

Values of circulating molecular biomarkers (microRNAs) for the evaluation of renal failure during urgent abdominal sepsis anaesthesia

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Background. Micro-ribonucleic acids (miRNAs) are small non-coding molecules important for gene regulation and management of physiological processes (1). Alterations in the expression of miRNAs are potential novel biomarkers for many diseases (2).

Materials and methods. Random patients who underwent emergency surgery for abdominal sepsis were enrolled into the study ($N = 27$). Patients were divided into three groups according to the renal function and into two groups depending on the presence or the absence of lethal outcomes during the hospitalization period. Relative expression levels of circulating serum miR-30d-5p, miR-23a-3p, miR-146a-5p were assessed with real-time quantitative polymerase chain reaction using the $2^{-\Delta\Delta Ct}$ method and compared between the groups.

Results. Expression levels of all three miRNAs did not differ significantly between patients with acute renal failure (ARF) ($n = 8$), chronic renal failure (CRF) ($n = 8$), and with a normal renal function (NRF) ($n = 11$). Estimated glomerular filtration rates (eGFR) were significantly lower ($p = 0.016$), the values of urea ($p = 0.007$) and red blood cell distribution width (RDW) ($p = 0.001$) were significantly higher in septic patients who died, but no significant correlation between RDW values and expression of miRNAs was found.

Conclusions. The expression levels of serum miR-30d-5p, miR-23a-3p, miR-146-5p did not significantly differ between three groups of patients who developed ARF, had CRF, or retained NRF. No significant association between the RDW value and expression of miRNAs was noted.

Keywords: MiRNA, acute renal failure, chronic renal failure, RDW, sepsis

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INTRODUCTION

The prevalence of sepsis is high and the condition remains one of the leading causes of death worldwide (3). In 2016, the definitions of sepsis and septic shock were updated (Sepsis-3). Sepsis was redefined as a life-threatening organ dysfunction resulting from dysregulated host response to infection (4). Septic shock is characterized as a subset of sepsis and has circulatory, cellular, and metabolic abnormalities which are profound enough to substantially increase the risk of mortality (5).

Sepsis-induced acute kidney injury (AKI) has become very common in the critically ill. According to a research by Ali et al., AKI occurs in up to 47% of patients with diagnosed sepsis (6). Patients with chronic kidney disease (CKD) have a higher risk of morbidity and mortality from sepsis (7). Furthermore, patients with renal failure undergoing surgery are at essential risk of increased perioperative morbidity and mortality (8).

Early diagnosis and treatment initiation are critical to improve the survival rate of septic patients. Although numerous biomarkers have been tested clinically to diagnose sepsis, none had sufficient specificity or sensitivity to be routinely employed in clinical practice (9). Therefore, it is important to continue the search. Molecular biomarkers have a potential to facilitate the diagnostic process (10, 11).

Micro-ribonucleic acids (miRNAs) are small non-coding molecules and play an important role in gene regulation and management of physiological processes (1). The markers can be detected in a stable form in various body fluids (12). Chen et al. found that serum contains large amounts of stable miRNAs and their expression profile can be used as a potential novel biomarker for sensitive, specific and early diagnosis of many diseases (2). Recent studies have already suggested the use of microRNAs to diagnose and stage sepsis in the critically ill (10, 11).

Many reports suggest the use of miR-30d-5p, miR-23a-3p, and miR-146-5p as sepsis biomarkers (13–15), but some authors suggest miR-30d-5p and miR-146-5p as markers of renal failure (16, 17). In this study the relative levels of expression of serum miR-30d-5p, miR-23a-3p, miR-146-5p and clinical blood test results were observed in patients with abdominal sepsis and renal failure during urgent anaesthesia.

MATERIALS AND METHODS

Random patients who underwent emergency surgery for abdominal sepsis (peritonitis) were enrolled into the study. Patients received treatment at the Lithuanian University of Health Sciences from July 2018 to September 2018. A written informed consent was obtained from all of them. The study was approved by Kaunas Regional Biomedical Research Ethics Committee (No. BE-2-19) and carried out in accordance with the approved guidelines. Data describing demographics, comorbidities, routine blood test results, serum creatinine levels, and lethal endpoints were collected. Acute renal failure (ARF) was considered as Estimated Glomerular Filtration Rate (eGFR) value of <60 ml/min/1.73 m². Chronic renal failure (CRF) diagnosis was detected through medical notations. Patients were divided into three groups depending on renal function: (a) septic patients with no medical notations of renal insufficiency who developed ARF on hospitalization; (b) septic patients with CRF; (c) septic patients, with normal renal function (NRF). Venous blood samples were drawn from all patients. Relative expression levels of circulating serum miR-30d-5p, miR-23a-3p, miR-146a-5p were assessed with real-time quantitative polymerase chain reaction (RT-qPCR) using the $2^{-\Delta\Delta Ct}$ method and compared between the groups. Cel-miR-39-3p was used for normalization of RNA values. Data of normal distribution were presented as mean \pm SD. The normality of data was assessed with Kolmogorov-Smirnov or Shapiro Wilk tests. Pearson's criterion was used to analyse the correlation between the values of the blood test result and relative expression of miRNAs. Student's *t*-test was used to compare means. Statistical analysis was performed using IBM SPSS Statistics software (v. 23.0 Chicago, IL, USA). A *p* value <0.05 was considered statistically significant.

RESULTS

We observed 30 potential candidates with abdominal sepsis; however, three were excluded due to haemolytic samples. Finally, 27 patients were enrolled into our study. Characteristics of these patients are shown in Table 1. ARF developed in eight (29.65%) patients, pre-existing CRF were detected in eight (29.65%) individuals, and 11

(40.7%) patients retained NRF. Of the 27 patients enrolled, 22 (81.5%) survived and five (18.5%) died during the hospitalization period.

Table 1. Characteristics of patients with abdominal sepsis enrolled into the study

Characteristics	N = 27
Male, n (%)	12 (44.4)
Female, n (%)	15 (55.6)
Age (year), mean (range)	62.81 (23–90)
Chronic renal insufficiency, n (%)	8 (29.65)
Acute renal insufficiency, n (%)	8 (29.65)
Normal renal function, n (%)	11 (40.7)

The expression levels of miR-30d-5p, miR-146a-5p, miR-23a-3p were respectively 1.189 ($p = 0.609$), 1.999 ($p = 0.308$) and 1.112 ($p = 0.854$) fold-higher in patients with NRF compared to patients with ARF, but there was no significant difference between these groups. Results are depicted in Fig. 1.

No significant difference in miR-30d-5p, miR-146a-5p, miR-23a-3p fold changes – respectively, -1.002 , ($p > 0.99$), -1.188 ($p = 0.837$), -1.123 ; ($p = 0.849$) – were detected between patients with CRF and patients with NRF (Fig. 2).

Furthermore, no significant fold change difference of miR-30d-5p, miR-146a-5p, miR-23a-3p expressions (respectively 1.191 ($p = 0.536$), 2.376

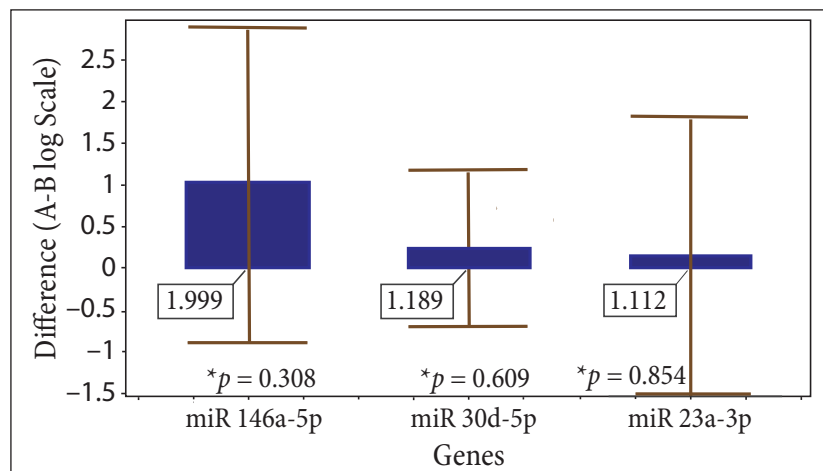


Fig. 1. Comparison of miR-146a-5p, miR-30d-5p, miR-23a-3p fold change between septic patients with ARF and septic patients with a normal renal function

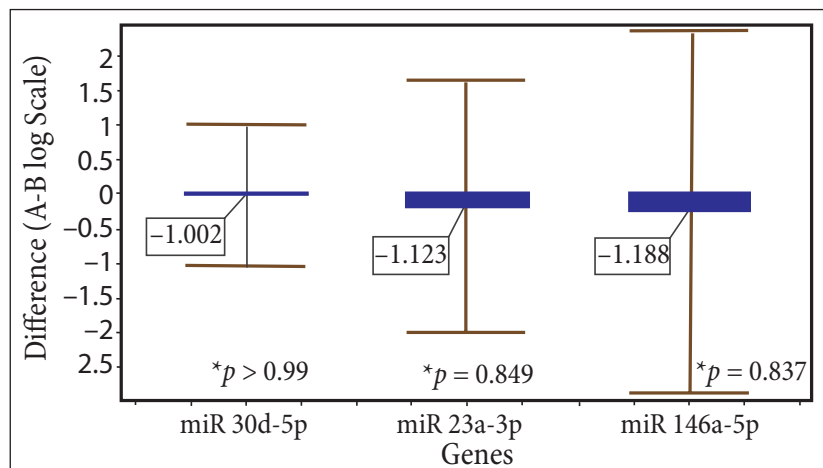


Fig. 2. Comparison of miR-30d-5p, miR-23a-3p, miR-146a-5p fold change between septic patients with CRF and septic patients with a normal renal function

($p = 0.308$), 1.249 ($p = 0.710$)) were found between patients with ARF and those with CRF (Fig. 3).

After analysing laboratory blood test results, significantly higher values of red blood cell distribution width (RDW), creatinine, C-reactive protein (CRP) (respectively $p = 0.013$, $p = 0.028$, $p = 0.014$) and a significantly lower value of eGFR ($p < 0.001$) were determined in patients with ARF and CRF compared to patients with NRF. Other analysed blood test results did not differ significantly between these groups (Table 2).

Urea ($p = 0.007$) and RDW ($p = 0.001$) values were significantly higher and eGFR was significantly lower ($p = 0.016$) in all septic patients who died during the hospitalization period compared to the patients who survived. Other analysed blood test results did not differ significantly (Table 3). However, no significant correlation between the RDW value and miRNAs expression levels was noted in the patients who survived ($r = 0.335$, $p = 0.082$) (Fig. 4) and in patients who had lethal endpoints ($r = 0.313$, $p = 0.608$).

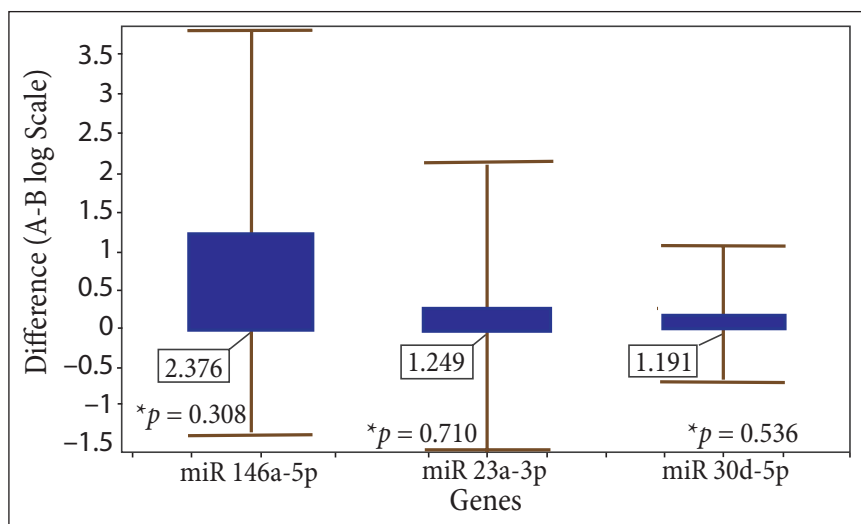


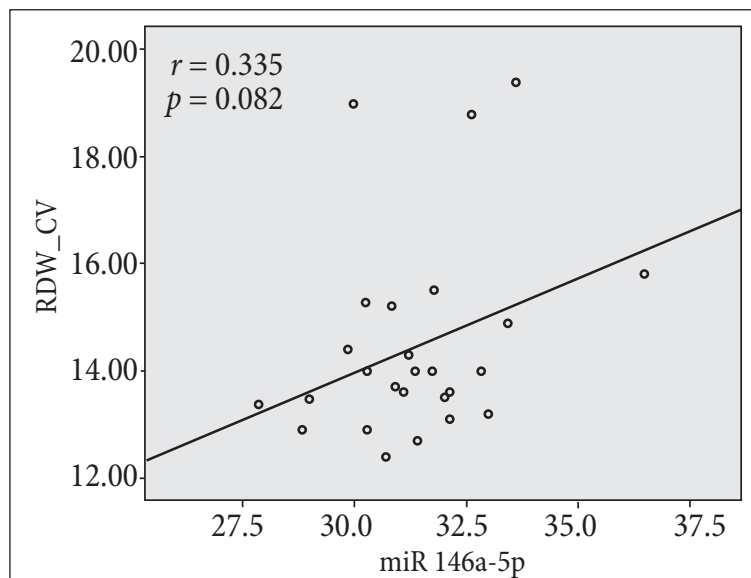
Fig. 3. Comparison of MiR-146a-5p, miR-23a-3p, miR-30d-5p fold change between septic patients with ARF and septic patients with CRF

Table 2. Comparison of blood test results between patients with renal insufficiency and patients with a normal renal function. INR – international normalized ratio; APTT – activated partial thromboplastin time

Variable	Renal insufficiency (ARF and CRF) (n = 16) Mean ± SD	Normal renal function (n = 11) Mean ± SD	p value
Haemoglobin (g/l)	114.25 ± 25.60	129.00 ± 32.00	0.196
RDW-CV (%)	15.35 ± 1.78	13.78 ± 0.88	0.013
White blood cells (×10 ⁹ /l)	11.80 ± 5.36	10.59 ± 5.00	0.559
Platelets (×10 ⁹ /l)	248.45 ± 116.15	270.36 ± 69.70	0.157
Creatinine (μmol/l)	195.74 ± 152.65	86.21 ± 36.02	0.028
eGFR (ml/min/1.73 m ²)	34.77 ± 16.58	88.80 ± 14.07	0.000
Urea (mmol/l)	24.05 ± 41.91	5.49 ± 3.08	0.158
Glucose (mmol/l)	6.33 ± 1.98	6.49 ± 2.12	0.841
CRP (mg/l)	262.00 ± 123.16	124.57 ± 145.41	0.014
INR	1.18 ± 0.14	1.19 ± 0.15	0.805
APTT(s)	36.60 ± 4.10	34.22 ± 4.82	0.181

Table 3. Comparison of blood test results between patients with lethal and non-lethal end points during hospitalization. INR – international normalized ratio; APTT, activated partial thromboplastin time

Variable	Survivors (n = 22) Mean ± SD	Non-survivors (n = 5) Mean ± SD	p value
Haemoglobin (g/l)	121.55 ± 29.95	144.60 ± 24.76	0.635
RDW-CV (%)	14.25 ± 1.39	16.78 ± 1.12	0.001
White blood cells (×10 ⁹ /l)	11.37 ± 5.11	11.05 ± 5.97	0.121
Platelets (×10 ⁹ /l)	242.68 ± 129.84	162.80 ± 109.14	0.215
Creatinine (µmol/l)	142.51 ± 141.38	189.00 ± 55.15	0.482
eGFR (ml/min/1.73 m ²)	63.44 ± 30.50	27.50 ± 9.23	0.016
Urea (mmol/l)	8.65 ± 7.18	50.98 ± 71.26	0.007
Glucose (mmol/l)	6.21 ± 1.87	7.23 ± 2.56	0.313
CRP (mg/l)	184.42 ± 141.04	301.00 ± 149.19	0.111
INR	1.17 ± 0.14	1.24 ± 0.21	0.386
APTT(s)	35.26 ± 4.18	37.32 ± 5.87	0.363

**Fig. 4.** Correlation between RDW values in septic patients and Cp of miR-146a-5p from blood serum in survivors

DISCUSSION

ARF has been described as a significant independent risk factor of mortality in patients with sepsis or septic shock (18). In our study, the eGFR was significantly lower in septic patients with fatal endpoints. Some authors previously reported, that the levels of miR-30d-5p and miR-146a-5p may change in patients with an impaired renal function, however, these studies did not include septic patients. For example, Rudnicki et al. found that miR-30d were downregulated in the cases of progressive chron-

ic kidney disease compared to stable chronic kidney disease cases (16). Amrouche et al. performed an *in vitro* analysis of renal allograft biopsy and an *in vivo* examination of urine samples ten days post kidney transplantation. A strong upregulation of miR-146a-5p was identified in renal allografts of the patients who displayed acute tubular necrosis compared to those with normal allograft biopsy results (17). However, we hypothesized that expression levels of miR-30d-5p, miR-23a-3p, and miR-146a-5p could be altered in septic patients and did not directly correlate with the development of ARF or CRE.

A potential role of miR-30d-5p, miR-23a-3p, and miR-146a-5p in sepsis was previously established by numerous reports. However, previous studies reported varying results considering the change in miRNA expression levels in septic patients and laboratory (*in-vitro*) models. For example, Wang et al. revealed a significant reduction of serum miR-146a expression in septic patients compared to healthy controls (19). Si et al. reported a significant *in vitro* downregulation of miR-23a after a stimulation of macrophages with lipopolysaccharide (LPS) that resulted in increased cell viability and suggested a pro-inflammatory role of miR-23a (20). Other authors reported, that in comparison to healthy controls the expression levels of circulating miR-30d-5p, miR-23a-3p, miR-146a-5p increased in septic patients – this suggested an anti-inflammatory role for these miRNAs (13–15).

The width of red blood cell distribution has been previously identified as a potential predictor of adverse outcomes in septic patients. Sakada et al. found that elevated RDW value on day 1 of septic shock is very strongly associated with the increased risk of hospital mortality and ICU mortality as increased RDW likely reflects the presence of proinflammatory cytokines and oxidative stress in septic shock (21). A retrospective analysis performed by Jo et al. determined, that levels of RDW at hospital admission are associated with 28-day mortality and severity of disease in patients with severe sepsis and septic shock (22). The results of a prospective observational study made by Jandial et al. showed that RDW was significantly associated with 30-day mortality in patients with severe sepsis non-related to age and comorbidities (23). We detected that overall mean RDW value was significantly higher in patients with abdominal sepsis who had lethal endpoints in comparison to analysed septic patients who survived. However, no correlation between RDW value and miRNAs expression level was noted in septic patients with and without lethal endpoints.

CONCLUSIONS

Expression levels of serum miR-30d-5p, miR-23a-3p, and miR-146-5p did not significantly differ between three groups of patients who developed

ARF, had CRF, or retained NRF. What is more, eGFR values were significantly lower and urea and RDW values were significantly higher in all patients who died during the hospitalization period compared to the patients who survived. However, no significant correlations between RDW values and miRNAs expression levels were noted in these two groups.

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**CIRKULIUOJANČIŲ MOLEKULINIŲ
BIOŽYMENŲ (MIKRORNR) REIKŠMĖ
VERTINANT INKSTŲ FUNKCIJOS
NEPAKANKAMŲ SKUBIOS ANESTEZIJOS
METU PACIENTAMS, SERGANTIEMS
ABDOMINALINIŲ SEPSIU**

Santrauka

Įvadas. Mikroribonukleorūgštys (miRNR) yra mažos genetinės informacijos nekoduojančios molekulės, reguliuojančios genų raišką ir fiziologinius organizmo procesus. MiRNR raiškos pokyčiai gali būti naudojami kaip potencialūs, nauji biologiniai žymenys nustatant daugelį ligų.

Tyrimo medžiaga ir metodai. Tirti atsitiktiniai pacientai, operuoti skubos tvarka dėl abdominalinės kilmės sepsio ($n = 27$). Analizuoti laboratorinių kraujo tyrimų žymenys, demografiniai rodikliai. Tiriamųjų imtis padalinta į tris grupes pagal inkstų funkcijos veiklą. Naudojant $2-\Delta\Delta C_t$ metodą, santykinės cirkuliuojančių kraujo serumo miR-30d-5p, miR-23a-3p ir miR-146a-5p raiškos įvertintos atliekant realaus laiko kiekybinės polimerazių grandinės reakcijos (RT-qPCR) tyrimą ir palygintos tarp grupių. Naudoti Kolmogorovo-Smirnovo ir Šapiro-Vilko testai, Pirsono koreliacijos, Stjudento t testo statistiniai metodai.

Rezultatai. Visų trijų tirtų miRNR raiškos statistiškai patikimai nesiskyrė tarp pacientų su ūminiu inks-

tų nepakankamumu (ŪIFN) ($n = 8$), su lėtiniu inkstų nepakankamumu (LIFN) ($n = 8$) ir pacientų, kurių inkstų funkcija išliko normali ($n = 11$). MiR-30d-5p, miR-146a-5p, miR-23a-3p raiškos atitinkamai buvo 1,189 ($p = 0,609$), 1,999 ($p = 0,308$), 1,112 ($p = 0,854$) kartų didesnės pacientų su išlikusia normalia inkstų funkcija, palyginti su pacientais, kuriems išsivystė ŪIFN. MiR-30d-5p, miR-146a-5p, miR-23a-3p raiškos atitinkamai buvo $-1,002$ ($p > 0,99$), $-1,188$ ($p = 0,837$), $-1,123$ ($p = 0,849$) kartų mažesnės pacientų su išlikusia normalia inkstų funkcija, palyginti su pacientais, kuriems nustatytas LIFN. MiR-30d-5p, miR-146a-5p, miR-23a-3p raiškos atitinkamai buvo 1,191 ($p = 0,536$), 2,376 ($p = 0,308$), 1,249 ($p = 0,710$) kartų didesnės pacientų su LIFN, palyginti su pacientais, kuriems išsivystė ŪIFN. Vis dėlto miRNR raiškos tarp grupių reikšmingai nesiskyrė. Apskaičiuotas glomerulų filtracijos greitis (aGFR) buvo reikšmingai mažesnis ($p = 0,016$), šlapalo ($p = 0,007$) ir raudonųjų kraujo ląstelių pasiskirstymo pločio (RDW) ($p = 0,001$) reikšmės buvo reikšmingai didesnės mirusių pacientų grupėje, tačiau tarp RDW ir miRNR raiškų statistiškai reikšmingo ryšio nenustatyta.

Išvados. Serumo miR-30d-5p, miR-23a-3p, miR-146a-5p raiškos nesiskyrė tarp pacientų, kuriems išsivystė IFN, turėjo LIFN ar inkstų funkcija išliko normali. Tarp RDW ir serumo miRNR raiškų statistiškai reikšmingo ryšio nenustatyta.

Raktažodžiai: miRNR, ūminis inkstų nepakankamumas, lėtinis inkstų funkcijos nepakankamumas, RDW, sepsis