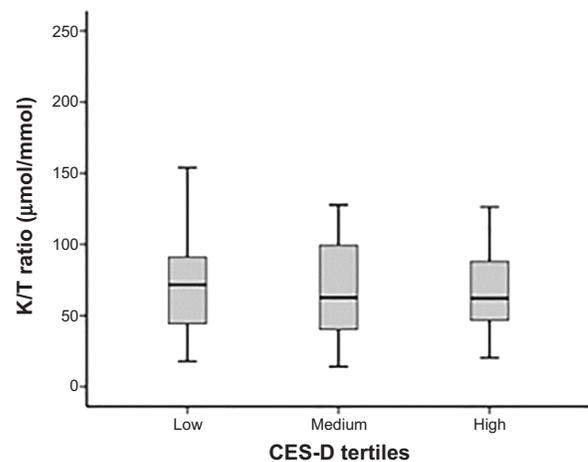
between tertiles, and there remained a trend for IL-18/IL-10 ($F_{2,46}=3.01$, $P=0.06$).

In patients without a premorbid history of depression (n=80), K/T ratios did not differ between CES-D scale tertiles ($F_{2,79}=0.41$, $P=0.67$). In those with cytokine assays (n=51), ratios of IFNγ/IL-10 ($F_{2,50}=3.87$, $P=0.03$), IL-18/IL-10 ($F_{2,50}=3.35$, $P=0.04$), IL-1β/IL-10 ($F_{2,50}=4.26$, $P=0.02$), and IL-1β concentrations ($F_{2,50}=4.01$, $P=0.03$) remained different between tertiles.

To facilitate comparison of our results with other studies, we repeated the main analyses using an established clinically important CES-D scale cutoff of ≥16. K/T ratios did not differ between groups ($F_1=2.05$, $P=0.15$) in a model adjusted as per the main analysis.

Discussion

In this study, no relationship was identified between the K/T ratio and CES-D scale scores. This finding was maintained after excluding those with antidepressant use or a history of depression. In the context of MDD, both positive[10–12] and negative findings with KYN metabolism have been reported.[49–51] As replicated in this study, a recent meta-analysis found disability to be among the most significant predictors of PSD,[2] suggesting a minor role for immunological contributions. However, the present findings should be interpreted in light of other known properties of the KYN pathway and additional observations. For instance, whereas the production of quinolinic acid and its intermediates by KYN monooxygenase in microglia may induce oxidative stress and excitotoxicity,[14] KYN catabolism into kynurenic acid by KYN aminotransferase II in astrocytes may be neuroprotective.[52] Therefore, the production of both neurotoxic and neuroprotective

Table 3 Differences between cytokines and cytokine ratios by CES-D tertiles (n=53)

Cytokines			Immunologic ratios		
	$F_{2,46}$*	*P*-value[†]		$F_{2,46}$*	*P*-value[†]
IL-6	3.04	0.06	IL-6/IL-10	0.88	0.42
IL-18	2.12	0.13	IL-18/IL-10	3.30	0.046[‡]
TNF	0.34	0.71	TNF/IL-10	0.69	0.51
IFNγ	2.54	0.09	IFNγ/IL-10	4.02	0.025[‡]
IL-1β	3.55	0.037[‡]	IL-1β/IL-10	4.34	0.019[‡]
IL-10	1.83	0.17	–	–	–

Notes: *F-statistics and corresponding [†]*P*-values ([‡]$P<0.05$) reflect results of independents in analysis-of-covariance models controlling for NIHSS score, age, antidepressant use, and hypertension. Controlling for smoking instead of hypertension showed similar results (for IL-1β, $P=0.11$; for IL-18/IL-10, $P=0.008$; for IFNγ/IL-10, $P=0.17$; for IL-1β/IL-10, $P=0.025$).
Abbreviations: CES-D, Center for Epidemiological Studies Depression; NIHSS, National Institutes of Health Stroke Scale.

metabolites when in balance may have no net effect on depressive symptoms.[10,53] Immunoregulatory effects of KYN may curb neuroinflammation,[54] resulting in the neuroprotective properties observed in ischemia models,[55] which might obscure deleterious roles of KYN metabolites.[54,55] Therefore, while the present study establishes that the peripheral K/T ratio is not a PSD biomarker, it does not preclude possible roles of KYN or its metabolites in the brain.

In the present study, higher K/T ratios and lower TRP concentrations were associated with older age. TRP concentrations were lower in subjects with a history of depression, although KYN concentrations were not different. Therefore, the present study replicates the relationship between low TRP and depressive diathesis seen in other populations, but does not provide evidence to support a role for KYN synthesis in this relationship. Mean K/T ratios in the present study (78 μmol/mmol) and another stroke study[8] were higher than those previously reported in MDD patients (25 μmol/mmol)[10] or in coronary artery disease patients (40 μmol/mmol), where depressive symptoms measured by the CES-D scale were associated with the K/T ratio.[13] Marked elevations in the K/T ratio poststroke may be above the levels associated with depressive symptoms and instead indicative of other poststroke sequelae.[8,56,57]

Relationships between cytokines (ie, IL-1β) and cytokine ratios (IL-18/IL-10, IL-1β/IL-10, and IFNγ/IL-10) with depressive symptoms were identified in agreement with some[24,26,27,29] but not all previous studies.[22,24,25] These exploratory results suggest that the risk of mild PSD symptoms might be better characterized by inflammatory imbalance; IL-1β and the immunologic ratios studied tended to be highest in the intermediate depressive symptoms tertile. These inverted U-shaped curves would be consistent with animal data, in which inflammatory activity produces "sickness

behaviors" that sometimes transition into a more severe syndrome reminiscent of human depression.[58]

The disagreement between studies that dichotomized patients into depressed or nondepressed categories may be partly explained by the heterogeneity in the presentation of depressive episodes poststroke; the present post hoc analyses agree with previous studies,[28,29,59] suggesting that somatic symptoms (ie, fatigue and sleep disturbance) may be associated with immune dysregulation, in addition to some core affective symptoms (ie, sadness, crying, and negative social bias). Clinically, cytokines have been associated with poststroke fatigue but not depression per se.[28,29] Our exploratory finding that cytokine ratios (IL-18/IL-10 and IL-1β/IL-10) as well as IL-1β were most consistently associated with CES-D scale item 7 ("everything was an effort") rather than mood items are consistent with this. This item, either with or without the inertia item ("could not get going"), has been operationalized to measure fatigue.[60,61] A recent study examined fatigue more specifically in patients poststroke, finding relationships with TRP availability and a KYN metabolite, but also finding no relationship with PSD.[62] Taken together, the results suggest a potential relationship between inflammatory cascades and fatigue and milder depressive symptoms, which may be qualitatively different from MDD.

As a possible limitation, inflammatory markers were not assessed in the acute phase of stroke and examined prospectively, which may have revealed different relationships; however, previous reports suggest that elevations in KYN and its metabolites persist for weeks to years following stroke or traumatic brain injury,[8,63] and the present findings suggest that elevations in other inflammatory markers are relevant to depressive symptoms in the subacute phase. These positive findings suggest that it would have been possible to observe a relationship if the K/T ratio was related to PSD. Although the CES-D scale is not a diagnostic instrument, the aim of this study was to characterize the relationship between the K/T ratio and depressive symptoms, for which the CES-D scale has been validated. Moreover, exploratory findings support the hypothesis that there may be nonlinear immunological relationships with depressive symptoms, suggesting the utility of nondichotomous assessment. While KYN and some KYN metabolites can cross the blood–brain barrier,[64] peripheral blood concentrations may not represent central concentrations and KYN metabolites were not assayed; however, peripheral KYN has been correlated with cerebrospinal fluid KYN and quinolinic acid concentrations[65] and with poststroke sequelae,[8,57] justifying its exploration as a biomarker for PSD. Finally, the main finding of the present study does
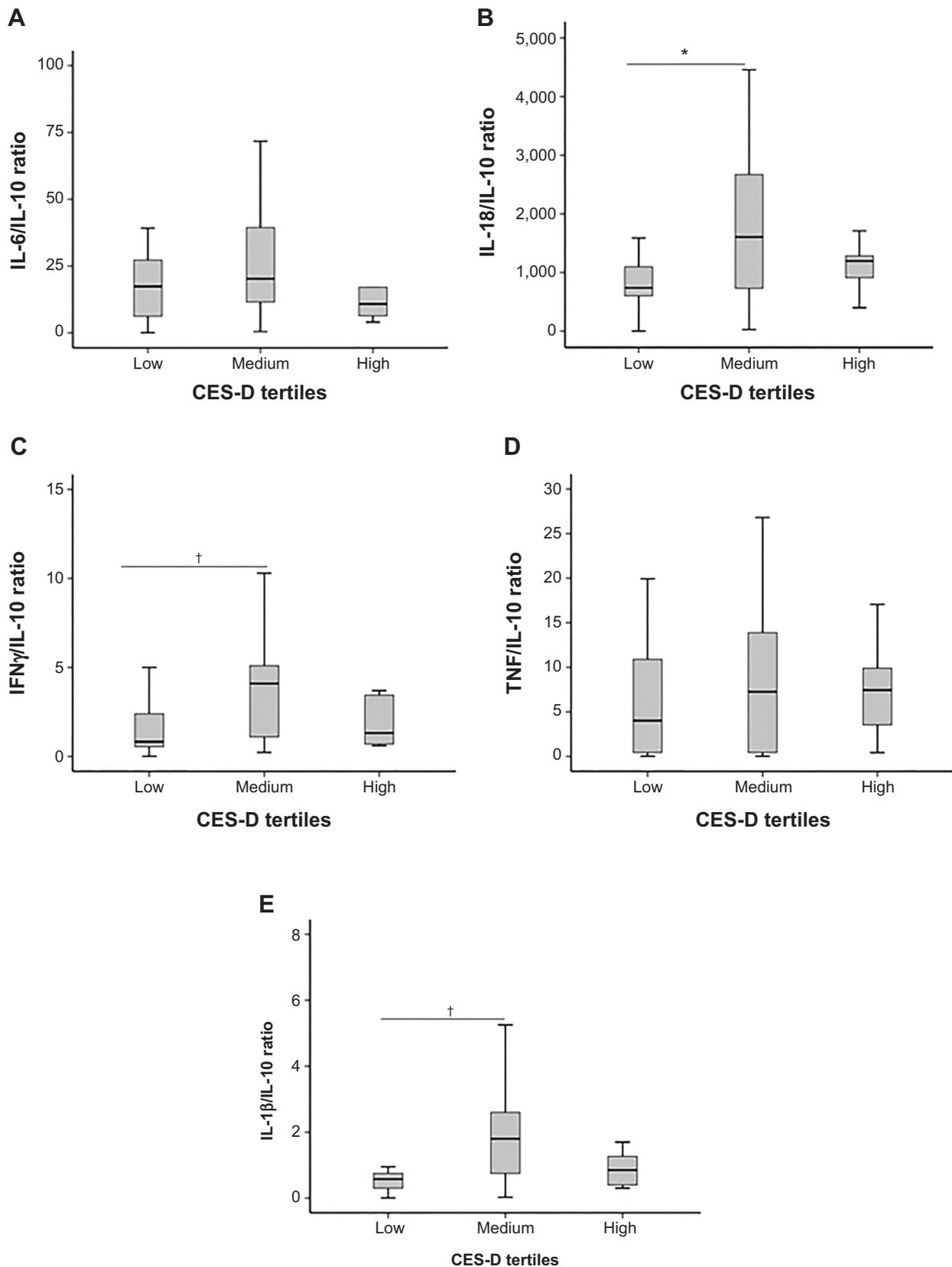
Figure 2 Mean serum cytokine/IL-10 ratios in low (0–6), medium (7–16), and high (17–47) Center for Epidemiological Studies Depression (CES-D) scale tertiles; n=53 for each panel.
Notes: (**A**) Mean serum IL-6/IL-10; (**B**) mean serum IL-18/IL-10; (**C**) mean serum IFNγ/IL-10; (**D**) mean serum TNF/IL-10; (**E**) mean serum IL-1β/IL-10 ratio in low (0–6), medium (7–16), and high (17–47) CES-D scale tertiles. *$P<0.0005$; †$P<0.05$.

not support the primary hypothesis, and the positive findings reported were based on exploratory and post hoc analyses in a subgroup of patients without correction for multiple comparisons. Although the relationship between cytokines and fatigue was determined based on a CES-D scale item, the validity of this item has been established,[60,61] and the results concur with those of other studies that used more nuanced fatigue assessments.[28,29] Although the exploratory results do not provide rigorous evidence, they are confluent with extant literature and provide an impetus for appropriately powered replicative and/or prospective studies. Finally, the associations observed in this study do not imply causation.

Conclusion

K/T ratios were not associated with depressive symptoms in the acute phase following an ischemic infarct, although associations between depressive symptoms and disability and vascular risk factors were confirmed, and relationships between serum TRP concentrations and histories of depression and age were found. Exploratory analyses suggested that inflammatory activity was associated with mild–moderate but not with severe depressive symptoms. Prospective studies might clarify relationships between immune imbalance and the evolution of depressive symptoms poststroke.

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

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