Universally available Innate and Adaptive immune cell counts in Acute Ischemic Stroke (AIS) in the context of shared pathobiology with COVID-19; Scoping Review

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Abstract: Stroke is one of the leading cause of adult disability and the second leading cause of death worldwide. The immune system actively participates in the pathobiological process of AIS, during the index event and during the repair process despite the limited attention drawn to this aspect in the existing stroke guidelines globally. The similar clinical course and similar circulating innate and adaptive immune cell counts in AIS and COVID-19 has created a renewed interest in these easily available biomarkers innate and adaptive immunological changes in AIS with potential diagnostic, prognostic, and therapeutic implications. The current scoping review aimed to assess the significance of circulating neutrophil and lymphocyte counts and their ratio (NLR) in AIS and explore their association with post-stroke recovery trajectory.

The Arksey and O'Malley methodological framework was employed to review the published papers on the neutrophil-lymphocyte ratio (NLR) and AIS in late November 2020. Only studies published in English from 2000-2020 were included in this scoping review.

Fifty-three published papers were reviewed. This review's key finding is that a canonical inflammatory response occurs in AIS just as in the case of COVID-19 and neurological involvements well described in the recent literature. An excessive circulating innate immune cells (neutrophils) and reduced circulating adaptive immune cells (lymphocytes) are associated with poorer outcomes during the acute interventions (reperfusion therapies) as well as the recovery trajectory. Main representatives of innate and adaptive immunity follow a canonical course in AIS and COVID-19. Exaggerated circulating innate (elevated neutrophils and elevated NLR) and reduced adaptive immune response (lymphopenia) correlate with the worse outcome in AIS and COVID-19. This scoping review's findings make the strongest case for a systems biology-based approach to the standard operating procedures in stroke care urgently.

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Keywords: stroke, neutrophil lymphocyte ratio, Systemic Immune Inflammatory Indea, sustainable stroke care

Introduction

On November 12th, 2020, the World Health Assembly (WHA), the global decision-making body of the World Health Organisation (WHO), went on to pass the landmark resolution WHA73.10 for 'global actions on epilepsy and other neurological disorders 'with 13 new paragraphs on key aspects of global brain health as a matter of high priority(1). The resolution confirmed the enormous impact of stroke-related death and disability(2) This was noted to be the main reason behind 80% death(3) and disability(4) in low to middle-income countries worldwide(5, 6). Furthermore, these long term underlying trends among the Emerging BRICS(7) and EM7 Markets play particularly sensitive role shaping the global landscape in epidemiology of stroke and consective challenge of financial sustainability(8).

Stroke is indeed one of the leading causes of disability, and the second leading cause of death worldwide(9). Approximately 85% of strokes are those of Ischaemic origin, and the rest is accounted for by intracerebral and subarachnoid haemorrhage and other types(9). Prognostic assessments are crucial for treatment selection in stroke and remains a challenging aspect for clinicians. Numerous prognostic factors validated by previous studies are not without limitations, including too much emphasis on motor systems, observer-dependent variability and cost(10). Inflammation plays a key role in AIS from pathobiology, index, and recovery (11-14). Neutrophils and lymphocytes are common markers of inflammation and representatives of innate and adaptive immunity, respectively(15). NLR and systemic immune-inflammatory response (SII) provide an excellent biomarker of the innate and adaptive immune response in AIS just as in the case of COVID-19(16-18). As a cost-effective(19) and easy to perform test in routine

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practice, the neutrophil-to-lymphocyte ratio (NLR) has been previously validated as a prognostic indicator in other conditions – cancer, cardiac disease, sepsis, coronary artery disease and metabolic syndrome(20). NLR is obtained from the differential white blood cell count and is calculated by absolute neutrophil count divided by total lymphocyte count.

Methodology

This review included published articles, abstracts, letters and commentaries of neutrophil studies to lymphocyte ratio and its relationship to stroke. In particular, studies were included if they looked into the relation of NLR and ischemic stroke, intracerebral haemorrhage, subarachnoid haemorrhage and post-stroke complications. There were no limitations on the sample size, duration and follow-up. Only studies in English and those that were published from the year 2000 onwards were included.

The following computerized databases were acceded: PUBMED, EMBASE, British Library, Library of Congress and National Lib of Medicine. Each article's bibliography was also manually searched for additional publications. We used the search terms: stroke; Neutrophil to lymphocyte ratio; intracerebral haemorrhage; subarachnoid hemorrhage and post-stroke complications. Two reviewers extracted the data (TW, CS), and a third reviewer (LK) resolved any conflicts for inclusion. The quality of the studies was also independently assessed.

Results

A total of 263 articles were generated from the electronic search. There were 50 duplicates, and the rest were screened on the title level. Further screening of the abstracts yielded 180 articles, of which 47 articles were deemed relevant and included in the study. Additional six studies were included from manual search of bibliographies. Figure 1 shows the PRISMA chart for the search.

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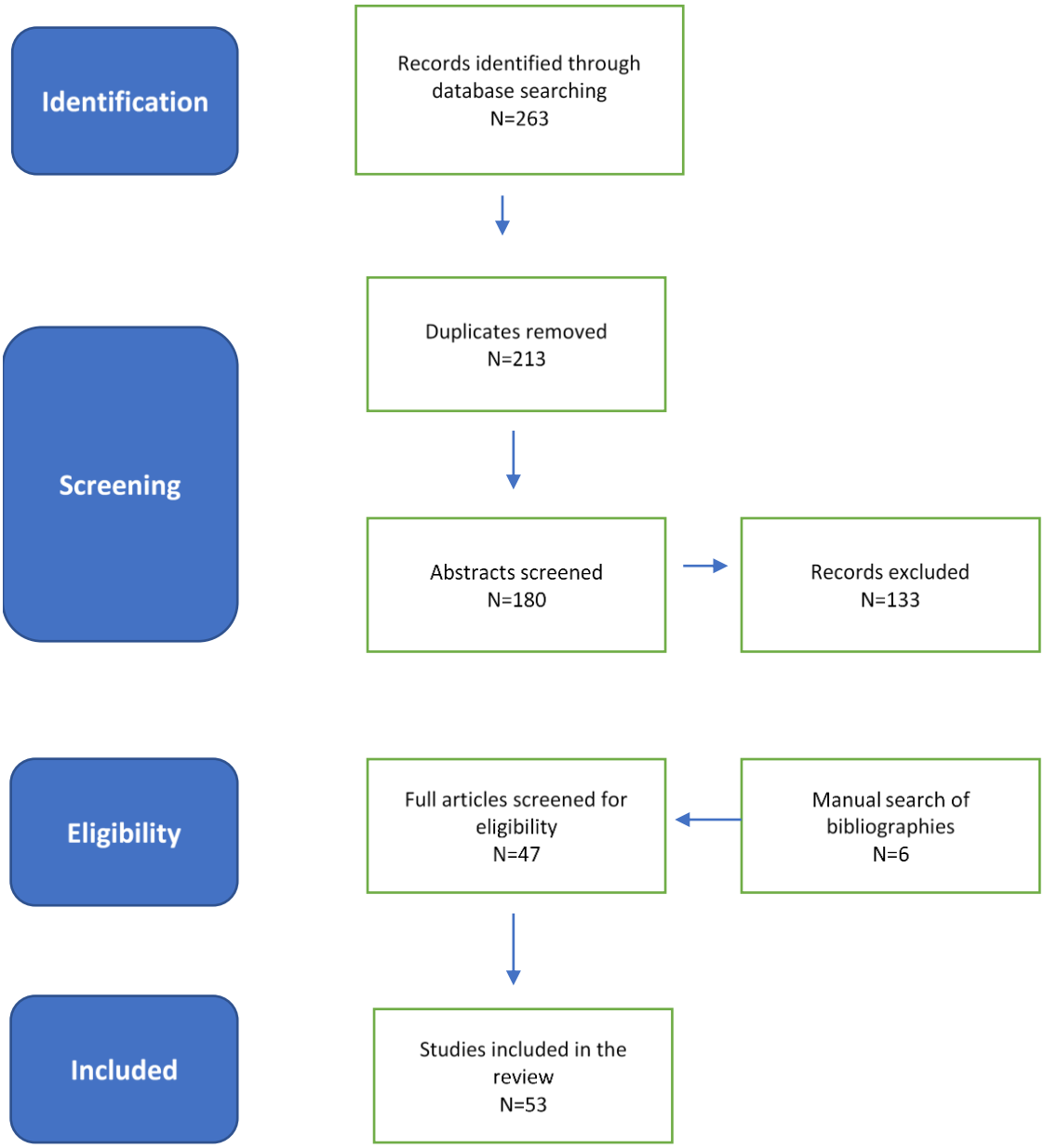
Figure 1. PRISMA chart



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Role of NLR in Acute Ischaemic Stroke

The role of INR has been most widely investigated in ischaemic strokes. Current interventions of acute ischaemic stroke (AIS) include intravenous thrombolysis and mechanical thrombectomy, which aim for recanalization of the cerebral arteries blocked by a clot or an embolus thus resulting in reperfusion of the hypoxic brain tissue. The National Institutes of Health Stroke Scale (NIHSS) and modified Rankin scale (mRS) are two prevalent, well-validated scoring systems used to measure stroke severity by quantifying neurological deficits in the former and the degree of dependence of daily activities in the latter.

NLR has been identified to be a useful clinical predictor of outcome in AIS patients being considered for mechanical thrombectomy, as NLR can predict functional dependence and mortality after adjustment for potential confounders that included vascular risk factors. Multiple analyses reveal NLR correlates with a greater functional disability at admission and at three months (increased mRS and NIHSS scores) and increased 90-day mortality (21-27) and was furthermore associated with length of hospital stay(28) and cost(29). Patients with NLR >3.3 were 2-times more likely to be at risk of death(24). This elucidates the role of NLR as a potential biomarker of prognosis, thereby providing an additional level of risk stratification in AIS. Interestingly, high NLR was also significantly correlated with the volume of infarct in anterior circulation strokes but not in those of posterior circulation origin(26). Besides, Switonska identified that elevated NLR was significantly higher in patients treated with both thrombolysis and thrombectomy than those who either received thrombolysis alone or were not

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treated. This confirmed that elevated NLR levels were associated with more severe and disabling strokes warranting aggressive treatment(23).

Current literature describes the role of NLR in predicting AIS complications. AIS patients are at risk of developing intracerebral haemorrhage as a complication in the presence or absence of thrombolytic treatment. NLR obtained at 12-18 hours after treatment with intravenous recombinant tissue plasminogen activator (rtPA) was positively associated with haemorrhagic transformation, characterized by symptomatic intracerebral haemorrhage or parenchymal haematomas(30). Many studies identified high NLR values translated to increased haemorrhagic complications (with thrombolysis often intensifying this risk) in AIS(31-33). Other complications with a potential link to high NLR include recurrent ischaemic strokes (34) and infectious sequelae(21, 35). NLR could be used as a predictive marker to monitor and prevent such complications. Promisingly, NLR was also an independent risk factor for the incidence of AIS in generally healthy adults in a study compared with other traditional cardiovascular risk factors, demonstrating the potential of NLR in primary prevention of stroke(36).

The role of INR in AIS and its co-morbid conditions have also been explored. Studies suggest that NLR may reflect the severity of carotid artery stenosis (37-39) and aortic arch calcification(40), both important risk factors of AIS. Nam et al. showed that high NLR could predict early neurological deterioration in cryptogenic stroke patients with active malignancy(41).

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Ischaemic injury to the brain triggers a systemic inflammatory response involving brain cells' activation in the ischaemic area, inducing leucocytes' migration from this area. Neutrophils are described as the first leucocytes to act on the infarcted cells contributing to an enlargement of the area. Lymphocytes accumulate later during the stroke recovery. The high NLR reflects both an amplified innate immune response characterized by higher neutrophils (polymorphonuclear cells) and an attenuated adaptive immune response as demonstrated by decreased lymphocytes(23, 24, 42, 43).

The role of NLR has been explored in neonates and results of a preliminary study demonstrated dynamic changes in NLR following ischaemic brain injury which represent a possible target for therapeutic intervention in this younger subset of patients(44).

NLR and its role in predisposition to Acute Ischemic Stroke

Low-grade systemic inflammation connects metabolic syndrome, cardiovascular diseases such as AIS and aging(45). AIS occurs in the context of a compromised vascular system very much akin to COVID-19 related neurological issues(18, 46-53). The evidence is overwhelming that

activation of innate immunity before and after the AIS and COVID-19 with adaptive immunity implications after the index events of AIS and COVID-19 share similar pathobiology and trajectory. These facts have already been documented so far in large populations of Eur-Asia with prominent public health consequences(54, 55). Unlike its innate counterpart, the adaptive immunity is critically important for the attenuating inflammatory response during the recovery process both in the context of AIS and COVID-19 (See the table one, with kind permission from Wijeratne et al., Frontiers in Neurology,2021, Under the creative common license(56).

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Table 1

Pathophysiology of COVID-19 and Acute Ischemic Stroke—Chronological Order
1. Binding of SARS-CoV-2 to ACE-2 on the surface of alveolar pneumocytes (ACE2 is the receptor for host cell entry of SARS-CoV-2. ACE-2 is widely expressed on the alveolar epithelial cells, endothelial cells, enterocytes of the small intestine and arterial smooth muscle cells) (46)
2. Viral infection activates intracellular pattern recognition receptors (PRRs) in the host. PRRs sense pathogen associated molecular patterns (PAMPs) [not this is very similar to pathophysiology of AIS where acute ischemia induced damage associated molecular patterns (DAMPs) perform exactly the same task] (10, 47–49)
3. PAMPs sets off cytolytic immune responses with type one interferon and natural killer cells (NK) with aim of removing the pathogens (50)
4. Endothelial activation secondary to direct viral activation
5. Innate immune response with the recruitment of macrophages, neutrophils, and monocytes to the alveoli.
6. Further innate immune reaction with T helper cells 1&17 T cells and ongoing recruitment of monocytes, macrophages and neutrophils (51)
7. Endothelial activation secondary to direct viral action
8. Endothelial activation secondary to persistent inflammation
9. Activation of cell adhesion molecules and further recruitment of T cells, monocytes and macrophages
10. Release of tissues factors from the activated endothelium
11. Recruitment of intravascular neutrophils
12. Release of neutrophil extracellular traps (NET) from the activated endothelium
13. Release of von Willebrand factor (vWF) and micro thrombosis (52)
14. Maladapted innate immune response and procoagulant activity lead to systemic cytokine rise
15. Increased platelet aggregation and thrombo-embolism
16. Activation of cell adhesion molecules and ongoing recruitment of monocytes, macrophages and neutrophils within the cerebral blood vessels
17. Endothelial activation in cerebral blood vessels
18. Endothelial dysfunction in the brain
19. Local immune and inflammatory activity in the brain
20. Disruption of the blood-brain barrier
21. Activation of microglia in the brain
22. Further recruitment of resident immune cells in the brain
23. Increased inflammatory activity in the brain
24. Micro-thrombosis, thrombo-embolism in the brain
25. Hypoperfusion of cerebral tissues
26. Acute Ischemic stroke

**Attempts have been made to assimilate all available current evidence to formulate this table. However, we are aware the pandemic is still evolving, and additional molecular mechanisms and pathways are likely to be discovered as time goes by.*

While neutrophils play a key role in the innate immune response, lymphocytes with their wide range of cellular phenotypes play a crucial role in regulating the adaptive immune response. The key role of NLR in pathobiology, progress and prognostication of AIS should be the expectation rather than the exception. While NLR has been used as a prognosticating index in patients with various stroke mechanisms, it has also been employed to determine stroke risk in healthy and among patients with other pathologic conditions. Elevated values, especially among normal subjects, highlight the importance of subtle and ongoing chronic inflammation and its predisposition to pathologies such as diabetes and stroke(36, 57).

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In a retrospective literature published by Suh et al., it has been demonstrated that among almost 25,000 normal subjects, elevated NLR is a risk factor for ischemic stroke(36). This study further concluded that the addition of NLR improved the Framingham risk for reclassification of ischemic stroke(36). On the other hand, patients with preexisting cardiovascular risk factors are also prone to stroke if NLR is elevated(58). A study of more than 32, 000 atrial fibrillation subjects without prior anticoagulation were prospectively followed up for the first-ever stroke and has been shown that independent of patients' CHADS-Vasc scores; there is a dose-dependent increase in the incidence of stroke with increasing NLR quartiles which were estimated to be 2.27, 2.72, 3.26 and 4.54 per 100 person-years, respectively(58). This is further corroborated in another study that compared patients with AF with and without stroke suggesting that patients belonging to the former had a higher NLR than their non-stoke counterparts(59). Moreover, Min et al. also concluded that aside from AF, NLR was also positively correlated to NIHSS and diabetes among ischemic stroke patients(59).

It is clear that both AF and stroke are related to a state of chronic inflammation which may be manifested serologically by the dissociation of neutrophils and lymphocytes(58, 59). The occurrence of stroke among patients with AF is linked to the formation of a cardiac thrombus, which is also linked to a higher NLR state.56 In animal models, there has been a success in using corticosteroids in minimizing atrial-tissue related inflammation which contributes to a pro-arrhythmic state, but this has yet to be fully evaluated in humans(60). There is also limited information on anti-inflammatory agents in stroke prevention, particularly in AF patients.

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NLR and its role in post-stroke complications

1.Stroke Associated Pneumonia

Stroke Associated Pneumonia (SAP), an occurrence in almost 14.% of all stroke patients, is one of the most significant acute stroke complications.58 It is an important risk factor for mortality at one and six months, with death rates at 19% and 44%, respectively(61). The Neutrophil to lymphocyte ratio on admission has also been explored as a prognostic indicator for pneumonia among stroke patients. Nam et al. observed that patients with higher NLR presented with more prolonged hospitalization and higher NIHSS(62). Furthermore, a higher frequency of mortality at one month and worse MRS at three months was observed among patients with higher NLR(62). Compared to other parameters such as white blood cell, absolute Neutrophil and lymphocyte count, an elevated NLR on admission was also associated with a higher risk of mortality after 90 days(63).

While impairment in swallowing mechanisms largely contributes to pneumonia among post-stroke patients, there is increasing evidence that stroke itself induces an immunocompromised state predisposing patients to infection(64). This is supported by observations of decreased lymphocyte number and an increase in neutrophil count in acute stroke patients(65). While targeted neutrophil therapy may have the theoretical advantage, Ruhnau recommends that therapy's potential benefits may be countered by its opposing effects in the brain and systemically(65). This is made true in a clinical trial of an anti-inflammatory drug Enlimomab on patients with ischemic stroke (Enlimomab)(66). Comparing the former with placebo, it has

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been shown that among Enlimomab-treated patients, there were more serious adverse events, particularly fever and infection, and higher mortality rates(66).

2. Post-stroke delirium

Delirium is another complication known to occur in 13% to 48% of patients with acute stroke(67). There is clinical data to suggest that risk of death, institutionalization and hospital stay is significantly higher among stroke patients with delirium(68). The use of the Neutrophil to lymphocyte ratio as a prognostic factor in patients with delirium was first explored by Egberts et al. in acutely ill patients(69). This study's results, which involves 86 patients, prove that patients who are clinically unwell and subsequently develop delirium have higher NLR(69).

A prospective observational study of 1001 patients with acute ischemic stroke diagnosed with delirium using the CAM-ICU (Confusion Assessment Method for Intensive Care Unit) corroborated this condition's increased incidence as previously observed in other studies(70). In the same study, along with NLR, clinical (hemianopia, aphasia, NIHSS) and laboratory variables (leukocytes and CRP) which comprise the Deliirium in Acute Ischemic Stroke (DELIAS) score, this has been shown to have good predictive value for detecting early-onset delirium and of moderate predictive value for delirium up to the fifth day from the stroke(70). On the other hand, a follow-up study of patients with first-ever acute ischemic stroke has shown that instead of NLR, platelet to white cell count ratio was associated with early delirium occurrence (71). These studies support previous evidence that acute brain injury may result in the loss of adaption of the immune cells as manifested by the disproportionate changes of hematologic parameters(70, 71).

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3.Post Stroke Depression

Depression is one of the leading causes of morbidity among stroke patients and significantly impacts all aspects of rehabilitation(72). A meta-analysis including seven studies involving more than 17,000 patients has shown that post-stroke depression (PSD) is associated with increased risk of mortality with a relative risk of 1.50 (95%CI: 1.28 to 1.75; $p < 0.001$)(73). One of the key implicated mechanisms is neurobiological systems' involvement, emphasizing post-stroke inflammation, which is the expectation rather than the exception(74-76). Grundy and colleagues have demonstrated that inflammatory mediators produced after acute stroke may have damaging effects on rat models(77). It has been further proposed that the production of IL-1 and other chemokines by mononuclear phagocytes attracts neutrophils, monocytes and T-lymphocytes which further play a role in the dysregulation of humoral and inflammatory systems and maybe indirectly correlated to its pathogenesis(75).

The Neutrophil's use to lymphocyte ratio as a predictor for depression among stroke patients has been well documented in the literature(78-80). In a prospective study involving 299 ischemic stroke patients, increased NLR during admission was correlated to PSD after one month, with diagnosis based on the 17-Hamilton rating scale(81). Using the Quick Inventory for Depression Symptomology (QIDS), Wolde et al. showed the positive correlation between high NLR on acute stroke presentation and the occurrence of depression after 30 days(79). A retrospective study of more than 300 patients also demonstrated that in conjunction with platelet to lymphocyte ratio, high admission NLR is associated with depression at six months after

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stroke(82).On the other hand, a correlation of depression severity has also been demonstrated with increasing NLR among patients without stroke but with significant vascular risk factors(83).

Future Potential Therapeutic Opportunities

The unmet needs in stroke prevention and post stroke rehabilitation continue to remain an immense challenge to the world. It is clear that the human immune system is quite complex with synergistic (innate immune cells and mechanisms) as well as antagonistic (adaptive immune cells and mechanisms) roles with different cells playing different roles at different time points of AIS with likely shared pathobiology with COVID-19, other vascular diseases such as ischemic heart disease, acute myocardial infarction in particular(84-87). Innate and adaptive immune cells and pathways should complement each other's for an effective and optimum recovery process after the index event of AIS. This aspect has not been considered or explored by any existing national or international stroke guidelines and standard operating procedures at present (Wijeratne et al., Manuscript in submission). Patients with an already compromised vascular system with AIS appear to show elevated NLR, suggesting an undue prominence of circulating neutrophils (innate immune cells) over lymphocytes (adaptive immune cells). This means the potential therapeutic targets such as reducing the innate immune system's hyperactivity while minimizing the depression of the adaptive immune system during the index event of AIS. There may be a role for safe, low cost,psycho-neuro-immuno-modulatory chemicals such as melatonin and curcumin with potential therapeutic benefits in disabling post-stroke complications post-stroke depression, post-stroke fatigue, post-stroke anxiety etc(88).

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To date, the use of anti-inflammatory agents in post-stroke depression is minimally explored. Studies have shown that SSRI, one of the mainstays for PSD treatment, exhibits an anti-inflammatory activity by upregulating neurotrophins and enhancing neurogenesis(89-91). A meta-analysis involving 14 publications with more than 6,000 patients, has shown that nonsteroidal inflammatory drugs, most notably Celecoxib, alleviate depressive symptoms compared to placebo(92). There is also evidence that Celecoxib and Infliximab's intake in patients with major depression has resulted in a concomitant decrease of the inflammatory markers, IL-6 and CRP, respectively(93, 94). While anti-inflammatory agents' role in depression seems to be promising, further studies are needed, especially among post-stroke patients, given the former's association with vascular events(95).

Conclusion

The Neutrophil to lymphocyte ratio is a cheap, effective, yet an underutilized biological marker in AIS. It provides information on prognosis and risk stratification among stroke patients and individuals who are at risk of developing neurovascular events. The NLR and immune-based simple algorithms such as systemic immune-inflammatory index (SII), Sunshine Prognostic Score (SPS) provide substantial, systems based comprehensive biological information on a stroke patient's degree of chronic inflammation be associated with different clinical outcomes and different therapeutic potentials(56, 96-98). While animal studies have proven the theoretical role of anti-inflammatory drugs in controlling the damaging the degree of systemic and neuro-inflammation, this has not translated significantly in human clinical trials.

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The recently concluded 73rd WHA confirmed the enormity of challenges ahead to the world. The 73rd WHA makes the strongest argument for synergy between researchers, academics, government and non-governmental sectors, hospitals to tackle the growing number of neurological disorders as a matter of high priority. Stroke remains the largest contributor to global death and disability. Systems biology-based approach in AIS management should provide the best synergy to bring the best care for AIS throughout the recovery trajectory.

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