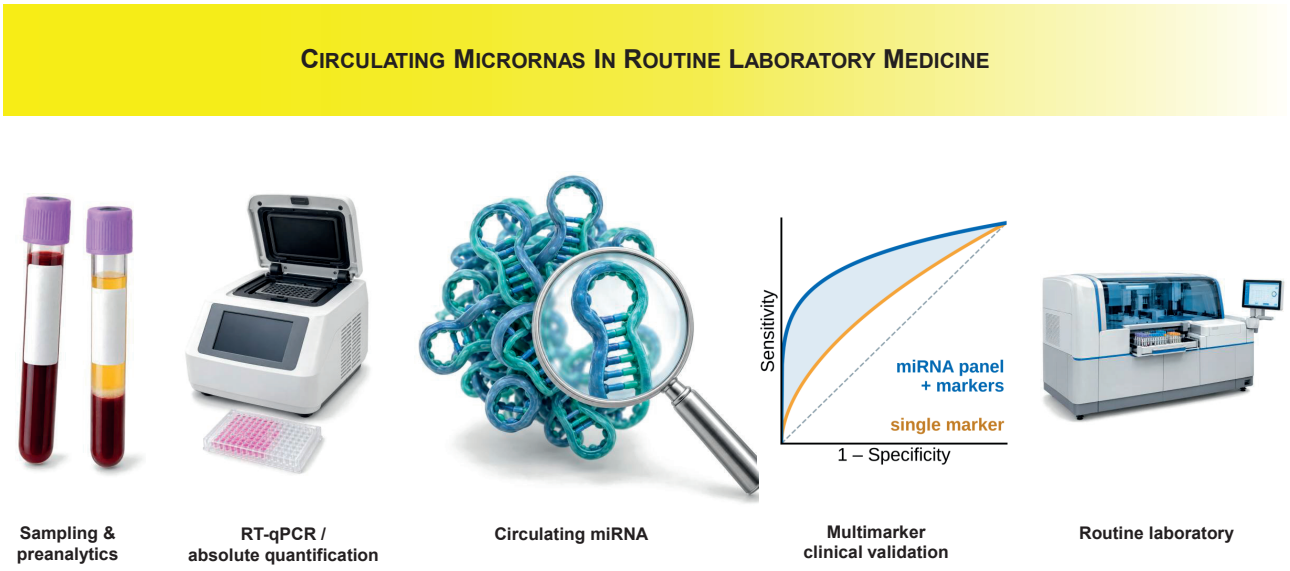
Translating microRNAs into clinical laboratory medicine: A critical review of biomarker potential, methodological barriers and implementation pathways

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This critical translational review evaluates the clinical significance of circulating microRNAs (miRNAs) across a broad spectrum of medical disciplines, identifies the main barriers to routine laboratory implementation and proposes a practical roadmap toward clinical use. A structured literature search of PubMed, Web of Science and Scopus covered English-language publications through May 2026. Evidence on preanalytical factors, analytical platforms, absolute quantification, reference interval derivation, clinical validation and translational feasibility of miRNA diagnostics was reviewed across oncology, neurodegeneration, cardiovascular disease, atherosclerosis, ophthalmology, acute kidney injury, sepsis, metabolic disease, liver disease, autoimmune disease, infectious disease, transplantation, reproductive medicine and toxicology. In multiple clinical domains, miRNAs may reflect pathophysiological processes earlier than established laboratory biomarkers; however, single miRNAs rarely offer disease-specific information. Multimarker panels combined with established biochemical markers demonstrate superior diagnostic and prognostic performance, whereas preanalytical standardization, absolute quantification and prospective clinical validation remain critical unmet needs. Reference intervals for eight cardiovascularly relevant miRNAs, determined in whole blood by the miREIA immunoassay, provide a first step toward routine implementation. Routine implementation of miRNA diagnostics therefore requires a five-stage laboratory medicine framework: preanalytical standardization, analytical validation with absolute quantification, biological reference data, prospective clinical validation and demonstration of clinical and economic utility beyond existing biomarkers.



Circulating miRNAs are complementary, not replacement, biomarkers. Routine use requires standardized preanalytics, absolute quantification, reference intervals and prospectively validated multimarker panels.

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INTRODUCTION

MicroRNAs (miRNAs) are endogenous, single-stranded, non-coding RNA molecules approximately 18–24 nucleotides long that regulate gene expression at the post-transcriptional level by degrading target messenger RNA or inhibiting translation[1]. They were first described in Caenorhabditis elegans in 1993 and subsequently identified in humans, with more than 2,600 mature human miRNAs catalogued in miRBase Release 22 (2019) (ref.[2,3]). Their functions span the full range of biological processes: proliferation, differentiation, apoptosis, inflammation, fibrosis and endothelial function – processes encoded by the approximately 60% of the protein-coding genome now recognized to be subject to miRNA regulation[4].

Their diagnostic potential became apparent with the discovery that miRNAs are stable in plasma, serum and other body fluids, protected from endogenous RNase degradation by encapsulation in exosomes, microvesicles or Argonaute protein complexes[5]. Current reviews of metazoan miRNA biology and historical work on animal miRNA functions further support their broad relevance as regulatory molecules[6,7]. Exosome-mediated transfer of RNAs provides a mechanistic basis for extracellular signaling[8]. This stability, combined with tissue-selective expression patterns[9], has inspired major interest in circulating miRNAs as fluid-based biomarkers accessible through minimally invasive sampling[10,11]. Extracellular miRNA transport via high-density lipoproteins adds a further dimension to their biological relevance as signaling molecules[12].

Yet clinically implemented miRNA tests remain uncommon[3,10]. The central problem is a translational gap: poor inter-study reproducibility arising from heterogeneous sample types, inconsistent normalization strategies, insufficient standardization of preanalytical handling, analytical workflows and normalization and, overinterpretation of disease specificity[3,13]. This critical review evaluates the conditions under which miRNAs can become robust laboratory tools, surveys the breadth of clinical areas in which they may contribute and proposes a realistic path toward routine implementation.

BIOLOGY OF MicroRNAs RELEVANT TO DIAGNOSTICS

Biogenesis and mechanism of action

MiRNA biogenesis begins with transcription of primary miRNA transcripts (pri-miRNAs) by RNA polymerase II, which are cleaved in the nucleus by the RNase III enzyme Drosha and its cofactor DGCR8 to produce precursor miRNAs (pre-miRNAs), exported to the cytoplasm and further processed by Dicer into approximately 22-nucleotide duplexes[14]. MiRNA genes may be located in intergenic regions or within introns and, less frequently, exons of protein-coding or non-coding genes, and may occur as individual loci or polycistronic clusters. Their transcription is influenced by transcription-factor activity and epigenetic regulation, including chromatin state and DNA methylation. The mature guide strand is incorporated into the RISC, where it directs sequence-specific binding to the 3′ untranslated regions of target mRNAs, leading to translational repression or mRNA degradation[7,9]. One miRNA may regulate hundreds of genes, and conversely one gene may be regulated by multiple miRNAs[4]. As a result, miRNA dysregulation in disease reflects broad reorganization of gene regulatory networks rather than single-target alterations – which also explains why individual miRNAs are often insufficient as disease-specific biomarkers[15].

Extracellular miRNAs and compartment biology

MiRNAs are released into body fluids through active secretion within exosomes and microvesicles, through apoptosis and necrosis, and as Argonaute-associated ribonucleoprotein complexes[5,8]. Exosome-mediated miRNA transfer between cells represents a biologically distinct paracrine communication pathway[8,16]. Circulating miRNA concentrations therefore represent a composite signal from blood cells, platelets, vascular endothelium and multiple tissue sources and may be influenced by cellular activation, tissue injury and clearance. The biological matrix selected for measurement fundamentally determines which compartment is sampled[17]: whole blood includes intracellular miRNA from blood cells, plasma and serum emphasize extracellular signals but differ in platelet-derived content, and cerebrospinal fluid (CSF), urine, saliva or tear fluid may provide local disease-site specificity[10,18]. These matrix-dependent differences are a major source of inter-study inconsistency[17].

PREANALYTICAL FACTORS

Preanalytical variability is consistently identified as the most critical, underaddressed bottleneck preventing miRNA translation to clinical routine[13,19].

Sample matrix and collection

EDTA anticoagulant is preferred for plasma-based assays; heparin inhibits reverse transcriptase and should be avoided[17]. Optimal conditions include whole-blood storage at room temperature for no longer than six hours, plasma storage at −80 °C for long-term preservation, and documentation of all procedures in a standardized protocol[19]. de Gonzalo-Calvo et al. recently proposed minimum preanalytical information requirements for miRNA biomarker publications, covering collection timing, centrifugation, storage, hemolysis, lipemia, medications and physical activity[20]. Intra- and inter-individual variability of circulating miRNAs often substantially exceeds analytical variation, making rigorous sampling standardization indispensable[13,21].

Hemolysis and stability

Erythrocytes contain high concentrations of specific miRNAs, notably hsa-miR-451a and hsa-miR-423-5p, and even visually non-apparent hemolysis can markedly elevate measured concentrations, generating false biological

interpretations[21]. Hemolysis control through spectrophotometry or hemolysis index calculation is mandatory for rigorous miRNA research using serum or plasma[21]. Each freeze–thaw cycle introduces additional losses; stability is best preserved at −80 °C (ref.[21]). The RNA isolation method substantially influences yield and recovery, accounting for a significant proportion of total analytical variability[22]. Internal standardization using spiked exogenous cel-miR-39 or cel-miR-54 controls is widely employed but does not correct for differential biological variability between samples[22,23].

ANALYTICAL METHODS AND QUANTIFICATION

RT-qPCR and normalization

RT-qPCR remains the most widely used analytical platform for miRNA measurement[10]. No universally accepted endogenous reference miRNA exists; commonly used references include hsa-miR-16-5p, hsa-miR-93-5p and U6 snRNA, but their stability across disease states and matrices is not guaranteed[13,24]. Normalization heterogeneity is a primary driver of inter-study inconsistency, and has been identified as the single largest source of irreproducibility in miRNA biomarker research[13]

Discovery platforms: NGS and microarrays

Microarray-based profiling and NGS enable hypothesis-free discovery of miRNA signatures and are primarily used for broad profiling and candidate discovery. Their complexity, turnaround time and cost limit routine diagnostic use[25,26].

Bead-based multiplex platforms

Bead-based multiplex technologies such as Luminex xMAP enable simultaneous measurement of multiple nucleic-acid targets and provide a scalable approach for panel-based assays[27]. Analytical sensitivity, calibration and inter-platform comparability remain important considerations for clinical implementation.

Digital PCR

Digital PCR (dPCR), including droplet digital PCR (ddPCR), is a targeted quantitative method that enables absolute copy-number estimation without an external standard curve and is analytically distinct from sequencing and microarray discovery platforms.

Absolute quantification – the miREIA approach

The miREIA (microRNA enzyme immunoassay) platform quantifies selected miRNAs in absolute picomolar units (pmol/L) using sandwich immunoassay principles with hybridization capture, analogous to protein biomarker immunoassays in routine laboratory practice[28]. This approach demonstrates strong correlation with RT-qPCR but offers higher technical reproducibility and enables construction of reference intervals in interpretable units[28]. A competitive magneto enzyme-linked immunoassay variant for hsa-miR-21-5p and hsa-miR-27a-3p has also been described, with limits of detection of 1.5 nM and 2.7 nM and format compatible with routine clinical laboratory workflows[29]. The miRQC international study demonstrated substantial platform-dependent variability for miRNA quantification, further supporting the need for absolute quantification methods[24].

Ring trials and inter-laboratory comparability

Inter-laboratory ring trials offer a strategic approach to address technical and biological variability in miRNA biomarker research[13]. A systematic methodological analysis identified the absence of standardized procedures, inconsistencies in reference gene selection, platform heterogeneity and inadequate data analysis as the main drivers of irreproducibility[13]. Ring trials have been underused in the field but will become essential for external quality assessment as clinical applications mature[13].

REFERENCE INTERVALS AND CLINICAL INTERPRETATION

Routine clinical diagnostics requires not only statistical significance between groups but clinically interpretable values: reference intervals, decision thresholds, and a clear understanding of biological and analytical variation[30]. Reference intervals for eight cardiovascularly relevant miRNAs (hsa-miR-21-5p, hsa-miR-23a-3p, hsa-miR-126-3p, hsa-miR-142-5p, hsa-miR-195-5p, hsa-miR-1-3p, hsa-miR-499a-5p and hsa-miR-29a-3p) were determined in whole blood from 99 apparently healthy adults using absolute quantification by miREIA, with no sex-related differences and only a weak age dependence for hsa-miR-21-5p (Spearman r = −0.208; P = 0.043) (ref.[28]). The established reference intervals (2.5th–97.5th percentile) for the panel were: hsa-miR-21-5p 1.45–96.3 pmol/L; hsa-miR-23a-3p 13.0–432 pmol/L; hsa-miR-126-3p 5.67–66.5 pmol/L; hsa-miR-142-5p 37.4–293 pmol/L; hsa-miR-195-5p 11.5–254 pmol/L; hsa-miR-1-3p 50.6–1800 pmol/L; hsa-miR-499a-5p 8.90–82.5 pmol/L; and hsa-miR-29a-3p 22.9–210 pmol/L (ref.[28]). These provide early population-based reference data for cardiovascularly relevant miRNAs measured by absolute immunoassay quantification[28]

ONCOLOGY

Cancer is the most extensively studied field for circulating miRNA diagnostics, driven by the need for minimally invasive early detection and the biological rationale that malignant transformation profoundly alters gene regulatory networks[15,31]. Multiple miRNAs have been identified with diagnostic, prognostic and treatment-monitoring potential across hematological malignancies and solid tumors: a landmark study by Lu et al. showed that miRNA expression profiles classify human cancers more accurately than protein-coding genes[32]. Circulating miRNAs were subsequently characterized as stable blood-based markers for

cancer detection[33]. In genitourinary cancers, circulating miRNAs show promising sensitivity for early-stage disease detection and therapy monitoring[34].

The liquid biopsy concept has transformed oncological diagnostics by enabling analysis of tumor-derived nucleic acids in blood without tissue biopsy[35]. MiRNAs complement ctDNA by reflecting not only tumor cell content but also stromal, immune and endothelial responses to the tumor microenvironment[35]. EV-miRNA subpopulations isolated from body fluids may further enhance diagnostic performance over total cell-free miRNA (ref.[36]). Multi-miRNA classifiers consistently outperform single analytes, and future oncological applications will center on early detection, molecular subtype classification, treatment response monitoring, and integration with multi-omics and artificial intelligence-driven classifiers[32,37,38].

Despite this extensive research activity, miRNAs currently have a limited role in routine oncological decision-making. Targeted therapies are predominantly selected according to validated genomic alterations identified in tissue or circulating tumor DNA, whereas immunotherapy selection commonly relies on established markers such as PD-L1 expression[35]. Future miRNA assays will therefore need to demonstrate incremental clinical value beyond these established molecular diagnostic paradigms.

NEURODEGENERATION

Neurodegenerative diseases – AD, PD, ALS, MS and Huntington's disease – share an urgent need for earlier, molecularly informed biomarkers[39]. Altered miRNA expression has been identified in brain tissue and circulating fractions across these conditions: in AD, hsa-miR-9-5p, the miR-29 family, hsa-miR-107-3p and hsa-miR-132-3p target pathways involved in beta-amyloid processing and tau phosphorylation; in PD, hsa-miR-331-5p and hsa-miR-16-2-3p show altered plasma profiles; in MS, miRNA profiles may distinguish disease subtypes and activity[40]. A critical translational challenge is matrix selection: CSF miRNAs reflect CNS processes more closely but require lumbar puncture, while plasma miRNAs carry substantial systemic noise from vascular disease, inflammation and metabolic comorbidities[39,40]. MiRNAs in neurodegeneration are most realistically used in multimarker integration with established CSF biomarkers (Aβ42/40, p-tau, neurofilament light chain), neuroimaging and cognitive phenotyping to enable early pre-symptomatic diagnosis[39].

ATHEROSCLEROSIS AND CARDIOVASCULAR DISEASE

The underlying processes of cardiovascular disease – endothelial activation, vascular inflammation, lipid dysregulation, plaque formation, myocardial remodeling and fibrosis – are deeply intertwined with miRNA signaling[41,42]. hsa-miR-126-3p is highly expressed in endothelial cells, promotes vascular integrity and angiogenesis through VEGF signaling inhibition[43,44] and its delivery by apoptotic bodies induces CXCL12-dependent vascular protection[44]. Emerging insights into miRNA-mediated regulation confirm the roles of hsa-miR-21-5p, hsa-miR-155-5p, hsa-miR-146a-5p and hsa-miR-126-3p in pro-inflammatory and oxidative stress signaling within the vessel wall[45]. The miR-33 family, hsa-miR-92a-3p and hsa-miR-155-5p are further identified as therapeutic targets modulating macrophage polarization and reverse cholesterol transport[46].

In a published case-control study from the Ostrava Cardiology program, serum levels of hsa-miR-21-5p, hsa-miR-23a-3p, hsa-miR-142-5p and hsa-miR-126-3p were all significantly higher in HFrEF patients compared with healthy controls ($P \leq 0.001$), measured by miREIA (ref.[47]). None of the four miRNAs correlated with NT-proBNP levels, consistent with the interpretation that miRNAs capture orthogonal pathophysiological dimensions – fibrosis, endothelial dysfunction and immune activation – rather than hemodynamic severity[47]. These findings are also consistent with a recent meta-analysis confirming that combining multi-miRNA panels with NT-proBNP and BNP improves diagnostic performance beyond either approach alone[48]. hsa-miR-423-5p was among the first identified circulating biomarkers for heart failure[49]. Fibrosis-associated hsa-miR-21-5p stimulates MAP kinase signalling in cardiac fibroblasts and promotes myocardial disease[50]. Stress-responsive miRNAs including hsa-miR-195-5p and the miR-208 family can evoke cardiac hypertrophy[42]. hsa-miR-210-3p has recently been associated with NT-proBNP, sST2 and galectin-3 in HF patients[51]. The therapeutic antagomiR CDR132L (anti-miR-132-3p) has completed a phase II clinical trial in HFrEF patients post-myocardial infarction[52], illustrating that the same miRNAs may serve as biomarkers and therapeutic targets. Microinjection of antagomiR-92a has also been shown to promote angiogenesis and prevent adverse infarct remodeling[53]. MiRNAs in heart failure are extensively reviewed in published literature[54,55]; their role as mediators in chronic kidney disease has been separately reviewed[56].

Circulating miRNAs in patients with coronary artery disease were first systematically characterized by Fichtlscherer et al.[57] and hsa-miR-499a-5p has been identified as a regulator of mitochondrial dynamics[58]. Plasma hsa-miR-133a-3p serves as a marker of both acute myocardial infarction and underlying coronary artery stenosis[59], while early diagnosis of acute myocardial infarction using circulating miRNA was validated by Wang et al.[60].

OPHTHALMOLOGY

DR, AMD and glaucoma involve angiogenesis, neurodegeneration, oxidative stress and microvascular inflammation, all closely linked to miRNA regulation[61]. Numerous miRNAs show altered expression in DR, modulating pro-inflammatory pathways (hsa-miR-155-5p, hsa-miR-21-5p), anti-inflammatory NF-κB signaling (hsa-miR-146a-5p), and endothelial integrity and angio-

genesis (hsa-miR-126-3p, hsa-miR-200b-3p) (ref.[61]). A targeted review conceptualizes miRNAs as master regulators integrating hyperglycemia-induced damage across multiple pathogenic pathways in DR (ref.[62]). In AMD, miRNAs participate in inflammatory signaling within the retinal pigment epithelium, with complement pathway and VEGF signaling as key targets[61]. Integration of miRNA biomarkers with optical coherence tomography imaging endpoints represents a promising translational direction[61,62].

ACUTE KIDNEY INJURY AND SEPSIS

Current AKI biomarkers are biologically late: serum creatinine rises only after significant nephron loss, while routinely used inflammatory markers do not distinguish immune activation from direct organ injury[63]. A systematic review and meta-analysis of 71 studies measuring miRNAs in AKI identified hsa-miR-21-5p as the most consistently reported, showing upregulation at the time of AKI diagnosis with significant pooled effect size in sepsis-related AKI (ref.[63]). A separate systematic review of 92 clinical studies confirmed 128 dysregulated miRNAs across AKI etiologies[64]. A comprehensive review confirmed miRNA involvement in ischemia-reperfusion, nephrotoxicity, sepsis and cisplatin-induced AKI, with translational potential conditioned on analytical standardization[65]. hsa-miR-210-3p has also been shown to independently predict survival in critically ill patients with AKI (ref.[66]).

A comprehensive review of the miRNA network in sepsis identified hsa-miR-150-5p, hsa-miR-146a-5p, hsa-miR-223-3p, hsa-miR-21-5p and hsa-miR-155-5p as among the most consistently reported, reflecting systemic immune dysregulation, endothelial injury and organ dysfunction[67]. Exosome-derived miRNAs have attracted particular interest as biomarkers of sepsis-associated intercellular communication[68]. A multicomponent biomarker platform based on extracellular vesicles incorporating miRNAs, proteins and lipids has been proposed as an integrated sepsis assessment approach[69]. A 2025 clinical validation study measuring selected plasma miRNAs at first clinical presentation in patients with sepsis-associated AKI reported limited overall diagnostic and prognostic performance, although hsa-miR-21-5p retained some association with sepsis-associated AKI or short-term outcomes, underscoring the need for prospective validation before clinical implementation[70]. The impact of sepsis on circulating exosomes as miRNA carriers has been reviewed recently[71]. Circulating miRNAs and plasma gelsolin have been simultaneously evaluated as composite biomarkers of sepsis severity[72].

Plasma miRNAs as sensitive and specific biomarkers of tissue injury were validated in preclinical models[73], and miRNA in body fluids represents a key source of disease biomarkers[74].

METABOLIC DISEASE, OBESITY AND MASLD/MASH

MiRNAs participate in adipose tissue-muscle-liver cross-talk, insulin signaling, lipid metabolism and inter-organ communication[75]. In obesity and type 2 diabetes, circulating hsa-miR-21-5p, hsa-miR-93-5p, the miR-103 family, hsa-miR-107-3p and hsa-miR-146a-5p reflect inflammatory activation, insulin resistance and adipose tissue remodeling[75]. An independent case-control validation study in adolescents with long-duration type 1 diabetes supports the feasibility of reproducible miRNA biomarker validation designs[76]. Circulating miRNAs including hsa-miR-122-5p, hsa-miR-34a-5p, hsa-miR-21-5p and hsa-miR-192-5p are consistently associated with metabolic dysfunction-associated steatotic liver disease (MASLD; previously NAFLD) and metabolic dysfunction-associated steatohepatitis (MASH; previously NASH) (ref.[77]): hsa-miR-122-5p is consistently increased in MASLD but decreased in cirrhosis; hsa-miR-34a-5p and hsa-miR-21-5p increase across the spectrum; hsa-miR-192-5p appears prominently in MASH (ref.[77]). A comprehensive review confirmed that miRNA alterations reflect MASLD progression from simple steatosis to steatohepatitis, fibrosis and hepatocellular carcinoma, and that miRNAs can serve both as noninvasive staging markers and hepatocellular signaling molecules[78]. An independent validation study re-examining 18 postulated serum miRNA biomarkers in MASLD demonstrated that cross-validated models combining clinical variables with miRNA data showed enhanced predictivity for MASH compared with ALT alone[79].

LIVER DISEASE AND DRUG-INDUCED LIVER INJURY

hsa-miR-122-5p constitutes approximately 70% of total hepatic miRNA content and is readily detectable in blood after hepatocellular injury[80,81]. A systematic review and meta-analysis confirmed that hsa-miR-122-5p has high diagnostic accuracy for DILI, with liver-specific expression giving it advantages over non-specific transaminase elevation[82]. A comprehensive review from the regulatory perspective confirmed that hsa-miR-122-5p offers enhanced sensitivity and specificity and the potential to differentiate lethal from non-lethal liver injury, but identified the lack of a standardized quantification method and insufficient confirmatory studies in human idiosyncratic DILI as major outstanding challenges[83]. MiRNAs as biomarkers for liver disease were also reviewed in the context of drug development and their roles in diagnosing and monitoring both clinical and preclinical DILI (ref.[81]). The promise of miRNA replacement therapy as a liver-targeted approach has been demonstrated in preclinical models[84].

AUTOIMMUNE AND INFLAMMATORY DISEASES

In RA, hsa-miR-146a-5p and hsa-miR-155-5p modulate NF-κB-driven inflammatory cascades and TNF-α signaling, representing both potential biomarkers and therapeutic targets[85]. A comprehensive review of miRNA biological functions in RA and SLE identified these miRNAs as key regulators of pathogenic inflammation[85]. A review of miRNA theranostic potentials across autoimmune diseases – RA, type 1 diabetes, MS, SLE and IBD – confirmed the utility of differentially expressed miRNAs as biosensor components and therapeutic targets[86]. In SLE, hsa-miR-21-5p, hsa-miR-146a-5p and hsa-miR-155-5p are consistently dysregulated and reflect interferon signaling and B-cell activation[85]. MiRNAs in systemic rheumatic diseases were among the earliest recognized examples of miRNA-mediated immune regulation[87].

INFECTIOUS DISEASE

MiRNAs modulate host-pathogen interactions, innate and adaptive immune responses, and organ injury cascades during infection, with both host and pathogen-derived miRNAs contributing to disease pathophysiology[18,67]. The most reproducibly reported sepsis-associated miRNAs – hsa-miR-150-5p, hsa-miR-146a-5p, hsa-miR-223-3p and hsa-miR-21-5p – link immune dysregulation, endothelial dysfunction and organ failure pathways[67]. Exosome-derived miRNAs serve as dynamic biomarkers of sepsis progression[68,71]. Beyond sepsis, miRNA biomarker research is expanding in viral hepatitis and respiratory infections[18].

LIVER TRANSPLANTATION

Allograft rejection, ischemia-reperfusion injury, immunosuppressant toxicity and chronic fibrosis may not be detected early enough by established laboratory parameters[88,89]. In a prospective study of 151 liver transplant recipients, plasma hsa-miR-155-5p and hsa-miR-181a-5p reflected tissue-level changes in T-cell-mediated rejection (TCMR), with a combined biomarker score (hsa-miR-155-5p + hsa-miR-181a-5p + CXCL-10) achieving AUROC of 0.94 for prediction and 0.99 for diagnosis of TCMR in an independent validation cohort[88]. A systematic review of miRNA after liver transplantation confirmed upregulation of hsa-miR-122-5p, hsa-miR-155-5p, the miR-181 family, hsa-miR-483-3p and hsa-miR-885-5p in association with acute cellular rejection across 13 studies, with standardization identified as the main outstanding challenge[89].

REPRODUCTIVE MEDICINE AND PREGNANCY

A comprehensive overview of miRNAs in preeclampsia identified them as critical regulators of impaired trophoblast invasion, aberrant angiogenesis and systemic inflammatory activation, and as potential early-pregnancy biomarkers and therapeutic targets[90]. Early-pregnancy profiling in a nested matched case-control cohort identified 16 differentially expressed miRNAs at 10–18 weeks of gestation associated with preeclampsia, with a connected CXCL8/CXCL10/MMP9/IGF1R subnetwork as the functional core[91]. A review of circulating miRNAs in preeclampsia described their association with impaired spiral artery remodeling, hypoxic placental signaling and systemic endothelial dysfunction from implantation to epigenetic regulation[92]. Translation of miRNA blood-based discovery to molecular testing for endometriosis diagnosis has been assessed for clinical feasibility in a prospective study, demonstrating proof-of-concept for miRNA panels as a non-invasive alternative to diagnostic laparoscopy[93].

MicroRNA THERAPEUTICS

MiRNA mimics restore the function of deficient tumor-suppressive miRNAs while antagomiRs silence overexpressed pathogenic miRNAs using LNA or other chemical backbone modifications[94,95]. A review of miRNA therapeutics in clinical trials summarized candidates for cancer, cardiovascular disease, viral infections and metabolic disease, noting that no miRNA-based therapy has yet received regulatory approval, but significant progress in nano-delivery and oligonucleotide chemistry continues[94,95]. Miravirsen (anti-miR-122) was the first

Table 1. Selected miRNA-based therapeutics in clinical trials (ref.[52,53,94-97]).

Drug	Target miRNA	Chemistry	Route	Disease	Phase	Status
Miravirsen	hsa-miR-122-5p	LNA	SC	Hepatitis C	IIa	Completed
CDR132L	hsa-miR-132-3p	LNA, PS	IV	HFrEF post-MI	II	Completed
Lademirsen	hsa-miR-21-5p	2′-MOE, PS	SC	Alport syndrome	II	Terminated
MRG-110	hsa-miR-92a-3p	LNA	IV	Cardiovascular	I	Completed
Remlarsen	hsa-miR-29b-3p	2′-OMe	Intradermal	Fibrotic scar	II	Discontinued
MRX34	hsa-miR-34a-5p	–	Liposome IV	Primary liver cancer	I	Terminated
MesomiR	hsa-miR-16-5p	2′-OMe	Minicell IV	Mesothelioma, NSCLC	I	Completed
RG-125/AZD4076	miR-103/107 family	GalNAc	SC	MASH/T2DM	I	Completed

LNA, Locked nucleic acid; PS, Phosphorothioate; IV, Intravenous; SC, Subcutaneous; 2′-OMe, 2′-O-methyl; 2′-MOE, 2′-O-methoxyethyl; GalNAc, N-acetylgalactosamine conjugate; MI, Myocardial infarction; NSCLC, Non-small cell lung cancer; MASH, Metabolic dysfunction-associated steatohepatitis; T2DM, Type 2 diabetes mellitus.

miRNA therapeutic to enter clinical trials, demonstrating sustained virological response in hepatitis C by targeting the essential hsa-miR-122-5p/HCV interaction[96]. Development of miRNA therapeutics is coming of age with increasing translation of concept to clinical study[97].

FROM SINGLE MARKERS TO MULTIMARKER PANELS

Single miRNAs rarely suffice for routine clinical use because they are pleiotropic, influenced by multiple tissues, disease pathways and physiological processes.[3,13] The same analyte may rise in cancer, inflammation, fibrosis and vascular disease – limiting diagnostic specificity for any single condition.[15] Extracellular miRNA: a new source of biomarkers was among the first frameworks to articulate the panel paradigm.[23] Multi-miRNA classifiers consistently outperform single analytes.[32,37] In heart failure, prognostic panels substantially improve prediction over single markers.[48] In sepsis and organ injury, panel thinking is essentially mandatory because systemic inflammation generates overlapping miRNA patterns across simultaneously active processes.[63,67] The combination of miRNAs with established biochemical markers, clinical variables and machine learning algorithms represents the most realistic path toward clinical utility.[38,98]

The clinically relevant combination is likely to be disease-specific rather than universal. In heart failure, miRNA panels may complement natriuretic peptides; in liver injury, hsa-miR-122-5p may be interpreted alongside established liver injury markers; in neurodegeneration, miRNA signatures are most plausibly integrated with established CSF or plasma biomarkers and clinical phenotyping; and in liver transplantation, combined miRNA and chemokine scores illustrate the potential of cross-class multimarker models. Such combinations should be evaluated prospectively against the relevant standard-of-care pathway rather than against healthy controls alone.

A LABORATORY MEDICINE ROADMAP TO IMPLEMENTATION

Moving miRNAs from experimental work to clinical routine requires five sequential stages[3,13,30]:

1. Preanalytical standardization – Define matrix, collection tubes (EDTA), timing, storage (−80 °C), hemolysis monitoring, freeze-thaw limits and RNA extraction workflow in a standard operating procedure[20].

2. Analytical validation – Demonstrate precision (CV%), linearity, sensitivity, specificity, interference (hemolysis, lipemia, bilirubin), platform comparability and absolute quantification capability[13,21,29].

3. Biological reference data – Establish reference intervals in clinically defined healthy populations stratified by age and sex; characterize intra-individual variation and major biological confounders[28,30].

4. Clinical validation – Conduct prospective multicenter studies demonstrating added clinical value over existing standard-of-care biomarkers; case-control retrospective studies alone are insufficient[3,13].

5. Clinical utility and economics – Demonstrate that miRNA testing leads to better clinical decisions with acceptable turnaround time, cost and accessibility; EQA and ring trial schemes are needed for inter-laboratory harmonization[13,30].

Table 2. Clinical readiness of miRNA diagnostics by domain.

Clinical domain	Best-supported candidates	Most advanced evidence	Main barrier
Oncology	hsa-miR-21-5p, hsa-miR-141-3p, tissue-specific panels	Multi-miRNA liquid biopsy panels[32,35,37]	Specificity; validation cohort size
Cardiovascular / HFrEF	hsa-miR-21-5p, hsa-miR-126-3p, hsa-miR-23a-3p, hsa-miR-142-5p	Reference intervals[28]; HFrEF case-control[47]	No prospective outcome study
Liver / DILI	hsa-miR-122-5p	Regulatory biomarker qualification[83]	Standardized quantification; idiosyncratic DILI
MASLD / MASH	hsa-miR-122-5p, hsa-miR-34a-5p, hsa-miR-21-5p, hsa-miR-192-5p	Independent validation[79]	Liver biopsy gold standard; no validated cut-off
AKI / Sepsis	hsa-miR-21-5p, hsa-miR-210-3p	Systematic reviews[63,64]; clinical validation study[70]	Methodological heterogeneity
Liver transplantation	hsa-miR-155-5p, hsa-miR-181a-5p, hsa-miR-122-5p	Prospective cohort, AUROC 0.94–0.99[88]	Standardization; combined with cfDNA needed
Neurodegeneration	hsa-miR-9-5p, the miR-29 family, hsa-miR-107-3p, hsa-miR-132-3p	Systematic reviews[39,40]	CSF vs. plasma; systemic noise
Preeclampsia	hsa-miR-210-3p, hsa-miR-155-5p	Early-pregnancy cohort[91,92]	Prospective validation; sampling timing
Diabetic retinopathy	hsa-miR-21-5p, hsa-miR-126-3p, hsa-miR-146a-5p	Recent reviews[61,62]	Blood vs. local ocular sampling
Autoimmune / RA	hsa-miR-146a-5p, hsa-miR-155-5p	Mechanistic/review evidence[85,86]	Disease specificity; polypharmacy
Endometriosis	miR-200 family	Clinical feasibility study[93]	Laparoscopy gold standard; small cohorts

DISCUSSION

MiRNAs should not be framed as replacements for established biomarkers but as complementary molecular indicators of pathophysiological processes often invisible to established clinical chemistry biomarkers – fibrosis, endothelial dysfunction, immune activation and cellular stress[1,28]. The observation that none of the four miRNAs measured in HFrEF correlated with NT-proBNP[47] should not be interpreted as failure: it suggests that miRNAs capture an orthogonal biological dimension relevant for long-term prognosis, phenotyping and therapeutic target identification[47,48].

The central translational implication is that a single-miRNA model is unlikely to be sufficient for routine laboratory medicine. The same circulating miRNA may reflect several tissues and overlapping processes, whereas clinically useful panels can combine complementary biological signals. Integration of multiple miRNAs with established laboratory biomarkers and clinical variables therefore offers a more plausible route to incremental diagnostic or prognostic value than replacement of current tests.

The field requires greater methodological discipline. Small single-center case-control studies with incompletely described preanalytics and overstated specificity claims have slowed translation[3,13]. Future work should prioritize harmonized multicenter designs, prospective validation against meaningful clinical endpoints, and honest comparison against current standard-of-care pathways[13,30]. MiRNAs in body fluids represent a genuinely new class of biomarkers[11,74], but the history of troponins, natriuretic peptides and NGAL shows that completing all five translational stages – not just biological discovery – is what determines clinical biomarker status.

Preanalytics remain a gatekeeper for this transition: matrix selection, collection and processing conditions, hemolysis, storage, RNA extraction and normalization can all alter the measured signal before clinical interpretation begins. The liver-enriched hsa-miR-122-5p illustrates both the promise and the remaining gap. There is substantial evidence that it is a sensitive marker of hepatocellular injury, including drug-induced liver injury but routine use still requires standardized quantification, validated decision limits and prospective demonstration of added value over established liver tests. This example closely follows the proposed laboratory roadmap from analytical control to clinical utility.

Future research should therefore prioritize prospective multicenter studies using harmonized preanalytical protocols, analytically validated assays and prespecified multimarker models. These should compare miRNA-based algorithms directly with current standard-of-care pathways, quantify incremental clinical value and address inter-laboratory comparability, reference data, external quality assessment and health-economic feasibility before routine implementation.

Breadth is equally important. Neurodegeneration, ophthalmology, nephrology, sepsis, metabolic disease, liver disease, autoimmunity, toxicology, transplantation and reproductive medicine all represent areas of genuine unmet clinical need[39,61,64,88,90]. The most impactful future applications may arise precisely where existing biomarkers are most inadequate[38,98].

CONCLUSION

MicroRNAs have substantial and broad clinical potential as biomarkers reflecting inflammation, fibrosis, endothelial dysfunction, oxidative stress, apoptosis and inter-organ communication across major human diseases[1,7,9]. Their stability in body fluids and mechanistic biological relevance make them attractive candidates for minimally invasive diagnostics, prognosis and therapeutic monitoring across more than a dozen clinical domains. Routine clinical implementation has been delayed by preanalytical variability, methodological heterogeneity, inadequate normalization, limited absolute quantification, a shortage of reference intervals and insufficient prospective validation[3,13]. Single miRNAs rarely provide adequate disease specificity for stand-alone tests, while multimarker panels integrated with established biomarkers and clinical context are more realistic[1,13]. The translational experience in absolute quantification, reference interval derivation and HFrEF supports a practical implementation path: standardize sample handling, validate analytically robust assays in absolute units, define biological reference data, test multimarker models prospectively, and focus on clinical questions where current diagnostics are genuinely incomplete[28,47].

Search strategy and selection criteria

A literature search was performed in PubMed, Web of Science and Scopus for English-language publications through May 2026. Search terms combined "microRNA", "miRNA", "circulating microRNA", "biomarker", "laboratory medicine", "absolute quantification", "preanalytical standardization", "ring trials" and "reference intervals" with clinical domain terms for all areas covered in the text. Priority was given to systematic reviews, meta-analyses, clinically oriented reviews, prospective human studies and laboratory medicine papers addressing analytical and translational implementation. References are limited to 100.

ABBREVIATIONS

AD, Alzheimer's disease; AKI, Acute kidney injury; ALS, Amyotrophic lateral sclerosis; AMD, Age-related macular degeneration; AUROC, Area under receiver operating characteristic curve; CNS, Central nervous system; CSF, Cerebrospinal fluid; ctDNA, Circulating tumor DNA; CV, Coefficient of variation; ddPCR, Droplet digital polymerase chain reaction; DILI, Drug-induced liver injury; DR, Diabetic retinopathy; EDTA, Ethylenediaminetetraacetic acid; EQA, External quality assessment; EV, Extracellular vesicle; HF, Heart failure; HFpEF, Heart failure with preserved ejection fraction; HFrEF, Heart failure with reduced ejection fraction;

LVEF, Left ventricular ejection fraction; miREIA, microRNA enzyme immunoassay; miRNA/miR, microRNA; mRNA, Messenger RNA; MS, Multiple sclerosis; MASLD, Metabolic dysfunction-associated steatotic liver disease; MASH, Metabolic dysfunction-associated steatohepatitis; NGS, Next-generation sequencing; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; PD, Parkinson's disease; pre-miRNA, Precursor microRNA; pri-miRNA, Primary microRNA; PTX3, Pentraxin-3; RA, Rheumatoid arthritis; RISC, RNA-induced silencing complex; RT-qPCR, Reverse transcription quantitative polymerase chain reaction; SLE, Systemic lupus erythematosus; VEGF, Vascular endothelial growth factor.

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Declaration of Generative AI and AI-Assisted Technologies: Perplexity AI (perplexity.ai, Council model, accessed May 2026) was used for language editing, text organization and formatting support during manuscript preparation. Literature selection, critical appraisal of evidence, clinical interpretation, numerical data, reference verification, and all scientific conclusions were performed and verified exclusively by the authors, who accept full responsibility for the accuracy and integrity of the final manuscript. The use of AI did not influence the scientific content of this work.

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