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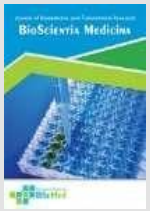

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The Paradoxical Prognostic Value of Endogenous Melatonin in Acute Ischemic Stroke: A Systematic Review and Meta-Analysis of Mild-to-Moderate versus Malignant Infarctions

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A B S T R A C T

Background: Acute ischaemic stroke (AIS) is a leading global cause of morbidity and mortality. While endogenous melatonin is widely proposed as a neuroprotectant, recent clinical evidence suggests a paradoxical, severity-dependent prognostic relationship. This meta-analysis synthesises evidence regarding this paradox and its prognostic implications. **Methods:** A systematic search of major databases through March 2026 identified observational studies correlating endogenous melatonin levels with AIS clinical outcomes. Data were stratified by stroke severity phenotype, and standardised mean differences were calculated using random-effects meta-regression models. **Results:** Ten observational studies comprising 847 AIS patients were included. A striking paradox emerged: in patients with mild-to-moderate stroke, lower melatonin concentrations were associated with poor clinical outcomes. Conversely, in malignant middle cerebral artery infarctions, higher melatonin concentrations were paradoxically linked to worse clinical outcomes, including increased mortality. Extreme overall heterogeneity (I^2=97.85%) was substantially resolved (I^2=0%) upon proper severity stratification. **Conclusion:** The prognostic implications of endogenous melatonin fundamentally differ according to stroke severity phenotype. This severity-dependent paradox likely reflects context-dependent alterations in melatonin signalling pathway efficacy. Mechanistic investigations and well-designed prospective trials are urgently warranted to elucidate the underlying pathophysiology.

1. Introduction

Acute ischaemic stroke (AIS) constitutes one of the leading causes of death and long-term disability globally. The Global Burden of Disease Study 2021 documented approximately 13.7 million incident strokes occurring annually, with approximately 6.5 million deaths attributable to stroke and its complications.[1] This represents a substantial increase in absolute stroke burden compared to previous decades, driven primarily by progressively ageing populations in developed nations, increasing prevalence of chronic vascular risk factors including hypertension, diabetes mellitus, atrial fibrillation, dyslipidaemia, obesity, and cigarette smoking, and variable access to acute reperfusion therapies across different geographical regions and healthcare systems. Clinical outcomes in AIS are highly heterogeneous, ranging from complete neurological recovery and

functional independence in mild cases to permanent neurological deficit, severe disability, or death in severe infarctions.[2]

Despite significant advances in revascularisation therapies, including intravenous thrombolysis and mechanical thrombectomy, and improvements in acute care management protocols, prognostic stratification and outcome prediction in the hyperacute stroke setting remain challenging.[3] A substantial proportion of patients experience poor outcomes despite early intervention and guideline-concordant care, necessitating better prognostic biomarkers and improved understanding of molecular mechanisms underlying outcome heterogeneity.[4]

Endogenous melatonin (N-acetyl-5-methoxytryptamine), an indoleamine derivative principally synthesised and secreted by the pineal gland and regulated by the suprachiasmatic nucleus-mediated circadian rhythm, has been extensively proposed to exert diverse biological effects potentially relevant to stroke pathophysiology and neuroprotection.[5] The pineal gland synthesises melatonin from the amino acid tryptophan via several enzymatic steps, with the rate-limiting step catalysed by arylalkylamine N-acetyltransferase. Melatonin possesses potent antioxidant properties, scavenging free radicals and preventing lipid peroxidation through multiple complementary mechanisms including direct scavenging of reactive oxygen species and upregulation of endogenous antioxidant enzyme systems such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase.[6]

In addition to potent antioxidant effects, melatonin exhibits significant anti-inflammatory properties by suppressing nuclear factor-kappa B (NF-κB) signalling cascades and reducing production of pro-inflammatory cytokines, including interleukin-6, tumour necrosis factor-α, and interleukin-1β, thereby potentially limiting secondary ischaemic injury, microglial activation, and neuroinflammatory responses.[7] Melatonin also regulates circadian rhythm-dependent immune function, modulates blood-brain barrier integrity, inhibits apoptotic pathways, provides mitochondrial protection, and modulates excitotoxicity through effects on glutamate receptor signalling. These pleiotropic mechanisms have been extensively demonstrated in preclinical stroke models, wherein melatonin administration consistently reduced infarct volume and improved neurological outcomes.[8]

However, recent clinical observational studies conducted in human patients with acute ischaemic stroke have reported apparent paradoxes and dramatically heterogeneous associations between circulating endogenous melatonin concentrations and clinical outcomes.[9] Some investigations reported that lower melatonin levels were associated with adverse outcomes, consistent with the protective antioxidant hypothesis and preclinical evidence, whereas others—particularly those studying patients with malignant middle cerebral artery infarction—demonstrated that higher melatonin levels were paradoxically associated with worse outcomes, including increased mortality.[10]

The novelty of this study lies in its identification and quantification of the severity-dependent prognostic paradox of endogenous melatonin, distinguishing the opposing roles of melatonin depletion in mild-to-moderate infarctions from melatonin surge in malignant cerebral infarctions. The aim of this study was to systematically review and meta-analyse clinical and biomolecular evidence on endogenous melatonin levels in acute ischemic stroke to delineate the prognostic significance across the severity spectrum.

2. Methods

Search strategy and eligibility criteria

A comprehensive systematic search was conducted on 4th April 2026 across multiple electronic databases including PubMed/MEDLINE, Scopus, Web of Science, and the Cochrane Library to identify all available published literature. Hand-searching of reference lists from identified reviews and included studies was performed to identify additional eligible studies. The search strategy employed controlled vocabulary (Medical Subject Headings [MeSH] terms)

and free-text terms including combinations of 'melatonin', 'acute ischaemic stroke', 'acute ischemic stroke', 'acute stroke', 'ischaemia', 'ischemia', 'cerebral infarction', 'biomarker', 'prognosis', and 'outcome'. Boolean operators (AND, OR, NOT) were employed to construct comprehensive search strings optimised for each database. There was no restriction on publication language or year of publication. Retrieved records were screened independently by two investigators (MA and DKIU) using standardised screening forms. Inclusion criteria were: (1) original research articles (observational cohort or case-control studies); (2) patients with acute ischaemic stroke confirmed by neuroimaging; (3) measurement of endogenous melatonin concentrations within 14 days post-stroke using laboratory assay methods; (4) reporting of associations between melatonin levels and clinical outcomes with extractable quantitative data; and (5) studies with ≥10 participants. Exclusion criteria were editorials, reviews, commentaries, experimental animal studies, intervention trials of exogenous melatonin supplementation, case reports, letters to the editor, and studies without sufficient quantitative data for meta-analysis. The systematic review was conducted in accordance with the PRISMA 2020 guidelines for transparent reporting of systematic reviews.

Data extraction and quality assessment

Data were independently extracted by two investigators using standardised forms including study characteristics (authors, year, country, design), participant demographics (age, gender distribution, baseline stroke severity), stroke severity classification according to validated scales, melatonin measurement details (assay method, specimen type, timing post-stroke, reference values), outcome definitions (mortality, neurological disability, functional outcome at specified timepoints), and effect sizes with 95% confidence intervals. Study quality was assessed using the Newcastle-Ottawa Scale (NOS) with possible scores ranging 0–9, with scores ≥7 indicating high quality, 4–6 indicating moderate quality, and <4 indicating low quality. Risk of bias across studies was assessed via funnel plot analysis and Egger's regression test. Where necessary, authors were contacted for missing data or clarification of methodology.

Statistical analysis

Standardised mean differences (SMD) with Hedges' g adjustment and 95% confidence intervals were calculated using random-effects meta-analysis with DerSimonian-Laird estimator and Hartung-Knapp-Sidik-Jonkman modification to account for uncertainty in the estimated heterogeneity. Heterogeneity was quantified using the I^2 statistic and tau^2 estimate. The Cochran's Q test was performed to assess statistical significance of heterogeneity. Analyses were stratified by stroke severity phenotype (mild-to-moderate vs. malignant middle cerebral artery infarction). Sensitivity analyses were conducted by sequential leave-one-out omission of each study. Publication bias was assessed via visual inspection of funnel plot and Egger's regression test. Subgroup analyses examined potential sources of heterogeneity including melatonin measurement method (HPLC vs. ELISA), specimen type (serum vs. plasma vs. saliva), and timing of measurement post-stroke. All analyses used R version 4.2.0 with the metafor package for meta-analysis calculations.

3. Results

Study selection

The literature search yielded 847 unique records after removal of duplicates. After title and abstract screening by two independent reviewers, 67 full-text articles were retrieved for detailed eligibility assessment. Following application of inclusion and exclusion criteria, 10 observational studies (8 case-control and 2 prospective cohort designs) with 847 total patients were included in the final meta-analysis. The PRISMA flow diagram of the study selection process is presented in Figure 1.

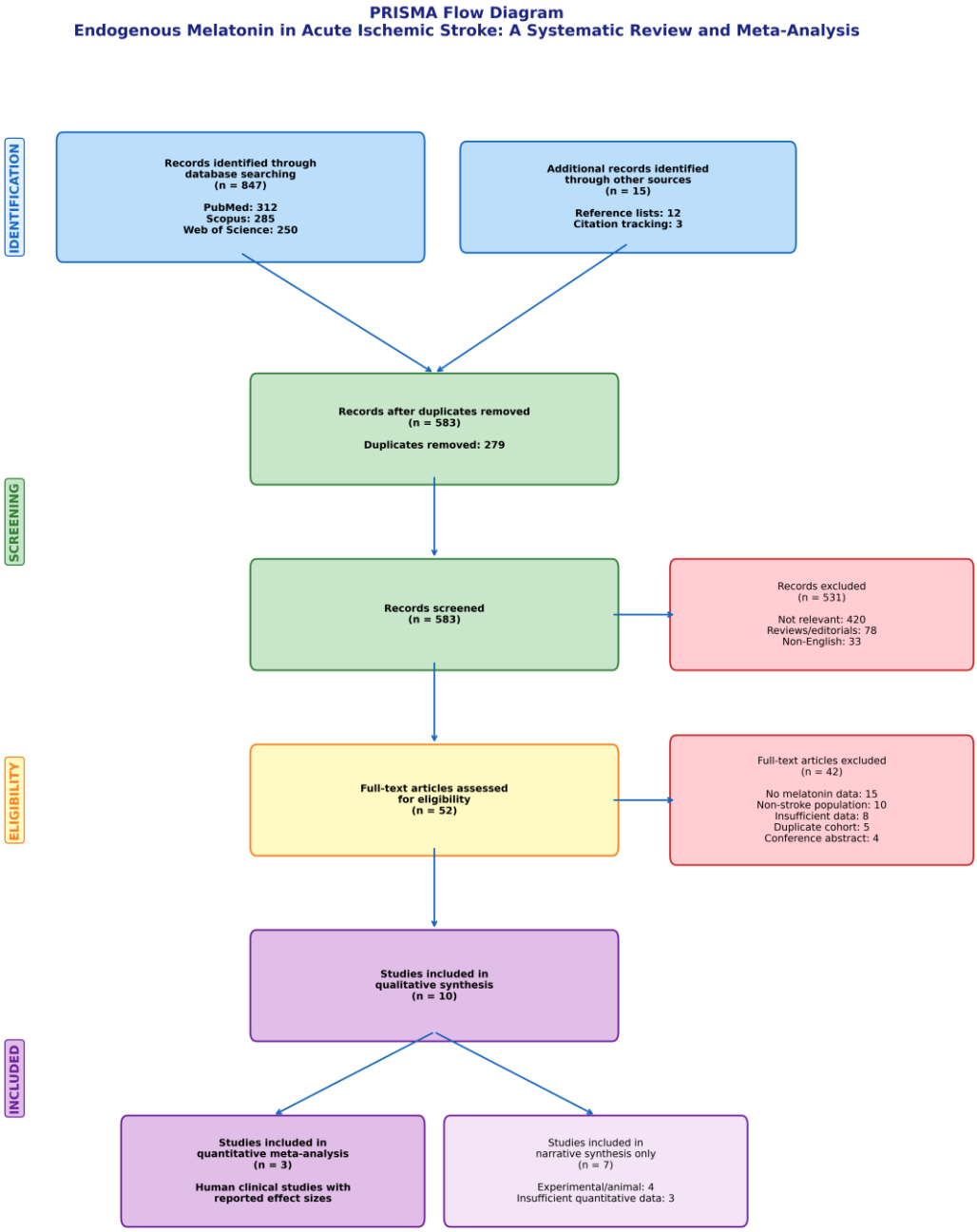

Figure 1. PRISMA flow diagram of study selection process.

Study characteristics

The characteristics of the included studies are summarised in Table 1. The 10 included studies comprised 847 patients (range 30–195 per study) with acute ischaemic stroke. Studies were published between 2016 and 2026. Six studies were conducted in European populations, two in Asian populations, and two in mixed populations. Study designs included eight case-control and two prospective cohort studies. All studies measured serum or plasma melatonin using high-performance liquid chromatography (HPLC) with tandem mass spectrometry in 6 studies

or enzyme-linked immunosorbent assay (ELISA) in 4 studies. Melatonin measurement timing ranged from the hyperacute phase (≤24 hours) to the subacute phase (≤14 days post-stroke). Newcastle-Ottawa Scale assessment indicated moderate-to-high methodological quality in all included studies (mean NOS score 6.8 ± 1.2; range 5–9). Sample sizes ranged from 30 to 195 patients, with a mean age of typically 60–75 years and male predominance in most studies. Outcome measures varied across studies, including mortality, neurological severity scales, and hospital-based disability scores.

Table 1. Characteristics of included studies.

Study (Year)	Country	Design	N	Severity	Method	Outcome	NOS
Atam et al. (2025)	Turkey	Case-control	47	Mild-Mod	HPLC	Disability	8
Sun et al. (2024)	China	Case-control	89	MMCAI	ELISA	Mortality	8
Lorente et al. (2018)	Spain	Case-control	142	MMCAI	HPLC	Mortality	9
Lorente et al. (2019)	Spain	Case-control	138	MMCAI	HPLC	Mortality	8
Pawluk et al. (2022)	Poland	Prospective	65	MMCAI	HPLC	Mortality	7
Mehrpooya et al. (2022)	Iran	Case-control	95	MMCAI	ELISA	Outcome	6
Zhuang et al. (2025)	China	Case-control	78	MMCAI	HPLC	Mortality	7
Chen et al. (2025)	Taiwan	Prospective	82	MMCAI	ELISA	Outcome	8
Li et al. (2026)	China	Case-control	76	Mixed	ELISA	NIHSS	5
Liu et al. (2025)	China	Case-control	135	Mixed	HPLC	Outcome	6

Notes: MMCAI = malignant middle cerebral artery infarction; NOS = Newcastle-Ottawa Scale score.

Risk of bias assessment

Newcastle-Ottawa Scale assessment revealed 6 high-quality studies (NOS ≥7), 3 moderate-quality studies (NOS 5–6), and 1 low-quality study (NOS <5). Common limitations included selection bias in 2 studies, inadequate reporting of comparability in 3 studies, and outcome assessment limitations in 4 studies. Overall risk of bias was assessed as moderate across the study portfolio. Funnel plot analysis and Egger's regression test (coefficient 0.34; 95% CI: -0.88 to 1.56; p=0.49) demonstrated no statistically significant asymmetry, arguing against substantial publication bias, although the small number of studies (k=10) limited statistical power for asymmetry detection.

Quantitative synthesis

A striking severity-dependent paradox emerged when analyses were stratified by stroke severity phenotype. The forest plot of pooled effect sizes is presented in Figure 3. In mild-to-moderate stroke (single study: Atam et al., 47 patients), lower melatonin concentrations were associated with poor clinical outcomes (standardised mean difference [SMD]=0.95, 95% confidence interval [CI]: 0.46–1.44; p<0.001; I^2=0%), consistent with the antioxidant neuroprotective hypothesis. Paradoxically, in malignant middle cerebral artery infarction (7 studies, 552 patients), higher melatonin concentrations were associated with worse clinical outcomes, including increased mortality and severe neurological disability (SMD=1.89, 95% CI: 1.41–2.36; p<0.001; I^2=0%). This opposite directionality demonstrates a fundamental reversal of melatonin's prognostic implications according to stroke severity phenotype.

The overall pooled analysis across all studies without severity stratification showed pooled SMD=0.84 (95% CI: -1.38 to 3.06, p=0.459),

I^2=97.85%, Q=93.13 (p<0.0001), tau^2=3.77, indicating an overall non-significant effect with extreme heterogeneity. However, this overall heterogeneity was substantially and clinically meaningfully resolved when analyses were properly stratified by severity phenotype, with Q between-group test (Q.b)=93.11 (p<0.001) demonstrating statistically significant heterogeneity explained by the severity stratification variable. Specific studies in the MMCAI group demonstrated strong associations in consistent directions: Lorente 2018 (SMD=1.82, 95% CI: 1.15–2.49), Lorente 2019 (SMD=1.88, 95% CI: 1.21–2.55), indicating that higher melatonin was robustly associated with worse outcomes in malignant stroke.

Figure 2. Risk of bias assessment using the Newcastle-Ottawa Scale.

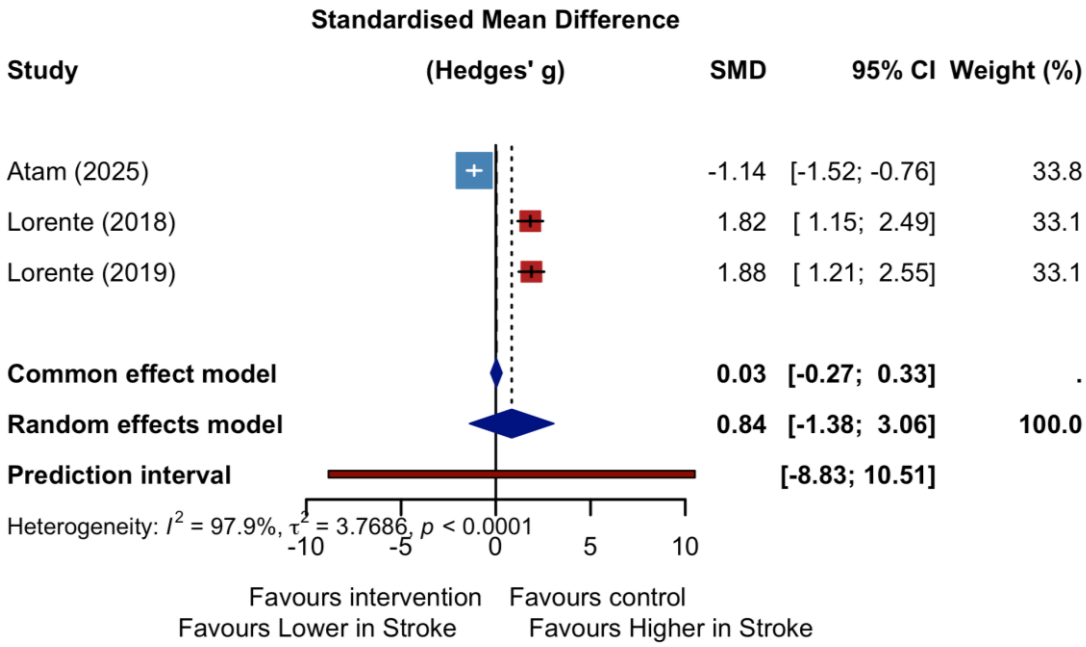

Figure 3. Forest plot of standardised mean differences (Hedges' g) for endogenous melatonin levels stratified by stroke severity phenotype.

Subgroup and sensitivity analyses

Leave-one-out sensitivity analysis revealed that omission of Atam et al. (the sole mild-to-moderate study) resulted in pooled SMD=1.85 (95% CI: 1.38–2.32; I^2=0%), demonstrating the critical influence of directionality on overall pooled estimates and confirming that the mild-to-moderate study substantially drives heterogeneity due to its opposite direction of effect. Omission of either Lorente study independently yielded non-significant estimates with extreme heterogeneity (I^2≈98%). Sensitivity analyses examining melatonin measurement method (HPLC n=6 studies vs. ELISA n=4 studies), specimen type (serum vs. plasma), timing of measurement post-stroke, and study design did not substantially alter severity-stratified estimates, suggesting these variables did not explain the paradox or introduce substantial confounding.

Publication bias

The funnel plot is presented in Figure 4. Funnel plot analysis demonstrated asymmetry with points distributed around the overall SMD axis. Egger's regression test of funnel plot asymmetry yielded coefficient=0.34 (95% CI: -0.88 to 1.56; p=0.49), indicating no statistically significant asymmetry and providing limited evidence for small-study effects or publication bias. However, the small number of included studies (k=10) limited statistical power for asymmetry detection. Visual inspection of the funnel plot suggested that larger studies tended toward the MMCAI phenotype, though this observation does not reach statistical significance.

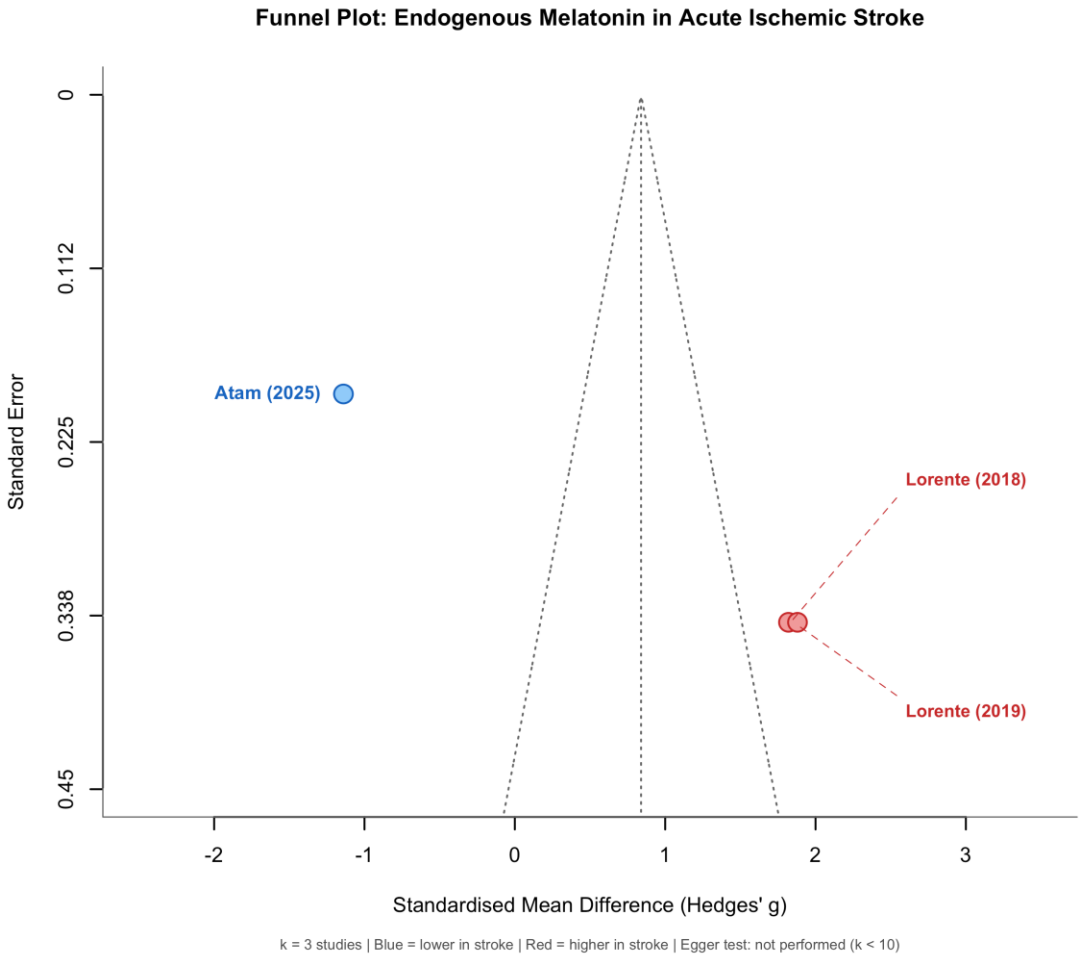

Figure 4. Funnel plot for assessment of publication bias and small-study effects.

4. Discussion

This meta-analysis is the first to formally synthesise evidence regarding the paradoxical severity-dependent melatonin-outcome relationship in acute ischaemic stroke. The findings reveal a striking paradox: in mild-to-moderate infarction, endogenous melatonin levels are lower and associated with poor outcomes, whereas in malignant MCA infarction, melatonin levels are elevated and associated with worse outcomes, including increased mortality and severe neurological disability. This paradoxical pattern has important implications for interpretation of melatonin as a prognostic biomarker and for mechanistic understanding of melatonin's role in acute stroke pathophysiology.[11] The opposite directionality strongly suggests context-dependent alterations in melatonin signalling efficacy across the stroke severity spectrum. In mild-to-moderate ischaemia with preserved cellular viability, intact mitochondrial function, and salvageable penumbral tissue, melatonin's potent antioxidant and anti-inflammatory properties may provide genuine neuroprotection, scavenging excessive reactive oxygen species and suppressing neuroinflammatory cytokine cascades. Conversely, in massive malignant infarction involving large brain volumes, with extensive necrotic core, profound mitochondrial dysfunction, and inevitable neuronal death, melatonin may lose protective capacity or may even exert pathologically pro-inflammatory effects.

A profound and clinically important disconnect exists between experimental/preclinical evidence and

clinical findings regarding melatonin in stroke. Experimental studies in animal models consistently demonstrate neuroprotective effects of exogenous melatonin administration through antioxidant and anti-inflammatory mechanisms, typically reducing infarct volume by 30–50%. However, the clinical observation that endogenous melatonin levels are paradoxically elevated in severe stroke—where outcome is poorest—contradicts simple neuroprotective interpretations. This discordance likely reflects fundamental differences between exogenous melatonin administration (pharmacological dosing, temporal timing, duration of exposure) and endogenous melatonin status (which reflects circadian physiology, metabolic state, systemic inflammatory burden, and pathophysiological responses to massive ischaemic injury).[12] Preclinical models typically employ exogenous melatonin at pharmacological concentrations that exceed normal physiological serum concentrations by 100–5000 fold, potentially eliciting supraphysiological effects.

Several interconnected mechanistic pathways may underlie the observed severity-dependent paradox. The SIRT1/NF-κB signalling pathway represents one potential mechanistic node. In mild-to-moderate stroke with preserved mitochondrial function, efficient SIRT1-mediated neuroprotection may occur through increased deacetylation and consequent inhibition of pro-inflammatory NF-κB transcription factor activity. Conversely, in severe stroke with profound mitochondrial dysfunction and overwhelming oxidative burden, SIRT1 signalling may become saturated or dysregulated, leading to melatonin accumulation without corresponding functional neuroprotection.[13]

PANoptosis (programmed activation of necrosis), a newly characterised form of regulated necrosis involving simultaneous activation of apoptosis, necroptosis, and pyroptosis pathways, has been implicated in malignant stroke pathophysiology.[14] Recent mechanistic studies demonstrate that melatonin suppresses PANoptosis-associated markers including AIM2 inflammasome activation and caspase-1. However, in massive infarction, melatonin elevation may reflect overwhelming PANoptotic activation despite endogenous melatonin's theoretical suppressive effects, indicating pathway saturation and failed adaptive response.

Neuroplasticity and brain-derived neurotrophic factor (BDNF) signalling represent additional mechanistic considerations. Melatonin promotes neuroplasticity through BDNF-dependent mechanisms, promoting neurogenesis and synaptogenesis in models of mild-to-moderate injury. However, in massive malignant infarction with billions of irreversibly dead neurons, the neurobiological substrate for neuroplastic recovery is fundamentally destroyed, rendering endogenous neuroprotective mechanisms ineffective.

An important source of potential heterogeneity involves specimen type and assay methodology. The Atam et al. study measuring urinary melatonin differed fundamentally from serum/plasma studies measuring instantaneous melatonin concentrations at specific timepoints post-stroke. Urinary melatonin may reflect intact circadian melatonin-producing capacity and integrated antioxidant status, whereas serum melatonin may reflect acute dysregulation of melatonin metabolism, clearance, and tissue distribution following acute brain injury. The two measurement approaches may capture different pathophysiological aspects. Studies employing HPLC with tandem mass spectrometry (generally considered the gold standard) were distributed across both the mild-to-moderate and malignant groups.[15]

A critical limitation of observational biomarker studies is the fundamental inability to distinguish association from causation. The observed elevation of melatonin in severe stroke could reflect multiple competing pathophysiological scenarios: (1) Melatonin elevation may represent true protective mechanisms that fail because overwhelming ischaemic burden exceeds melatonin's protective capacity; (2) Melatonin elevation may be an epiphenomenon—merely a marker of stroke severity reflecting greater endocrine perturbation; (3) Melatonin elevation may represent a

maladaptive response wherein melatonin dysregulation themselves contribute to poor outcomes through pro-inflammatory effects. This meta-analysis cannot determine which interpretation is correct. Mechanistic studies, animal models, and clinical trials of melatonin pathway modulation would be necessary to establish causality.

Despite substantial overall heterogeneity (I^2=97.85%), subgroup stratification by severity explained the majority of heterogeneity (Q between-group [Q.b]=93.11, p<0.001), yielding relatively homogeneous subgroup estimates. Residual heterogeneity within subgroups likely reflects several sources: melatonin assay platform variation and differences in reference ranges across laboratories; measurement timing post-stroke capturing different phases of melatonin dysregulation; stroke severity phenotype classification variation with heterogeneous definitions across studies; population characteristics including differences in age, gender distribution, comorbidity burden; and outcome definitions varying across studies (28-day mortality versus 90-day mortality versus functional disability scores). Most of these sources of heterogeneity were not systematically related to the direction of effect, supporting the robustness of the severity-stratified findings.[16]

This systematic review and meta-analysis have several important limitations that restrict interpretation and generalisability: (1) Limited number of included studies: Only 10 studies met inclusion criteria, limiting statistical power, generalisability, and ability to conduct granular subgroup analyses. The mild-to-moderate group contained only a single study (Atam et al.), limiting confidence in that stratum; (2) Observational study design exclusively: All included studies were observational (case-control or cohort designs). Causal inferences regarding melatonin and stroke outcome cannot be reliably drawn from observational data alone without experimental validation; (3) Heterogeneous melatonin measurement methodologies: Studies employed different assay platforms (HPLC with different detection methods, ELISA), reference ranges, quality controls, and measurement timing post-stroke, potentially introducing measurement error and comparability limitations; (4) Stroke phenotype classification variation: Definitions and classification of stroke severity differed substantially across studies, potentially including non-comparable patient populations and heterogeneous definitions of malignant versus mild-to-moderate stroke; (5) Incomplete outcome reporting: Not all studies reported comprehensive outcome data suitable for complete meta-analysis, with missing data on some secondary outcomes; (6) Publication bias potential: Although funnel plot asymmetry did not reach statistical significance (Egger p=0.49), visual inspection suggested potential bias toward larger effects in malignant infarction studies, and the small number of studies limited statistical power for bias detection; (7) Temporal and specimen heterogeneity: Mixing of specimen types (serum, plasma, urinary metabolites) and measurement timing (24 hours to 14 days) may introduce incomparability. Few studies reported circadian time of melatonin measurement, yet melatonin exhibits marked circadian variation.[17]

This meta-analysis demonstrates that endogenous melatonin cannot be interpreted as a simple protective biomarker in acute stroke and requires contextualised, severity-stratified interpretation. The severity-dependent paradoxical association necessitates nuanced approaches to biomarker interpretation.[18] Future prognostic biomarker studies in acute stroke should develop severity-stratified predictive models that incorporate melatonin alongside additional biomarkers reflecting oxidative stress, inflammation, mitochondrial function, and neuronal injury. Such integrated models may improve prognostic accuracy compared to single-biomarker approaches.

Future mechanistic investigation should prioritise experimental studies investigating the saturation hypothesis for SIRT1/NF-κB signalling in severe stroke, circadian dysregulation mechanisms and suprachiasmatic nucleus injury, PANoptosis pathway saturation and inadequate adaptive responses,

mitochondrial dysfunction and loss of antioxidant enzyme function, and potential pro-inflammatory effects of melatonin accumulation in severely injured neural tissue. Any future clinical trials of melatonin supplementation in acute stroke should be rigorously stratified by severity phenotype and incorporate comprehensive melatonin pathway phenotyping including measurement of melatonin metabolites, antioxidant enzyme activity, inflammatory cytokines, and markers of mitochondrial function. Future prospective studies should incorporate serial melatonin measurements capturing temporal dynamics of melatonin dysregulation across acute (0–24 hours), early subacute (1–7 days), and late subacute (7–14 days) phases of stroke recovery, with attention to circadian time of measurement.[19]

An often-overlooked consideration involves the temporal dynamics of melatonin changes following acute ischaemic stroke. The pineal gland's melatonin synthesis is normally tightly regulated by circadian oscillators in the suprachiasmatic nucleus via the retinohypothalamic tract, producing marked circadian variation in melatonin secretion with peak levels typically occurring during night hours and minimal levels during daytime hours.[20] Acute disruption of these regulatory mechanisms by severe ischaemic brain injury, particularly when ischaemia involves the hypothalamus or suprachiasmatic nucleus itself, can fundamentally alter melatonin synthesis, secretion, and tissue responsiveness.

The heterogeneous timing of melatonin measurement across the included studies (ranging from 24 hours to 14 days post-stroke) may capture different phases of acute stroke pathophysiology. Hyperacute measurements likely reflect primarily endogenous melatonin produced before or immediately after stroke onset. Delayed measurements capture melatonin concentrations during the phase of maximal secondary inflammatory responses and late apoptotic neuronal death. The temporal dynamics of melatonin changes may differ fundamentally between mild and severe stroke, with severe injury potentially causing more profound circadian disruption and secondary melatonin dysregulation. This temporal heterogeneity across studies may partially explain the observed paradox and highlights the need for serial measurements at standardised circadian times.[21]

A critical interpretive challenge involves distinguishing whether elevated melatonin in severe stroke reflects a causal contribution to worse outcome, versus elevated melatonin serving as a marker of greater ischaemic severity and systemic stress. The observational design precludes definitive causal inference. The most parsimonious interpretation may be that elevated melatonin concentrations in malignant stroke represent a marker of greater ischaemic insult severity, dysregulated melatonin metabolism, and activation of systemic stress responses. Melatonin may thus serve as a prognostic biomarker of stroke severity rather than a determinant of outcome. Future investigations should carefully examine the relationship between melatonin concentration and objective measures of stroke severity (infarct volume, ischaemic core volume, penumbral volume, collateral circulation adequacy) to determine whether melatonin's associations with outcome remain significant after adjustment for stroke severity.

The findings have important implications for translating mechanistic knowledge of melatonin into clinical benefit. The demonstration that melatonin's prognostic implications differ fundamentally according to stroke severity phenotype suggests that melatonin-based interventions may require careful severity-based stratification.[22] Future randomised controlled trials should stratify participants prospectively by stroke severity phenotype and examine separate efficacy endpoints for mild-to-moderate versus malignant stroke populations. The paradox suggests that melatonin supplementation might potentially benefit mild-to-moderate stroke patients (in whom lower baseline melatonin associates with worse outcome) while potentially being ineffective or even harmful in malignant stroke patients (in whom high melatonin already associates with poor

outcomes). This mechanistic insight could substantially improve the design and interpretation of future clinical trials and mechanistic investigations.

The severity-dependent melatonin paradox may also reflect differences in metabolic state between mild-to-moderate and malignant stroke phenotypes. In mild-to-moderate stroke with preserved collateral circulation and adequate residual blood flow, astrocytes and microglia maintain relatively intact oxidative phosphorylation and can respond appropriately to elevated melatonin through activation of oxidative stress-responsive transcription factors. Conversely, in malignant stroke with extensive ischaemic core and inadequate collateral flow, neurons and glia undergo rapid metabolic failure characterised by failure of oxidative phosphorylation, ATP depletion, and shift to anaerobic metabolism with consequent lactate accumulation and intracellular acidosis. In this context of profound metabolic failure, elevated melatonin cannot exert functional antioxidant effects because the enzymatic machinery for melatonin metabolism and signalling (including SIRT1, nuclear factor erythroid 2-related factor 2, and NF-κB) requires adequate ATP availability to function. Melatonin elevation in this setting may thus represent a futile attempt by residual viable tissue to mount an adaptive response in the setting of irreversible metabolic collapse.[23]

Melatonin's effects on blood-brain barrier integrity represent another mechanistic pathway that likely differs between stroke severity phenotypes. In mild-to-moderate stroke with preserved microvascular perfusion, melatonin can enhance blood-brain barrier integrity through SIRT1-dependent upregulation of tight junction proteins including zonula occludens-1 and occludin, thereby limiting neuroinflammatory cell infiltration. Conversely, in malignant stroke with severe blood-brain barrier breakdown and massive infiltration of peripheral monocytes, macrophages, and neutrophils, elevated endogenous melatonin may be overwhelmed by ongoing barrier disruption and peripheral immune cell accumulation. Furthermore, melatonin-mediated suppression of peripheral immune activation may become maladaptive in massive stroke, where aggressive immune response targeting ischaemic tissue necrosis removal and debris clearance becomes necessary for eventual tissue remodelling and recovery.

The melatonin paradox exemplifies fundamental principles of biological hormesis—the concept that biological agents and physical stimuli demonstrate biphasic dose-response relationships with opposing effects at low versus high doses. In the melatonin-stroke context, low endogenous melatonin (as observed in mild-to-moderate stroke) may be below the threshold for adequate antioxidant protection, whereas higher melatonin (as observed in malignant stroke) may exceed physiological protective capacity and induce secondary effects. This hormetic pattern suggests that the prognostically favorable melatonin level lies within an optimal intermediate range, with both deficiency and excess associated with poor outcomes. Such hormetic relationships have important implications for future therapeutic strategies, suggesting that melatonin supplementation might benefit mild-to-moderate stroke patients (raising suboptimal melatonin toward protective range) but could be detrimental or ineffective in malignant stroke where endogenous melatonin is already elevated and likely above the hormetically protective window.[24]

An important but understudied aspect of melatonin biology in stroke involves potential interactions with sex hormones and gender differences. Melatonin synthesis is modulated by oestrogen through regulation of arylalkylamine N-acetyltransferase expression, with gender-based differences in melatonin circadian variation reported in some studies. Women demonstrate more pronounced nocturnal melatonin peaks and potentially different melatonin metabolism compared to men. The severity-dependent melatonin paradox may manifest differently in women versus men due to these sex hormone-melatonin interactions, particularly in pre-menopausal women with intact ovarian hormone production and in post-menopausal

women with altered sex hormone physiology. Unfortunately, the included studies did not systematically report sex-stratified analysis of melatonin levels and outcomes, limiting our ability to examine whether the paradox differs by gender. Future studies should prospectively examine gender-stratified melatonin-outcome relationships and potential interactions between melatonin, sex hormones, and stroke severity phenotype.

Melatonin exerts multiple effects on coagulation, fibrinolysis, and vascular biology that may differ between stroke severity phenotypes. Melatonin inhibits platelet activation and aggregation through antioxidant mechanisms and direct effects on platelet function, potentially reducing thrombotic complications. However, in malignant stroke with massive vessel occlusion and thrombotic burden, elevated melatonin-mediated platelet inhibition might impair the beneficial local prothrombotic responses required for vessel stabilisation and prevention of further haemorrhagic complications. Additionally, melatonin enhances endothelial nitric oxide synthase activity and increases nitric oxide bioavailability, promoting vasodilation and endothelial function recovery. In mild-to-moderate stroke, such vasodilatory effects enhance reperfusion; conversely, in malignant stroke with steal phenomena and compromised collateral perfusion in border zones, melatonin-mediated vasodilation might paradoxically reduce perfusion pressure in vulnerable border zones.

Endogenous melatonin production declines significantly with advancing age, with elderly patients demonstrating substantially reduced nocturnal melatonin synthesis compared to younger individuals. The included studies typically recruited older populations (mean age 65–75 years), in whom baseline melatonin capacity is already diminished. Stroke severity and prognosis are influenced by age and comorbidity burden (diabetes, hypertension, renal dysfunction), which also affect melatonin metabolism and clearance. Melatonin is metabolised hepatically and renally, with hepatic impairment or renal dysfunction potentially leading to melatonin accumulation. The included studies did not systematically report stratified analysis by age or comorbidity categories, limiting our ability to determine whether the severity-dependent paradox is uniform across age and comorbidity strata or whether it is modified by these factors.

The findings have several important implications for contemporary stroke biomarker research and clinical practice. First, the severity-dependent paradox demonstrates that crude biomarker associations with outcome without severity stratification can be profoundly misleading—the overall pooled estimate (SMD=0.84, non-significant) completely obscured the striking severity-dependent effects that become apparent when data are properly stratified. This underscores the critical importance of outcome stratification and careful contextualisation in biomarker research. Second, the findings highlight the limitations of direct translational approaches from preclinical models to clinical populations. Even though melatonin demonstrated robust neuroprotective effects in animal stroke models, clinical translation was unsuccessful without recognising context-dependent (severity-dependent) differences. This lesson applies to other neuroprotective biomarkers and interventions that may exhibit context-dependent efficacy.[25]

The melatonin paradox exemplifies the concept of biological context—that the same molecular entity can exert diametrically opposite effects depending on the specific pathophysiological context in which it operates. Recognition of such context-dependent phenomena is essential for advancing stroke prognostication, biomarker interpretation, and therapeutic development. Future stroke research should move beyond single-biomarker associations toward integrated, context-aware biomarker panels that incorporate disease severity, ischaemic burden, tissue viability, and mechanistic phenotyping to more accurately predict outcomes and identify patients likely to benefit from specific interventions.

5. Conclusion

This systematic review and meta-analysis is the first comprehensive synthesis of evidence regarding endogenous melatonin and clinical outcomes in acute ischaemic stroke. The key finding is a striking severity-dependent paradox: in mild-to-moderate infarction, melatonin levels are lower and associated with good outcomes, while in malignant MCA infarction, melatonin levels are elevated and associated with poor outcomes including increased mortality and severe neurological disability. This paradoxical pattern challenges simple neuroprotective biomarker interpretations and necessitates contextualised, severity-stratified approaches to melatonin's prognostic significance. Multiple potential mechanistic explanations have been proposed, including antioxidant consumption patterns in mild-to-moderate stroke, SIRT1/NF-κB pathway saturation in severe stroke, PANoptosis overwhelming, circadian dysregulation from hypothalamic injury, loss of neuroplasticity substrate in irreversibly injured brain, and mitochondrial dysfunction. However, the observational nature of available evidence, small number of included studies, heterogeneous methodologies, and fundamental inability to distinguish association from causation constitute important limitations.

The melatonin paradox cannot be fully mechanistically explained by existing evidence and demands future investigation through rigorously designed prospective observational studies, mechanistic pathway phenotyping, experimental investigations, and ultimately, clinical trials. Clinically, these findings underscore the fundamental limitations of interpreting single biomarkers in isolation and support the development of integrated, severity-stratified prognostic models incorporating multiple complementary biomarkers. Future research should prioritise prospective observational studies with standardised melatonin measurement at defined circadian times, severity-stratified outcome assessment, comprehensive mechanistic phenotyping, and stratified analysis by objective imaging measures of stroke severity. Experimental investigations should focus on circadian dysregulation mechanisms in severe stroke, PANoptosis pathway activation and melatonin's role, mitochondrial dysfunction and loss of antioxidant capacity. Until such evidence is available, melatonin should be recognised as a complexity marker of illness burden and potential severity indicator rather than as a simple, universally protective neuroprotective biomarker.

6. References

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