

Comparative evaluation of 15-minute rapid diagnosis of ischemic heart disease by high-sensitivity quantification of cardiac biomarkers

SHENGNAN HE^{*}, LONG QU^{*}, XI HE, DAN ZHANG and NI XIE

Institute of Translational Medicine, Health Science Center, Shenzhen Second People's Hospital, The First Affiliated Hospital of Shenzhen University, Shenzhen, Guangdong 518035, P.R. China

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Abstract. A rapid clinical diagnosis may efficiently improve the survival rate and prognosis of patients with ischemic heart disease (IHD). Therefore, a one-step, rapid and inexpensive analysis for the quick diagnosis of IHD was investigated in the present study. Consecutive patients who were subjected to myoglobin, cardiac troponin I and creatine kinase-MB isoenzyme assessment at the Emergency Department of Shenzhen Second People's Hospital (Shenzhen, China) between December 2017 and March 2018 prior to treatment were screened. Clinically applicable disposable strips were employed for quantification of the cardiac biomarkers. The analytical performance of the strips was evaluated by receiver operating characteristic (ROC) curves and compared with the traditional chemiluminescence immunoassay (CLIA) method. The data of 391 participants were collected. At the baseline, 57 patients were diagnosed with IHD and 334 patients were diagnosed with other diseases. The area under the ROC curve (AUC) of the CLIA model was 0.787 (95% CI, 0.709-0.865) with a specificity of 76.7% and a sensitivity of 71.9%. At the optimal cutoff value of -1.867, the negative and the positive predictive value were 94.1 and 34.5%, respectively. The AUC of the disposable

strip model was 0.792 (95% CI, 0.729-0.855). At the cutoff value of -1.820 or below, the negative predictive value was 94.9%, the positive predictive value was 28.9%, the specificity was 66.8% and the sensitivity was 79.0%. The P-value of the ROCs was 0.858, indicating no statistically significant difference between the two assay methods. The cost of the disposable strip was 50% of that of the CLIA method and it took only 25% of the time that was required for the quantification of the three cardiac markers by CLIA. In conclusion, the disposable strip provides a platform for point-of-care testing and may be an easy, rapid, reliable and cost-saving method for the diagnosis of IHD.

Introduction

Ischemic heart disease (IHD) is an important global public health concern, which is the most common cause of mortality and disability worldwide (1-3). An estimated 110.55 million prevalent cases of IHD were present globally in 2015 (4). The World Health Organization estimates further increases in IHD-associated mortality by 2030 in all country income strata (5). Therefore, IHD remains a huge burden on health-care systems.

Early diagnosis may efficiently improve the survival rate and prognosis of patients with IHD, which relies on medical history, electrocardiogram and cardiac biomarkers (6). As clinical history and physical examination of patients are limited and 12-lead electrocardiogram is only used for diagnosis in a small proportion of cases, the use of specific biomarkers as diagnostic indicators has added value for rapidly diagnosis of IHD in populations (7-11). Myoglobin (Myo), cardiac troponin I (cTnI) and creatine kinase-MB isoenzyme (CK-MB) are common biomarkers frequently used for IHD diagnosis in hospital settings (8,12-14). The concentration of these cardiac biomarkers may be determined by radioimmunoassay, ELISA and chemiluminescence immunoassay (CLIA) with excellent specificity and sensitivity (15,16). At present, CLIA is commonly used in hospitals. However, the complex procedures take a large amount of time and rely on bulky instruments, which may delay the rescue of patients and are inconvenient to perform. Therefore, the rapid detection of these biomarkers with a portable device may be an efficient approach for better prognostic prediction.

Correspondence to: Professor Ni Xie, Institute of Translational Medicine, Health Science Center, Shenzhen Second People's Hospital, The First Affiliated Hospital of Shenzhen University, 3002 Sungang West Road, Shenzhen, Guangdong 518035, P.R. China
E-mail: xn100@szu.edu.cn

^{*}Contributed equally

Abbreviations: AUC, area under the ROC curve; CLIA, chemiluminescence immunoassay; CK-MB, creatine kinase-MB isoenzyme; cTnI, cardiac troponin I; IHD, ischemic heart disease; Myo, myoglobin; POCT, point-of-care testing; RIA, radioimmunoassay; ROC, receiver operating characteristic

Key words: ischemic heart disease, cardiac biomarkers, rapid diagnosis, disposable strips

Disposable strips based on a fluorescent immunoassay are generally used for the quantitative determination of specific markers (17). This method allows for a one-step, rapid and inexpensive analysis, which is easy to perform at community health centers and in under-developed regions. In the present study, the strips for the quantification of Myo, cTnI and CK-MB were employed for a substantial reduction in the time of IHD diagnosis.

Materials and methods

Materials. Myo, cTnI and CK-MB disposable strips and a UNICELL immunofluorescence reader were obtained from Shenzhen YHLO Biotech.

UNICELL-cTnI/CK-MB/Myo employs an immuno-chromatography sandwich assay that generates a fluorescence signal, which is proportional to the level of cTnI/CK-MB/Myo in the blood specimen. Corresponding mouse anti-cTnI/CK-MB/Myo antibody is coated on the test line (T line) of the testing strip and anti-avidin antibody is coated on the control line (C line) of the testing strip. Fluorescence particles labeled anti-cTnI/CK-MB/Myo antibody and avidin are coated on the binding pad (Shenzhen YHLO Biotech Co. Ltd.).

Whole blood samples were obtained from patients at Shenzhen Second People's Hospital (Shenzhen, China). Testing was performed by adding 100 μ l whole blood, serum or plasma into the sample well. If cTnI/CK-MB/Myo was present in the specimen, an immune complex is formed by binding cTnI/CK-MB/Myo with fluorescence particle-labelled anti-cTnI/CK-MB/Myo antibody. Subsequently, through the capillary effect, this immune complex moves forward to the T line and is captured by another anti-cTnI/CK-MB/Myo antibody on it. At the same time, the fluorescence particle-labeled avidin on the binding pad passes through the C line and binds with the anti-avidin antibody coated on the C line.

The UNICELL immunofluorescence reader was used to determine the signal intensity of fluorescence of the T line and the C line. The concentration of cTnI/CK-MB/Myo in the specimen was automatically calculated according to the master curve that was stored in the ID card and the result was displayed in the units of ng/ml.

Patient recruitment and data collection. For the present clinical validation study, consecutive patients who had been subjected to assessment of Myo, cTnI and CK-MB by CLIA at the Emergency Department of Shenzhen Second People's Hospital (Shenzhen, China) between December 2017 and March 2018 were subjected to screening with the test strips. Patients were excluded if they had received medical treatment prior to blood collection or were without complete data. A total of 391 randomized patients were enrolled in the study, ranging from 15 to 96 years. A total of 62.40% of the patients were male. Baseline data were collected by trained research staff according to a standard operating procedure. The data included gender, age, the concentrations of Myo, cTnI and CK-MB quantified by CLIA and disposable strips. The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Shenzhen Second People's Hospital

Table I. Baseline characteristics of the patients (n=391).

Clinical characteristic	Value
Age (years)	62.9 (49.5-77)
Male sex	244 (62.40)
Diagnosed diseases	
Ischemic heart disease	57 (14.58)
Acute myocardial infarction	23 (5.88)
Acute coronary syndrome	21 (5.37)
Acute heart failure	4 (1.02)
Acute myopericarditis	1 (0.26)
Cardiogenic shock	1 (0.26)
Cardiopulmonary arrest	1 (0.26)
Other diseases	334 (85.42)

Values are expressed as n (%) or median (25th-75th percentile).

(Shenzhen, China; approval no. 20170811015). During data collection, all patients were informed to fully understand the study and signed the consent documents referenced from the Chinese Clinical Trial registry (<http://www.chictr.org.cn/>, no. ChiCTR2000032549). All of the experiments were performed in accordance with relevant guidelines and regulations.

Quantification methods. The concentrations of Myo/cTnI/CK-MB in the blood samples were determined with the disposable strips and quantitatively evaluated with the UNICELL immunofluorescence reader. The procedure was as follows: All of the blood samples were stored at 4°C for 24 h. The samples were left to reach room temperature for 10 min prior to further testing. Each sample (100 μ l) was dispensed into the sample well and the strips were then inserted into the reader. The fluorescent signal intensities of the T line and the C line were recorded 15 min later, which were used to calculate the concentrations of Myo/cTnI/CK-MB in the samples according to the master curve that is stored in the ID Card. The values (ng/ml) were displayed automatically on the screen of the device. The Myo/cTnI/CK-MB quantification results by the CLIA method were obtained from the department of laboratory medicine of Shenzhen Second People's Hospital (Shenzhen, China).

Statistical analysis. In the setting of the present study, a total of 57 patients diagnosed with IHD would provide a statistical power of 99% to validate the use of test strips for cardiac biomarkers. Baseline characteristics, including gender, age and the concentrations of Myo, cTnI and CK-MB quantified by CLIA and disposable strips, were analysed to construct the receiver operating characteristic (ROC) curves, which were used to determine the optimal cut-off concentrations and compare the sensitivity and specificity of different cardiac biomarkers and their predictive value to diagnose IHD. The ROC curves with or without bootstrap resampling (time=500) and a nomogram were established with EmpowerStats software (www.empowerstats.com, X&Y solutions, Inc.; updated on 28/02/2020).

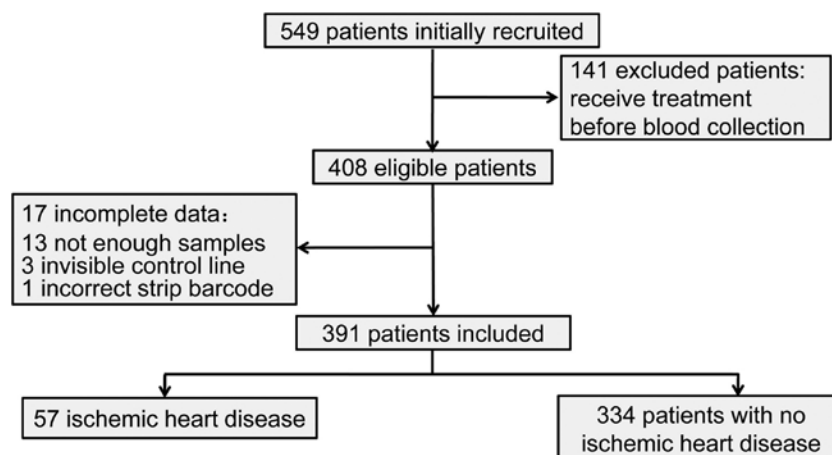


Figure 1. Flow chart of participant recruitment. There were 549 patients initially recruited, and 141 patients were excluded because they received treatment before blood collection. There were 17 participants excluded due to an insufficient blood volume for completing the test, invisible control line or incorrect strip barcode that cannot be recognized by the immunofluorescence reader. The final study population comprised 391 patients, and 57 patients were diagnosed with IHD at the baseline. IHD, ischemic heart disease.

Results

Patients. A total of 549 participants from the Emergency Department received Myo/cTnI/CK-MB tests during the study period and 141 of them were excluded due to having received treatment prior to blood collection. Another 17 participants were excluded due to an insufficient blood volume for completing the test, invisible control line or incorrect barcode. The final study population comprised 391 patients. The median age was 62.9 ± 17.21 years and 62.40% of the patients were male (Table I). At baseline, 57 patients were diagnosed with IHD, including 23 cases of acute myocardial infarction, 21 of acute coronary syndrome, 4 cases of acute heart failure, 1 of acute myopericarditis, 1 of cardiogenic shock and 1 case of cardiopulmonary arrest. The remaining 334 patients were diagnosed with other diseases (Fig. 1; Table I).

Comparison of the CLIA model and disposable strip model.

A total of 2 different multivariable logistic regression models were constructed. Model 1 was constructed with baseline predictors, including gender, age and concentration of Myo/cTnI/CK-MB assessed by CLIA method. Model 2 included gender, age and concentration of Myo/cTnI/CK-MB quantified by disposable strip. The regression coefficients from the multivariate logistic model were used to construct the model for predicting the presence of IHD. The scoring model 1 was as follows: $-1.22037 + 0.17693 \times \text{gender} - 0.01179 \times \text{age} + 0.46488 \times \text{cTnI} - 0.00207 \times \text{Myo} + 0.00102 \times \text{CK-MB}$. The scoring model 2 was as follows: $-0.95456 + 0.16130 \times \text{gender} - 0.00966 \times \text{age} + 0.19297 \times \text{cTnI} - 0.01119 \times \text{Myo} + 0.02895 \times \text{CK-MB}$ (sex: '0' for male and '1' for female; 'years' for age; 'ng/ml' for the laboratory parameters). ROC analysis was used to identify the area under the ROC curve (AUC) of each model (Fig. 2A). The AUC of model 1 was 0.787 (95% CI, 0.709-0.865) with a specificity of 76.7% and a sensitivity of 71.9% at a cutoff value of -1.867. The AUC of model 2 was 0.792 (95% CI, 0.728-0.855). The specificity was 66.8% and the sensitivity was 79.0%. The optimal cutoff value was

determined to be -1.820. There was no significant difference between model 1 and model 2 in terms of IHD diagnosis ($P=0.858$). Parameters regarding the predictive value of the disposable strip test for IHD are presented in Table II. The negative predictive value at the cutoff value of -1.820 or below for the scoring model 2 was 94.9% and the positive predictive value was 28.9%. The results indicated that with a cutoff value of -7.008, the sensitivity and negative predictive value for the diagnosis of IHD were 98.3 and 98.8%, respectively.

ROC curves for the diagnosis of IHD in each model using Bootstrap resampling (times=500) indicated that the AUC of model 1 was 0.78 (95% CI, 0.6899-0.8519; specificity, 76.65%; sensitivity, 71.93%). The AUC of model 2 was slightly higher than that of model 1 (AUC=0.79; 95% CI, 0.6899-0.8519; specificity, 66.77%; sensitivity, 78.95%). The P-value was 0.7465 ($P>0.05$), indicating that the disposable strip model shared comparable accuracy in IHD diagnosis with the CLIA model (Fig. 2B-D). ROC curves for the individual biomarkers quantified by disposable strip are provided in Fig. 3. Compared with the combined use of the biomarkers (AUC=0.792), the AUC for single use of Myo, cTnI and CK-MB was 0.685 ($P<0.001$), 0.706 ($P=0.0092$) and 0.662 ($P<0.001$), respectively. The result suggested that the predictive performance of Myo, cTnI and CK-MB, used separately, was not superior to the predictive performance of the combination use. To predict the presence of IHD, a prognostic nomogram was established through Cox regression model analysis according to all significant independent indicators, including gender, age and the concentration of Myo, cTnI and CK-MB quantified by the disposable strip. Each factor in the nomogram was assigned a weighted number of points and the sum of points for each patient reflected the probability of the presence of IHD (Fig. 4).

The disposable strip is a cost-saving and rapid method for IHD diagnosis. The cost of the two methods was compared. The patients paid 47.18 US\$ for the quantification of Myo/cTnI/CK-MB by CLIA, while the disposable

Table II. Proportions of patients with positive samples, sensitivities, specificities, positive and negative predictive values and likelihood ratios for the disposable strip to identify patients with ischemic heart disease.

Cut-off value	Specificity (%)	Sensitivity (%)	PPV (%)	NPV (%)	+ likelihood ratio	- likelihood ratio
-7.008	23.7	98.3	18.0	98.8	1.293	0.072
-2.119	48.2	89.5	22.8	96.4	1.733	0.217
-1.820	66.8	79.0	28.9	94.9	2.382	0.324
-1.641	74.3	68.4	31.2	93.2	2.658	0.432
-1.601	78.4	59.7	32.1	92.9	2.766	0.514

The scoring model was as follows: $-0.95456 + 0.16130 \times \text{gender} - 0.00966 \times \text{age} + 0.19297 \times \text{cTnI} - 0.01119 \times \text{Myo} + 0.02895 \times \text{CK-MB}$ (sex: '0' for male and '1' for female; 'years' for age; 'ng/ml' for the laboratory parameters). PPV, positive predictive value; NPV, negative predictive value; CK-MB, creatine kinase-MB isoenzyme; cTnI, cardiac troponin I; Myo, myoglobin.

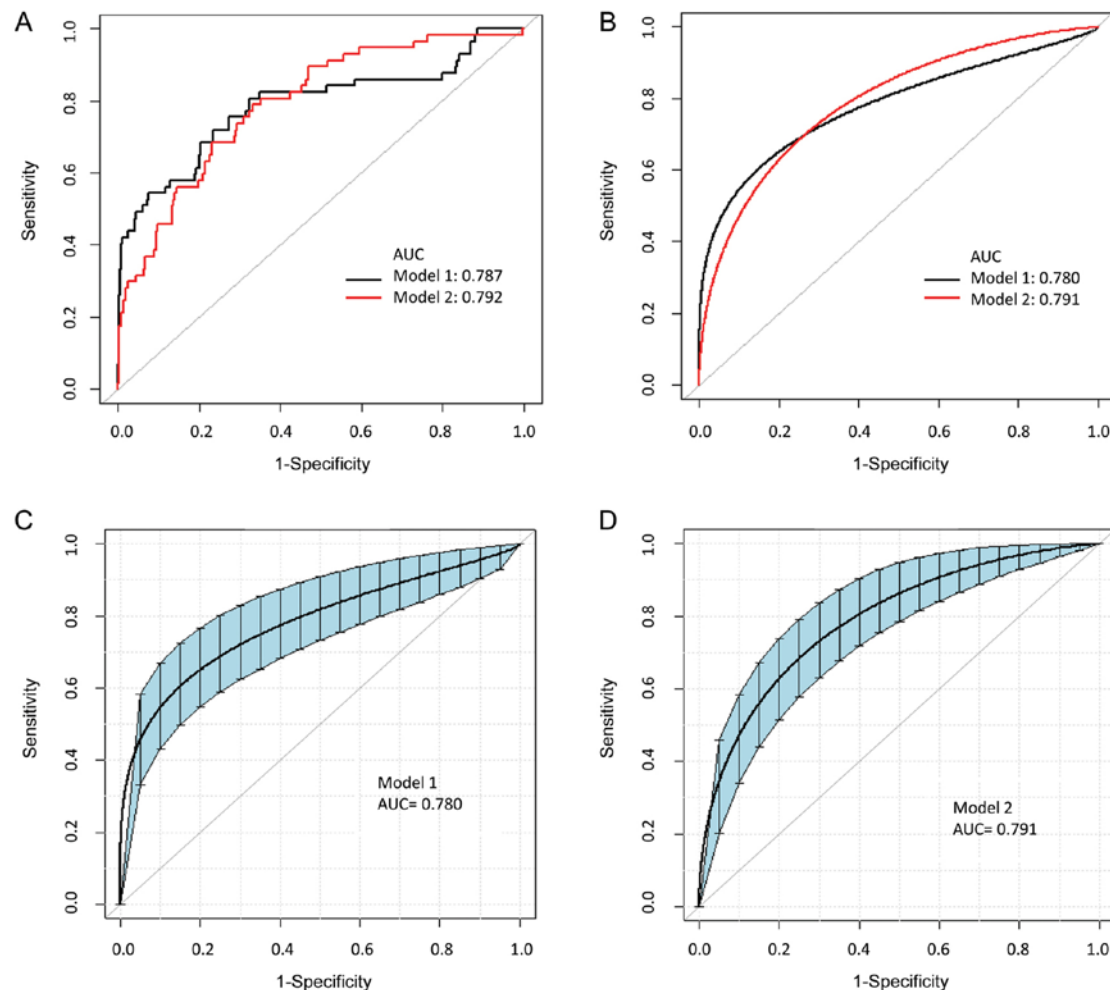


Figure 2. ROC curve for diagnosis of IHD. (A) Model 1 (black) is for the CLIA model including gender, age, concentration of Myo/cTnI/CK-MB assessed by the CLIA method. Model 2 (red) is for the disposable strip model including gender, age and concentration of Myo/cTnI/CK-MB quantified by disposable strips. The AUCs for model 1 and model 2 for predicting IHD were 0.787 and 0.792. There was no significant difference between the two models. The optimal cutoff values of model 1 and model 2 were -1.867 and -1.820. (B) ROC curves for the diagnosis of IHD in each model using Bootstrap resampling (times=500). The AUCs for model 1 (black) and model 2 (red) for predicting IHD were 0.780 and 0.791. (C) ROC curves for the diagnosis of IHD in model 1 using Bootstrap resampling (times=500). The optimal cutoff value was -1.867. The blue area represented 95% CI (0.710-0.854) of the AUC. (D) ROC curves for the diagnosis of IHD in model 2 using Bootstrap resampling (times=500). The optimal cutoff value was -1.820. The blue area represented 95% CI (0.729-0.845) of the AUC. ROC, receiver operating characteristic; AUC, area under the ROC curve; CLIA, chemiluminescence immunoassay; CK-MB, creatine kinase-MB isoenzyme; cTnI, cardiac troponin I; IHD, ischemic heart disease; Myo, myoglobin.

strip method only cost 21.91 US\$, saving >50% compared to the CLIA method. The time spent on each method was also

compared in the present study. The doctors of the emergency department usually waited for >1 h to get the results of the

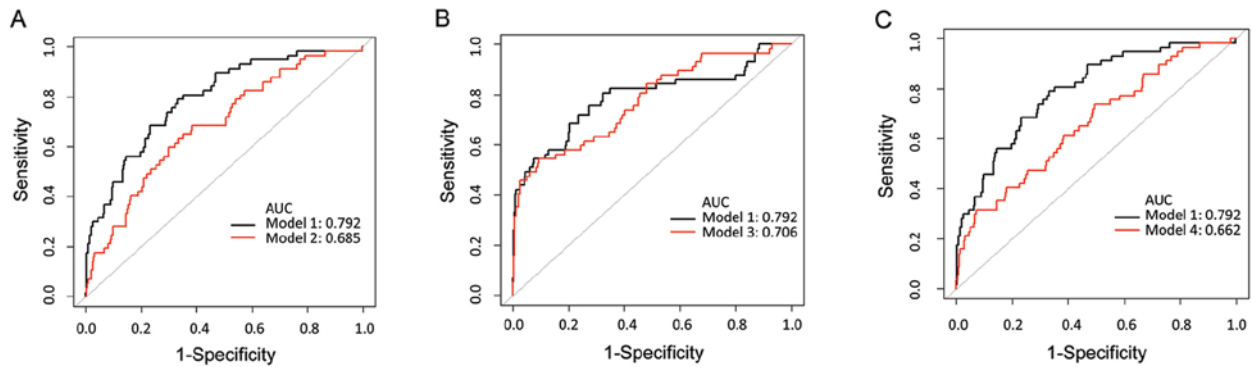


Figure 3. Comparison of the biomarkers applied individually and collectively. Model 1 (black) is the combination model including gender, age and concentration of Myo/cTnI/CK-MB quantified by disposable strip. Model 2, model 3 and model 4 (red) are single models including gender, age and concentration of (A) Myo, (B) cTnI or (C) CK-MB quantified by a disposable strip, separately. ROC, receiver operating characteristic; AUC, area under the ROC curve; CK-MB, creatine kinase-MB isoenzyme; cTnI, cardiac troponin I; Myo, myoglobin.

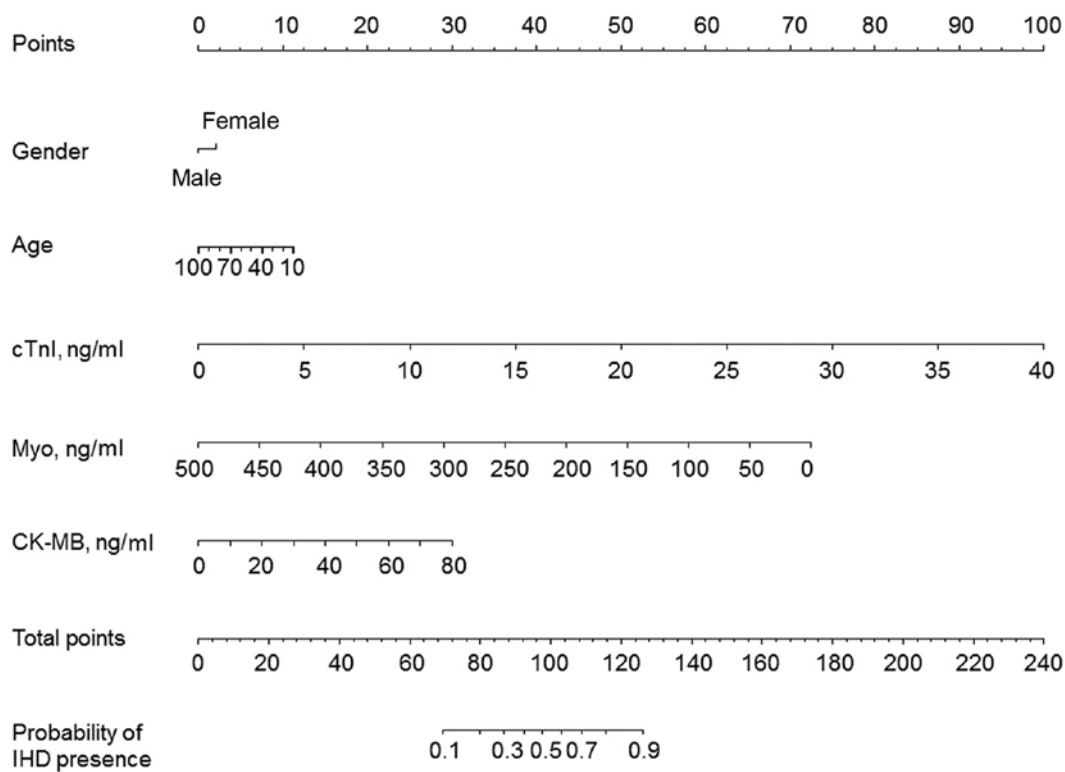


Figure 4. Nomogram for estimating the risk of IHD presence in the cohort by disposable strip method. To use the nomogram, draw an upward vertical line from each variable axis to the 'Points' bar to determine the points of each variable. The points from all of the variables were added and a downward vertical line from the 'Total points' axis was drawn to determine the IHD probabilities at the lower line of the nomogram. The cardiac parameters were quantified by the disposable strip. CK-MB, creatine kinase-MB isoenzyme; cTnI, cardiac troponin I; IHD, ischemic heart disease; Myo, myoglobin.

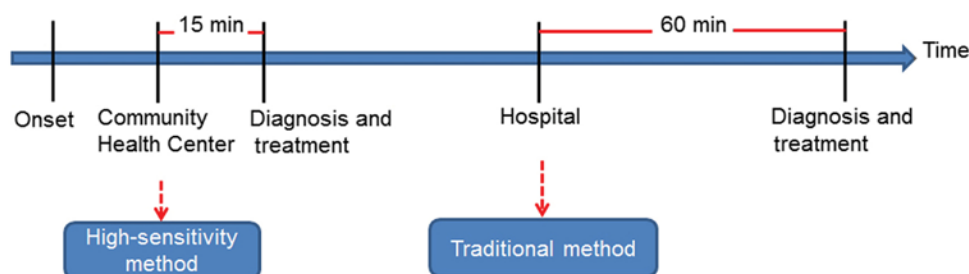


Figure 5. Scheme of the CLIA and disposable strip methods for the diagnosis of ischemic heart disease. The high-sensitivity method is quantification of Myo/cTnI/CK-MB by disposable strips; the traditional method is quantification of Myo/cTnI/CK-MB by CLIA. CLIA, chemiluminescence immunoassay; CK-MB, creatine kinase-MB isoenzyme; cTnI, cardiac troponin I; Myo, myoglobin.

Myo/cTnI/CK-MB tests by CLIA, while it only took 15 min for the disposable strip method, which saved 75% of the time to diagnose and treat the patients with IHD earlier (Fig. 5).

Discussion

Disposable strips based on a fluorescent immunoassay have received considerable attention due to their portability, rapid performance, cost effectiveness and ease of use (16,17). This qualitative assay is easy to perform and evaluate based on the fluorescence intensity of the band on the test zone, and the quantitative data may be obtained by recording the optical responses with a hand-held 'strip reader'. It has been derived to recognize a diverse range of targets, including metal ions (18), protein (19), cells (20) and toxicants (21), with high specificity and sensitivity. In the present study, a method to rapidly diagnose IHD was established using patient gender, age and the concentration of Myo/cTnI/CK-MB assessed by disposable strips. It exhibited a similar diagnostic capability for IHD compared with the traditional CLIA method frequently used in hospitals. Furthermore, the method of the present study had the following advantages: First, since cardiac myocytes may withstand ischemia for only 30 min prior to the occurrence of permanent injury, reliable and rapid tests for myocardial damage urgently require inclusion for rapid treatment. According to clinical guidelines, the test information of cardiac biomarkers is required within 1 h to diagnose patients and manage their treatment (22). Furthermore, the National Academy of Clinical Biochemistry Laboratory Medicine Practice guidelines have also suggested that laboratory testing for cardiac markers should be performed with a turnaround time of 1 h, optimally 30 min or less (23,24). In institutions unable to meet the 1-h turnaround time requirement, quantitative point-of-care testing (POCT) should be implemented (23,24). As the conventional approach for IHD diagnosis takes >1 h (25), the 15-min rapid method provided by the present study is time-saving, which greatly facilitates faster decision-making and interventional therapy. This assay is likely to improve the survival rate and prognosis of patients with IHD. Furthermore, the CLIA method requires bulky instruments, which is in conflict with the bedside-testing purpose of portable devices, while the system of the present study perfectly meets the requirement for POCT and demonstrated a reliable analytical performance. To this end, the disposable strip-based method of the present study may be widely applied in under-developed regions and community health service centers and at marathon races. In addition, disposable strip has the advantage of easy manufacture and low cost, making their use an inexpensive analysis compared with the traditional method. Finally, the performance of complex instruments applied in traditional CLIA methods depends on skilled and properly trained technicians, while the ease of performing the assessment with the present disposable strip-based method makes it a suitable system for POCT that does not require prior training.

The present analysis only took factors associated with technical success into account, without considering any risk factors, including hypertension, smoking and family history of coronary artery disease (26). It is necessary to validate the results with the inclusion of these risk factors in further

investigations. Although the present study only included limited factors, it had similar accuracy in the diagnosis of IHD to that of the traditional CLIA method.

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Availability of data and materials

The data used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

SH and NX conceived and designed the study. LQ made the draft and SH revised the manuscript. SH, LQ and DZ performed the experiments. SH and XH analysed the data. All authors read and approved the manuscript.

Ethics approval and consent to participate

The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Shenzhen Second People's Hospital (Shenzhen, China). During data collection, all patients were informed to fully understand the study and signed the consent documents referenced from the Chinese Clinical Trial registry (<http://www.chictr.org.cn/>, no. ChiCTR2000032549). All of the experiments were performed in accordance with relevant guidelines and regulations.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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