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Circulating microRNA-146a and microRNA-146b exhibit potential to serve as markers for acute pancreatitis management and prognosis

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Abstract. – OBJECTIVE: This study aimed to explore the association of microRNA (miR)-146a and miR-146b expressions with risk, severity, in-hospital death of acute pancreatitis (AP).

PATIENTS AND METHODS: 50 severe AP (SAP) patients, 50 moderate-severe AP (MSAP) patients, 50 mild AP (MAP) patients and 50 healthy controls (HCs) were enrolled. Plasma samples were collected after the enrollment, then miR-146a and miR-146b expressions were detected by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Ranson's score, Acute Physiology and Chronic Health Evaluation (APACHE) II score, sequential organ failure assessment (SOFA) score, C-reactive protein (CRP) as well as in-hospital mortality were assessed in AP patients.

RESULTS: Both miR-146a and miR-146b expressions were the highest in SAP patients, followed by MSAP patients, MAP patients and HCs. Meanwhile, they distinguished SAP, MSAP, MAP patients from HCs, and also distinguished SAP, MSAP and MAP patients from each other. In SAP, MSAP and MAP patients, MiR-146a positively correlated with Ranson's score, APACHE II score, SOFA score and CRP. Besides, miR-146b positively correlated with Ranson's score, APACHE II score, SOFA score and CRP in SAP patients; correlated with Ranson's score, APACHE II score and CRP in MSAP patients; and correlated with Ranson's score and SOFA score in MAP patients. Notably, miR-146a predicted increased in-hospital death risk of both SAP and MSAP patients, while miR-146b predicted raised in-hospital death risk of SAP patients but not MSAP patients.

CONCLUSIONS: Circulating miR-146a and miR-146b exhibit potential as markers for AP management and prognosis.

Key Words:

Acute pancreatitis, MicroRNA-146a, MicroRNA-146b, Disease condition, In-hospital death.

Introduction

Acute pancreatitis (AP) is one of the most common gastrointestinal disorders requiring hospitalization worldwide, and the in-hospital mortality of AP is around 5%-15%[1-3]. AP is generally classified into mild acute pancreatitis (MAP), moderate-severe acute pancreatitis (MSAP) and severe acute pancreatitis (SAP), and its clinical characteristics vary from transient abdominal discomfort to persistent organ failures[1,2]. The recent advances in MR and analytic techniques may help quantify potential signal changes that reflect the underlying tissue abnormalities, however, once the systemic inflammatory response syndrome initiates, there is limited available modality to reverse the progression of inflammation in AP, and prognosis for AP patients is still dismal[4,5]. It has been recognized that the exploration of convincing diagnostic or prognostic biomarkers would improve AP treatment outcomes.

MicroRNA (miR)-146a and miR-146b, belonging to the miR-146 family, are reported to be closely implicated in the onset and progress of severe inflammatory diseases. Notably, miR-146a promotes sepsis-induced inflammation and cardiomyopathy by regulating Toll-like receptor 4 (TLR-4)/ nuclear factor kappa-B (NF-κB) pathway in a negative feedback mechanism[6], and it regulates inflammation-related multiple organ injuries, such as lung injury, kidney injury, liver injury, etc.[7-9]; As for miR-146b, it promotes inflammation in septic cellular models and regulates inflammation-induced lung injury and liver injury[10,11]. Furthermore, both miR-146a and miR-146b participate in pathology of several pancreas-related diseases, such as pancreatic cancer, chronic pancreatitis, insulin secretion disorder,

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etc.[12-14]. Considering the close correlation of miR-146a and miR-146b with severe inflammation, multiple organ injury as well as their participation in pancreas-related diseases, we suggested that they might have potential to be biomarkers for AP management, while no related study has been reported. Therefore, we conducted this study to explore the association of miR-146a and miR-146b expressions with risk, severity and in-hospital death of AP.

Patients and Methods

Participants

In this study, 50 SAP patients, 50 MSAP patients and 50 MAP patients were recruited from our hospital between July 2016 and June 2019, respectively, resulting in a total of 150 AP patients. The inclusion criteria of SAP patients were: (1) diagnosed as AP according to 2012 revised Atlanta classification of AP[15]; (2) persistent organ failure >48 h (single organ failure or multiple organ failure); (3) age above 18 years. The exclusion criteria of SAP patients were as follows: complicated with hematological malignancies or solid tumors or chronic inflammatory diseases. The inclusion criteria of MSAP patients were: (1) diagnosed as AP according to 2012 revised Atlanta classification of AP[15]; (2) transient organ failure (organ failure that was resolved within 48 hours) or local/systemic complications in the absence of persistent organ failure; (3) age above 18 years. The exclusion criteria of MSAP patients were as follows: complicated with hematological malignancies or solid tumors or chronic inflammatory diseases. The inclusion criteria of MAP patients were: (1) diagnosed as AP according to 2012 revised Atlanta classification of AP[15]; (2) without organ failure and local/systemic complications; (3) age above 18 years. The exclusion criteria of MAP patients were as follows: complicated with hematological malignancies or solid tumors or chronic inflammatory diseases. In addition, 50 age- and gender-matched adult healthy subjects whose healthy conditions were confirmed by physical examination and had no history of pancreatic or bile duct diseases were enrolled as healthy controls (HCs) during June 2019. Written informed consents were provided by the participants or their guardians before enrollment. This study was approved by the Ethics Committee of our hospital.

Clinical Data Collection and Severity Assessment

Demographic characteristics, C-reactive protein (CRP) level and etiology of AP patients were recorded after admission to our hospital. Besides, the general disease condition of AP patients was assessed within 48 hours after admission, which was measured using Ranson's score, Acute Physiology and Chronic Health Evaluation (APACHE) II score and sequential organ failure assessment (SOFA) score. Demographic characteristics and CRP level of HCs were also documented.

Sample Collection and Detection

Peripheral blood samples of AP patients were collected after admission, and peripheral blood samples of HCs were collected at enrollment. After collection, plasma was isolated from the blood samples immediately by centrifugation at 3000 g for 15min under 4°C. Then, the plasma was stored at -80°C until detections. The relative expression of miR-146a and miR-146b in plasma was detected by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Firstly, total RNA was extracted using TRIzol™ Reagent (Thermo Fisher Scientific, Waltham, MA, USA), and reverse transcription to cDNA was conducted with QuantiNova Reverse Transcription Kit (Qiagen, Duesseldorf, Nordrhein-Westfalen, German). Then, with application of QuantiNova SYBR Green PCR Kit (Qiagen, Duesseldorf, Nordrhein-Westfalen, German), the qPCR process was performed. Additionally, U6 was applied as the internal reference of miRNAs. And the sequence information of primers was displayed as follows: miR-146a, forward primer: ACACTCCAGCTGGGTGAGAACTGAATTCCA, reverse primer: TGTCGTGGAGTCGGCAATTC; miR-146b, forward primer: ACACTCCAGCTGGGTGAGAACTGAATTCCA, reverse primer: TGTCGTGGAGTCGGCAATTC; U6, forward primer: CTCGCTTCGGCAGCACATATACTA, reverse primer: ACGAATTTGCGTGTCATCCTTGC.

Follow-Up

The daily follow-up was carried out for all AP patients until they died in hospital or discharged from hospital. The AP patients who died in hospital were recorded during follow-up for calculation of in-hospital mortality. All AP patients were divided into in-hospital death group and survivor group based on the survival status in hospital.

Statistical Analysis

Statistical analyses in this study were performed using SPSS statistical software (IBM, Armonk, NY, USA) versions 24.0. Figures in this study were plotted with the use of GraphPad Prism software (GraphPad Software Inc, San Diego, CA, USA) versions 7.00. Comparison among three/four groups was determined by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test (pot-hoc test), Chi-square test, or Kruskal-Wallis H rank sum test followed by Dunn's multiple comparisons test (post-hoc test). Comparison between two groups was determined by Wilcoxon rank sum test. Correlation between variables was analyzed by Spearman's rank correlation test. The ability of variables in discriminating different subjects or in predicting in-hospital death risk was illuminated by Receiver operating characteristic (ROC) curve and the area under the curve (AUC) with 95% confidence interval (CI). p value<0.05 was considered significant.

Results

Clinical Features

No difference of age (p = 0.493) or gender (p < 0.742) was found among SAP patients, MSAP patients, MAP patients and HCs (Table I), whereas the CRP level differed among them: the highest CRP level was observed in SAP patients, and the lowest CRP level was observed in HCs (p < 0.001). In AP patients, Ranson's score (p < 0.001), APACHE II score (p < 0.001) and SOFA score (p < 0.001) were associated with increased AP severity. As to etiology, it was similar among SAP patients, MSAP patients and MAP

Table I. Clinical features.

Items	HCs (N = 50)	MAP patients (N = 50)	MSAP patients (N = 50)	SAP patients (N = 50)	*p*-value
Age (years)					0.493
Mean ± SD	60.3 ± 13.8	57.5 ± 12.0	57.3 ± 14.5	60.6 ± 14.8	
Range	20.0-80.0	29.0-80.0	26.0-80.0	26.0-80.0	
Gender, No.					0.742
Female	22 (44.0)	18 (36.0)	17 (34.0)	20 (40.0)	
Male	28 (56.0)	32 (64.0)	33 (66.0)	30 (60.0)	
CRP (mg/L)					< 0.001
Median	3.2	44.2	101.2	157.0	
(IQR)	(1.2-4.6)	(27.8-62.2)	(80.0-137.5)	(98.8-209.1)	
Range	0.2-9.8	9.8-166.3	26.2-511.6	36.7-513.9	
Etiology, No. (%)					0.426
BAP	-	20 (40.0)	25 (50.0)	27 (54.0)	
AAP	-	2 (4.0)	6 (12.0)	3 (6.0)	
HTGAP	-	20 (40.0)	15 (30.0)	15 (30.0)	
Others	-	8 (16.0)	4 (8.0)	5 (10.0)	
Ranson's score					< 0.001
Mean ± SD	-	1.2±0.5	2.7 ± 0.9	3.8 ± 1.2	
Range	-	1.0-3.0	1.0-5.0	3.0-8.0	
APACHE II score					< 0.001
Mean ± SD	-	4.4 ± 2.2	11.8 ± 5.1	15.1 ± 7.3	
Range	-	1.0-9.0	2.0-25.0	4.0-30.0	
SOFA score					< 0.001
Mean ± SD	-	2.0 ± 0.6	4.9 ± 2.0	6.9 ± 2.2	
Range	-	1.0-3.0	2.0-9.0	4.0-12.0	
Treatment, No. (%)					< 0.001
Conservative	-	47 (94.0)	39 (78.0)	29 (58.0)	
Percutaneous drainage	-	3 (6.0)	6 (12.0)	6 (12.0)	
Laparotomy	-	0 (0.0)	5 (10.0)	15 (30.0)	

Abbreviations: HCs, healthy controls; MAP, mild acute pancreatitis; MSAP, moderate-severe acute pancreatitis; SAP, severe acute pancreatitis; SD, standard deviation; CRP, C-reactive protein; IQR, interquartile range; BAP, biliary acute pancreatitis; AAP, alcohol-induced acute pancreatitis; HTGAP, hypertriglyceridemic acute pancreatitis; APACHE II, acute physiology and chronic health evaluation II; SOFA, sequential organ failure assessment.

patients (p = 0.426). Treatment choice was different among SAP patients, MSAP patients and MAP patients (p < 0.001).

Comparison of miR-146a and miR-146b Expressions Among SAP Patients, MSAP Patients, MAP Patients and HCs

MiR-146a expression was different among SAP patients, MSAP patients, MAP patients and HCs: the highest miR-146a expression was observed in SAP patients, followed by MSAP patients, MAP patients and HCs (p < 0.001) (Figure 1A). For miR-146b expression, it showed a similar trend as that of miR-146a among these patients (p < 0.001) (Figure 1B). Moreover, miR-146a expression was positively correlated with miR-146b expression in SAP patients (p < 0.001, r = 0.479), MSAP patients (p < 0.001, r = 0.453), MAP patients (p = 0.002, r = 0.423) and HCs (p = 0.013, r = 0.348) (Figure 1C-F).

The Abilities of miR-146a and miR-146b to Distinguish SAP Patients, MSAP Patients, MAP Patients and HCs

MiR-146a was able to distinguish SAP patients from MSAP patients (AUC: 0.744, 95% CI: 0.647-0.840; Youden index at the best cut-off point: 0.420) (Figure 2A), SAP patients from MAP patients (AUC: 0.851, 95% CI: 0.778-0.923; Youden index at the best cut-off point: 0.620) (Figure

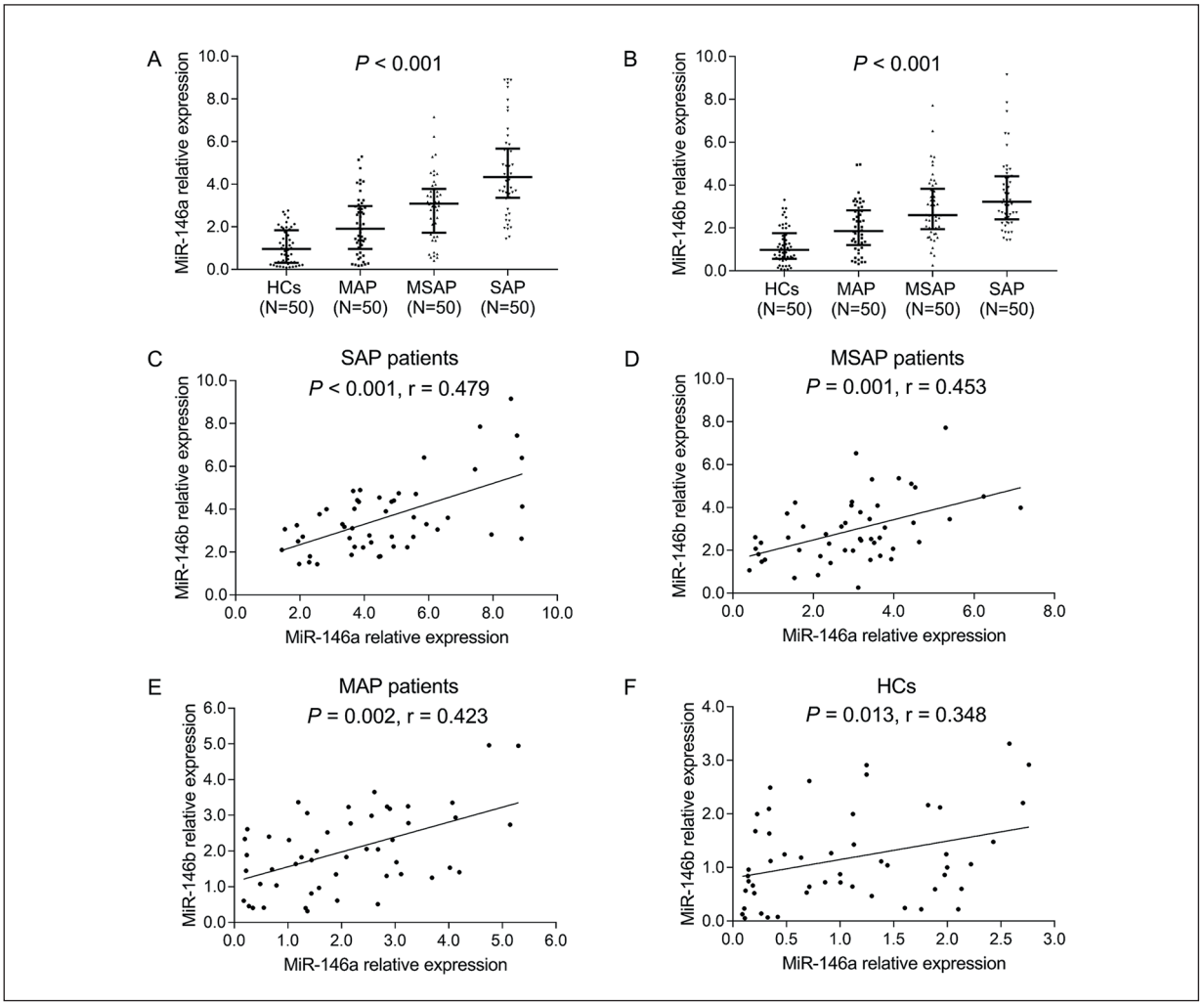

Figure 1. miRNA-146a and miR-146b expressions among SAP patients, MSAP patients, MAP patients and HCs. MiRNA-146a expressions (**A**) and miR-146b expressions (**B**) in SAP patients, MSAP patients, MAP patients and HCs. Correlations of miR-146a expression and miR-146b expression in SAP patients (**C**), MSAP patients (**D**), MAP patients (**E**) and HCs (**F**). miR-146a, microRNA-146a; miR-146b, microRNA-146b; SAP, severe acute pancreatitis; MSAP, moderate-severe acute pancreatitis; MAP, mild acute pancreatitis; HCs, healthy controls.

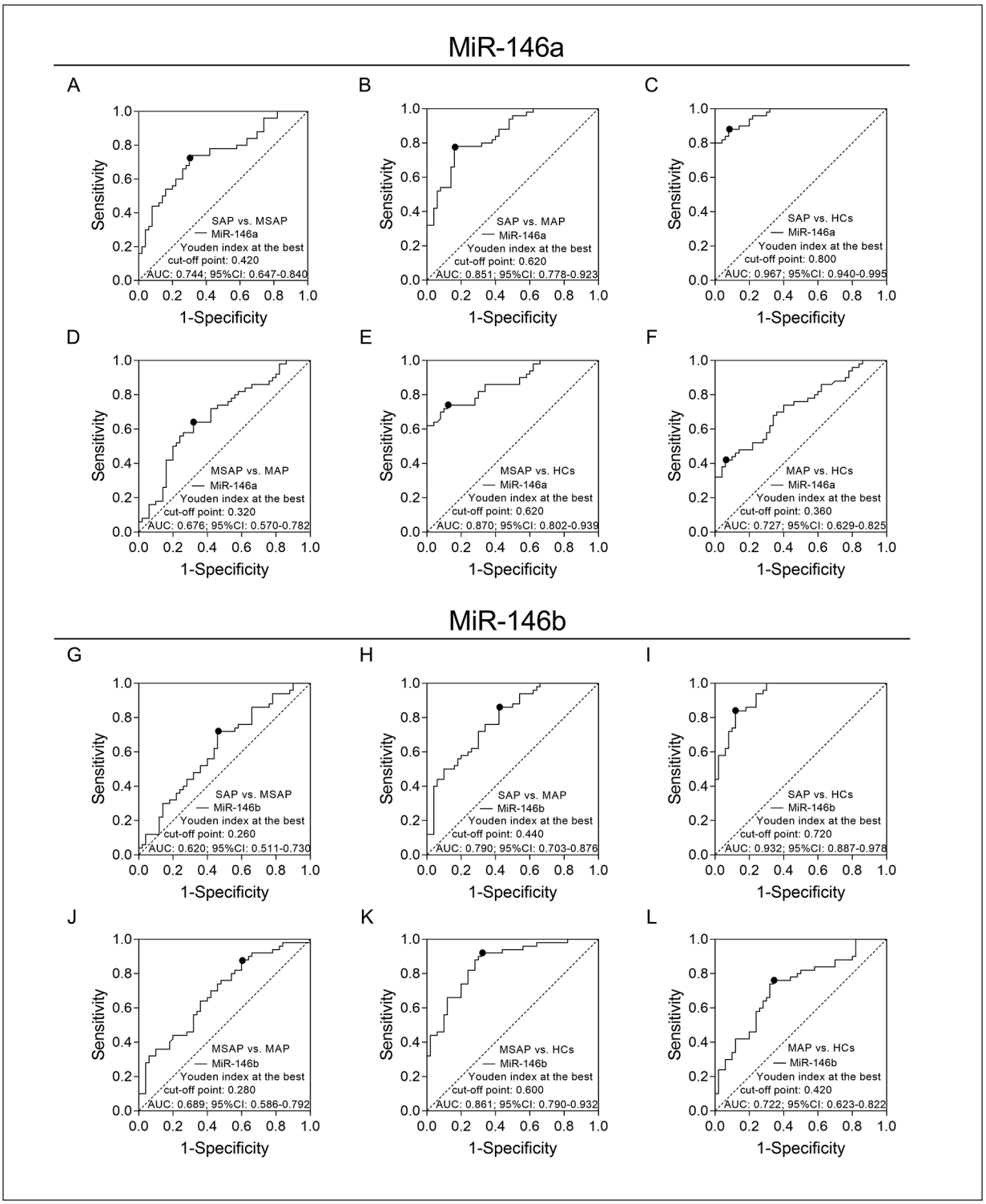

Figure 2. ROC curves for SAP, MSAP and MAP risks. ROC curves for assessing the ability of miR-146a to distinguish SAP patients from MSAP patients (**A**), SAP patients from MAP patients (**B**), SAP patients from HCs (**C**), MSAP patients from MAP patients (**D**), MSAP patients from HCs (**E**), and MAP patients from HCs (**F**). ROC curves for assessing the ability of miR-146b to distinguish SAP patients from MSAP patients (**G**), SAP patients from MAP patients (**H**), SAP patients from HCs (**I**), MSAP patients from MAP patients (**J**), MSAP patients from HCs (**K**) and MAP patients from HCs (**L**). ROC, Receiver operating characteristic; AP, acute pancreatitis; SAP, severe acute pancreatitis; MSAP, moderate-severe acute pancreatitis; MAP, mild acute pancreatitis; miR-146a, microRNA-146a; miR-146b, microRNA-146b.

2B), SAP patients from HCs (AUC: 0.967, 95% CI: 0.940-0.995; Youden index at the best cut-off point: 0.800) (Figure 2C), MSAP patients from MAP patients (AUC: 0.676, 95% CI: 0.570-0.782; Youden index at the best cut-off point: 0.320) (Figure 2D), MSAP patients from HCs (AUC: 0.870, 95% CI: 0.802-0.939; Youden index at the best cut-off point: 0.620) (Figure 2E), and MAP patients from HCs (AUC: 0.727, 95% CI: 0.629-0.825; Youden index at the best cut-off point: 0.436) (Figure 2F). Regarding miR-146b, it could distinguish SAP patients from MSAP patients (AUC: 0.620, 95% CI: 0.511-0.730; Youden index at the best cut-off point: 0.260) (Figure 2G), SAP patients from MAP patients (AUC: 0.790, 95% CI: 0.703-0.876; Youden index at the best cut-off point: 0.440) (Figure 2H), SAP patients from HCs (AUC: 0.932, 95% CI: 0.887-0.978; Youden index at the best cut-off point: 0.720) (Figure 2I), MSAP patients form MAP patients (AUC: 0.689, 95% CI: 0.586-0.792; Youden index at the best cut-off point: 0.280) (Figure 2J), MSAP patients from HCs (AUC: 0.861, 95% CI: 0.790-0.932; Youden index at the best cut-off point: 0.600) (Figure 2K), and MAP patients from HCs (AUC: 0.722, 95% CI: 0.623-0.822; Youden index at the best cut-off point: 0.420) (Figure 2L).

Correlation of miR-146a and miR-146b with Disease Severity Assessments in SAP Patients, MSAP Patients and MAP Patients

In SAP patients, miR-146a expression was positively correlated with Ranson's score ($p = 0.001$, r = 0.453), APACHE II score ($p < 0.001$, r = 0.551), SOFA score ($p < 0.001$, r = 0.603) and CRP ($p = 0.001$, r = 0.438) (Table II). MiR-146b expression was also positively correlated with Ranson's score ($p < 0.001$, r = 0.518), APACHE II score ($p = 0.014$, r = 0.347), SOFA score ($p < 0.001$, r = 0.485) and CRP ($p < 0.001$, r = 0.520). In MSAP patients, miR-146a expression was positively correlated with Ranson's score ($p < 0.001$, r = 0.558), APACHE II score ($p = 0.044$, r = 0.286), SOFA score ($p = 0.032$, r = 0.304) and CRP ($p = 0.005$, r = 0.393). Meanwhile, miR-146b expression was positively correlated with Ranson's score ($p = 0.001$, r = 0.454), APACHE II score ($p = 0.004$, r = 0.399) and CRP ($p = 0.039$, r = 0.293) but not correlated with SOFA score ($p = 0.089$, r = 0.243). In MAP patients, positive correlation of miR-146a expression with Ranson's score ($p = 0.004$, r = 0.398), APACHE II score ($p = 0.013$, r =0.350), SOFA score ($p = 0.036$, r = 0.298) and CRP ($p = 0.002$, r = 0.436) were observed. Besides, miR-146b expression was positively correlated with Ranson's score ($p = 0.007$, r = 0.375) and SOFA score ($p = 0.011$, r = 0.358) but not correlated with APACHE II score ($p = 0.314$, r = 0.145) or CRP ($p = 0.143$, r = 0.210).

Correlation of miR-146a and miR-146b Expressions with In-Hospital Death Risk in SAP Patients and MSAP Patients

The percentage of in-hospital death in SAP patients, MSAP patients and MAP patients were 24.0% (n=12), 10.0% (n=5) and 0.0% (n=0), respectively (Figure 3A). In SAP patients, miR-146a expression ($p < 0.001$) (Figure 3B) and miR-146b expression ($p = 0.002$) (Figure 3C) were dramatically elevated in in-hospital death group compared to survivor group, and both of them had good predictive values for increased in-hospital death risk (for miR-146a, AUC: 0.868 (95% CI: 0.749-0.988) ; Youden index at the best cut-off point: 0.623; for miR-146b, AUC: 0.800 (95% CI: 0.652-0.949) ; Youden index at the

Table II. Correlation of miR-146a/b with Ranson's score, APACHE II score, SOFA Score and CRP.

Items		SAP patients		MSAP patients		MAP patients	
		MiR-146a	MiR-146b	MiR-146a	MiR-146b	MiR-146a	MiR-146b
Ranson's score	r	0.453	0.518	0.558	0.454	0.398	0.375
	p-value	0.001	< 0.001	< 0.001	0.001	0.004	0.007
APACHE II score	r	0.551	0.347	0.286	0.399	0.350	0.145
	p-value	< 0.001	0.014	0.044	0.004	0.013	0.314
SOFA score	r	0.603	0.485	0.304	0.243	0.298	0.358
	p-value	< 0.001	< 0.001	0.032	0.089	0.036	0.011
CRP	r	0.438	0.520	0.393	0.293	0.436	0.210
	p-value	0.001	< 0.001	0.005	0.039	0.002	0.143

Abbreviations: APACHE II, acute physiology and chronic health evaluation II; SOFA, sequential organ failure assessment; CRP, C-reactive protein.

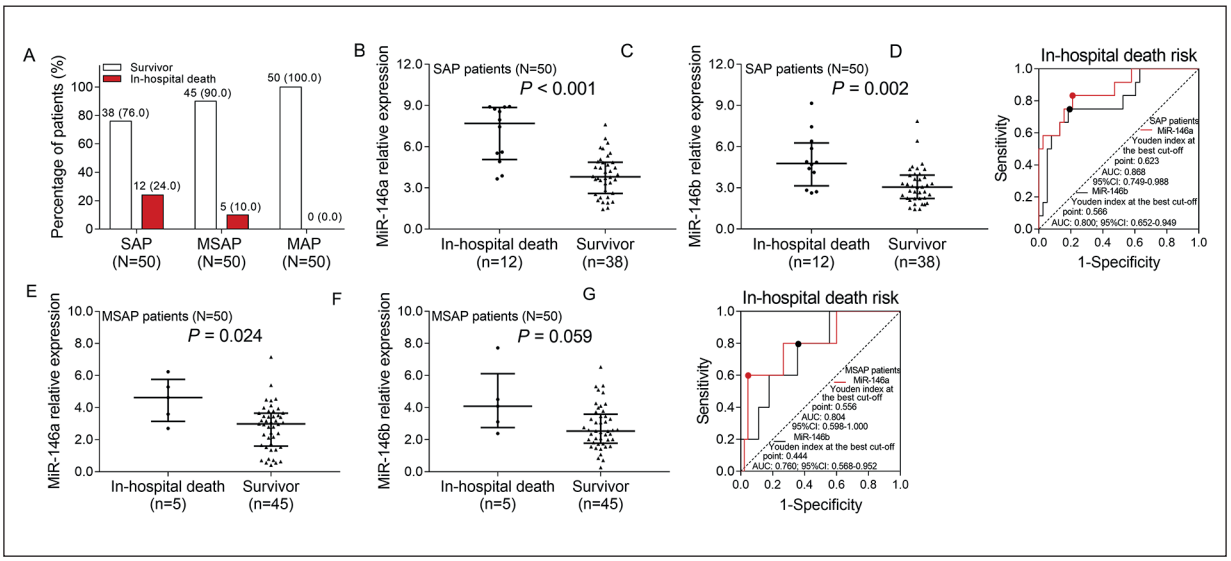

Figure 3. Association of miR-146a and miR-146b expressions with in-hospital death risk in SAP and MSAP patients. Percentages of survivors and in-hospital deaths in SAP patients, MSAP patients and MAP patients (**A**). MiR-146a expression (**B**) and miR-146b expression (**C**) in in-hospital death group and survivor group in SAP patients. Predictive values of miR-146a and miR-146b for in-hospital death risk in SAP patients (**D**). miR-146a expression (**E**) and miR-146b (**F**) expression in in-hospital death group and survivor group in MSAP patients. Predictive values of miR-146a and miR-146b for in-hospital death risk in MSAP patients (**G**). miR-146a, microRNA-146a; miR-146b, microRNA-146b; SAP, severe acute pancreatitis; MSAP, moderate-severe acute pancreatitis; MAP, mild acute pancreatitis.

best cut-off point: 0.566) (Figure 3D). In MSAP patients, miR-146a expression was elevated in in-hospital death group compared to survivor group (p = 0.024) (Figure 3E), and it predicted raised in-hospital death risk (AUC: 0.804 (95% CI: 0.598-1.000); Youden index at the best cut-off point: 0.556) (Figure 3G). Whereas miR-146b expression was similar between in-hospital death group and survivor group (numerically higher in in-hospital death group compared to survivor group) (p = 0.059) (Figure 3F), and it could predict increased in-hospital death risk to some extent (AUC: 0.760 (95% CI: 0.568-0.952); Youden index at the best cut-off point: 0.444) (Figure 3G). However, no in-hospital death occurred in MAP patients, thus, the correlation of miR-146a and miR-146b expressions with in-hospital death in MAP patients could not be assessed.

Predictive Values of Ranson's Score, APACHE II Score, SOFA Score and CRP for In-Hospital Death Risk in SAP Patients and MSAP Patients

In SAP patients, Ranson's score (AUC: 0.905 (95% CI: 0.824-0.985); Youden index at the best cut-off point: 0.737) presented with a good predictive value for the in-hospital death risk, and APACHE II score (AUC: 0.794 (95% CI: 0.649-0.939) ; Youden index at the best cut-off point: 0.491), SOFA score (AUC: 0.797 (95% CI: 0.657-0.937) ; Youden index at the best cut-off point: 0.447) and CRP (AUC: 0.770 (95% CI: 0.615-0.925) ; Youden index at the best cut-off point: 0.447) also could predict in-hospital death risk (Figure 4A). In MSAP patients, these factors including Ranson's score (AUC: 0.882 (95% CI: 0.762-1.000); Youden index at the best cut-off point: 0.644), APACHE II score (AUC: 0.904 (95% CI: 0.819-0.990); Youden index at the best cut-off point: 0.800), SOFA score (AUC: 0.938 (95% CI: 0.867-1.000); Youden index at the best cut-off point: 0.822) and CRP (AUC: 0.742 (95% CI: 0.596-0.888); Youden index at the best cut-off point: 0.556) all could predict in-hospital death risk (Figure 4B). Interestingly, the predictive value of miR-146a and miR-146b for in-hospital risk was similar to those of Ranson's score, APACHE II score, SOFA score and CRP in SAP patients and MSAP patients.

Discussion

MiRNAs regulate expression of numerous genes and participate in a wide range of biological functions, such as cell metabolism, immune responses

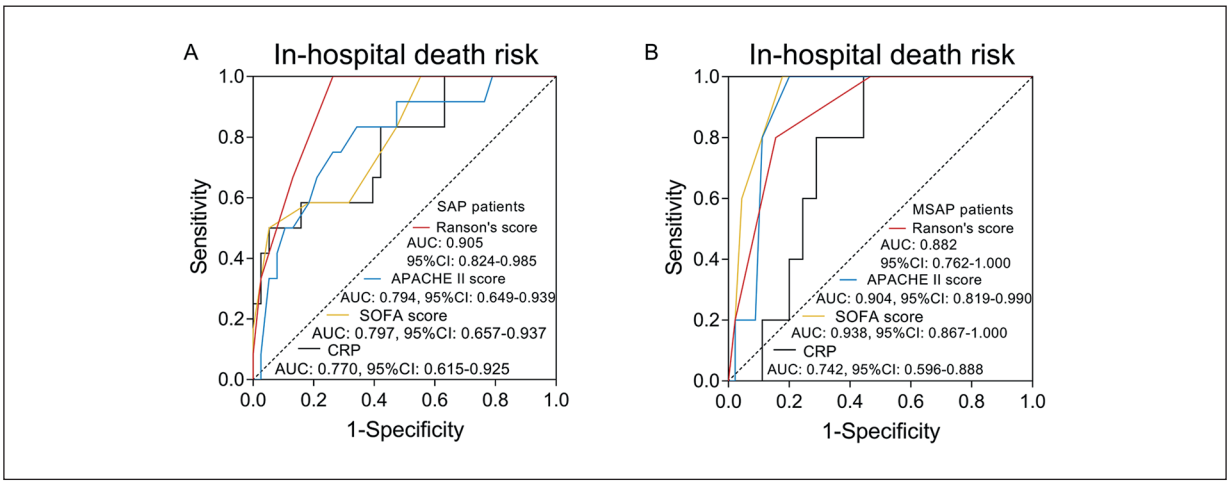

Figure 4. Predictive values of common severity-assessment scores and inflammation for in-hospital death risk in SAP and MSAP patients. Predictive values of Ranson's score, APACHE II score, SOFA sore and CRP for in-hospital death risk in SAP patients (**A**) and MSAP patients (**B**). CRP, C-reactive protein; APACHE II, Acute Physiology and Chronic Health Care Evaluation (APACHE) II; SAP, severe acute pancreatitis; MSAP, moderate-severe acute pancreatitis.

and carcinogenesis[16,17]. MiR-146a, as a frequently-investigated miRNA, is reported to involve in the modulation of inflammatory responses via regulating target gene expression, such as interleukin (IL)-1 receptor-associated kinase 1 (IRAK1) and tumor necrosis factor (TNF) receptor-associated factors (TRAFs)[18,19]. It is shown that miR-146a is overexpressed in circulating plasma of kidney injury mice model and increases the pro-inflammatory cytokine levels in cerebrospinal fluid of sepsis mice model, which indicates that miR-146a may be involved in the pathology of inflammatory disorders and organ injuries[20,21]. In clinical practices, the overexpression of miR-146a has been found in bronchoalveolar lavage fluid of asthma children and kidney samples of the diabetic nephropathy patients[22,23]. Besides, miR-146a high expression is correlated with periodontal injury severity in generalized aggressive periodontitis patients and is associated with pulmonary histological score in acute lung injury patients as well[24,25]. These previous observations illustrate that miR-146a may participate in the regulation of inflammation and inflammation-induced organ injuries. As for pancreatic diseases, only one study displays that miR-146a may facilitate the chronic pancreatitis genetic susceptibility to further promote the disease developments, implying that miR-146a may enhance the occurrence and progression of pancreatitis[13].

Based on these indications, it seems fairly natural to speculate that miR-146a may also be involved in the AP development. However, the relevant evidence in AP has not been reported yet. In our study, we enrolled 50 SAP patients, 50 MSAP patients, 50 MAP patients and 50 HCs, to investigate the correlation of miR-146a expression with AP risk. We found that miR-146a expression could distinguish SAP patients, MSAP patients and MAP patients from HCs, and also could distinguish SAP, MSAP and MAP patients from each other. Meanwhile, miR-146a expression was positively correlated with Ranson's score, APACHE II score, SOFA score and CRP in SAP, MSAP and MAP patients. These results might be due to: (1) miR-146a might promote the TLR-4-induced inflammatory responses and further enhanced the occurrence of systemic inflammatory response syndrome as well as organ failures, which indicated worse AP severity. Therefore, the miR-146 high expression predicted elevated SAP risk, MSAP risk and MAP risk, and also distinguished different AP severity from each other[26]; (2) miR-146a might increase the Decorin and then deactivated the phosphatidylinositol 3 kinase (PI3K)/ serine/threonine kinase 1 (AKT)/ mammalian target of rapamycin (mTOR) pathway, which further promoted the excessive inflammatory responses and inflammation-induced injuries, thereby leading to aggravated organ injuries and further increased the occurrence of severe multiple organ dysfunctions. Thus miR-146a was positively correlated with Ranson's score, APACHE II score, SOFA score and CRP in AP patients[27]. Besides, we also found that miR-146a

expression predicted increased in-hospital death risk in SAP patients and MSAP patients, which might be account for that: miR-146a promoted the inflammatory responses and eventually enhanced organ injuries, leading to less opportunity to survive in miR-146a high expression patients. Thus, miR-146a high expression predicted increased in in-hospital death risk[13].

MiR-146b is also a well-known immunomodulatory member of miR-146 family, and it participates in pathology of inflammatory disorders and organ injuries[28,29]. In particular, miRNA-146b increases the inflammatory cytokine expressions (IL-6 and IL-8) in a sepsis model, moreover, it is overexpressed in acute kidney injury mice and contributes to cisplatin-induced apoptosis of renal tubular epithelial cells *in vitro*[30,31]. Also, its dysregulation may reflect specific abnormities, thereby functioning as an indicator for disease conditions of inflammatory disorders and organ injuries in clinical practices[28,32]. MiR-146b expression is overexpressed and it positively correlates with plasma CRP, infarct volume and National Institute of Health stroke scale (NIHSS) score in acute ischemic stroke patients; furthermore, it is upregulated in kidney tissue samples from acute kidney injury patients[28,32].

Nevertheless, limited data reveals the role of miR-146b in AP. In our study, we observed that miR-146b was overexpressed in SAP patients, MSAP patients and MAP patients compared to HCs, and it distinguished these patients from HCs. Also, miR-146b distinguished SAP, MSAP and MAP patients from each other. In addition, it positively correlated with Ranson's score, APACHE II score, SOFA score and CRP in SAP patients; with Ranson's score, APACHE II score and CRP in MSAP patients; with Ranson's score and SOFA score in MAP patients. The following reasons might explain these findings. (1) MiR-146b might promote the release of inflammatory cytokines, leading to excess immune responses and eventually raised inflammation level as well as organ failures in AP patients. Thus, miR-146 could predict raised SAP risk, MSAP risk, MAP risk, and could distinguish different AP severity from each other[33]; (2) MiR-146b might participate in the TNF-α-induced inflammatory responses to facilitate the inflammation-induced necroptosis, thereby promoted the damage of pancreatic tissues and resulted in multiple organ disorders, which would be reflected by assessment scores. Thus, miR-146b expression was positively correlated with the various assessment scores as well as inflammatory factor CRP level in AP patients[34]. In addition, miR-146b high expression was correlated with raised in-hospital death risk in SAP patients as well as MSAP patients. These results might be on account for that miR-146b overexpression were correlated to enhanced disease progression (as we discussed above), which eventually resulted in less survival possibility. Thus, miR-146b correlated with higher in-hospital death risk in AP patients. Interestingly, we also discovered that compared to miR-146a, miR-146b showed relatively less predictive value in predicting AP risk and in-hospital death risk, as well as relatively weaker correlations with disease conditions and inflammation in AP patients (according to the AUC values and correlation analyses in our study), indicating that miR-146a might have a higher potential to serve as a biomarker for assisting the risks of SAP, MSAP and MAP as well as predicting the prognosis of AP patients.

Some limitations existed in this present study. (1) The sample size was relatively small, thereby the statistical power might be relatively low. (2) This was a single center study, thus there might be some selective biases. (3) Despite the correlation of miR-146a and miR-146b with risk, severity and in-hospital death of AP, the regulatory effects of miR-146a and miR-146b on AP pathology needed deeper investigations.

Conclusions

Briefly, the above results demonstrated that circulating miR-146a and miR-146b exhibit potential to serve as markers for AP management and prognosis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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