A Novel Serum Exosomal MicroRNA Signature in the Early Prediction of Persistent Organ Failure in Patients With Acute Pancreatitis

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Objective: To investigate whether serum exosomal microRNAs (miRNAs) could be potential biomarkers in predicting acute pancreatitis (AP) with persistent organ failure (POF) at an early phase.

Background: Novel biomarkers are sorely needed for early prediction of POF in patients with AP.

Methods: In the discovery stage, exosomal miRNAs were profiled in sera from APs with or without POF (5 vs 5) using microarrays. POF-associated miRNA signatures then were assessed in the training cohort (n = 227) and further validated in 3 independent cohorts (n = 516), including one nested case-control cohort.

Results: A total of 743 APs were recruited in this large-scale biomarker identification study with a nested case-control study. Data from the discovery cohort demonstrated that 90 exosomal miRNAs were significantly dysregulated in APs with POF compared with controls. One miRNA classifier (Cmi) comprising 3 miRNAs (miR-4265, 1208, 3127-5p) was identified in the training cohort and was further evaluated in 2 validation cohorts for their predictive value for POF. Area under the curves for Cmi ranged from 0.88 to 0.90, which was statistically superior to area under the curves of Acute Physiology and Chronic Health Examination–II and bedside index for severity in AP, and outperformed blood urea nitrogen and creatinine in POF prediction across all cohorts ($P < 0.05$). Higher levels of Cmi indicated an increased need for intensive care unit admission, prolonged hospitalization, and elevated mortality rate, thus poor prognosis. In the nested case-control study, Cmi could help identify prediagnostic POF in postendoscopic retrograde cholangiopancreatography pancreatitis cases within "golden hours" after endoscopic retrograde cholangiopancreatography with high efficacy.

Conclusions: Serum exosomal Cmi may be an early predictor for POF in AP, even within "golden hours" after AP onset.

Trail registration: ClinicalTrials.gov (NCT02602808).

Key Words: acute pancreatitis, biomarker, exosome, persistent organ failure

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BACKGROUND

Acute pancreatitis (AP) is the most common cause of gastrointestinal-related hospitalization, with a rising incidence annually worldwide, which loads heavy burdens on the current health care system.[1,2] Persistent organ failure (POF), developing in 10%~20% of patients with AP, is acknowledged as the prime determinant for poor outcomes, with mortality reaching 30%.[3,4] It is crucial to identify patients with AP who are at risk of POF early in the course of the disease, especially in the "golden hours,"[5] for providing patients with timely and appropriate clinical management, such as triage to an intensive care unit (ICU), vigorous fluid resuscitation, and correction of metabolic abnormalities, which may significantly improve the prognosis of patients with AP.[6]

Certain scoring systems, such as the Ranson scoring system, Acute Physiology and Chronic Health Examination (APACHE)-II, and bedside index for severity in AP (BISAP) were recommended to predict AP severity.[6–9] However, those systems suffer many shortcomings and disadvantages, including moderate accuracy, low efficacy, time-consuming, and cumbersome to use.[3] Recent attention has been focused on the role of individual laboratory parameters, such as blood urea nitrogen (BUN) and creatinine, in predicting the severity of AP.[10–12] However, no studies have reported any methods performing significantly better than good clinical judgment so far.[1,3] The development of novel biomarkers for the early prediction of POF in patients with AP is,

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This study was approved by the Institutional Ethics Committee of Changhai Hospital, and informed consent was obtained. All authors had access to the study data and reviewed and approved the final manuscript.

L.L. and X.K.: study concept and design. L.L., F.L., F.K., P.X., F.S., Q.Z., and H.Y.: acquisition of data. L.L., X.K., Y.F.,J Zhao : analysis and interpretation of data. L.L.: drafting of the manuscript and statistical analysis. L.L., J. Zhou, S.W., and S.Z.: critical revision of the manuscript for important intellectual content. X.K., Y.D., and Z.L.: administrative, technical, material support, and study supervision.

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therefore, a major clinical need, which would allow improved triage and management of these patients.

MicroRNAs (miRNAs) are a class of endogenous noncoding RNAs that regulate gene expression posttranscriptionally. Some studies have profiled circulating miRNAs and identified certain miRNAs correlated with AP severity and outcomes.[13–15] Meanwhile, accumulating evidence suggested that exosomes are highly enriched in miRNAs. Furthermore, it is thought that exosomal miRNAs are of higher value with increased sensitivity, compared with nonexosomal miRNAs.[16–18] Thus, we hypothesize that exosomal miRNAs may be potentially used to noninvasively predict APs with POF earlier.

In the current multi-institutional prospective study, we investigated the differences in exosomal miRNA levels between APs with and without POF. In addition, we determined and validated the accuracy of the identified exosomal miRNAs in predicting POF at admission, compared with APACHE-II and BISAP, as well as other objective parameters, BUN and creatinine from 2 external cohorts. Furthermore, as the status of different markers in serum is dramatically changing over time in AP, we also evaluated the diagnostic efficacy of exosomal miRNAs for POF at the timepoint of 24 hours after endoscopic retrograde cholangiopancreatography (ERCP), which is commonly accepted as the "golden hours" for AP management.[5] The aim of the current study was to develop a noninvasive liquid biopsy method for the early prediction of POF in patients with AP.

METHODS

Study Design and Participants

A total of 743 serum samples from patients with AP with symptoms <72 hours were obtained for this study (Fig. 1). This multi-institutional prospective study included 3 stages. In the discovery stage, 10 patients were enrolled between December 1, 2015 and February 29, 2016, at Changhai Clinical Research Unit. In the training stage, 227 patients were enrolled between December 1, 2015 and May 31, 2017, at Changhai Clinical Research Unit. Three independent cohorts were involved in the validation stage. Validation cohort 1 (V1) enrolled participants between December 1, 2015 and December 31, 2017, at the Trauma Emergency Rescue Center of Naval Military Medical University. Validation cohort 2 (V2) was composed of participants at the Changhai Clinical Research Unit between June 1, 2017 and December 31, 2018. As serum markers are drastically changing in AP, to test miRNAs' ability to predict POF at consistent timing points, we validated the accuracy of exosomal miRNAs in a nested case-control study (V3), in which patients were from a surveillance program for post-ERCP pancreatitis (PEP) detection between May 1, 2016 and May 31, 2019 in Digestive Endoscopy Center of Changhai Hospital. The study was approved by the Institutional Ethics Committee of Changhai Hospital, and informed consent was obtained. All authors had access to the study data and reviewed and approved the final manuscript.

Criteria for diagnosis of AP, PEP, and definition of POF are listed in the Supplemental Materials (Supplemental Digital Content 1, http://links.lww.com/SLA/F11). Detailed inclusion and exclusion criteria for AP are listed in Supplemental Table 1 (Supplemental Digital Content 1, http://links.lww.com/SLA/F11). Patients were subgrouped by the AP severity according to the revised Atlanta classification.[19] We further evaluated levels of miRNAs in various groups from different cohorts.

APACHE-II and BISAP were calculated at admission in training, V1 and V2 cohorts. Both scores were calculated using a table developed by the senior author (X.K.). Initial and follow-up meetings were held by investigators (L.L., F.L., and P.X.) to resolve emerging discrepancies and to confirm that the same rules were applied to all calculations. Each clinical score was calculated based on the worst (most extreme) laboratory data and vital signs obtained at admission. For transferred patients, clinical scores analysis was performed by the data on initial admissions from outside hospitals. Missing values were considered normal. Two routine laboratory values (BUN and creatinine), which were significantly associated with AP severity, were also assessed individually.[10]

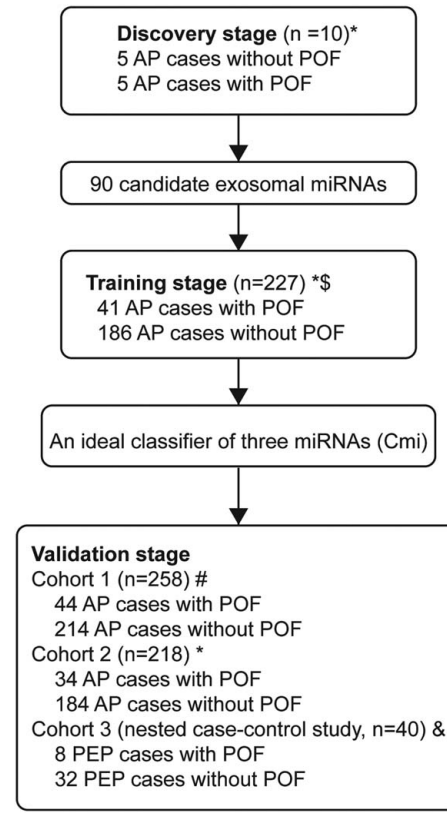

FIGURE 1. Study design. "*" indicates Changhai Clinical Research Unit; "$," including 10 samples used in the discovery stage; "#," Trauma Emergency Rescue Center of Naval Military Medical University and Digestive Endoscopy Center of Changhai Hospital.

Procedures

Procedures for exosome isolation, visualization and verification, RNA extraction, microarray profiling, and miRNA quantification are described in the Supplemental Materials (Supplemental Digital Content 1, http://links.lww.com/SLA/F11).

The microarray data have been deposited in the National Center for Biotechnology Information's Gene Expression Omnibus (GSE136030). The normalized data sets were then analyzed to identify different expression patterns of exosomal miRNAs between individuals with and without POF (5 vs 5; Supplemental Digital Content Fig. 1, http://links.lww.com/SLA/F11). To find specific markers for POF with lower variability and robust results, 35 candidate miRNAs were chosen according to the following criteria: average raw signal intensity ≥ 10 in the POF group, fold change > 8, and $P < 0.05$.

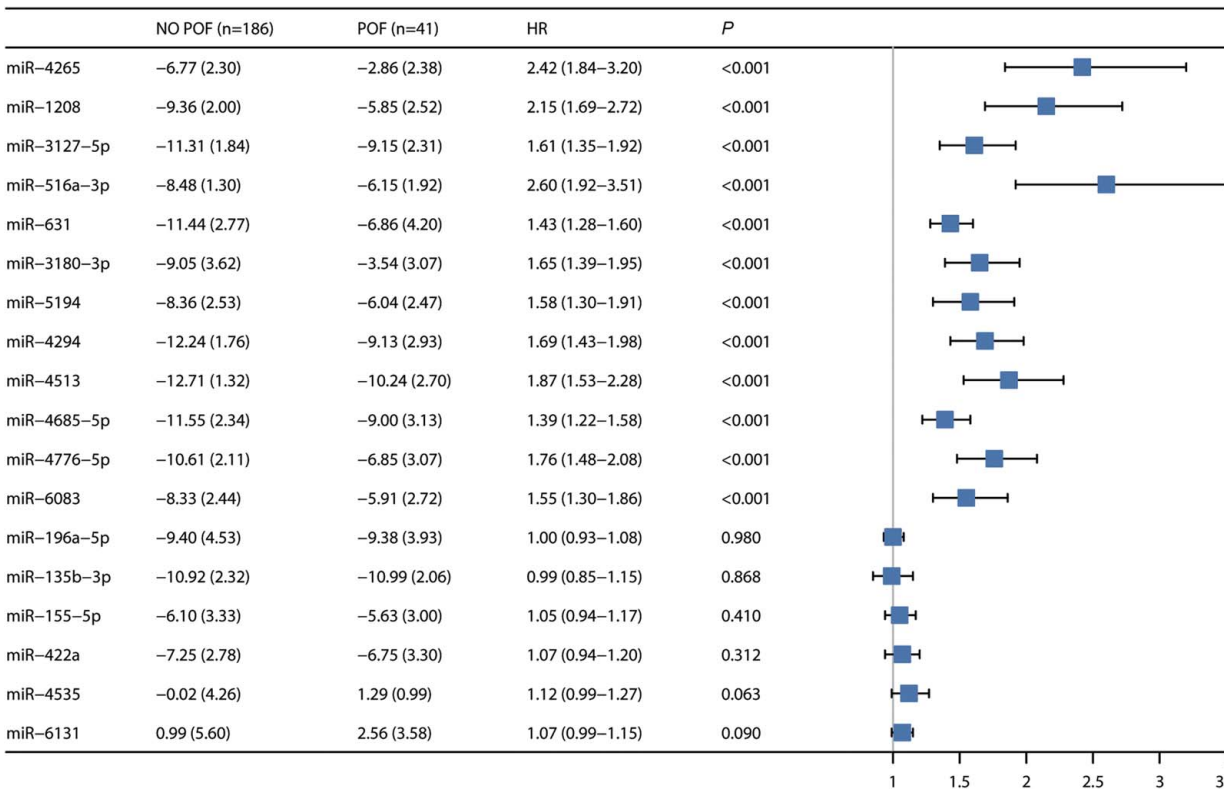

FIGURE 2. Comparison of levels of 18 candidate miRNAs in serum exosome from patients with AP, with versus without POF in the training cohort.

In the training stage, we assessed the levels of the 35 candidate miRNAs in 41 patients with AP with POF and 186 controls. Exosomal miRNAs were analyzed by quantitative polymerase chain reaction with a spiked-in non-human miRNA, cel-miR-39, as a reference control.[20] A logistic regression model was employed to construct a miRNA classifier (Cmi) to distinguish APs with POF from controls.

Using Cmi, we evaluated and presented the performance for the training, and 2 validation cohorts by means of sensitivity, specificity, area under the curve (AUC), and corresponding 95% CIs. For further validating Cmi, we compared its predictive accuracy with that of APACHE-II, BISAP, BUN, and creatinine, which are routinely used scoring systems/laboratory parameters for AP severity prediction in the clinic.[10] As POF is the key determinant for poor prognosis, we also evaluated the association between Cmi with those clinical outcomes: need for ICU care, length of hospitalization, and death.

We also carried out a nested case-control study in the third validation cohort. In this study, we followed 8537 patients who underwent ERCP. Serum samples were collected at the time-point of 24 hours after ERCP for amylase and Cmi analysis. Those patients who developed POF were identified using the criteria mentioned previously. Four age and sex-matched PEPs without POF in the same surveillance program were selected as controls for each case of PEP with POF identified.[21]

Statistical Analyses

Our power calculation for the number of participants in the discovery stage is described in Supplemental Materials (Supplemental Digital Content 1, http://links.lww.com/SLA/F11) and was around 95% with 5 APs with POF and 5 without POF. To improve the power of the nested case-control study, we raised the ratio of controls to cases to 4 to 1.[22]

For the array analysis, differentially detected miRNAs were identified through fold change, as well as P value was calculated using an unpaired t test. The threshold set for up and downregulated miRNAs was fold change ≥ 2.0 and P value <0.05. We did hierarchical clustering analysis with the Euclidean distance matric and completed the linkage method with MEV version 4.9.

Normally distributed data are presented as mean ± SD. χ^2 test or 2-sided Fisher exact test was used for all comparisons of proportions. The independent Student t test was used to compare normally distributed continuous variables. The Mann-Whitney test was used to compare non-normally distributed continuous variables. Receiver-operating characteristic curve was compared by using MedCalc for Windows version 11.4.2.0 software. Other statistics were performed using SPSS 16.0. We considered a P value <0.05 as statistically significant.

RESULTS

Patients' Characteristics

The study design strategy is shown in Figure 1. A total of 743 patients with AP were prospectively recruited for this study. Supplemental Table 2 (Supplemental Digital Content 1, http://links.lww.com/SLA/F11) lists the clinical characteristics of patients in the training and 3 independent validation cohorts. Patients with AP with POF had poorer outcomes, higher scores for 2 clinical scoring systems, and higher individual laboratory

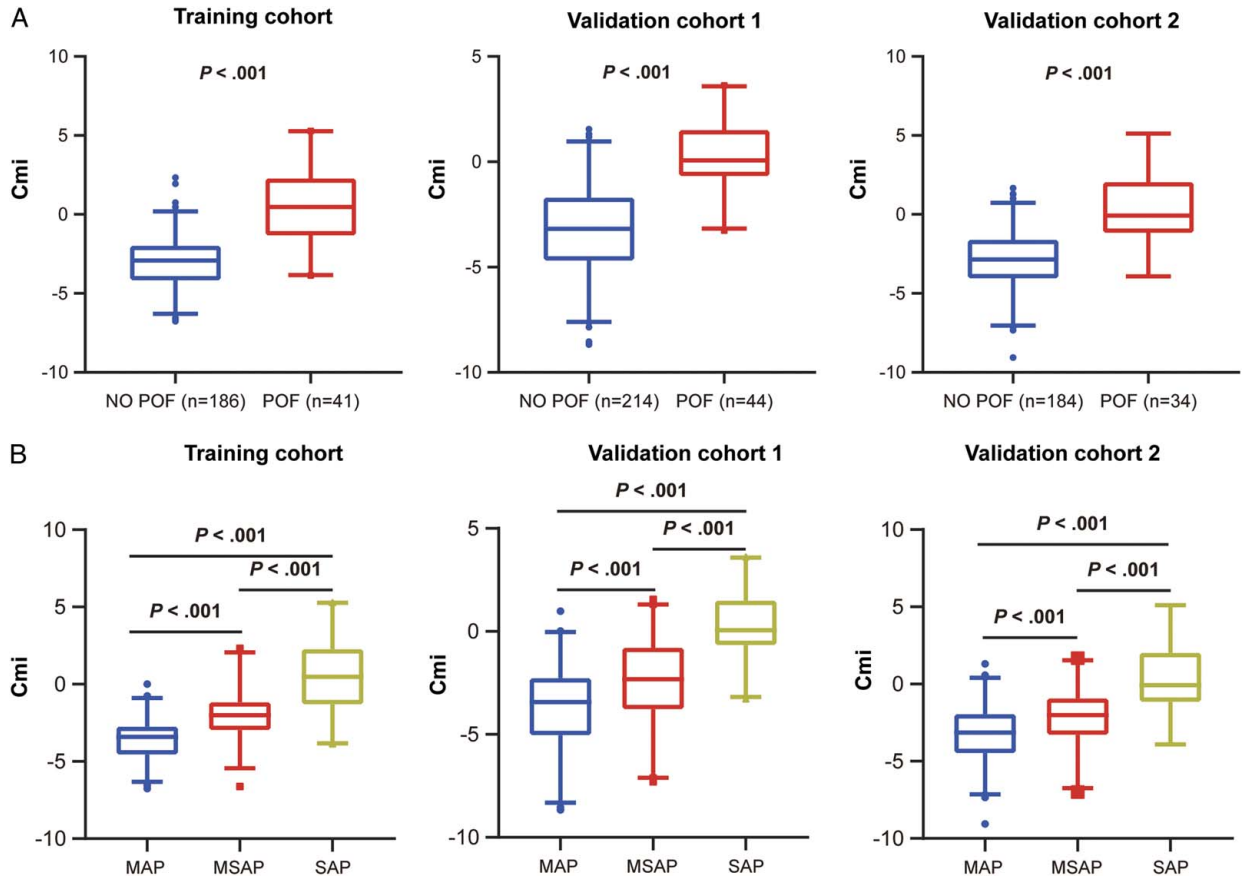

FIGURE 3. Association of Cmi with POF or severity status in AP samples from training, V1 and V2 cohorts. Patients with AP were stratified into with and without POF subsets (A), or MAP, MSAP, and SAP subsets according to the revised Atlanta classification criteria (B).

parameters (BUN and creatinine) as compared with those without POF in different cohorts.

Inspection and Confirmation of Exosomes Isolated From Serum Samples

Electron microscopy analysis of serum pellets obtained from AP samples showed the presence of round-shaped particles with double layer membrane structure (Supplemental Digital Content Fig. 2A, http://links.lww.com/SLA/F11). Nanoparticle tracking analysis of serum demonstrated that the diameters of most particles were within the range of 30 to 150 nm (Supplemental Digital Content Fig. 2B, http://links.lww.com/SLA/F11). Western blot analysis showed that 2 exosome markers (TSG101 and CD9) were detected in pellet samples but little in the centrifuged supernatant (Supplemental Digital Content Fig. 2C, http://links.lww.com/SLA/F11). The previously mentioned analysis confirmed our efficient extraction of serum exosomes.

Variation of Exosomal MicroRNA Profiles Between Acute Pancreatitis With and Without Persistent Organ Failure

In the discovery stage, we identified 90 miRNAs that were differentially detected between samples from APs with and without POF (5 vs 5, $P < 0.05$; Supplemental Digital Content Fig. 1, http://links.lww.com/SLA/F11, Supplemental Digital Content Table 3, http://links.lww.com/SLA/F11) by microarrays. For this 90-miRNA profile, clustering analysis displayed a perfect separation between AP samples with and without POF, indicating the potential predictive power of serum exosomal miRNAs for POF (Supplemental Digital Content Fig. 1, http://links.lww.com/SLA/F11). To minimize the risk of false-positive results, 55 miRNAs were excluded according to the following criteria: average signal intensity <10 in the POF group or fold change <8. We further investigated the remaining 35 miRNAs in the training cohort.

Identification of One MicroRNA Classifier as Potential Predictive Marker for Persistent Organ Failure

In the training stage, we verified the 35 miRNAs by quantitative polymerase chain reaction in 227 serum samples from 41 APs with POF and 186 APs without POF. Seventeen miRNAs were excluded because of the low detection rate (< 75%) in APs with POF. Accordingly, 12 of the remaining 18 miRNAs were found significantly associated with POF status (Fig. 2; $P < 0.001$). Box plots of the relative levels of these miRNAs in APs with and without POF in the training cohort are shown in Supplemental Figure 3 (Supplemental Digital Content 1, http://links.lww.com/SLA/F11). Then we performed a binary logistic regression for 12 miRNAs. Three miRNAs were selected in the final model. The equation of the model (classifier, Cmi) is the following: Cmi = 6.043+0.244×miR-3127-5p+0.511×miR-4265+0.309×miR-1208.

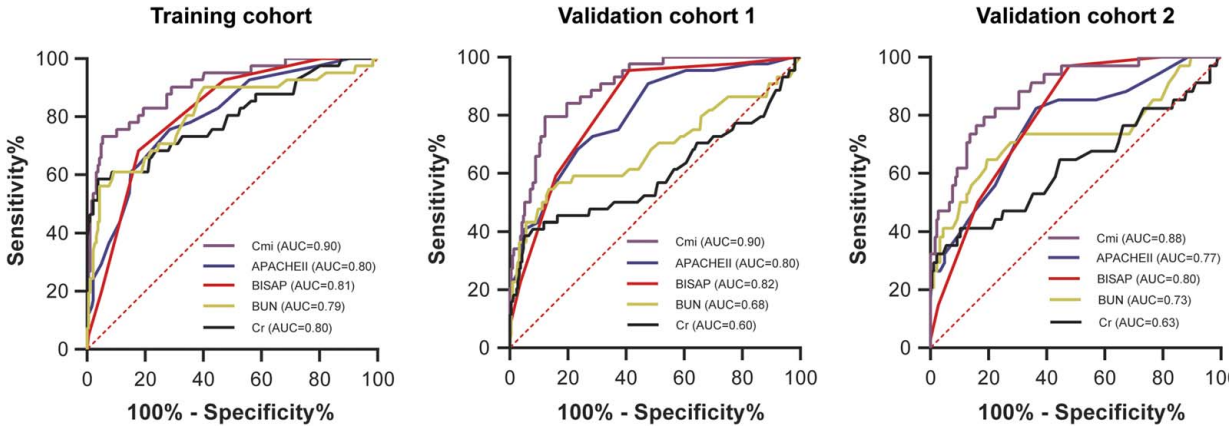

FIGURE 4. ROC curves for performances of Cmi, APACHE-II, BISAP, BUN, and creatinine in the training, V1, and V2 cohorts (please refer to Supplemental Digital Content Table 5, http://links.lww.com/SLA/F11 for the detailed comparisons of these markers). ROC indicates receiver-operating characteristic.

Validation of the Predictive Value of MicroRNA Classifier for Persistent Organ Failure Status

Box plots of the relative levels of Cmi in the training, V1 and V2 cohorts appear in Figure 3A. Levels of Cmi progressively increased with the severity of APs across all cohorts (Fig. 3B). Area under the receiver operating characteristic (AUROC) for Cmi was 0.90 (95% CI: 0.85–0.96) in the training cohort, 0.90 (95% CI: 0.85–0.94) and 0.88 (95% CI: 0.82–0.94) in V1 and V2 cohorts, respectively (Fig. 4, Supplemental Digital Content Table 4, http://links.lww.com/SLA/F11). In the training cohort, at the best cutoff value of −0.788, the sensitivity of Cmi for POF reached 73%, whereas the specificity reached 95%. At the same cutoff value, the sensitivity reached 80% and 65%, whereas the specificity reached 87% and 88% in the V1 and V2 cohorts, respectively (Supplemental Digital Content Table 4, http://links.lww.com/SLA/F11). These data further validated the predictive value of Cmi for POF status.

Association Between MicroRNA Classifier With Clinical Outcomes

Given that POF is the key determinant for AP severity and is strongly associated with a poorer prognosis, we further investigated the association between Cmi and clinical outcomes of AP. As shown in Supplemental Figure 4 (Supplemental Digital Content 1, http://links.lww.com/SLA/F11), patients with AP with ICU admission, prolonged hospitalization (>7 days), or death had significantly higher levels of Cmi compared with controls (all $P < 0.01$). Furthermore, levels of Cmi progressively increased from MAP, MSAP to SAP, which demonstrated that its elevation was severity stage-dependent (Fig. 3B). All these results indicated that Cmi was associated with poor prognosis of patients with AP.

Comparisons of Predictive Performance of MicroRNA Classifier With Commonly Used Scoring Systems/ Laboratory Parameters

To further evaluate the predictive power of our newly developed marker for POF, we compared the predictive accuracy of Cmi with 2 "traditional" multifactorial scoring systems: APACHE-II and BISAP, and 2 individual laboratory parameters: BUN and creatinine.[10] Notably, AUCs of Cmi were significantly greater than those of BUN and creatinine in the training, V1 and V2 cohorts ($P < 0.05$; Fig. 4, Supplemental Digital Content Table 5, http://links.lww.com/SLA/F11). In comparison with the 2 scoring systems, AUCs for Cmi were consistently larger than APACHE-II and BISAP across all cohorts (all $P < 0.05$; Fig. 4, Supplemental Digital Content Table 5, http://links.lww.com/SLA/F11). As Cmi was a single objective marker, which involves no subjective judgment, it might be more reliable and practical than current scoring systems for POF prediction in clinical AP management.

Predictive Potential of MicroRNA Classifier for Persistent Organ Failure Within "Golden Hours" After Acute Pancreatitis Onset

During the first 24 hours, namely "golden hours," intensive care is crucial to reduce the morbidity and mortality associated with the AP process.[5] As circulating miRNAs are reported to inform disease status earlier than traditional biomarkers,[23–25] as well as no objective markers are available for predicting POF in "golden hours," we sought to determine whether Cmi was potentially qualified.

In the V3 cohort from a nested case-control study, we employed PEP, an ideal model for evaluating markers in the early response to AP,[26] to examine the predictive values of Cmi for POF within 24 hours after ERCP. A total of 8537 patients underwent ERCP and 222 PEPs occurred. Eight PEPs with POF were identified after follow-up, and 32 PEPs without POF, matched by age and sex were enrolled as controls from the same study. Consistently, Cmi showed good performance in predicting POF at the timepoint of 24 hours after ERCP, with AUC values of 0.96 (95% CI: 0.91–1.00, $P < 0.0001$; Fig. 5A). Of note, 6 out of 8 prediagnostic PEPs which later developed POF had a Cmi value >-0.788 (Fig. 5B, cutoff value of −0.788 was previously identified in Supplemental Digital Content Table 4, http://links.lww.com/SLA/F11). Since these 6 cases were "POF free" at the timepoint of 24 hours after the ERCP procedure, this result strongly suggests that our Cmi might be capable of predicting POF within the "golden hours" after AP initiation, which may help clinicians be more proactive in triage and management of patients with AP.[5]

DISCUSSION

To our knowledge, this is the largest sample size of an AP biomarker study to date to identify specific serum exosomal

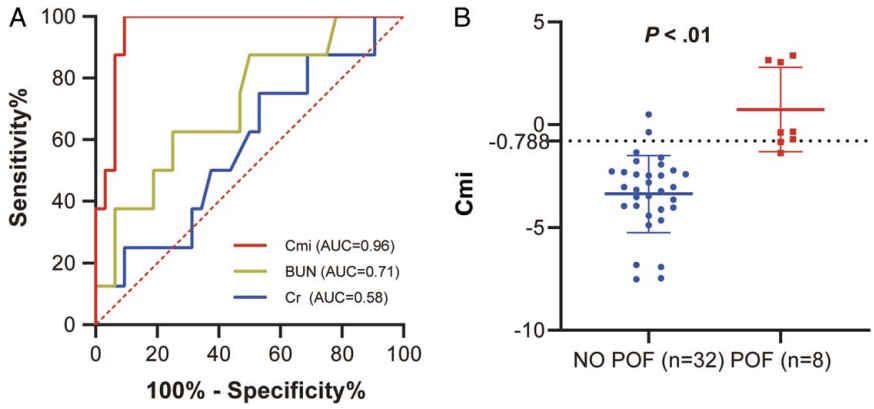

FIGURE 5. A and B, Performances of Cmi, BUN, and creatinine in the nested case-control study (V3; please refer to Supplemental Digital Content Table 5, http://links.lww.com/SLA/F11 for the detailed comparisons of these markers).

miRNA biomarkers for POF prediction. We found that 3 Cmi were significantly elevated in APs who developed POF at admission. Of note, in comparisons with previously established predictive models or individual markers, Cmi was statistically superior to APACHE-II and BISAP and significantly outperformed BUN and creatinine throughout all cohorts. Most importantly, Cmi could identify PEP cases developing POF within "golden hours." Furthermore, elevated levels of Cmi were positively associated with AP severity. Higher levels of Cmi correlated with increased need for ICU admission, prolonged hospitalization, and elevated mortality rate, thus poor prognosis. All these findings highlight the potential of Cmi as an early predictive marker for POF in APs.

POF is acknowledged as the prime determinant for poor outcomes in patients with AP.[3,4] Clinical scoring systems have been the focus of the prediction of AP severity for 4 decades, with a plethora of scores reported in the literature. However, all existing scoring systems seemed to have reached their maximal predictive efficacy, with only moderate accuracy in predicting POF.[1,10] Furthermore, current available scoring systems are complex and frequently cumbersome to calculate in clinical practice.[27] In addition, individual laboratory parameters, such as BUN and creatinine, have raised interest in predicting POF in APs, recently. However, no approaches have been proven significantly better than good clinical judgment.[1,3]

There are several advantages to screening miRNAs in exosomes for POF prediction. Firstly, evidence has revealed that exosomes serve as protective vesicles that shield miRNAs from RNase-rich environments, rendering exosomal miRNAs stable and reproducible as biological markers. Secondly, isolating the enriched miRNAs in exosomes from serum will remove insignificant circulating nonexosomal miRNA, which will definitely elevate the signal-to-noise ratio and increase the sensitivity of miRNA detection. Thirdly, unlike other circulating miRNAs that are passively released from apoptotic and necrotic cells, exosomes are actively secreted into the peripheral blood by different cell types and are biologically relevant to disease pathogenesis, including AP.[28,29] Therefore, exosomal miRNAs may truly become the specific molecular biomarkers, in contrast with cell-free miRNAs. Nevertheless, no exosomal miRNAs have yet been reported to be associated with POF prediction.

There are several potential limitations to the current study. Firstly, as no effect estimates of exosomal miRNAs in serum in patients with AP have previously been reported, our study cannot be designed with a statistical power that was certain to detect differences in exosomal miRNA levels between APs with and without POF. Special care is necessary when interpreting the lack of significance in subgroups. Secondly, recent studies are highlighting the value of the variation of certain markers over time for POF prediction, whereas current research lacks the exploration of the levels of Cmi over time. Future work will be required to investigate the variation of Cmi in APs. Thirdly, patients included in the training, V1 and V2 cohorts differed in a number of characteristics. However, Cmi in various cohorts showed similar great predictive performance, further confirming that our findings were robust. Last but not least, we observed significant overlap in Cmi levels among patients with or without POF in the training, V1 and V2 cohorts, which means that an isolated measurement of Cmi might offer limited guidance for treating individual patients with AP in these cohorts. However, as the status of different markers in serum is dramatically changing over time in AP, we speculate that this overlap may result from the heterogeneous stages and timing of AP cases in our study. To address this point, we utilized PEP, an ideal model for AP, for evaluating Cmi for predicting POF consistently 24 hours after AP onset (V3 cohort). Our findings revealed a small overlap in Cmi levels between patients with AP with or without POF, with patients with POF consistently exhibiting higher Cmi levels than most (30/32) patients with non-POF (Fig. 5B). This suggests that Cmi could aid clinicians in proactive triage and management of patients with AP during the critical "golden hours."

Despite these limitations, there are several strengths in this study, including a relatively large number of patients with AP included in each cohort, a high-throughput screening algorithm for candidate miRNAs in the discovery cohort, and 3 independent cohorts applied to validate the biomarker. In particular, we evaluated the predictive power of Cmi for POF in patients with PEP, which tends to give more valuable evidence for AP management within "golden hours." In addition, the application of the exosomal Cmi would avoid the influence of subjective factors, such as interpretation of chest radiography and patients' signs, in predicting performance. Thus, such biomarkers are thought to be more reliable, practical, and convenient in future clinical practice than other prediction models.

CONCLUSIONS

This study possessed the largest sample size to identify exosomal biomarkers for POF prediction in AP. The serum

exosomal Cmi showed good performance beyond those routine parameters in POF prediction accuracy. In addition, our results indicate that Cmi could predict POF within "golden hours" after AP onset, which may allow physicians to be proactive in AP management and may significantly improve the prognosis of patients with AP.

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REFERENCES

1. Mederos MA, Reber HA, Girgis MD. Acute pancreatitis: a review. *JAMA*. 2021;325:382–390.
2. Lankisch PG, Apte M, Banks PA. Acute pancreatitis. *Lancet*. 2015;386: 85–96.
3. Forsmark CE, Swaroop Vege S, Wilcox CM. Acute pancreatitis. *N Engl J Med*. 2016;375:1972–1981.
4. Schepers NJ, Bakker OJ, Besselink MG, et al. Impact of characteristics of organ failure and infected necrosis on mortality in necrotising pancreatitis. *Gut*. 2019;68:1044–1051.
5. Fisher JM, Gardner TB. The "golden hours" of management in acute pancreatitis. *Am J Gastroenterol*. 2012;107:1146–1150.
6. Forsmark CE, Baillie J. AGA Institute technical review on acute pancreatitis. *Gastroenterology*. 2007;132:2022–2044.
7. Working Party of the British Society of Gastroenterology; Association of Surgeons of Great Britain and Ireland; Pancreatic Society of Great Britain and Ireland; Association of Upper GI Surgeons of Great Britain and Ireland. UK guidelines for the management of acute pancreatitis. *Gut*. 2005;54(suppl 3):iii1–iii9.
8. Larvin M, McMahon MJ. APACHE-II score for assessment and monitoring of acute pancreatitis. *Lancet*. 1989;2:201–205.
9. Wu BU, Johannes RS, Sun X, et al. The early prediction of mortality in acute pancreatitis: a large. *Gut*. 2008;57:1698–1703.
10. Mounzer R, Langmead CJ, Wu BU, et al. Comparison of existing clinical scoring systems to predict persistent organ failure in patients with acute pancreatitis. *Gastroenterology*. 2012;142:1476–1482; quiz e15-16.
11. Wu BU, Bakker OJ, Papachristou GI, et al. Blood urea nitrogen in the early assessment of acute pancreatitis: an international validation study. *Arch Intern Med*. 2011;171:669–676.
12. Muddana V, Whitcomb DC, Khalid A, et al. Elevated serum creatinine as a marker of pancreatic necrosis in acute pancreatitis. *Am J Gastroenterol*. 2009;104:164–170.
13. Kusnierz-Cabala B, Nowak E, Sporek M, et al. Serum levels of unique miR-551-5p and endothelial-specific miR-126a-5p allow discrimination of patients in the early phase of acute pancreatitis. *Pancreatology*. 2015; 15:344–351.
14. Liu P, Xia L, Zhang WL, et al. Identification of serum microRNAs as diagnostic and prognostic biomarkers for acute pancreatitis. *Pancreatology*. 2014;14:159–166.
15. Kong XY, Du YQ, Li L, et al. Plasma miR-216a as a potential marker of pancreatic injury in a rat model of acute pancreatitis. *World J Gastroenterol*. 2010;16:4599–4604.
16. Boukouris S, Mathivanan S. Exosomes in bodily fluids are a highly stable resource of disease biomarkers. *Proteomics Clin Appl*. 2015;9: 358–367.
17. Gallo A, Tandon M, Alevizos I, et al. The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. *PLoS One*. 2012;7:e30679.
18. Cheng L, Doecke JD, Sharples RA, et al. Prognostic serum miRNA biomarkers associated with Alzheimer's disease shows concordance with neuropsychological and neuroimaging assessment. *Mol Psychiatry*. 2015; 20:1188–1196.
19. Banks PA, Bollen TL, Dervenis C, et al. Classification of acute pancreatitis–2012: revision of the Atlanta classification and definitions by international consensus.. *Gut*. 2013;62:102–111.
20. Kong X, Du Y, Wang G, et al. Detection of differentially expressed microRNAs in serum of pancreatic ductal adenocarcinoma patients: miR-196a could be a potential marker for poor prognosis. *Dig Dis Sci*. 2011;56:602–609.
21. Lin XJ, Chong Y, Guo ZW, et al. A serum microRNA classifier for early detection of hepatocellular carcinoma: a multicentre, retrospective, longitudinal biomarker identification study with a nested case-control study. *Lancet Oncol*. 2015;16:804–815.
22. Grimes DA, Schulz KF. Compared to what? Finding controls for case-control studies. *Lancet*. 2005;365:1429–1433.
23. Wang GK, Zhu JQ, Zhang JT, et al. Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. *Eur Heart J*. 2010;31:659–666.
24. Xin Y, Yang C, Han Z. Circulating miR-499 as a potential biomarker for acute myocardial infarction. *Ann Transl Med*. 2016;4:135.
25. Chen X, Hu Z, Wang W, et al. Identification of ten serum microRNAs from a genome-wide serum microRNA expression profile as novel noninvasive biomarkers for nonsmall cell lung cancer diagnosis. *Int J Cancer*. 2012;130:1620–1628.
26. Messmann H, Vogt W, Holstege A, et al. Post-ERP pancreatitis as a model for cytokine-induced acute phase response in acute pancreatitis. *Gut*. 1997;40:80–85.
27. Papachristou GI, Muddana V, Yadav D, et al. Comparison of BISAP, Ranson's, APACHE-II, and CTSI scores in predicting organ failure, complications, and mortality in acute pancreatitis. *Am J Gastroenterol*. 2010;105:435–441; quiz 442.
28. Bonjoch L, Casas V, Carrascal M, et al. Involvement of exosomes in lung inflammation associated with experimental acute pancreatitis. *J Pathol*. 2016;240:235–245.
29. Mori MA, Ludwig RG, Garcia-Martin R, et al. Extracellular miRNAs: from biomarkers to mediators of physiology and disease. *Cell Metab*. 2019;30:656–673.