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Original Article

Ambient Mass Spectrometry and Machine Learning-Based Diagnosis System for Acute Coronary Syndrome

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Aims: The purpose of this study is to establish a novel diagnosis system in early acute coronary syndrome (ACS) using probe electrospray ionization-mass spectrometry (PESI-MS) and machine learning (ML) and to validate the diagnostic accuracy.
Methods: A total of 32 serum samples derived from 16 ACS patients and 16 control patients were analyzed by PESI-MS. The acquired mass spectrum dataset was subsequently analyzed by partial least squares (PLS) regression to find the relationship between the two groups. A support vector machine, an ML method, was applied to the dataset to construct the diagnostic algorithm.
Results: Control and ACS groups were separated into the two clusters in the PLS plot, indicating ACS patients differed from the control in the profile of serum composition obtained by PESI-MS. The sensitivity, specificity, and accuracy of our diagnostic system were all 93.8%, and the area under the receiver operating characteristic curve showed 0.965 (95% CI: 0.84–1).
Conclusion: The PESI-MS and ML-based diagnosis system are likely an optimal solution to assist physicians in ACS diagnosis with its remarkably predictive accuracy.

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Keywords: acute coronary syndrome, chest pain, machine learning, predictive accuracy

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1. INTRODUCTION

Acute coronary syndrome (ACS) is characterized by an abrupt and sudden constriction or occlusion of the lumen of the coronary artery, induced by the rupture or erosion of atheromatous plaques in the arterial wall, resulting in the formation of thrombi that lead to myocardial ischemia and necrosis.[1)] ACS represents a symptomatic manifestation of coronary heart disease (CHD) and accounts for over 30% of all deaths in above-35-year-old adults.[1)] The symptoms of ACS may manifest as either typical, such as substernal chest pain, or atypical, such as non-specific chest discomfort.[1)] Thus, obtaining a comprehensive medical history is of paramount importance in the diagnosis of ACS. Significantly, risk stratification plays a pivotal role in approaching patients with suspected ACS through clinical assessment, electrocardiography (ECG), cardiac biomarkers, and cardiac imaging.[1)] In fact, in line with the widespread utilization of high-sensitivity assays like high-sensitive cardiac troponin (hs-cTn), the traditional clinical triage decision-making process, which incorporates patient history, physical examination, and ECG, has been augmented to maximize the diagnostic value, thereby aiding in reducing the door-to-balloon time for ST-elevation myocardial infarction (STEMI) and classifying non-ST-elevation myocardial infarction (NSTEMI) based on risk scores.[2)] That being said, although the assessment of hs-cTn kinetics or serial hs-cTn measurements is crucial in the evaluation of suspected ACS,[2)] the application of this theory poses challenges in various clinical scenarios, including patients at intermediate risk who require additional evaluations and prolonged hospital stays, as well as those with comorbidities such as chronic kidney

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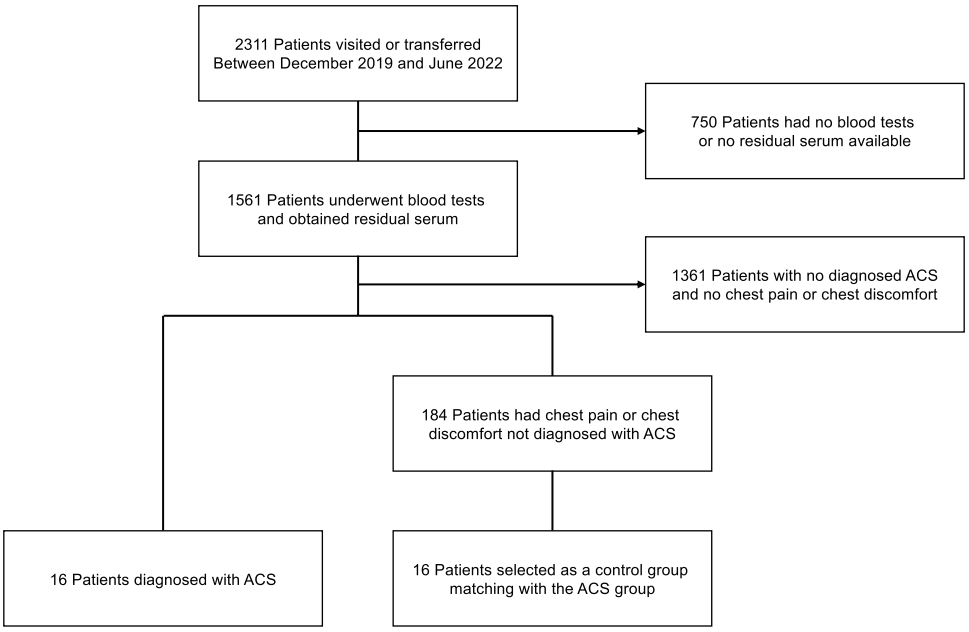

Fig. 1. Selection and exclusion criteria and number of patients in this study. From a population of 2311 patients, 16 controls and 16 ACS groups were collected. Sera obtained from 32 patients were analyzed using mass spectrometry. ACS, acute coronary syndrome.

disease, which is highly likely to be a confounding factor.[3,4] Currently, alternative diagnostic methods are not available in clinical practice, underscoring the need for a more accurate, time-efficient, and objective diagnostic approach for ACS.

The ACS etiology, in which inflammatory pathways play a central role,[5] has the potential to induce alterations in plasma metabolites. A non-targeted approach employing liquid chromatography coupled with mass spectrometry (LC-MS) possesses the capability to identify these changes and thereby facilitates the early diagnosis of ACS.[6,7] However, LC-MS requires column purification steps and time of its processing, so it is less practical in terms of the handling and speed required in clinical settings. Probe electrospray ionization mass spectrometry (PESI-MS) is a method capable of analyzing nearly "raw" samples without the need for extensive pretreatment.[8] PESI-MS represents an ionization mass spectrometry (MS) technique that surpasses conventional electrospray ionization methods in terms of equipment and procedural simplicity, as well as exhibiting a low cost per sample.[8] Furthermore, the combination of PESI-MS and machine learning (ML) in rapid analyses of biological samples for a novel method of cancer discrimination has been demonstrated.[9–11]

Regarding the ML approach for early diagnosis of ACS, Than et al. share a similar perspective but employ the cardiac troponin biomarker.[12] Specifically, an algorithm incorporating factors such as age, gender, and hs-cTn may prove valuable in identifying low-risk and high-risk patients.[12] However, as previously mentioned, the serial measurement of a high-sensitivity variable, required within a complex clinical context, could impede timely diagnosis. Hence, PESI-MS and ML, with their ability to comprehensively explore molecular profiles and effectively delineate clusters within ACS inflammatory pathways, hold promise as decision-making tools in the future. The objective of this study was to assess the availability of the diagnostic system in the early ACS through PESI-MS analyses of serum metabolomic profiles and ML for discrimination tasks.

2. METHODS

2.1. Research ethics

Procedures were followed in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975.

2.2. Sample selection

The study employed a prospective design, focusing on patients who presented to our institute or were transferred via ambulance during the designated emergency rotation hours every Tuesday (excluding public holidays) from December 11th, 2019 to June 15th, 2022.

Figure 1 illustrates the flowchart outlining the sample selection process in the present study. Within the research period, a total of 2311 patients were admitted to the emergency department (ED) of our institute. We excluded 750 patients due to the unavailability of blood samples, resulting in 1561 serum samples from the patients who met the inclusion criteria. Out of them, 1361 patients were reported with no ACS and no symptoms of chest pain and/or chest discomfort, then excluded from our target. In the clinical pathway, utilizing real-time ECG and cardiac biomarker assays such as Troponin T and/or echocardiography, we identified 16 patients with confirmed ACS and 184 patients with diagnoses of non-ischemic chest pain. To assess the diagnostic accuracy of the PESI-MS and ML-based diagnosis system in ACS, we selected a control group of 16 patients with non-ischemic chest pain who were age- and sex-matched with the 16 ACS patients.

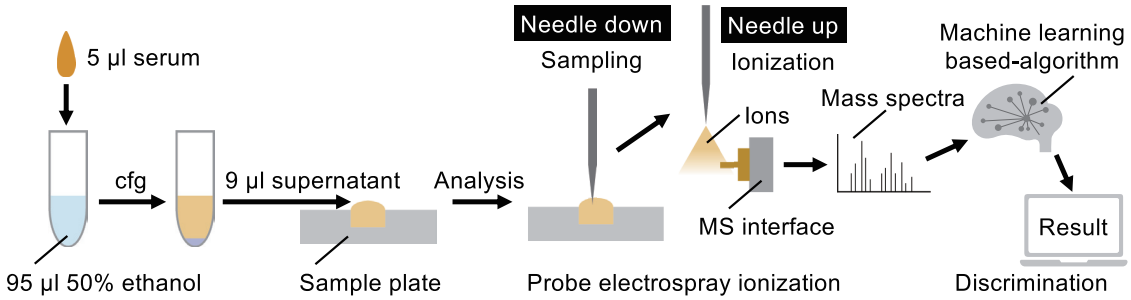

Fig. 2. Schematic diagram of sample preparation, PESI-MS analysis, and diagnosis. Five microliters of serum was used for analysis. The results of the analysis using mass spectrometry were obtained in 2 min, and an algorithm was used to discriminate the results to obtain a diagnostic result for ACS. ACS, acute coronary syndrome; cfg, centrifugation; MS, mass spectrometry; PESI-MS, probe electrospray ionization-mass spectrometry.

2.3. Mass spectrometry

The 32 samples were processed following the identical protocol. Figure 2 shows an overview. A volume of 5 µL of residual serum sample was combined with 95 µL of 50% ethanol, subjected to 5 min of mixing, and subsequently chilled on ice for an additional 5 min. Subsequently, centrifugation was performed using a himac CT15RE (Hitachi Koki Co., Ltd., Ibaraki Prefecture, Japan.) at 21,500×*g* for 5 min. Finally, 9 µL of supernatant was mounted on a sample plate for PESI-MS analysis. Analysis was performed using a single quadrupole mass spectrometer (DPiMS-2020, Shimadzu Corporation, Tokyo, Japan) with positive ion mode, a detector voltage of 1.30 kV, a dissolvent line temperature of 250˚C, and a heat block temperature of 50˚C. Data acquisitions were required for 2 min per sample.

2.4. Statistical analysis and discriminant analysis

The value of ion intensity from the mass spectra was summed with the corresponding *m/z* to an integer bin, and the data were normalized by the median in each sample. The data were analyzed using MetaboAnalyst 5.0 Xia Lab @ McGill (http://www.metaboanalyst.ca). Partial least squares (PLS) regression was used to visually understand the difference in the mass spectral profiles of the control and the ACS groups. PLS regression is one of the statistical methods to project high-dimensional data into a series of linear subspaces of the explanatory variables. The most discriminating one in the new variables is defined as Component 1, the second most discriminating one is defined as Component 2, and the third most discriminating one is defined as Component 3.

The discriminant study was performed using a support vector machine (SVM). The variables used for the analysis by SVM were determined as follows: the explanatory variables (represented as *m/z*) that differ between the control and the ACS groups for the objective variables were found by Student's *t*-test and sorted in *p*-value order. The SVM model was optimized by sequentially adding the corresponding explanatory variables in order from the lowest *p*-value. The optimized SVM model answered the possibility score of each serum sample as a continuous value between 0 and 1, corresponding to the control and the ACS patients, respectively. To calculate the diagnostic accuracy, the threshold value used for the judgment was set to 0.5; that is, if the value was closer to 1 than 0.5, it was diagnosed with the ACS, and if it was closer to 0 than 0.5, it was diagnosed with the control. This possibility score was evaluated by a random sub-sampling method, a kind of cross-validation. Consequently, the receiver operating characteristic (ROC) curve and the area under the curve (AUC) were employed to evaluate the diagnostic accuracy of the diagnostic algorithm.[13] To visualize the data extracted from MetaboAnalyst, we employed Chart Studio on the Plotly website (https://chart-studio.plotly.com) to generate 2-dimensional (2D) and 3-dimensional (3D) Scatter plots. Categorical and continuous variables were used for descriptive statistics. Hypothesis testing was conducted, respectively, using Fisher's exact test and *t*-test.

3. RESULTS

There were no statistically significant differences in the mean age between the two groups under study (ACS group: mean = 65, SD = 14.70; control group: mean = 65.31, SD = 14.71; 0.48) (Table 1). Most other parameters also exhibited no significant differences except for diastolic blood pressure (dBP) and white blood cell count (WBC). The mean dBP of the ACS group was 89.64 ± 18.68 and the control group was 73.43 ± 7.21. The mean WBC of the ACS group was 9677.14 ± 3251.73 and the control group was 6957.14 ± 1443.78.

Supplementary Table 1 presents an overview of the patient's characteristics upon admission to the ED. The majority of the ACS group consisted of patients with the STEMI clinical subtype (10/16 patients), followed by coronary plaque angina (2/16 patients), NSTEMI (2/16 patients), and unstable angina pectoris (2/16 patients) (Supplementary Figure 1).

We analyzed 32 samples using PESI-MS and obtained their mass spectra. We compared the averaged mass spectra of the control and ACS groups, and any obvious differences were not found because of the many features (Fig. 3). We then conducted the dimensional reduction by PLS regression to visually represent the two cohorts of patient samples based on their PESI-MS-based database (Fig. 4). The 3D plot using Component 1 (19.7%), Component 2 (6.2%), and Component 3 (7.8%) axes revealed a difference between the two clusters of the control and ACS groups, indicating that serum composition obtained by PESI-MS can find the different characteristics of the control and ACS patients.

We evaluated the accuracy of mass spectrum output in ACS diagnosis first by ROC curve analysis. As a result, the ROC curve and the AUC could utilize analyses in the evaluation of PESI-MS in terms of diagnostic accuracy, which ruled out the prevalence of the disease. Likewise, ROC analysis with its conceivable curve could show the cutoff value with robustness.[13]

Table 1. Demographics of two cohorts.

Criteria	Characteristics	Control group (n = 16)	ACS group (n = 16)	p-value (95% CI)
Demography				
	Age	65.31 ± 14.71	65.00 ± 14.70	0.48
	Sex (♀)	6 (37.50%)	2 (12.50%)	0.09
Medical history				
	Diabetes	6 (42.86%)	4 (25%)	0.23
	Chronic kidney disease	0	1 (6.25%)	0.50
	Hypertension	4 (25%)	9 (56.25%)	0.06
Symptoms				
	Headache	2 (12.5%)	1 (6.25%)	0.39
	Dizziness	2 (12.5%)	1 (6.25%)	0.39
	Nausea	2 (12.5%)	1 (6.25%)	0.39
	Vomit	0	3 (18.75%)	0.11
	Confused/disoriented*	0	2 (12.5%)	0.24
	Transient unconscious	0	1 (6.25%)	0.50
	Numbness	1 (6.25%)	0	0.50
	Chest pain	10 (62.5%)	12 (75%)	0.23
	Chest discomfort	9 (56.25%)	8 (50%)	0.26
	Body temperature (°C)	36.42 ± 0.68 (n = 15)	36.41 ± 0.51 (n = 15)	0.49
	Systolic BP (mmHg)	141.71 ± 21.15 (n = 7)	145.21 ± 33.15 (n = 14)	0.40
	Diastolic BP (mmHg)	73.43 ± 7.21 (n = 7)	89.64 ± 18.68 (n = 14)	0.02
Laboratory tests				
	WBC (per mL)	6957.14 ± 1443.78 (n = 7)	9677.14 ± 3251.73 (n = 14)	0.03
	Total protein (g/dL)	7.21 ± 0.52 (n = 7)	7.02 ± 0.58 (n = 14)	0.23
	Albumin (g/dL)	4.14 ± 0.21 (n = 7)	4.06 ± 0.38 (n = 14)	0.30

*Glasgow Coma Score (GCS): 9–14.
ACS, acute coronary syndrome; BP, blood pressure; WBC, white blood cell count.

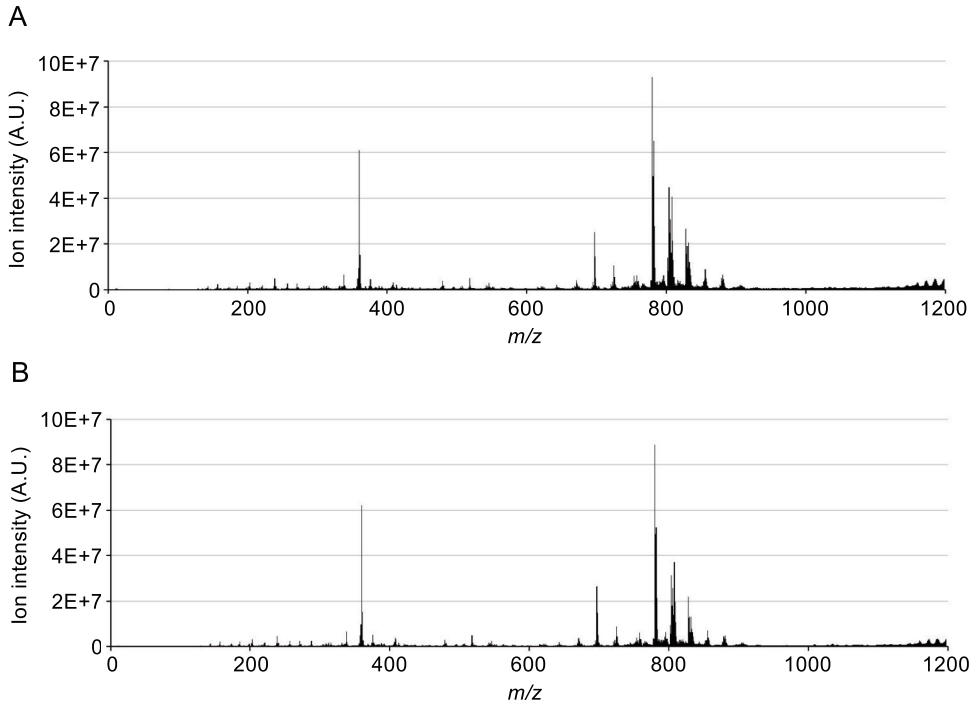

Fig. 3. Normalized mean mass spectra using PESI-MS. Normalized mean mass spectra of serum composition derived from 16 patients in the control group (A) and 16 ACS group (B) are shown. ACS, acute coronary syndrome; PESI-MS, probe electrospray ionization-mass spectrometry.

The diagnostic efficacy between ACS and control derived by our diagnostic algorithm was remarkable, with an area under the ROC of 0.965 (95% CI: 0.84–1), a sensitivity of 93.8%, a specificity of 93.8%, and an accuracy of 93.8%.

4. DISCUSSION

When scrutinizing a patient exhibiting thoracic discomfort, thoracic pain, or other related symptoms, it holds paramount significance to discriminate against ACS.[1)] Given that these symptoms lack specificity for ACS, the identification of patients with ACS from those presenting with nonspecific manifestations necessitates the performance of ECG, hematological assessments, and echocardiography in those individuals. In addition, there are subtypes of ACS that cannot be diagnosed by a single symptom or laboratory finding, such as atypical chest pain or NSTEMI, making their diagnosis and treatment more complex. Clinically, this diagnostic

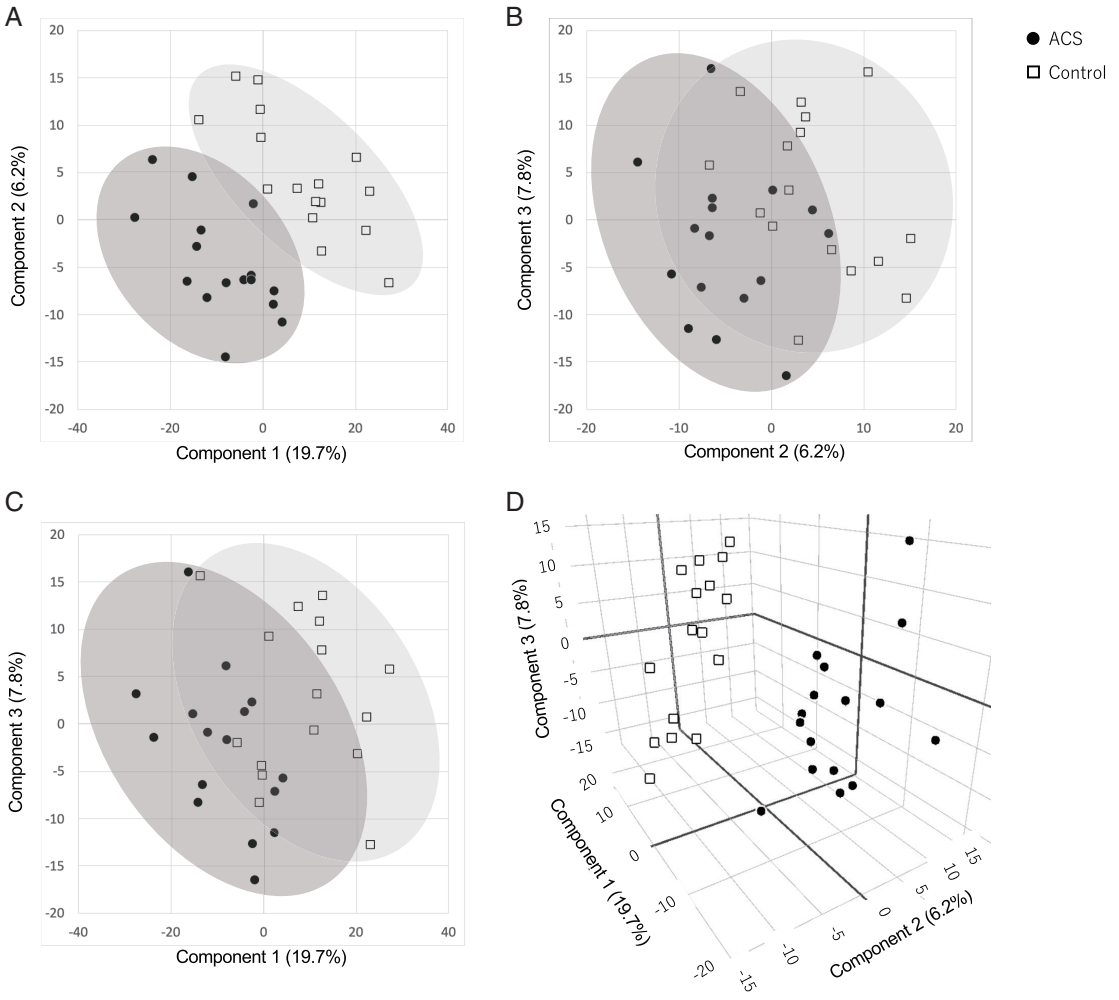

Fig. 4. PLS regression of mass spectra by PESI-MS. PLS regression was performed using normalized mass spectra. PLS score plots for comparison of 16 control groups (white square) and 16 diagnosed ACS groups (black dot) show good separation. PLS score plot of Components 1 *vs.* 2 (A), 2 and 3 (B), 1 and 3 (C), and all components (D) are shown. Gray circles represent 95% CI. 19.7%, 6.2%, and 7.8% are the score of Component 1, Component 2, and Component 3, respectively. ACS, acute coronary syndrome; CI, confidence interval; PESI-MS, probe electrospray ionization-mass spectrometry; PLS, partial least squares.

process mandates the involvement of a cardiologist specialized in the domain of cardiology. Nonetheless, the initial point of care for ACS, which may manifest at any time of the day, may not always involve a cardiologist. Furthermore, while the survival rate of ACS hinges on the expeditiousness of diagnosis and the prompt attention provided by a specialist, it is inappropriate to refer all patients with suspected ACS symptoms to a specialist. Thus, it is imperative to judiciously select patients with a high likelihood of ACS from the pool of individuals presenting with suspected ACS symptoms. The current investigation underscores the benefits of integrating MS data into the traditional diagnostic framework for ACS, encompassing ECG, hematological analyses, and echocardiography.

Herein, recent studies revealed the beneficial application of MS into the traditional diagnostic framework for ACS.[6,7] Zhong et al. showed the value of LC-MS in ACS diagnosis with respect to the detection of a combination of phosphatity-lethanolamin Lyso (16:0) and Lys phosphatidylcholine (20:4) in a diagnostic model that had the highest AUC of 0.905.[6] This was consistent with the study of Lee et al. with an increase of two-fold phosphatidylethanolamines (38:5 and 40:5) in plasma samples of ACS patients compared to those of their stable coronary artery disease counterparts.[7] Nevertheless, LC-MS entails time-consuming procedures, rendering it potentially unsuitable for expeditious diagnosis of ACS in the ED settings, where promptness is imperative. In other words, PESI-MS offers advantages over LC-MS in terms of ease and speed of implementation.[8] Consequently, in this study, we employed PESI-MS, a simpler technique that yields results in a more concise timeframe. Indeed, over the past decade, the utilization of PESI-MS for compositional analysis, in conjunction with ML for data analysis, has demonstrated its diagnostic accuracy in the field of oncology.[9,10] For instance, MS has proven its ability to differentiate breast cancer from normal breast tissues,[9] as well as to delineate the serum samples of patients diagnosed with pancreatic ductal adenocarcinoma[10] or the borders of hepatocellular carcinoma.[11]

In the current study, we have developed a novel diagnosis method for ACS disease, which incorporates both PESI-MS and ML techniques. Importantly, this method has demonstrated its practical validity in a clinical setting. As a result, the utilization of this method is likely to offer advantages over

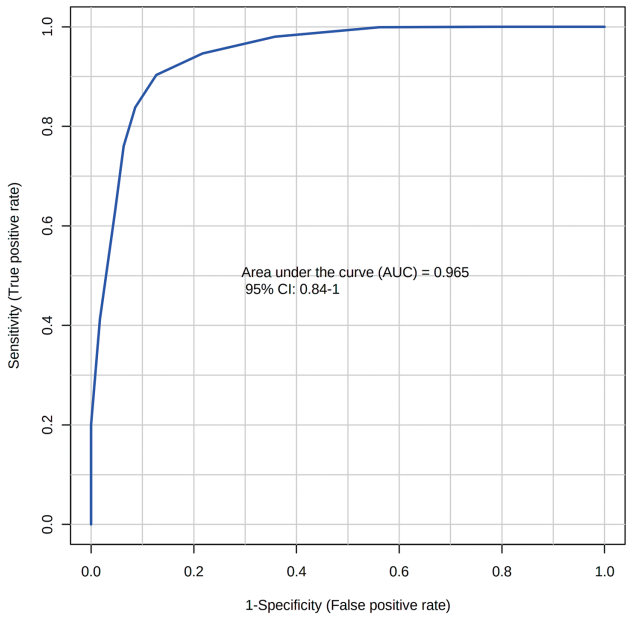

Fig. 5. ROC curve of probability obtained from machine learning-based diagnostic algorithm. The AUC is 0.965. AUC, area under the curve; ROC, receiver operating characteristic.

the traditional approach in complex scenarios, particularly given that this method needs a small portion of serum and simple handling and provides accurate diagnosis. Put differently, irrespective of the operator, the test yields highly accurate and reliable results, rendering it particularly suitable for implementation in emergency settings. It would be helpful to aid physicians in ACS-challenging cases like unclassified chest pain and NSTEMI diagnoses that require follow-up. The importance of the current study is that this exploratory study has pioneered the utilization of PESI-MS in ACS diagnosis; the combination of PESI-MS and ML demonstrated remarkable performance of the diagnosis (Fig. 5) for future other applications. For example, our method can be applied to predicting high- and low-risk CHD patients, which was analyzed using the perspective of ML by Than et al.[12] This study reveals that machine-learned algorithms, built upon PESI-MS analysis outcomes, rapidly distinguish ACS in patients with vague chest symptoms, even from a limited blood sample. These findings suggest potential in facilitating referrals of patients genuinely requiring medical attention to ACS-capable physicians, even in the absence of specialized expertise in the general ED. These algorithms, reliant on PESI-MS analysis, not only reduce healthcare costs but also ensure suitable patient care. The approach's advantages over traditional methods in intricate scenarios arise from its minimal serum requirement, ease of use, and precision. Operator-independent, it is ideal for emergencies, aiding in challenging ACS cases like unclassified chest pain. Ultimately, understanding the PESI-MS and ML-based system promises not only insights into mechanisms but also ACS prognosis assessment.[5]

Noteworthy, diagnostic results can be obtained very quickly because pretreatment simply suspends the serum in 50% ethanol (Fig. 2). The PESI-based MS streamlines real-time analysis and reduces the time from serum to spectrum acquisition.[8] Although 2 minutes was set as the analysis time in this experimental design, it was confirmed that mass spectra could be obtained even with 10–30 seconds of analysis (not shown), making it possible to construct a more rapid diagnostic system in the future. The diverse range of mass spectral datasets was applied to ML to construct discriminant models and test diagnosis in ACS patients (Fig. 2). Furthermore, through cluster visualization, it was observed that the spectrum database exhibits the difference between ACS-diagnosed samples and control samples (Fig. 4). The current study demonstrates the remarkable accuracy of the PESI-MS and ML-based diagnosis system in distinguishing ACS and control samples, as evidenced by an AUC of 0.965 (95% CI: 0.84–1) (Fig. 5). This finding represents the first evidence of the diagnostic ability of our system in ACS. In short, the results of the current study reveal the usefulness of PESI-MS and ML-based diagnosis systems in delineating the ACS patients among people who had chief complaints of chest pain and/or discomfort. This merit, coming along with the simple and less time-consuming characteristic of PESI-MS compared to its antecedent LC-MS, can support clinical decisions for timely and appropriate interventions. Likewise, the PESI-MS-based approach can reduce medical costs and shorten wait times for serial cardiac biomarker tests, which help to improve medical care to patients.

This study has several limitations. First, the small sample size represents an apparent weakness, potentially introducing selection bias in the sample selection process. Consequently, the diagnostic accuracy of ACS subtypes, including STEMI, NSTEMI, and unstable angina, is not sufficient for actual use. Thus, future investigations encompassing larger sample sizes can explore additional pathophysiological insights within various clinical contexts of ACS by examining diverse molecular spectra obtained through the PESI-MS. Second, the accuracy of the PESI-MS and ML-based diagnosis system in the current study was assessed using labeled samples in a pilot study with a retrospective design. Thus, future prospective studies are necessary for sufficient validation in real-time clinicals. It is also essential to conduct further investigations to determine whether the findings of this study can be generalized to other populations of ACS patients.

5. CONCLUSION

The findings of this pilot study imply that the integration of serum composition analysis employing PESI-MS and ML holds substantial significance in facilitating the prompt detection of ACS. The PESI-MS-based diagnostic method emerges as an optimal approach to assist medical practitioners in the diagnosis of ACS, given its notably high predictive accuracy: AUC = 0.965, sensitivity = 93.8%, specificity = 93.8%, and accuracy = 93.8%.

ETHICS STATEMENT

Approval of the research protocol: the study was approved by the Institutional Ethics Committee of our institute with details as below:

Chairman: Zentaro Yamagata

Approval number: 2086

Approved date: July 8, 2019

Study title: Establishment of a system to determine the severity and urgency of emergency patients.

Informed Consent: N/A. Based on the nature of the current study, the protocol did not entail any medical interventions or invasions aside from utilizing residual specimens from routine blood collection tests and extracting and analyzing information from medical records. However, to comply with the provisions outlined in the Ethical Guidelines for Life Science and Medical Research Involving Human Subjects, pertaining to the disclosure of "matters that should be disclosed regarding the conduct of such research in the event that informed consent is not obtained," and to offer subjects or their proxies the opportunity to decline participation in the research, materials outlining the option to opt-out were made available. Furthermore, data from subjects who have opted out will be expunged from the analysis and promptly discarded.

Registry and the Registration No. of the study/Trial: UMIN000053009.

Animal Studies: N/A.

AUTHOR CONTRIBUTIONS

Q.N.N.T. and M.U. analyzed and interpreted the patient data regarding acute coronary syndrome (ACS). K.Y., Q.N.N.T., and M.U. performed the MS analyses of samples. T.I., Q.N.N.T., and M.U. analyzed statistics using ML. Q.N.N.T was a major contributor to writing the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest for this article.

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