Transcriptomic Horizons: The Emergence of RNA-Based Biomarkers for Prognostication After Cardiac Arrest.

Abstract

Cardiac arrest (CA) remains a leading cause of mortality and long-term neurological disability worldwide. Accurate early prognostication—particularly prediction of neurological outcomes—represents a critical unmet need in post-resuscitation care. Traditional clinical parameters and protein biomarkers exhibit significant limitations in sensitivity and timing. Recent advances in transcriptomic technologies have enabled comprehensive characterization of RNA molecules as dynamic indicators of the systemic and cerebral responses to global ischemia-reperfusion injury. This review synthesizes current evidence on novel transcriptomic markers following CA, encompassing messenger RNAs, non-coding RNAs, and epitranscriptomic modifications. We evaluate the prognostic utility of specific candidates including SLC2A1, microRNA panels, and emerging long non-coding RNAs. The review further examines analytical approaches, from targeted validation to machine learning-driven multi-omic integration, and discusses challenges in clinical translation. Emerging evidence supports the potential of transcriptomic signatures to enhance prognostic accuracy and provide mechanistic insights into post-cardiac arrest pathophysiology, though prospective validation in diverse cohorts remains essential.

Keywords: *SLC2A1, Cardiac arrest (CA), microRNA, non-codingRNAs, Messenger RNA*

1. Introduction

Cardiac arrest (CA) represents a catastrophic failure of circulatory function, precipitating global ischemia that rapidly compromises highly metabolically active organs, particularly the brain. Despite advances in resuscitation protocols and post-arrest care, survival rates remain modest, with out-of-hospital CA survival typically below 10% in many regions, and a substantial proportion of survivors experiencing significant neurological impairment . The pathophysiology of post-CA injury is complex, encompassing ischemia-reperfusion injury, cerebral edema, neuroinflammation, and mitochondrial dysfunction, with secondary injury cascades evolving over hours to days.

Accurate early prediction of neurological outcomes is paramount for clinical decision-making—guiding intensity of care, informing family discussions, and identifying patients who may benefit from targeted interventions. Current prognostication relies on a combination of clinical examination, neuroimaging, electrophysiology, and serum biomarkers such as neuron-specific enolase (NSE) and neurofilament light (NfL) . However, these approaches have significant limitations: clinical examination is confounded by sedation and hypothermia; neuroimaging may require transport of unstable patients; and protein biomarkers often lack sensitivity and specificity during early time windows.

Multi-omics technologies have revolutionized our capacity to investigate biological systems following CA, offering mechanistic insights across genomic, transcriptomic, proteomic, and metabolic dimensions . Among these layers, transcriptomics—the comprehensive study of RNA molecules—has emerged as particularly promising for biomarker discovery. RNA species are dynamically regulated in response to cellular stress, exhibit tissue-specific

expression patterns, and can be detected in peripheral blood, providing a readily accessible window into systemic pathophysiology.

This review synthesizes the current landscape of transcriptomic markers for prognostication following CA. We examine the biological rationale for RNA-based biomarkers, evaluate candidate markers across RNA classes, discuss analytical methodologies, and identify challenges and future directions for translation into clinical practice [1].

2. The Pathophysiological Rationale for Transcriptomic Biomarkers

2.1 The Post-Cardiac Arrest Inflammatory and Ischemic Cascade

The period following return of spontaneous circulation (ROSC) is characterized by a complex interplay of systemic and cerebral processes. Ischemia-reperfusion injury triggers a sterile inflammatory response, with activation of innate immunity, complement cascades, and release of damage-associated molecular patterns. Concurrently, the brain experiences secondary injury through excitotoxicity, oxidative stress, mitochondrial failure, and disruption of the blood-brain barrier.

These pathophysiological processes are reflected at the transcriptional level. Transcriptomic analyses have identified coordinated changes in gene expression involving immune signaling pathways, mitochondrial function, and cellular stress responses. Importantly, these changes are detectable in peripheral blood, which may serve as a "liquid biopsy" reflecting systemic injury burden and, potentially, the severity of cerebral damage [1,2].

2.2 The Advantage of RNA Over Protein Biomarkers

RNA biomarkers offer several theoretical advantages over traditional protein markers. First, the transcriptome is more dynamic than the proteome, with changes detectable within hours of injury, enabling earlier prognostication . Second, high-throughput sequencing technologies allow simultaneous assessment of thousands of transcripts, facilitating discovery of composite signatures rather than reliance on single markers . Third, diverse RNA species—including messenger RNAs (mRNAs), long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), and microRNAs (miRNAs)—provide complementary information across regulatory layers.

The emergence of epitranscriptomics has added further complexity, with RNA modifications such as N6-methyladenosine (m6A) and N1-methyladenosine (m1A) influencing RNA stability and translation, and potentially contributing to post-arrest pathophysiology [1,3].

3. Messenger RNA Biomarkers: From Discovery to Validation

3.1 SLC2A1: A Replicated Prognostic Marker

The most robustly validated mRNA biomarker to emerge from recent transcriptomic studies is solute carrier family 2 member 1 (SLC2A1), encoding the glucose transporter GLUT1—a critical component of the blood-brain barrier responsible for cerebral glucose delivery . In a discovery study using direct RNA sequencing of whole blood from 50 CA patients (25 with favorable neurological outcome, 25 with severe impairment or death), Stopa and colleagues identified SLC2A1 as significantly upregulated in patients with poor outcomes.

This finding was replicated in two independent cohorts: the North Pole cohort (n=233) and the Target Temperature Management (TTM) trial cohort (n=511). Across both cohorts, elevated SLC2A1 expression at 48 hours post-ROSC independently predicted neurological sequelae or death, with an odds ratio of 2.06 (95% CI 1.31–3.4, p=0.0028) when added to a baseline clinical model. The gene's expression demonstrated incremental predictive value, suggesting potential utility as an adjunct to existing prognostic tools.

The biological significance of SLC2A1 upregulation merits consideration. GLUT1 is the primary glucose transporter at the blood-brain barrier; its upregulation may represent a compensatory response to cerebral metabolic failure, paralleling the "neuroenergetic failure" described in post-arrest pathophysiology . However, as the authors acknowledge, the widespread expression of GLUT1 beyond the brain may limit its specificity as a purely cerebral injury marker, and functional studies are needed to determine whether SLC2A1 is merely a marker or a mediator of injury [3].

3.2 Bioinformatic Identification of Hub Genes

Complementary to targeted discovery approaches, integrative bioinformatics analyses have identified additional candidate mRNA biomarkers. Analysis of the GSE200117 next-generation sequencing dataset identified 844 differentially expressed genes between CA patients and controls, with enrichment in pathways related to organonitrogen compound metabolism, response to stimulus, translation, and immune system function.

Through protein-protein interaction network analysis and module identification, ten hub genes were prioritized: up-regulated HSPA8, HOXA1, INCA1, and TP53; and down-regulated HSPB1, LMNA, SNCA, ADAMTSL4, and PDLIM7 . Several of these genes have plausible mechanistic roles: HSPA8 and HSPB1 encode heat shock proteins involved in cellular stress responses; TP53 is a master regulator of apoptosis; and SNCA encodes alpha-synuclein, implicated in neurodegeneration. Regulatory network analysis further predicted microRNAs (hsa-mir-1914-5p, hsa-mir-598-3p) and transcription factors (JUN, PRRX2) targeting these hub genes, suggesting potential upstream regulatory mechanisms [3].

3.3 Preclinical Insights into mRNA Dynamics

Animal models have provided complementary insights into the temporal dynamics and tissue-specificity of transcriptional responses. In a murine model of asphyxial CA, Chen and colleagues identified 1,162 mRNAs and 1,920 long non-coding RNAs regulated in the hippocampus, implicating pathways of neuronal apoptosis and inflammation . These findings highlight the brain region-specific transcriptional responses that may be obscured in peripheral blood analyses.

In a rat model of CA-CPR, heparin treatment—investigated for its potential neuroprotective effects—was associated with differential expression of 757 genes, with restoration of mitochondrial function-related genes (Epsa1, Idh2, Hif3a) and attenuation of oxidative stress . This study illustrates how transcriptomic profiling can not only identify biomarkers but also elucidate mechanisms of therapeutic interventions [1,3].

4. The Non-Coding RNA Landscape: MicroRNAs and Beyond

4.1 MicroRNAs: The Most Extensively Studied Class

MicroRNAs (miRNAs)—small non-coding RNAs that post-transcriptionally regulate gene expression—have received the most intensive investigation as prognostic biomarkers following CA. A systematic review by Mellon and Bahilo-Martinez identified ten clinical studies examining miRNA expression in relation to neurological outcomes, comprising four primary biomarker studies and six post-hoc analyses from the TTM trial.

Eleven miRNAs were found to be differentially expressed within 72 hours of ROSC. The most replicated finding was up-regulation of miR-124-3p at 6 hours post-ROSC in patients with unfavorable outcomes—a pattern consistent with the brain-enriched expression of this miRNA and its release following neuronal injury . Notably, miR-124-3p correlated positively with NSE levels, supporting its interpretation as a marker of neuronal damage.

Predictive accuracy varied across candidates. Strong predictors included:

· miR-6511b-5p at 6 hours (AUC=0.85)
· miR-191-5p at 48 hours (AUC=0.89)
· miR-124 at 48 hours (AUC=0.89)

These values compare favorably with those of established protein biomarkers, suggesting potential clinical utility [2,3].

4.2 Mechanistic Insights from miRNA Studies

Beyond their prognostic utility, miRNA expression patterns offer mechanistic insights. miR-122-5p—primarily expressed in the liver—was paradoxically identified as a positive prognostic marker, potentially reflecting the systemic response to ischemia-reperfusion rather than isolated cerebral injury . Elevated levels of miR-21-5p and miR-122-5p have been associated with poor neurological outcomes, implicating pathways of inflammation and tissue injury.

The brain-enriched miRNAs (miR-9-3p, miR-124-3p, miR-129-5p) display positive correlations with NSE measurements, supporting their potential as specific neuronal injury markers . However, as with mRNA biomarkers, the specificity of circulating miRNAs to brain injury is not absolute, as these molecules are also expressed in other tissues and may be released from peripheral sources [3].

4.3 Long Non-Coding and Circular RNAs

Long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs) represent emerging classes of regulatory RNA with biomarker potential. In the murine CA model described above, nearly 2,000 lncRNAs were differentially expressed in the hippocampus, with potential involvement in neuronal apoptosis and inflammation.

Using RNA sequencing, Stefanizzi and colleagues identified upregulation of the circular RNA circNFAT5 in CA patients with poor neurological outcomes. When combined with clinical data and established biomarkers, circNFAT5 expression enhanced prognostic performance, suggesting potential utility as part of a multi-marker panel. The stability of circRNAs—attributed to their covalently closed loop structure—may confer advantages for clinical detection, though validation in larger cohorts is needed [2,3].

5. Analytical Approaches and Technological Advances

5.1 High-Throughput Platforms

Transcriptomic biomarker discovery has been catalyzed by advances in sequencing technologies. RNA sequencing (RNA-seq) provides comprehensive characterization of the transcriptome, including quantification of all RNA species, detection of novel transcripts, and assessment of splicing patterns . Direct RNA sequencing, as employed in the SLC2A1 discovery study, offers the advantage of avoiding PCR amplification bias and preserving RNA modification information.

Microarray technology, while historically important, has limitations including restricted dynamic range, cross-hybridization, and potential quantification inaccuracies. However, microarrays remain cost-effective for hypothesis-driven studies with predefined targets.

Targeted methodologies—including quantitative PCR, northern blotting, and fluorescence in situ hybridization—complement discovery platforms for validation and functional investigation . Quantitative PCR, in particular, has been widely used to validate RNA-seq findings, as in the SLC2A1 study, providing a more accessible approach for clinical translation [2,4].

5.2 Bioinformatic Integration and Machine Learning

The high-dimensional nature of transcriptomic data necessitates sophisticated analytical approaches. Bioinformatics pipelines integrate differential expression analysis, pathway enrichment, protein-protein interaction networks, and regulatory network construction to identify biologically meaningful signals.

Machine learning approaches have further enhanced biomarker discovery. Li and colleagues used machine learning to re-analyze publicly available datasets, identifying RNA interaction networks that could guide selection of potential biomarkers . Heat-map clustering and volcano plot visualization have been employed to distinguish survivors from non-survivors based on transcriptomic signatures.

Multi-omic integration represents the next frontier. By combining transcriptomic data with proteomic, metabolomic, and genomic layers, researchers can construct more comprehensive predictive models and elucidate causal relationships . Artificial intelligence may facilitate this integration, identifying patterns across high-dimensional datasets that would be undetectable with conventional approaches [4].

6. The Emerging Field of Epitranscriptomics

6.1 RNA Modifications in Post-Arrest Injury

Beyond RNA abundance, post-transcriptional modifications add a further layer of regulatory complexity. Over 170 RNA modifications have been identified, with N6-methyladenosine (m6A) being the most extensively studied. m6A influences RNA processing, nuclear export, stability, and translation, and has emerged as a player in cardiovascular disease.

The role of RNA modifications in CA prognostication has been less explored than that of transcript abundance. A recent study by Wang and colleagues investigated N1-methyladenosine (m1A) modifications in relation to outcomes after CA, suggesting that epitranscriptomic marks may provide additional prognostic information. Adenosine-to-inosine RNA editing is also under investigation, though studies in the post-CA context remain limited [5].

6.2 Technological Challenges

Detection of RNA modifications requires specialized sequencing approaches. Traditional RNA-seq protocols do not preserve modification information, necessitating chemical or enzymatic methods that can distinguish modified from unmodified nucleotides. These technologies remain less widely accessible than standard RNA-seq, though their application to critical illness is growing.

The integration of epitranscriptomic data with transcript abundance and protein-level measurements promises to provide a more complete picture of post-arrest molecular changes. However, analytical pipelines for such integration are still evolving, and the clinical utility of epitranscriptomic marks remains to be established [6].

7. Challenges in Clinical Translation

7.1 Replication and Validation

Despite promising discoveries, the translation of transcriptomic biomarkers to clinical practice faces substantial hurdles. A recurring theme in the literature is the need for larger, multicenter validation studies. Many candidate markers have been identified in single-center cohorts with limited sample sizes; replication across diverse patient populations is essential to establish robustness.

Variability in study design further complicates interpretation. Differences in sample collection timing, RNA extraction methods, normalization strategies, outcome definitions, and statistical approaches make direct comparisons difficult. Standardization of methodologies, as advocated in the systematic review of miRNAs, is critical for advancing the field [6].

7.2 Specificity and Confounding Factors

Peripheral blood transcriptomic signatures may be influenced by numerous factors unrelated to cerebral injury. The cellular composition of whole blood—particularly the proportion of erythrocytes, leukocytes, and platelets—can affect transcript measurements. Systemic conditions such as dysglycemia, infection, and pre-existing cardiovascular disease may confound biomarker relationships.

The specificity of blood-based markers for brain injury is a particular concern. While brain-enriched RNAs such as miR-124-3p offer theoretical specificity, even these molecules may be released from peripheral sources under certain conditions. Integration of cerebrospinal fluid analyses, where feasible, may provide additional confirmation of the cerebral origin of candidate markers [7].

7.3 Timing and Temporal Dynamics

The kinetics of RNA changes following ROSC are not fully characterized. SLC2A1 was measured at 48 hours post-ROSC; miRNAs have been assessed at various time points from 6 to 72 hours . The optimal sampling window for each marker may differ, and temporal trajectories may provide more information than single time-point measurements.

Longitudinal sampling is complicated by early mortality: patients who die soon after ROSC are systematically missing from later samples, potentially biasing analyses toward survivors. Unbalanced longitudinal designs and survivor-enriched samples remain a challenge for time-course transcriptomic studies [2,4].

7.4 From Marker to Mediator

An important conceptual distinction exists between biomarkers—which correlate with outcomes—and mediators—which causally contribute to injury. As Stopa and colleagues note regarding SLC2A1, "our study does not establish a causal role for GLUT1 in driving neurological damage". Distinguishing markers from mediators requires functional studies, such as genetic manipulations or pharmacological interventions in animal models.

This distinction has therapeutic implications. Biomarkers may aid prognostication without informing treatment; mediators could represent therapeutic targets. The demonstration that heparin, through multi-target regulation of mitochondrial genes, improves neurological outcomes in a rat model, illustrates how transcriptomic insights can bridge from biomarker discovery to mechanistic understanding and therapeutic development [3,5].

8. Future Directions and Clinical Perspectives

8.1 Multi-Omic Integration

The consensus emerging from recent reviews is that multi-omic integration offers the greatest promise for improving prognostication. Transcriptomic, proteomic, metabolomic, and genomic layers provide complementary information: transcripts reflect dynamic cellular responses, proteins indicate functional output, metabolites capture downstream biochemical consequences, and genetic variants may influence individual susceptibility.

Machine learning approaches are likely to be essential for integrating these high-dimensional datasets. Already, studies have employed heat-map clustering, volcano plots, and feature-importance analysis to identify multi-omic signatures. Prospective validation of such integrated models in real-world clinical settings represents the next logical step [1,5].

8.2 Point-of-Care Translation

For transcriptomic biomarkers to impact clinical care, they must be accessible at the bedside. Current technologies—requiring RNA extraction, amplification, and sequencing or quantitative PCR—are not compatible with point-of-care testing. However, advances in digital PCR, isothermal amplification, and CRISPR-based detection may eventually enable rapid, low-cost RNA measurement in clinical settings.

Interim strategies may involve central laboratory testing with clinically actionable turnaround times. The 48-hour sampling window for SLC2A1 aligns with typical post-arrest prognostication timelines, suggesting potential integration into existing workflows [3,4].

8.3 Therapeutic Targeting

Beyond prognostication, transcriptomic signatures may identify therapeutic targets. The demonstration that heparin modulates mitochondrial gene expression suggests that transcriptomic profiling could guide pharmacological interventions . Inflammatory pathways identified through transcriptomic analyses may be amenable to immunomodulatory therapies, as suggested by studies of IL-6 blockade.

As our understanding of post-arrest transcriptomic responses deepens, opportunities for precision medicine in critical care may emerge. Patients with distinct molecular phenotypes—for example, predominant inflammatory versus mitochondrial dysfunction signatures—might benefit from targeted therapeutic approaches [7].

9. Conclusion

Transcriptomic biomarkers represent a frontier in post-cardiac arrest prognostication. Messenger RNA, microRNA, long non-coding RNA, and circular RNA species offer dynamic, accessible windows into the systemic and cerebral responses to ischemia-reperfusion injury. Among candidate markers, SLC2A1 mRNA has demonstrated consistent prognostic value across independent cohorts, while microRNAs—particularly miR-124-3p—show replicated associations with neurological outcomes [3,5,8].

The convergence of high-throughput sequencing, bioinformatics, and machine learning has accelerated discovery, enabling identification of coordinated pathway-level signatures rather than isolated markers. Epitranscriptomic modifications add further regulatory complexity and may provide additional prognostic information [4,7].

However, substantial challenges remain. Validation in larger, multicenter cohorts with standardized methodologies is essential. The confounding effects of cellular composition and systemic conditions must be addressed. Longitudinal studies are needed to characterize temporal trajectories and identify optimal sampling windows. Most critically, the translation of transcriptomic markers to clinically actionable point-of-care tests will require technological innovation [3,5].

Despite these challenges, the trajectory is encouraging. Transcriptomic biomarkers are increasingly being integrated into multi-omic models that capture the systems-level response to cardiac arrest. As analytical methods mature and prospective validation accumulates, RNA-based markers may soon complement—or perhaps surpass—traditional clinical and protein-based approaches, enabling more precise prognostication and, ultimately, personalized post-resuscitation care. [6-8].

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