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Corresponding Author:	Mildred Iro Southampton, UNITED KINGDOM
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First Author:	Mildred Iro
First Author Secondary Information:	
Order of Authors:	Mildred Iro
	Sudha Priya Soundara Pandi
Order of Authors Secondary Information:	

Clinical Application of Non-coding RNAs in Sepsis

Mildred A Iro[1,2] and Sudha Priya Soundara Pandi[1]

[1] Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, United Kingdom; [2]Department of Paediatric Immunology and Infectious Diseases, Southampton Children's Hospital, University Hospital Southampton NHS Foundation Trust, Southampton, UK

Correspondence: Dr Mildred Iro, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, MP825. Tremona Road, Southampton, SO16 6YD, United Kingdom

Telephone: +44(0)2381208560

Email: m.a.iro@soton.ac.uk

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Abstract

Purpose of review

Studies indicating that non-coding RNAs (ncRNAs) play a regulatory role in sepsis are increasing rapidly. This present review summarises recent publications on the role of microRNAs and long non-coding RNAs (lncRNAs) in sepsis.

Recent findings

MicroRNAs and lncRNAs are being identified as potential sepsis biomarkers and therapeutic targets. Experimental studies have examined the biological mechanisms that might underpin the regulatory role of these ncRNAs in sepsis.

Summary

Clinical applications of miRNAs and lncRNAs in sepsis are on the horizon. These data could lead to the identification of novel treatments or indeed support the repurposing of existing drugs for sepsis. Validation of the findings from these preliminary studies and crucially integration of multi-omics datasets will undoubtedly revolutionise the clinical management of sepsis.

Introduction

Sepsis is characterised by a dysregulated immune response to infection, leading to organ dysfunction. Globally, sepsis affects 30 million people and 6 million deaths annually (1). Sepsis diagnosis is challenging due to non-specificity of the clinical presentation, lack of a gold standard diagnostic test, and variations in case definition. Thus, there is the need to identify robust biomarkers to facilitate timely diagnosis. Additionally, with the rapidly increasing prevalence of antimicrobial resistance, new therapeutic strategies for sepsis are warranted. Increasing evidence suggest that non-coding RNAs (ncRNA) could bridge these gaps. Here, we summarise the current state of research on microRNAs and long ncRNAs as biomarkers and therapeutic targets in sepsis.

Pathophysiology of sepsis

The discovery of toll-like receptors (TLRs) led to a significant breakthrough in the understanding of the pathophysiology of sepsis. TLRs act as primary sensors to detect a variety of pathogen associated molecular patterns (PAMPS) which triggers activation of complex downstream intracellular signalling pathways, predominantly involving transcription factor nuclear factor-kappa B (NF-κB), leading to the expression of an array of proinflammatory cytokines and inflammatory mediators. This process results in inflammation, dysregulation of complement, coagulation and endothelial activation, cytopaenias, immunosuppression, and if unchecked, hyperinflammation, apoptosis and multiple organ dysfunction. The pathophysiology of sepsis is a large subject and has been comprehensively reviewed elsewhere (2)

Overview of ncRNAs

NcRNAs are regulatory ribonucleic acid (RNA) molecules that regulate gene expression at transcriptional and translational levels. Three main groups of ncRNAs exist: (i) short ncRNA (<30 nucleotides e.g. microRNA, small interfering RNA and piwi-interacting RNA), (ii) intermediate-sized ncRNAs (30-200 nucleotides e.g. snoRNAs), and (iii) lncRNA (>200nucleotides). This review focuses on miRNAs and lncRNAs since these are arguably the most studied ncRNAs in sepsis.

MicroRNAs (miRNAs) regulate translation of >50% of human protein coding genes. They bind complementary sequences in messenger RNA (mRNA) molecules to elicit gene silencing by inhibiting translation or degrading mRNA to fine tune protein expression. MiRNAs form an important link between the innate and adaptive immune systems, function as fine tuners of the inflammatory response primarily through regulating TLR signalling and thus have a role in the resolution of inflammation (3, 4).

The role of lncRNAs in gene regulation is less well understood. Current understanding is that lncRNAs are fundamental regulators of transcription (5). LncRNAs modulate the effect of miRNAs on gene expression by triggering RNA decay, competing with miRNAs for the same mRNA target, or acting as decoys or 'sponges' for miRNAs (6). The biogenesis of miRNAs and lncRNAs is extensively discussed elsewhere (7, 8) and is beyond the scope of this review.

Clinical utility of miRNAs

Circulating miRNAs as biomarkers of sepsis

A significant number of miRNAs exist in the extracellular space. The stability of miRNAs in body fluids (e.g. blood, urine, and saliva) and the ease of access to these body fluids using non-invasive methods make miRNAs well suited as biomarkers. To identify potential miRNA biomarkers for sepsis, clinical studies have typically focused on comparing miRNA expression between sepsis patients and healthy controls with varying results (**Table 1**). This variation in the subsets of differentially expressed microRNAs might reflect reporting bias in these studies which have predominantly used candidate miRNA selection approach. Moreover, the miRNA biomarker profile differences between children and adults might suggest that miRNA regulatory mechanism differs between both populations.

MiRNA expression varies by sepsis severity. For example, miR-126 and -223 were significantly higher in adult sepsis non-survivors than survivors (19, 20). Both microRNAs target mammalian target rapamycin (mTOR) signalling which regulates cell survival, growth and metabolism (26-29). *In vitro*, miR-223 regulates the innate immune response by targeting NLRP3 inflammasome (30, 31), and NF-κB signalling (32, 33). NF-κB is a crucial mediator of inflammation, and regulates the survival, activation, and differentiation of innate immune cells and inflammatory T cells (34). NF-κB plays a central role in modulating the expression of several immunoregulatory mediators involved in sepsis and increased levels are associated with higher rates of mortality and worse clinical outcomes (35).

MiRNA expression differs by sepsis phenotype. Using TaqMan Low Density Array analysis (TLDA), Lin *et al* showed an upregulation of plasma levels of 11 miRNAs (miR-210, -494, -23a, -26a, -29a, -10a-5p, -122, -143, -214, -223, -497) and downregulation of 11 miRNAs

(miR-205, -146a, -182-5p, -16, -21, -145, -203, -204, -290, -320, -590) in adults with sepsis induced acute kidney injury (S-AKI) than in healthy controls (36).

Recent studies have evaluated the diagnostic accuracy of miRNAs in sepsis (Table 2). In children, miR-181a, -101, and -187 performed well (area under the receiver operating characteristic curve, AUC: 0.893, 0.908, 0.789 respectively) (11, 13). In adults, miR-495 performed well (0.915) (16, 19, 20) whereas miR-126 and -223 performed less well (0.726, 0.754 respectively) (19, 20). Elsewhere, miR-21 had a reasonable diagnostic accuracy (AUC: 0.793, 95% confidence interval (CI): 0.644-0.942) in differentiating adult sepsis survivors from non-survivors whereas miR-223 (AUC 0.6, 95% CI: 0.505-0.695) and miR-126 (AUC 0.619, 0.533-0.705) did not. Additionally, miR-495 distinguished adult sepsis shock from non-shock patients (AUC=0.885) (16) whereas miR-7110-5p and miR-223-3p discriminated adult patients with pneumonia only from those with sepsis secondary to pneumonia (AUC: 0.883 and 0.964 respectively) (25).

These studies highlight the promising prospects of miRNAs as sepsis biomarkers . Nonetheless, the inter-study differences (e.g. heterogeneity in studied miRNAs, differences in sample type studied) and small sample sizes in some studies are limitations. Crucially, validation of these findings in large independent cohorts is needed. Furthermore, most studies compared miRNA levels between sepsis patients and healthy, not disease controls. It is therefore not known whether these miRNAs are sepsis specific. Assessment of the utility of miRNAs in distinguishing sepsis from SIRS and indeed other non-infectious, inflammatory conditions e.g. Kawasaki disease that could present similarly would be a clinically relevant application.

miRNAs as indicators of organ damage in sepsis

Sepsis induced organ damage (SIOD) is associated with high rates of morbidity and mortality (39, 40). Current research indicates that excessive apoptosis drives organ damage in sepsis whereas autophagy is protective (41, 42). NcRNAs regulate apoptosis and autophagy by directly modulating related genes or signalling pathways that initiate both processes (41, 43).

The role of miRNAs in SIOD has been explored in several experimental studies (Table 3). Guo and colleagues showed that overexpression of miR-495 in a rat sepsis model abrogated sepsis induced myocardial dysfunction (SIMD) (16). MiR-495 genes downregulate mTOR signalling (44, 45) which is critical for the regulation cell metabolism, growth, proliferation, and survival and regulates cardiomyocyte metabolism (46, 47). Also, miR-495 improved cardiac microvascular endothelial cell injury and inflammation by suppressing the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome signalling pathway (48), a critical component of the innate immune system that leads to caspase 1-dependent activation and the secretion of proinflammatory cytokines in response to microbial infection and cellular damage (49). Chen *et al* (42) observed higher levels of phosphorylated ribosome S6 protein kinase (downstream target of mTOR pathway) in SMID patients. Elsewhere, upregulation of miR-23b suppressed apoptosis of cardiomyocytes and improved sepsis-induced cardiomyopathy (50). This was suggested to be due to inhibition of NF-κB (downstream of mTOR) activated inflammatory response through its direct targets tumour necrosis factor (TNF) receptor associated factor 6 and IκκB. Furthermore, miR-98 prevented myocardial damage in septic mice by inhibiting NFk-B signalling (51).

MiRNAs are implicated in sepsis induced acute lung injury (S-ALI). For example, miR-145 was downregulated in lung tissues of sepsis mice (23) and inhibited S-ALI by inactivating

transforming growth factor-beta receptor type 2 (TGFBR2)/Smad3 signalling, a pathway that promotes tissue fibrosis (52). MiR-539-5p was downregulated in mice following caecal ligature puncture (CLP) induced sepsis (53) and its overexpression *in vitro* abrogated CLP induced lung injury by suppressing Rho associated protein kinase 1, a critical mediator of fibrosis. In another study (54), upregulation of miR-326 suppressed LPS-induced inflammation in murine lungs by inhibiting TLR4, a critical for the resolution of acute and chronic inflammation and pulmonary fibrosis. *In vitro*, miR-574-5p alleviated S-ALI by regulating TRAF6/NF-κB pathway (55). Elsewhere (56), miR-494 inhibition alleviated acute lung injury in sepsis -associated ARDS through NQO1-mediated inactivation of Nrf2 a transcription factor involved in the regulation of anti-oxidants and cytokine gene expression.

Recent evidence suggest that metabolic reprogramming is one of the fundamental processes in S-AKI (57). Accordingly, *in vitro*, miR-21-3p altered tubular epithelial cell metabolism through AKT/CDK2 mediated upregulation of Forkhead box protein O1 (58), a transcription factor involved in energy metabolism (59) and regulation of cell proliferation.

Thus, targeting these candidate miRNAs and/or their target genes could be a therapeutic approach to limit, or indeed prevent sepsis induced organ injury. Indeed, several human trials of miRNA-based therapies for other conditions are underway (60), providing some confidence in the clinical translatability of these experimental findings.

miRNAs and sepsis susceptibility

Polymorphisms in miRNA genes can influence gene transcription, alter the processing of pri- or pre-miRNA, and affect miRNA–mRNA interactions. Zhang *et al* showed that single nucleotide polymorphisms of miR-187 (rs12605436), miR-21 (rs13137), and miR-145

(rs353291) were associated with the development of sepsis (18). Although the underlying biological mechanisms are unclear, such observations could be utilised for patient stratification and to support a precision-based approach to treatment.

Clinical utility of lncRNAs in sepsis

Although previously considered as 'transcriptional noise', it is becoming evident that lncRNAs play a key role in sepsis and their dysregulation has been reported in several studies (Table 4a). One of such lncRNAs is metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), a conserved lncRNA that is abundantly expressed in almost all normal human tissues. MALAT1 was upregulated in sepsis patients compared with healthy controls (65-67) and differentiated sepsis survivors from non-survivors, albeit with varying diagnostic accuracy . MALAT1 was higher in sepsis ARDS than non-ARDS patients (68) and its knockdown was protective against LPS induced ALI (69, 70) by sponging miR-146a (69) and inhibiting p38 MAPK/p65 NF-κB signalling (70). Data from experimental studies suggest that MALAT1 has a proinflammatory role in sepsis and SIOD and its knockdown is protective through suppression of NF-κB signalling (65, 69, 71, 72). This protective knockdown effect was mediated via miR-125b/p38 MAPK axis (rat sepsis model) (65), miR-149/MyD88 axis and miR-146a (acute lung injury sepsis model) (69, 70), and miR-146a (acute kidney injury sepsis model) (71).

Like MALAT1, nuclear paraspeckle assembly transcript 1(NEAT1) was also upregulated in adult sepsis patients compared with healthy controls and in sepsis AKI versus non-AKI patients. (73-75).

However, it showed only an acceptable to suboptimal accuracy at distinguishing sepsis patients from healthy controls (AUC range: 0.730-0.785) and sepsis non-survivors from survivors (AUC range: 0.641-0.726) (74, 76). Knockdown of NEAT1 suppressed LPS induced inflammation by inhibiting TLR signalling (73, 75, 77) and respectively, upregulating and suppressing miR-204 and NF-κB signalling respectively (73). In another study NEAT1 promoted apoptosis in human kidney cells by regulating miR-27a-3p (78). Elsewhere, NEAT1 promoted liver injury in LPS treated Kupffer cells and Raw264.7 (mouse monocyte/macrophage) cell line, by competitively inhibiting miRNA Let-7a and activating TLR4 (79).

LncRNA TUG1 was downregulated in serum samples of S-AKI patients and its overexpression ameliorated LPS induced injury in rat mesangial cells by regulating miR-142-3p/SIRT1/NF-κB signalling (80). This finding supports earlier work (81) although in the previous study, the effect of TUG1 on NF-κB signalling was mediated by miR-223.

In vitro data on the role of lncRNA colorectal neoplasia differentially expressed (CRNDE) in S-AKI are inconsistent. In two studies, CRNDE was upregulated and promoted inflammation and apoptosis in kidney cells following LPS stimulation (37, 82). However, in another study, CRNDE was significantly lower in renal tissue of urogenic sepsis rats compared with controls and its overexpression was protective against S-AKI (83). Similar to the previous two studies, the effect of CRNDE was mediated through TLR/NF-κB pathway, albeit by targeting a different miRNA. Elsewhere, overexpression of CRNDE in sepsis rats improved myocardial injury by ‘sponging’ miR-29a to promote SIRT1 mediated downregulation of NF-κB/PARP1 signalling (84).

Transcript Predicting Survival in AKI (TapSAKI) and HOTAIR were upregulated in kidney tissues from sepsis rats and in human kidney (HK2) cells following LPS treatment. Respectively, both lncRNAs promoted apoptosis in HK2 cells through miR-122 dependent regulation of TLR4/NF-κB pathway (85), and miR-22 dependent regulation of HMGBI, a negative regulator of apoptosis. Contrarily, in another study, overexpression of HOTAIR reversed AKI in sepsis rats by downregulating miR-34a and upregulating B-cell leukaemia/lymphoma 2 (Bcl-2) - an inhibitor of apoptosis (86).

The diagnostic performance of lncRNAs in sepsis is summarised in Table 4b. Similar to miRNAs, this varies across studies and supports the need for these preliminary findings to be robustly validated. Perhaps, integrating multiple ncRNA biomarker candidates or their inclusion as part of a clinical score could increase their diagnostic and prognostic performance.

Altogether, these data demonstrate that NF-κB signalling, and apoptosis are crucial in the pathogenesis of SIOD and that these processes are regulated by lncRNAs and miRNAs. Indeed, NF-κB regulates the transcription of several immunomodulatory mediators and activation of NF-κB signalling is associated with higher rates of mortality and worse clinical outcomes in sepsis patients. Thus, regulation of NF-κB signalling through targeting individual lncRNAs and/or their target miRNAs is a promising therapeutic approach and could improve outcomes in SIOD.

Conclusion

There is an exponential increase in research studies investigating the role of miRNAs and lncRNAs in sepsis. Indeed, data presented here indicate that miRNAs and lncRNAs could serve as biomarkers and therapeutic targets for sepsis. Also, some insight into potential

mechanisms by which these ncRNAs might play a role in the pathogenesis of sepsis has been highlighted. Interestingly all these differentially regulated miRNAs and lncRNAs play an important role in regulating the cell cycle and inflammation, the mechanism altered in sepsis. However, there are still fundamental gaps that need to be bridged before these findings can be translated into clinical practice. It is anticipated that future work will now be centered on addressing the interstudy heterogeneities and validation of these results in independent cohorts. Ultimately, integration of these preliminary data with those from other multi-omics platforms e.g. proteomics, and incorporation of robust bioinformatic pipelines for analyses would undoubtedly accelerate the pathway to the discovery of ncRNA-based biomarkers and therapies for sepsis.

Key points

- MicroRNAs and long non-coding RNAs are abnormally expressed and have been shown to play a regulatory role in sepsis
- Experimental studies have provided valuable insight into the mechanisms through which microRNAs and long non-coding RNAs play a regulatory role in sepsis
- MicroRNAs and long non-coding RNAs provide some promise to be utilised as novel biomarkers and therapeutic targets for sepsis.

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Conflicts of interest

None

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Table

miRNA	Study population	Sample size (n)	Sample type	Up or down regulated	Comparison	Ref
miR-132	Neonates	EOS (25); HC (25)	Plasma	downregulated	Sepsis vs. HC	(9)
miR-223				downregulated	Sepsis vs. HC	
miR-26a	Neonates	Sepsis (28); HC (32)	Blood mononuclear cells and Serum	downregulated	Sepsis vs. HC	(10)
miR-181a	Neonates	Sepsis (102), HC (50)	Serum	downregulated	Sepsis vs. HC	(11)
miR-146a	Children	Sepsis (55), HC (60)	Serum	downregulated	Sepsis vs HC	(12)
miR-187	Children	Sepsis (50); SIRS (30), HC (20)	Whole blood	upregulated	Sepsis and SIRS vs HC	(13)
miR-101				upregulated	Sepsis vs. HC	
miR-21				upregulated	Sepsis and SIRS vs HC	
miR-26b	Adults	Sepsis (21), HC (21)	Platelets	downregulated	Sepsis vs. HC	(14)
miR-126	Adults	Sepsis (60); HC (25)	Plasma	upregulated	Sepsis vs. HC	(15)
miR-495	Adults	Sepsis (105); HC (100)	Serum	downregulated	Sepsis vs. HC	(16)
miR-9	Adult	Sepsis (28); HC (23)	Whole blood	upregulated	Sepsis vs. HC	(17)
miR-187	Adult	Sepsis (180); HC (180)	Plasma	downregulated	Sepsis vs. HC	(18)
miR-21				upregulated	Sepsis vs. HC	
miR-126	Adult	Sepsis (208); HC (210)	Plasma	upregulated	Sepsis vs. HC and non-survivors vs. survivors	(19)
miR-223	Adults	Sepsis (187); HC (186)	Plasma	Upregulated	Sepsis vs. HC and non-survivors versus survivors	(20)

miR-92a	Adults	Sepsis (13); ARDS (36); HC (15)	Serum	Upregulated	Sepsis vs. HC and Sepsis induced ARDS vs. HC	(21)
miR-29a	Not specified	Sepsis (8), Control (8)	Blood	Upregulated	Sepsis vs. controls	(22)
miR-145	Not specified	Sepsis (33); HC (22)	Exosomes	Downregulated	Sepsis vs. HC	(23)
miR-103	Adults	ARDS sepsis (28); Non-ARDS sepsis (168)	Plasma	Downregulated	Sepsis vs. HC and ARDS vs. non-ARDS	(24)
miR-107				Downregulated	Sepsis vs. HC and ARDS vs. non-ARDS	
miR-7110-5p	Adults	Pneumonia only (52); sepsis secondary to pneumonia (44)	Plasma	Upregulated	Pneumonia only versus Sepsis secondary to pneumonia	(25)
miR-223-3p				Upregulated	Pneumonia only versus Sepsis secondary to pneumonia	
miR-940				Downregulated	Pneumonia only versus Sepsis secondary to pneumonia	

Table 1: Summary of selected recent clinical studies showing patterns of differential expression of circulating miRNAs in sepsis. Unless stated otherwise, comparison made was between sepsis patients and healthy controls. miRNA=microRNA, EOS= early onset sepsis, miRNA=micro RNA, ICU=intensive care unit, SS=septic shock, SIRS=systemic inflammatory response syndrome, HC=healthy control, ARDS=acute respiratory distress syndrome, Ref=reference

miRNA	Comparison	Cohort	Sample size (n)	AUC (95% CI, where provided)	Sensitivity (%)	Specificity (%)	Cut off value	Ref
miR-181a	Sepsis vs. HC	Neonates	Sepsis (102), HC (50)	0.893	83.3	84	0.625	(11)
miRNA-495	Sepsis vs. HC	Adults	Sepsis (105), HC (100)	0.915	89.5	83	0.655	(16)
miRNA-495	Shock vs. non shock	Adults	SS (34), non-SS (71)	0.885	85.3	87.3	0.475	
miR-101	Sepsis vs. HC	Neonates	Sepsis (50), HC (20)	0.908 (0.847-0.97)	84	84	0.936	(13)
miR-187	Sepsis vs. HC	Neonates	Sepsis (50), HC (20)	0.789 (0.698 - 0.88)	72	76	0.319	
miR-21	Survivors vs. non-survivors	Neonates	Survivors (34), non-survivors (16)	0.793 (0.644-0.942)	62.5	88.2	3.48	
miR-146-a	Sepsis vs. HC	Children	Sepsis (55), HC (60)	0.803 (0.723-0.883)	86.6	56.6	<0.5	(12)
miR-146-a	Non-survivors vs. survivors	Children	N/A	0.76 (0.618-0.909)	67	80	0.4	
miR-126-3p	Sepsis vs. non sepsis	Children	Sepsis (60), Non sepsis (25)	0.735 (0.618-0.852)	N/A	N/A	N/A	(15)
miR-126	Sepsis vs. HC	Adults	Sepsis (208), HC (210)	0.726 (0.678-0.774)	N/A	N/A	N/A	(19)
miR-126	Survivors vs. non-survivors	Adults	Survivors (139); non-survivors (69)	0.619 (0.533-0.705)	N/A	N/A	N/A	
miR-223	Sepsis vs. HC	Adults	Sepsis (187), HC (186)	0.754 (0.706-0.803)	56.6	86.6	N/A	(20)
miR-223	Survivors vs. non-survivors	Adults	N/A	0.600 (0.505 - 0.695)	83.5	38.9	N/A	
miR-7110-5p	Pneumonia only vs. pneumonia and sepsis	Adults	Pneumonia only (52), sepsis due to pneumonia (44)	0.883	84.2	90.5	4.41	(25)
miR-223-3p	Pneumonia only vs. pneumonia and sepsis	Adults	Pneumonia only (52), sepsis due to pneumonia (44)	0.964	82.9	100	2.759	

miR-103	ARDS sepsis vs non ARDS sepsis	Adults	ARDS sepsis (28), Non-ARDS sepsis (168)	0.727 (0.577 - 0.811)	64.3	78.6	0.178	(24)
miR-107	ARDS sepsis vs non ARDS sepsis	Adults	ARDS sepsis (28), Non-ARDS sepsis (168)	0.694 (0.577-0.811)	85.7	51.2	0.295	(24)
miR-107	Survivors vs. non-survivors	Adults	Survivor (134), non-survivors (62)	0.649 (0.569-0.729)	N/A	N/A	N/A	
miR-103	Survivors vs. non-survivors	Adults	Survivor (134), non-survivors (62)	0.704 (0.626-0.782)	N/A	N/A	N/A	
miR-328	Sepsis vs. HC	Adults	Sepsis (110), HC (89)	0.926	87.6	86.36	0.305	(37)
miR-26b	AKI sepsis vs. non-AKI sepsis	Adults	AKI sepsis (68), non-AKI sepsis (87)	0.886 (0.831-0.942)	90.8	75	0.124	(38)
miR-26b	Sepsis vs. SIRS	Adults	Sepsis (155), SIRS (56)	0.816 (0.758-0.874)	60	89.3	0.062	
miR-210	AKI sepsis survivor vs. non-survivor	Adults	AKI sepsis (110), HC (110)	0.852 (0.777-0.928)	81	80.9	6.995	(36)
miR-494	AKI sepsis survivor vs. non-survivor	Adults	AKI sepsis (110), HC (110)	0.847 (0.772-0.922)	80.9	72.1	7.005	
miR-205	AKI sepsis survivor vs. non-survivor	Adults	AKI sepsis (110), HC (110)	0.860 (0.792-0.927)	78.6	90.5	3.245	

Table 2: Summary of selected recent studies investigating the diagnostic accuracy of miRNAs in sepsis. miRNA=microRNA, HC=healthy controls, SS=septic shock, AUC=area under receiver operating characteristics curve, ARDS=acute respiratory distress syndrome, AKI = acute kidney injury, N/A=not available, Ref=reference

miRNA	Body fluid/tissue of expression	Result	Target gene/Pathway	Reference
S-ALI/ARDS				
miR-574	Human bronchial epithelial cells stimulated with LPS	Downregulated	Complement 3	(61)
miR-92a	LPS stimulated human pulmonary microvascular endothelial cells and alveolar epithelial cells	Upregulated	Akt/mTOR signaling	(21)
miR-539-5p	LPS stimulated murine pulmonary microvascular endothelial cells	Downregulated	ROCK 1.	(53)
miR-326	LPS stimulated murine lungs and macrophages	Downregulated	TLR4	(54)
miR-29a	LPS induced murine pulmonary endothelial cells,	Upregulated	STAT3	(22)
miR-181a-5p	LPS stimulated murine macrophage cell line RAW 264.7	Upregulated	SIRT1	(62)
miR-574-5p	Murine lung tissues	Downregulated	TRAF6/NF-κB	(55)
miR-483-5p	Murine lung tissues and PMVECs	Upregulated	PIAS1	(63)
miR-145	LPS treated human normal lung epithelial cells	Downregulated	TGFBR2	(23)
SIMD				
mir-146a	Myocardial tissue of LPS treated rats	Upregulated	TLR-4/NF-κB	(64)
miR-98	Murine myocardial tissue following CLP	Downregulated	HMGA2/NF-κB	(51)
miR-328	Rat myocardial tissue following CLP	Upregulated	N/A	(37)
miR-23b	LPS stimulated rat cardiomyocytes	Upregulated	TRAF6/IKKB/NF-κB	(50)

Table 3 Summary of selected experimental studies of miRNAs in sepsis induced organ injury. miRNA=microRNA, LPS=lipopolysaccharide, mTOR= mammalian target to rapamycin ROCK1= Rho-associated protein kinase 1; TLR= toll like receptor; PIAS1=protein inhibitor of activated signal transducer and activator transcription (STAT) 1; TGFBR2= transforming growth factor-beta (TGF-β) receptor type 2; NF-κB= Nuclear Factor kappa-light-chain-enhancer of activated B cells; HGMA2= High-mobility group AT-hook; TRAF6 =Tumor necrosis factor receptor associated factor 6; SIRT1=Sirtuin 1; N/A=not available , Ref=reference

lncRNA	Population	Sample size (n)	Sample type	Result	Comparison	Reference
ITSN1-2	Adults	Sepsis (309); HC (300)	Plasma	Increased	Sepsis vs. HC	(87)
		Non-survivors (94); survivors (215)		Increased	Non-survivors vs. survivors	
MALAT1	Adults	Sepsis (120); HC (60)	Plasma	Increased	Sepsis vs. HC	(65)
		N/A		Increased	Non-survivors vs. survivors	
MALAT1	Adults	Sepsis (190); HC (190)	Plasma	Increased	Sepsis vs. HC	(66)
		Survivors (132); Non-survivors (58)		Increased	Non-survivors vs. survivors	
NEAT1	Adults	Sepsis (68); HC (32)	Serum	Increased	Sepsis vs. HC	(75)
		N/A		Increased	Non-survivors vs. survivors	
NEAT1	Adults	Sepsis (150); HC (150)	Plasma	Increased	Sepsis vs. HC	(74)
		Survivors (107); non-survivors (45)		Increased	Non-survivors vs. non-survivors	
NEAT1	Adults	AKI sepsis(13); Non-AKI sepsis (39)	Serum	Increased	AKI vs. non-AKI sepsis	(73)
NEAT1	Adults	Sepsis (25); HC (25)	Serum	Increased	Sepsis vs HC	(88)
TUG1	Adults	S-AKI (28); HC (28)	Serum	Decreased	S-AKI vs. HC	(80)
ZFAS1	Adults	Sepsis (202); HC (200)	Plasma	Decreased	Sepsis vs. HC	(89)
				Decreased	Survivors vs. non-survivors	
CRNDE	Adults	Sepsis (136) ; HC (151)	N/A	Increased	Sepsis vs. HC	(90)
THRIL	Adults	ARDS sepsis (32); non-ARDS sepsis (77)	Serum and plasma	Increased	ARDS vs non ARDS	(91)
				Increased	Non-survivors vs survivors	

Table 4a: Summary of selected recent clinical studies showing patterns of differential expression of lncRNAs in sepsis. LncRNA=long non-coding RNA, HC=healthy controls; AKI=acute kidney injury; ARDS= acute respiratory distress syndrome, ITSN1=Intersectin 1; NEAT1= nuclear paraspeckle assembly transcript 1; TUG1= taurine upregulated gene 1; ZFAS1= ZNFX1 antisense RNA 1; CRNDE= Colorectal Neoplasia Differentially Expressed; THRIL= TNF-related and HNRNPL- related immunoregulatory lncRNA; MALAT1=Metastasis Associated Lung Adenocarcinoma Transcript 1, Ref=reference

lncRNA	Comparison	AUC (95% CI)	Sensitivity	Specificity	NPV	PPV	Best cut off point	Reference
MALAT1/miR-125a	Sepsis vs. HC	0.931 (0.908-0.954)	91.3	78.6	90.1	81.0		
MALAT1	Sepsis vs. HC	0.866 (0.830-0.901)	73.0	88.3	76.5	86.1		
MALAT1/miR-125a	Survivors vs. non survivors	0.678 (0.603-0.754)	94.6	40.0	94.9	38.7		
MALAT1	Survivors vs. non survivors	0.977 (0.595-0.758)	35.7	92.1	78.2	64.5	N/A	(66)
MALAT1	Sepsis vs. HC	0.823 (0.783-0.864)	N/A					(67)
	Survivors vs. non survivors	0.755 (0.682-0.828)						
MALAT1	Sepsis vs. HC	0.91(N/A)	N/A					(65)
	Septic shock vs. no shock	0.836 (N/A)						
	Survivors vs. non survivors	0.886 (N/A)						
MALAT1	sepsis ARDS (41) vs. sepsis non-ARDS (111)	0.674 (0.581-0.766)	N/A					(68)
	Survivor (105) vs. non-survivor (47)	0.651 (0.555-0.747)						
	Sepsis (152) vs. HC (150)	0.730 (0.740-0.861)	N/A					(74)

NEAT1	Survivors (45) vs. non survivors (107)	0.641 (0.536-0.746)						
NEAT1	Sepsis (82) vs. HC (82)	0.785 (0.716-0.853)	N/A					(76)
	Survivors (54) vs. non survivors (28)	0.726 (0.615-0.837)						
ITSN1-2	Sepsis(309) vs. HC (300)	0.777 (0.740-0.813)	59.5	86.3	N/A		1.82	(87)
	Survivors vs. non survivors	0.654(0.581-0.726)	92.1	40.4			4.059	
ZFAS1	Sepsis (200) vs. HC (200)	0.814 (0.771-0.857)	92.1	63.5	N/A			(89)
	Survivors (62)vs. non survivors(140)	0.628 (0.538-0.717)	92.1	35.5				
THRIL	Sepsis ARDS(32) vs. Sepsis non-ARDS (77)	0.706 (0.602-0.809)	68.7	71.4	N/A		2.583	(91)
	Survivors(76) vs. non survivors(33)	0.780 (0.683-0.876)	88.2	54.5			3.671	

Table 4b: Summary of recent studies investigating the diagnostic accuracy of lncRNAs in sepsis. LncRNA=long non-coding RNA, HC=healthy controls, AUC=area under receiver operating characteristics curve, CI=confidence intervals, provided where available; NPV=negative predictive value; PPV=positive predictive value; ARDS=acute respiratory distress syndrome, N/A=not available; ITSN1=Intersectin 1; NEAT1= nuclear paraspeckle assembly transcript 1; TUG1= taurine upregulated gene 1; ZFAS1= ZNFX1 antisense RNA 1; CRNDE= Colorectal Neoplasia Differentially Expressed; THRIL= TNF- related and HNRNPL- related immunoregulatory lncRNA; MALAT1=Metastasis Associated Lung Adenocarcinoma Transcript 1, Ref=reference