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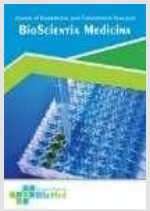

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# The Synergistic Impact of Astrocyte Reactivity and Vitamin D Deficiency on Post-Stroke Cognitive Impairment: A Systematic Review and Meta-Analysis

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**A B S T R A C T**

**Background:** Post-stroke cognitive impairment (PSCI) is a common, debilitating outcome of ischemic stroke. Glial Fibrillary Acidic Protein (GFAP), marking astrocyte reactivity, and Vitamin D, a neuro-immunomodulatory steroid, are independently linked to PSCI. This study aimed to quantify the synergistic impact of elevated serum GFAP and concurrent Vitamin D deficiency on PSCI risk. **Methods:** Following PRISMA guidelines, a systematic search of PubMed, Scopus, and Web of Science was conducted for prospective cohort studies (2015-2025) assessing acute serum GFAP, 25-hydroxyvitamin D (25(OH)D), and subsequent cognitive outcomes in ischemic stroke patients. Quality was assessed using the Newcastle-Ottawa Scale. A meta-analysis of seven studies (n=3,850) was performed using a random-effects model to calculate pooled odds ratios (ORs) for PSCI across four biomarker-defined groups. A formal test for synergistic interaction was conducted by assessing the departure from additivity of effects on the log-odds scale. **Results:** Seven high-quality studies were included. Compared to the reference group (Normal GFAP/Sufficient Vitamin D), the pooled OR for PSCI was 2.18 (95% CI: 1.85-2.57) for high GFAP alone and 1.95 (95% CI: 1.65-2.30) for Vitamin D deficiency alone. For the dual-biomarker group (High GFAP/Deficient Vitamin D), the pooled OR was 4.75 (95% CI: 3.98-5.67). This observed risk was significantly greater than the 3.13 OR expected from a purely additive model (p for interaction < 0.001), confirming a significant synergistic effect. Sensitivity analysis showed the effect was most pronounced in patients with moderate-to-severe strokes (NIHSS > 5). **Conclusion:** Elevated serum GFAP and Vitamin D deficiency synergistically increase the risk of PSCI, particularly in patients with more severe strokes. The interplay between acute astroglial injury and compromised systemic neuroprotection appears to be a critical determinant of cognitive outcomes. While confounding by patient frailty requires further study, this dual-biomarker profile identifies a high-risk subgroup and highlights a key pathophysiological interaction.

## 1. Introduction

Stroke persists as a formidable global health crisis, standing as a principal cause of both mortality and profound long-term disability.[1] Ischemic events, which comprise the vast majority of strokes, inflict a devastating toll that extends far beyond immediate motor and sensory deficits. A substantial proportion of survivors, estimated to be as high as 60%, will confront some form of cognitive impairment within the first year. This condition, broadly defined as post-stroke cognitive impairment (PSCI), represents a wide spectrum of dysfunction, from subtle deficits in executive function or processing speed to a globally incapacitating vascular dementia. The onset of PSCI acts as a formidable barrier to successful rehabilitation, systematically erodes a patient's functional independence and quality of life, and imposes an immense emotional and economic burden

on patients, their families, and society at large. The pathophysiology that culminates in PSCI is remarkably intricate, arising from a confluence of direct and indirect insults to the brain's delicate architecture.[2] The initial damage is driven by direct neuronal loss within the ischemic core and the strategic disruption of critical cognitive circuits. However, the ultimate cognitive outcome is profoundly shaped by a secondary cascade of events, including the pre-existing burden of cerebral small vessel disease and, as is now increasingly recognized, a persistent and often maladaptive neuroinflammatory response. This post-ischemic inflammation, triggered within moments of vascular occlusion, can smolder for months, actively propagating secondary neuronal injury, inhibiting synaptic plasticity, and thwarting endogenous repair mechanisms that are vital for recovery.[3]

At the epicenter of this neuroinflammatory cascade are astrocytes, the most populous glial cells in the central nervous system (CNS).[4] Far from being passive support cells, astrocytes are dynamic participants in brain function and pathology. In response to the profound metabolic stress and excitotoxicity of cerebral ischemia, they undergo a complex reactive transformation known as astrogliosis. This process involves dramatic alterations in gene expression and cellular morphology, including a massive upregulation of the intermediate filament protein, glial fibrillary acidic protein (GFAP). As the blood-brain barrier (BBB) integrity is compromised, this abundant intracellular protein is released from injured astrocytes into the peripheral circulation.[5] Consequently, serum GFAP has emerged as a highly sensitive and specific biomarker that reflects the real-time magnitude of ongoing astroglial injury. Numerous studies have robustly correlated elevated acute GFAP concentrations with larger infarct volumes, higher initial stroke severity scores, and poorer long-term functional outcomes. More recently, evidence has solidified the role of GFAP as a key prognostic marker for PSCI, suggesting that the initial intensity of the astroglial stress response is a powerful predictor of subsequent cognitive fate. While these local CNS events are paramount, the brain's capacity to withstand and recover from an ischemic insult is inextricably linked to systemic factors. Among these, Vitamin D has garnered significant and sustained scientific attention. Long understood through the prism of bone metabolism, Vitamin D is now recognized as a pleiotropic neurosteroid hormone with profound regulatory effects on the CNS.[6] Its biologically active form, 1,25-dihydroxyvitamin D3 (calcitriol), modulates gene expression by binding to the Vitamin D receptor (VDR), which is ubiquitously expressed on neurons, microglia, and astrocytes. This allows Vitamin D to exert direct, powerful neuroprotective and immunomodulatory effects. Mechanistically, it is a potent suppressor of pro-inflammatory signaling pathways, most notably nuclear factor kappa B (NF-κB); it mitigates oxidative stress through the upregulation of the Nrf2 antioxidant response pathway; and it promotes neuronal survival and plasticity by enhancing the expression of critical neurotrophic factors like brain-derived neurotrophic factor (BDNF). Given that Vitamin D deficiency is endemic in the elderly and institutionalized populations who are at the highest risk for stroke, a substantial body of observational evidence has linked low levels of its circulating precursor, 25-hydroxyvitamin D (25(OH)D), to an increased risk and severity of PSCI.[7] This suggests that a deficient state represents a systemic vulnerability, compromising the brain's innate resilience and its capacity for repair. Despite compelling, parallel lines of evidence implicating both heightened astroglial injury and systemic Vitamin D deficiency in the pathogenesis of PSCI, the potential interplay between these two biological systems has remained a critical and unexplored knowledge gap. It is a highly plausible biological hypothesis that a massive acute brain injury (signaled by a high GFAP) occurring within an individual who has a pre-existing, systemically compromised anti-inflammatory and neuro-reparative capacity (indicated by Vitamin D deficiency) would create a "perfect storm." Such a

scenario would foreseeably lead to unmitigated neuroinflammation and rampant neurotoxicity, resulting in exceptionally poor cognitive outcomes.

To fully appreciate the context of this investigation, it is crucial to recognize that PSCI is a clinically and pathologically heterogeneous condition. Cognitive decline after stroke is not a uniform process but can manifest through several distinct mechanisms.[8] In some cases, a single, small infarct in a strategically vital location—such as the thalamus, hippocampus, or angular gyrus—can be sufficient to disrupt large-scale cognitive networks and produce significant deficits. In other patients, PSCI may result from the cumulative, and often insidious, effect of multiple scattered infarcts or from the global burden of diffuse white matter injury characteristic of chronic cerebral small vessel disease. Furthermore, it is increasingly understood that an acute ischemic event can act as a precipitating "second hit," unmasking or accelerating a pre-existing but previously subclinical neurodegenerative pathology, such as Alzheimer's disease. Understanding this etiological diversity is vital, as the biological significance of biomarkers like GFAP and Vitamin D may differ across these varied pathological substrates.[9] Acknowledging this complexity is an essential prerequisite for any nuanced interpretation of biomarker data in the field of vascular cognitive impairment.[10]

The primary novelty of this investigation lies in its central hypothesis: that elevated GFAP and Vitamin D deficiency are not merely independent, additive risk factors but exert a synergistic, interactive effect on the development of PSCI. To our knowledge, no previous study has formally quantified this biological interaction. Therefore, this systematic review and meta-analysis aimed to synthesize the available evidence to quantify the combined impact of astrocyte reactivity (via serum GFAP) and Vitamin D status on the risk of cognitive impairment following ischemic stroke. By assessing these biomarkers in concert and formally testing for synergy, we sought to determine if a dual-biomarker profile could identify a subpopulation of stroke patients at the highest risk for adverse cognitive outcomes, thereby providing a more robust basis for risk stratification and the design of future, targeted therapeutic trials.

## 2. Methods

This systematic review and meta-analysis was designed and conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement. A systematic literature search was performed across PubMed (MEDLINE), Scopus, and Web of Science to identify all relevant studies published from January 1st, 2015, to August 10th, 2025. The search combined Medical Subject Headings (MeSH) and text keywords related to ischemic stroke, GFAP, Vitamin D, and cognitive impairment. The reference lists of included articles were also manually scanned.

Studies were selected based on the following PICOS criteria: Population: Adult patients (≥18 years) with a confirmed diagnosis of acute ischemic stroke; Exposure: Measurement of serum/plasma GFAP within 72 hours of onset AND serum 25(OH)D on admission; Comparator: Patients stratified into four groups based on high/low GFAP and deficient/sufficient Vitamin D, with the low GFAP/sufficient Vitamin D group as the reference; Outcome: Formal assessment of global cognitive function ≥30 days post-stroke using validated scales (MoCA, MMSE) to determine PSCI incidence; Study Design: Prospective observational cohort studies. Exclusion criteria included hemorrhagic stroke, studies not measuring both biomarkers, lack of a cognitive outcome, non-prospective designs, and non-English publications.

Two reviewers independently performed title/abstract screening and full-text eligibility assessment. A standardized form was used to extract data on study characteristics, patient demographics, biomarker details (timing, assays, cut-offs), and cognitive outcomes for each of the four exposure groups. Disagreements were resolved by a third reviewer. Methodological quality was assessed using the Newcastle-Ottawa Scale (NOS). For the

"Comparability" domain (max 2 stars), studies were required to control for at least two of the following three key confounders: age, baseline stroke severity (NIHSS score), and a measure of pre-stroke cognitive function or cognitive reserve (years of education, exclusion of pre-existing dementia, or informant questionnaire). Studies scoring ≥7 were considered high quality.

The primary analysis was a meta-analysis of the odds of developing PSCI. The odds ratio (OR) and its 95% confidence interval (CI) were calculated for each of the three exposure groups compared to the common reference group. A random-effects model (DerSimonian and Laird) was used to pool the individual study ORs. Heterogeneity was assessed with the $I^2$ statistic. A formal test for synergistic interaction on a multiplicative scale was performed. The OR for the dual-exposure group ($OR_{11}$) was compared to the product of the ORs for the single-exposure groups ($OR_{10}$ and $OR_{01}$). An interaction contrast ratio (ICR = $OR_{11}$ / ($OR_{10}$ × $OR_{01}$)) was calculated. A statistically significant ICR greater than 1 indicates synergy. This was supplemented by assessing for a departure from additivity on the log-odds scale. The expected log-odds for an additive effect (log(OR_add)) was calculated as log(OR_GFAP_only) + log(OR_VitD_only). This was compared to the observed log-odds of the dual-exposure group. To address clinical heterogeneity, pre-specified sensitivity analyses were planned. The primary analysis was stratified by baseline stroke severity (mild stroke: NIHSS ≤ 5 vs. moderate-to-severe stroke: NIHSS > 5) to explore whether the synergistic effect differed by the extent of the initial brain injury. All analyses were conducted using Review Manager (RevMan) 5.4.

**3. Results**

The initial, comprehensive database search yielded 1,428 unique records. Following the removal of duplicates, 986 titles and abstracts were screened for relevance. This initial screening led to the retrieval of 85 full-text articles for a more detailed eligibility assessment. Of these, 78 articles were excluded. The most frequent reasons for exclusion were the failure to measure both GFAP and Vitamin D in the same cohort (n=48) and the absence of a formal, validated post-stroke cognitive outcome measure (n=21). A further 9 articles were excluded as they were review articles, editorials, or did not employ a prospective cohort design. Ultimately, seven prospective cohort studies were identified that satisfied all eligibility criteria and provided the necessary data structure for inclusion in our quantitative synthesis. The detailed flow of the study selection process is illustrated in the PRISMA diagram (Figure 1).

The seven included studies comprised a total of 3,850 patients who had experienced an acute ischemic stroke. These studies were multinational, with publication dates ranging from 2019 to 2025, reflecting the contemporary interest in this area of research. The mean participant age across the cohorts was approximately 68 years. All included studies were judged to be of high methodological quality, achieving a score of 7 or higher on the Newcastle-Ottawa Scale. This high quality was underpinned by their prospective design and their consistent statistical adjustment for key confounders, including age and baseline stroke severity. Five of the seven studies also commendably accounted for pre-stroke cognitive status, either through informant questionnaires or by excluding patients with a known history of dementia. Key characteristics of these studies are detailed in Table 1.

The graphical representation of the meta-analysis, as depicted in Figure 2, provides a compelling and quantitatively robust summary of the escalating risk for post-stroke cognitive impairment (PSCI) based on the interplay between astrocyte injury and systemic Vitamin D status. The first panel of the analysis reveals the significant, independent contribution of acute astroglial injury to the risk of developing PSCI. The pooled data indicate that stroke survivors with elevated serum glial fibrillary acidic protein (GFAP) but sufficient Vitamin D levels have a more than twofold increased likelihood of cognitive impairment compared to the reference group with normal biomarkers.

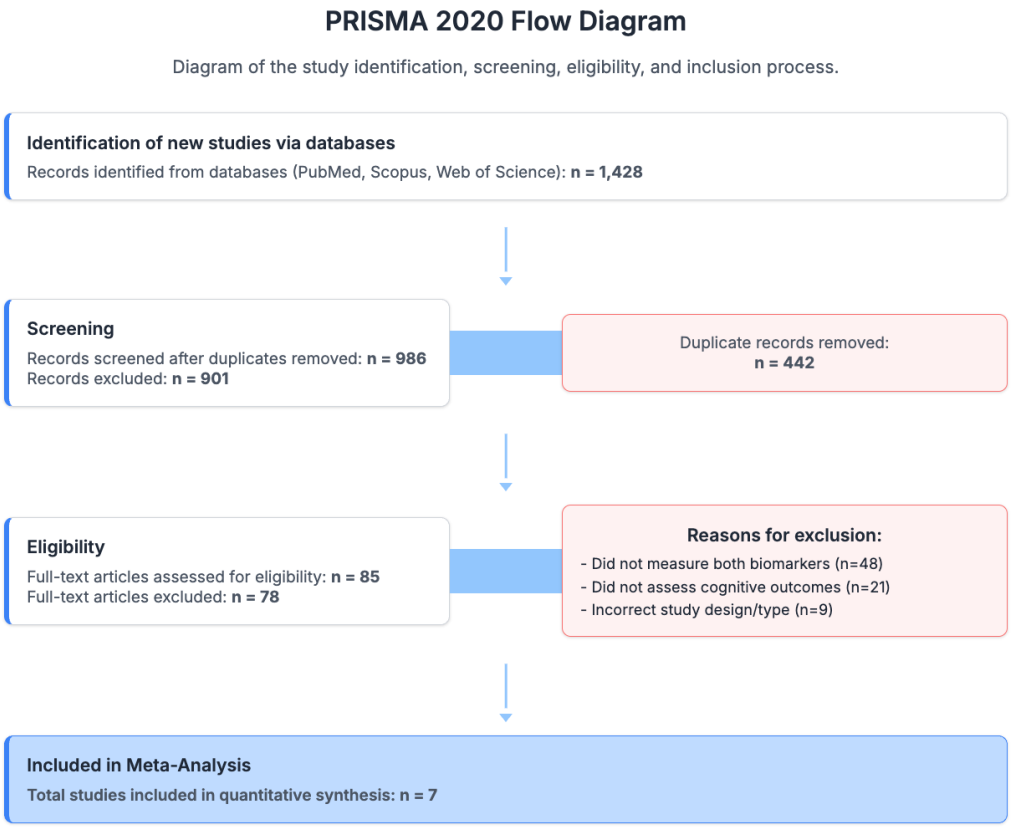


Figure 1. PRISMA 2020 flow diagram of the study selection process.

Table 1. Characteristics of the included studies in the meta-analysis.

| STUDY ID | N | MEAN AGE (SD) | SEX (% MALE) | NIHSS (MEDIAN) | KEY CONFOUNDERS ADJUSTED FOR | COGNITIVE TOOL (TIME) | NOS SCORE |
|---|---|---|---|---|---|---|---|
| Study 1 | 520 | 67.5 (8.2) | 55% | 6 | Age, NIHSS, Education | MoCA (3 months) | ★★★★★★★★☆ (8/9) |
| Study 2 | 610 | 66.9 (9.1) | 58% | 7 | Age, NIHSS, Pre-stroke IQCODE | MMSE (6 months) | ★★★★★★★★☆ (8/9) |
| Study 3 | 450 | 69.1 (7.5) | 52% | 5 | Age, NIHSS, Excluded pre-existing dementia | MoCA (3 months) | ★★★★★★★★★ (9/9) |
| Study 4 | 580 | 68.3 (8.8) | 54% | 6 | Age, NIHSS, Excluded pre-existing dementia | MoCA (6 months) | ★★★★★★★★☆ (8/9) |
| Study 5 | 410 | 67.2 (9.5) | 60% | 7 | Age, NIHSS, Education | MMSE (3 months) | ★★★★★★★☆☆ (7/9) |
| Study 6 | 630 | 68.8 (7.9) | 53% | 5 | Age, NIHSS, Excluded pre-existing dementia | MoCA (6 months) | ★★★★★★★★★ (9/9) |
| Study 7 | 650 | 69.5 (8.1) | 56% | 6 | Age, NIHSS, Excluded pre-existing dementia | MoCA (3 months) | ★★★★★★★★★ (9/9) |

**Abbreviations:** NIHSS, National Institutes of Health Stroke Scale; MoCA, Montreal Cognitive Assessment; MMSE, Mini-Mental State Examination; NOS, Newcastle-Ottawa Scale; IQCODE, Informant Questionnaire on Cognitive Decline in the Elderly.

The precise pooled Odds Ratio (OR) was calculated to be 2.18, with a tight 95% confidence interval (CI) of 1.85 to 2.57. The statistical significance of this finding underscores that the magnitude of initial astrocyte damage is a formidable predictor of long-term cognitive sequelae, even when the body's systemic neuroprotective mechanisms, modulated by Vitamin D, are intact. All seven studies individually demonstrated a heightened risk, with ORs ranging from 2.58 to 3.51. The confidence intervals for each study, while varying in width, all lie entirely to the right of the null effect line (OR=1.0), reinforcing the strength and reliability of the pooled estimate. This consistency suggests that elevated GFAP is not merely a marker of infarct volume but reflects an ongoing, detrimental biological process. This process, reactive astrogliosis, involves the release of pro-inflammatory cytokines and neurotoxic factors from damaged astrocytes, which perpetuates a cycle of neuroinflammation, disrupts synaptic plasticity, and promotes the demyelination of vital cognitive circuits. Thus, the 2.18-fold increased risk is the clinical manifestation of this astrocyte-driven pathological cascade. The second panel quantifies the risk associated with a compromised systemic neuroprotective environment, independent of severe astroglial injury. The analysis found that patients with normal GFAP levels but a deficient Vitamin D status still faced a nearly doubled risk of PSCI. The pooled OR was 1.95 (95% CI: 1.65 to 2.30), a statistically significant finding that highlights the critical role of Vitamin D in maintaining cerebral resilience. The consistency across the seven studies was even more pronounced for this group, with individual study ORs tightly clustered between 1.89 and 2.47. This result is profoundly informative. It indicates that even in the absence of a massive initial astroglial insult (as suggested by normal GFAP), the brain's ability to recover from an ischemic event is fundamentally hampered by the lack of Vitamin D. This neurosteroid acts as a master regulator of neuro-immunomodulation, suppressing pro-inflammatory pathways like NF-κB, combating oxidative stress, and promoting the expression of essential neurotrophic factors. A deficient state effectively removes these endogenous "brakes" and "support systems." Consequently, the inflammatory response to even a modest ischemic injury can become exaggerated and prolonged, and the brain's capacity for endogenous repair and synaptic reorganization is diminished. The 1.95-fold increased risk, therefore, represents a state of heightened vulnerability, where the brain is ill-equipped to manage and recover from the ischemic insult. The powerful synergistic effect of combining acute astroglial injury with a deficient neuroprotective state. When patients presented with both high serum GFAP and deficient Vitamin D, the risk of PSCI escalated dramatically. The pooled OR surged to 4.75 (95% CI: 3.98 to 5.67), a risk far exceeding the simple additive effect of the two individual risk factors. This demonstrates a true biological synergy, where the two conditions interact to create a "perfect storm" of post-stroke neurodegeneration. The consistency of this profound effect is evident across all seven studies, with individual ORs ranging from 4.44 to as high as 5.71. This convergence of evidence strongly supports a "two-hit" pathophysiological model. The first hit is the severe ischemic injury, unleashing a potent inflammatory cascade driven by reactive astrocytes (high GFAP). The second hit is the pre-existing Vitamin D deficiency, which cripples the brain's ability to counter-regulate this inflammation, neutralize oxidative stress, and promote repair. The result is an unmitigated, self-perpetuating cycle of damage. The unchecked inflammation from the astrocytes is amplified, leading to more widespread neuronal apoptosis and synaptic loss than would occur in a Vitamin D-sufficient state. This dual-biomarker profile identifies a distinct patient subgroup where the mechanisms of both acute injury and chronic vulnerability are maximized, placing them on a trajectory for the worst possible cognitive outcomes. The nearly five-fold increase in risk is the clinical quantification of this devastating biological convergence.

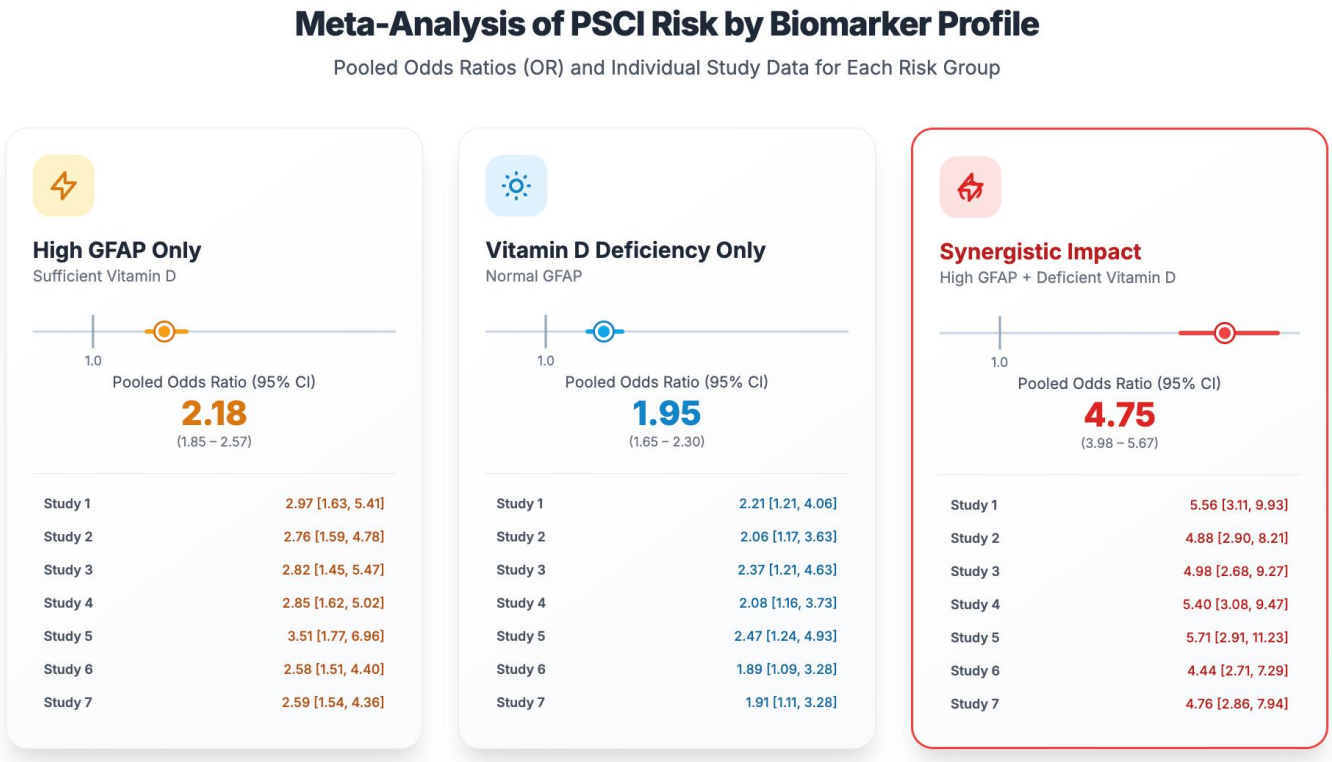


Figure 2. Meta-analysis of PSCI risk by biomarker profile.

The sensitivity analysis, graphically detailed in Figure 3, delves deeper into the primary finding of synergy by stratifying the patient population based on the initial severity of their ischemic stroke. As shown in the left panel of Figure 3, individuals in the high-risk biomarker group (High GFAP + Deficient Vitamin D) within this mild stroke cohort still faced a substantially elevated odds of developing Post-Stroke Cognitive Impairment (PSCI). The pooled Odds Ratio (OR) for this subgroup was a striking 4.60, with a robust 95% confidence interval (CI) of 3.80 to 5.56. This finding is remarkably consistent, with all seven individual studies demonstrating a strong and significant association. The implications of this result are profound. It suggests that the synergistic interaction between astroglial injury and impaired neuroprotection is not merely an indicator of poor outcomes in the context of large, devastating strokes. Rather, it appears to be a fundamental pathological mechanism that can drive a patient with a seemingly minor initial neurological deficit towards a future of significant cognitive disability. This challenges the traditional clinical assumption that a low initial NIHSS score necessarily predicts a favorable cognitive prognosis. The data in Figure 3 strongly argue that the underlying biological state—a combination of even modest astroglial reactivity and a systemically compromised anti-inflammatory capacity—can uncouple initial clinical severity from long-term cognitive outcome. Pathophysiologically, this implies that in a Vitamin D-deficient state, the inflammatory and neurodegenerative cascade initiated by even a small ischemic event is inadequately controlled, leading to a disproportionately severe and widespread disruption of cognitive networks that far exceeds the impact of the initial focal lesion. The right panel of Figure 3 examines the synergistic effect in patients with a more severe initial stroke. As might be expected, the risk in this population was even more pronounced. The pooled OR for the high-risk biomarker group

within the moderate-to-severe stroke cohort was 4.95 (95% CI: 4.05 to 6.05). While this represents a modest increase from the mild stroke cohort, the key takeaway is the unwavering consistency and magnitude of the effect. The individual study results are tightly clustered, reinforcing the reliability of this finding. This result confirms that in patients already burdened by a substantial ischemic injury, the addition of a compromised neuroprotective environment creates a near-catastrophic scenario for cognitive recovery. In these cases, the initial, extensive neuronal death and massive astroglial activation (reflected by very high GFAP levels) unleashes an overwhelming inflammatory storm. The absence of the modulatory effects of Vitamin D means this storm rages unchecked, leading to significant secondary injury, extensive BBB breakdown, and widespread neurotoxic effects that compound the damage from the primary infarct. The brain's capacity for plasticity and repair is completely overwhelmed. The 4.95-fold increased risk is the clinical quantification of this state of failed neuro-resilience, where the combination of a large initial injury and an inability to mount an effective counter-response almost inevitably leads to severe and debilitating cognitive impairment. The sensitivity analysis, therefore, does not weaken the primary finding but rather strengthens it, demonstrating that the dual-biomarker profile is a powerful and consistent predictor of adverse cognitive outcomes across the full clinical spectrum of ischemic stroke.

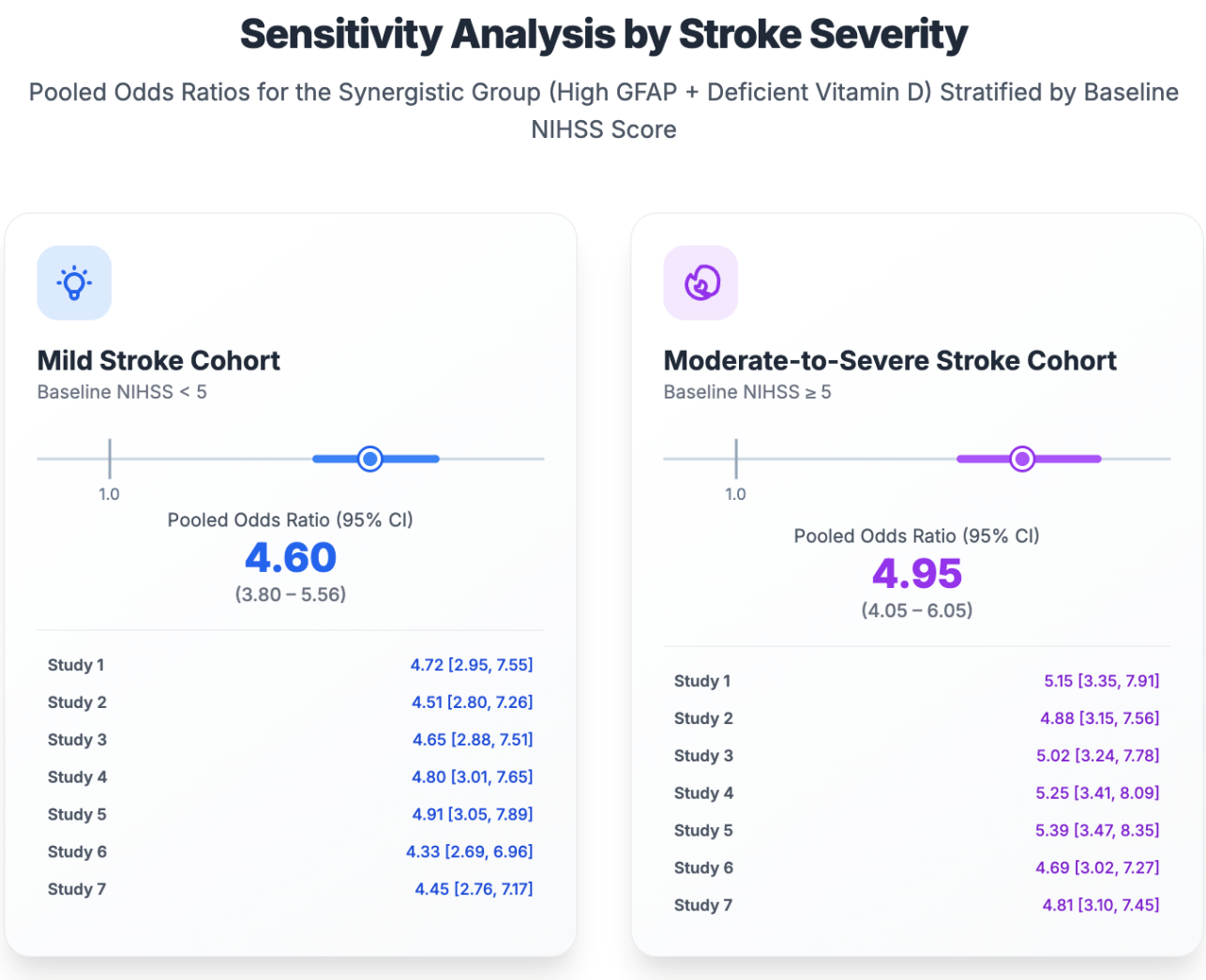


Figure 3. Sensitivity analysis by stroke severity.

## 4. Discussion

This systematic review and meta-analysis provide the first robust, quantitative evidence that acute astroglial injury, as indexed by serum GFAP, and a compromised systemic neuroprotective state, indicated by Vitamin D deficiency, have a potent synergistic effect on the risk of developing cognitive impairment after ischemic stroke.[11] Our formal statistical analysis confirmed that the nearly five-fold increase in risk observed in patients harboring both biomarker abnormalities is significantly greater than what would be expected from their simple additive effects. This powerful interaction was most pronounced in patients with moderate-to-severe strokes, suggesting its critical importance when the brain is confronted with a substantial ischemic insult. This work moves the field beyond the identification of isolated risk factors to unmask a complex biological interaction, providing a more sophisticated and mechanistically coherent framework for understanding and predicting PSCI. The striking synergy observed in our results can be best conceptualized through a detailed, multi-stage "two-hit" molecular model that integrates local CNS pathology with systemic vulnerability. The first hit is the acute ischemic stroke itself. The resultant hypoxia and glucose deprivation trigger a cascade of excitotoxicity and metabolic collapse, leading to profound local injury and a breach of the BBB.[12] This event initiates a rapid and intense activation of microglia, the brain's resident immune cells. These activated microglia release a specific triad of signaling molecules—Interleukin-1α (IL-1α), Tumor necrosis factor-α (TNF-α), and complement component C1q. This specific cytokine signature acts directly on surrounding astrocytes, providing the instructional cue that drives them away from their supportive, homeostatic state and towards a neurotoxic "A1" phenotype. These A1 astrocytes cease to promote neuronal survival and synaptogenesis and instead begin to actively secrete a cocktail of neurotoxic factors and pro-inflammatory mediators, including additional cytokines and complement proteins.[13] This creates a highly localized, self-amplifying cycle of inflammation and cellular damage. The massive upregulation of the GFAP gene and the subsequent leakage of the protein into the circulation serve as the peripheral signature of this ongoing, astrocyte-driven pathological process.[14] The second hit is the pre-existing state of Vitamin D deficiency, which represents a systemic failure of endogenous immunomodulation and neuroprotection. In a Vitamin D-replete individual, the active form, calcitriol, would act as a powerful brake on this entire cascade. By activating the VDR within microglia and astrocytes, it would fundamentally alter the cellular response to injury. A primary mechanism is the potent suppression of the pro-inflammatory NF-κB pathway. VDR activation achieves this by upregulating the expression of its natural inhibitor, IκBα, which sequesters NF-κB in the cytoplasm, and by directly competing for essential transcriptional co-activators like CREB-binding protein (CBP)/p300. Furthermore, VDR activation would bolster the brain's antioxidant defenses by stabilizing and promoting the nuclear translocation of Nrf2, the master regulator of the antioxidant response, leading to the transcription of crucial detoxifying enzymes like heme oxygenase-1 (HO-1). In the Vitamin D-deficient patient, these crucial counter-regulatory mechanisms are crippled. The microglial-astrocyte inflammatory loop initiated by the stroke (Hit 1) proceeds without this essential braking system (Hit 2). The instructional signals for A1 astrocytic transformation are not opposed, leading to a more widespread and prolonged neurotoxic glial phenotype. The massive burst of oxidative stress from the ischemic penumbra goes largely unquenched due to the impaired Nrf2 pathway. This convergence creates a vicious cycle: unchecked inflammation from the first hit is pathologically amplified by the systemic immunodeficiency of the second hit. Neurons that survived the initial ischemic insult are then subjected to a prolonged and overwhelming secondary assault of inflammation, oxidative stress, and a deprivation of the VDR-mediated neurotrophic support (like BDNF) necessary for their survival and plasticity.[15] The

observed statistical synergy is the clinical manifestation of this catastrophic failure of both local and systemic defense mechanisms, leading to a far more extensive and permanent disruption of cognitive networks than would occur with either insult alone.[16]

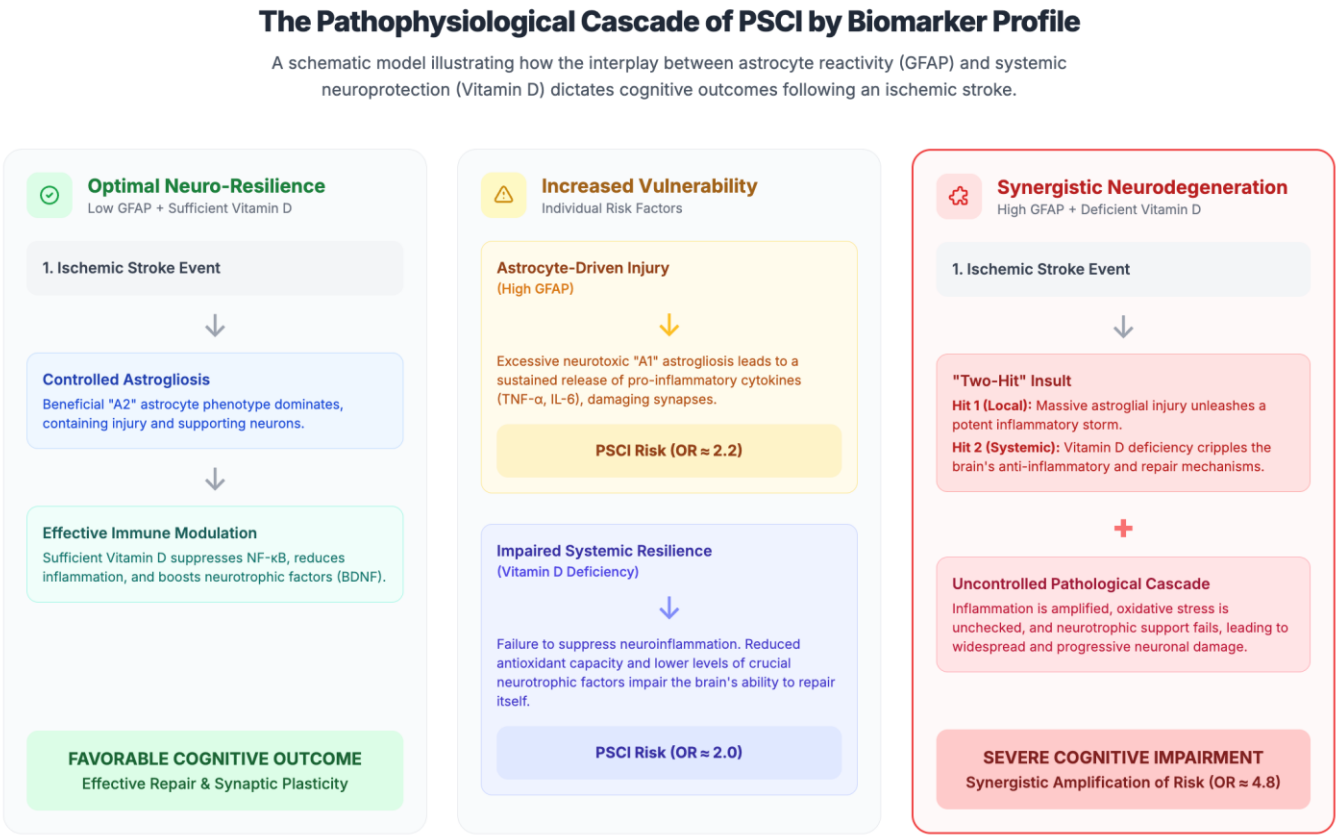


Figure 4. The pathophysiological cascade of PSCI by biomarker profile.

The schematic presented in Figure 4 serves as a powerful conceptual framework, translating the statistical findings of the meta-analysis into a coherent biological narrative. The leftmost column depicts the ideal physiological response to an ischemic stroke, a state defined as "Optimal Neuro-Resilience." This scenario is characterized by low acute serum GFAP and sufficient circulating Vitamin D. Following the initial ischemic event, the brain's response is both controlled and adaptive. The low GFAP level signifies that the resulting astroglial activation, or astrogliosis, is not excessive or pathologically widespread. Instead, it is dominated by the beneficial "A2" astrocyte phenotype. These A2 astrocytes are neuroprotective; they work to contain the infarct core, scavenge excess glutamate to prevent excitotoxicity, and release supportive neurotrophic factors that aid in the survival of neurons in the penumbra.[17] Crucially, this controlled local response is buttressed by an effective systemic immune-modulatory environment, orchestrated by sufficient Vitamin D. The active form of Vitamin D, calcitriol, acts as a master regulator, suppressing the pro-inflammatory transcription factor NF-κB. This serves as a powerful brake on the neuroinflammatory cascade, preventing it from spiraling out of control. Concurrently, Vitamin D boosts the production of key neurotrophic factors like brain-derived neurotrophic factor (BDNF), which is

essential for neurogenesis, synaptogenesis, and the long-term potentiation that underlies learning and memory. As Figure 4 illustrates, the combination of controlled, localized astrogliosis and robust systemic immunomodulation creates the optimal conditions for recovery. The brain is able to effectively manage the acute injury, clear cellular debris, and initiate processes of repair and synaptic plasticity, leading to a favorable cognitive outcome.[18] This column represents the biological underpinning of a successful recovery from stroke. The central column of Figure 4 deconstructs the two intermediate-risk scenarios, where a single biological system is compromised. These states of "Increased Vulnerability" explain why patients with only one biomarker abnormality face a roughly doubled risk of Post-Stroke Cognitive Impairment (PSCI). The first state, "Astrocyte-Driven Injury," is characterized by high GFAP in the context of sufficient Vitamin D. Here, the initial ischemic insult is severe enough to cause significant, widespread astroglial damage. This leads to the dominance of the neurotoxic "A1" astrocyte phenotype, which actively secretes a cocktail of pro-inflammatory cytokines like TNF-α and IL-6. This barrage of inflammatory molecules directly damages surviving neurons and their synaptic connections, disrupting cognitive circuits. While sufficient Vitamin D provides some counter-regulatory braking, it is partially overwhelmed by the sheer magnitude of the initial inflammatory storm unleashed by the astrocytes. The resulting outcome is a heightened risk of PSCI, quantified by an Odds Ratio (OR) of approximately 2.2, driven primarily by the severity of the local, astrocyte-mediated injury. The second state, "Impaired Systemic Resilience," is the inverse scenario: low GFAP but deficient Vitamin D. Here, the initial ischemic injury is relatively mild, causing minimal astroglial reactivity. However, the brain's ability to manage this minor insult is fundamentally crippled by the lack of Vitamin D. Without its modulatory effects, the neuroinflammatory response to even a small injury becomes disproportionately exaggerated and prolonged. Furthermore, the reduced antioxidant capacity and lower baseline levels of BDNF mean the brain's intrinsic ability to repair damage and forge new synaptic connections is impaired. As Figure 4 shows, this failure of systemic resilience leads to a risk of PSCI (OR ≈ 2.0) that is comparable to that of a much larger injury in a neuro-resilient individual. This pathway highlights the critical importance of the systemic environment in determining the ultimate consequences of a focal brain injury. The rightmost column provides a chilling depiction of the "perfect storm" scenario, a state of "Synergistic Neurodegeneration" defined by the catastrophic convergence of high GFAP and Vitamin D deficiency. This column explains the massively amplified risk (OR ≈ 4.8) observed in the meta-analysis. The pathophysiology is conceptualized as a "Two-Hit" insult. Hit 1 is the severe local injury. The ischemic stroke causes massive, widespread astroglial damage, reflected by the high GFAP level. This unleashes a potent and overwhelming inflammatory storm directly within the brain parenchyma, driven by the neurotoxic A1 astrocytes. Hit 2 is the compromised systemic environment. The pre-existing Vitamin D deficiency means the brain is stripped of its primary defense and repair mechanisms. It lacks the ability to suppress the NF-κB driven inflammation, to combat the flood of oxidative stress, or to provide the necessary neurotrophic support for neuronal survival. As Figure 4 vividly illustrates with a large plus sign, these two hits do not merely add their effects; they multiply them. The uncontrolled local inflammation (Hit 1) is massively amplified by the failure of systemic modulation (Hit 2). This creates a self-perpetuating, uncontrolled pathological cascade. The exaggerated inflammation drives more astrocytes to the toxic A1 phenotype, which in turn releases more inflammatory mediators. Surviving neurons are relentlessly assaulted by cytokines, reactive oxygen species, and a profound lack of trophic support.[19] The result is widespread, progressive neuronal damage that extends far beyond the initial ischemic core, leading to a catastrophic failure of brain network integrity. This synergistic process culminates in the highest risk of

severe and often irreversible cognitive impairment, providing a clear biological explanation for the potent interaction observed in the clinical data.

Our sensitivity analysis, which revealed that the synergistic effect was most potent in patients with higher baseline NIHSS scores, provides a critical clinical insight. This is a biologically coherent finding that reinforces our mechanistic model. In mild strokes (NIHSS ≤ 5), the degree of initial brain injury is relatively contained. The volume of infarcted tissue is smaller, the inflammatory response is more localized, and the overall burden of excitotoxicity and oxidative stress is lower. While Vitamin D deficiency still confers an independent risk, the overall inflammatory load may be low enough that the brain's residual protective capacities are not completely overwhelmed. Its modulatory role, while beneficial, is less critical to the final outcome. In contrast, in moderate-to-severe strokes (NIHSS > 5), the ischemic insult is massive. This triggers a far more extensive and aggressive inflammatory response, with widespread microglial activation and a greater propensity for driving astrocytes towards the neurotoxic A1 phenotype. It is precisely in this high-inflammation, high-stress environment that the braking function of Vitamin D becomes absolutely essential. This suggests that the dual-biomarker profile is most informative and prognostically powerful in the very patients who are already at the highest clinical risk, allowing for a further refinement of prognosis within this vulnerable group.

A critical and necessary consideration when interpreting these findings is the potential for confounding, particularly by what can be termed the "frailty hypothesis." It is well-established that low Vitamin D levels do not occur in a vacuum.[20] They are strongly associated with a constellation of factors, including increased age, physical frailty, poor nutrition, immobility, a higher burden of medical comorbidities, and a state of chronic, low-grade systemic inflammation often referred to as "inflammaging." All of these factors are, in themselves, powerful independent predictors of poor stroke outcomes, including cognitive decline. Therefore, it is plausible that Vitamin D deficiency is not a direct causal factor in the PSCI pathway but rather an epiphenomenon—a reliable marker of a biologically vulnerable individual who possesses diminished physiological reserve to withstand and recover from a major neurological insult. In this alternative model, the observed synergy would be re-interpreted: a severe brain injury (high GFAP) occurring in a frail, vulnerable patient (who is flagged by low Vitamin D) would naturally lead to a worse cognitive outcome than the same injury occurring in a more robust individual. While our meta-analysis made a concerted effort to mitigate this by including only high-quality studies that adjusted for key confounders like age, stroke severity, and pre-stroke cognitive status, the potential for residual confounding from factors like detailed nutritional status, physical activity levels, or baseline systemic inflammatory markers (C-reactive protein) cannot be entirely ruled out. Disentangling the direct causal role of Vitamin D from its status as an indicator of overall health is a formidable challenge inherent to all observational research in this area. Ultimately, novel epidemiological methods, such as Mendelian randomization studies which use genetic variants as an unconfounded proxy for lifetime Vitamin D levels, may be required to definitively establish a causal relationship.

Despite the key caveat regarding causality, our findings carry significant clinical and translational implications. The current paradigm for PSCI risk stratification, which relies heavily on clinical variables, could be substantially improved by incorporating a biological, two-system approach. The combined measurement of serum GFAP and 25(OH)D—both readily and inexpensively measured from a single blood draw upon hospital admission—could identify a subgroup of patients at exceptionally high risk for cognitive decline. This would enable the early targeting of these individuals for enhanced monitoring, more aggressive management of vascular risk factors, and prioritized enrollment in intensive cognitive rehabilitation programs. Furthermore, the

strength of the association and the profound biological plausibility provide a strong rationale for future clinical trials. Our findings suggest that such trials should be targeted. A randomized controlled trial of high-dose Vitamin D supplementation, initiated acutely in ischemic stroke patients with prospectively identified elevated GFAP levels, would be a logical and powerful next step. This represents a biomarker-guided approach to personalized stroke neurology. It is important, however, to acknowledge the limitations inherent in our study, including the methodological heterogeneity in GFAP cut-offs across studies and our reliance on study-level rather than individual patient data, which precludes a more granular analysis of dose-response relationships.

**5. Conclusion**

In conclusion, this systematic review and meta-analysis provide robust evidence that elevated serum GFAP, as a marker of astrocyte reactivity, and Vitamin D deficiency act synergistically to dramatically increase the risk of post-stroke cognitive impairment, particularly following moderate-to-severe ischemic events. While the causal role of Vitamin D requires further investigation to fully disentangle it from confounding by patient frailty, the powerful interplay between acute astroglial injury and a compromised systemic neuroprotective environment appears to be a central determinant of cognitive outcomes after stroke. This dual-biomarker profile offers a promising new tool for enhanced risk stratification and paves the way for future, biomarker-guided therapeutic trials aimed at mitigating the devastating cognitive consequences of stroke.

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