Original Article

Consistency analysis of high-sensitivity cardiac troponin I in peripheral blood and venous blood by quantum dot immunofluorescence assay and clinical application in acute myocardial infarction

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Background: Troponin is an important marker for the diagnosis of acute myocardial infarction (AMI). The detection of troponin in peripheral blood is simpler and more convenient than that in venous blood, which has attracted more and more clinical attention. The purpose of this study is to establish a novel method for the rapid detection of high-sensitivity troponin I (hs-cTnI) in peripheral blood by quantum dot fluorescence immunoassay and evaluated the clinical accuracy of the method.

Methods: A total of 90 patients with chest pain admitted to Wuxi Second People's Hospital of Nanjing Medical University had peripheral blood and venous blood samples collected for detection of hs-cTnI by rapid quantum dot fluorescence immunoassay. The differences between the two methods were evaluated, as well as the analytical performance and clinical diagnostic efficacy of hs-cTnI detection by quantum dot fluorescence immunoassay. The final diagnosis was determined by two independent cardiologists.

Results: This study verified the precision, linear range and sensitivity of the novel detection method. There was good correlation between the results of hs-cTnI quantum dot fluorescence immunoassay for peripheral blood and the results for venous blood (regression equation Y=1.026x+0.521, R^2=0.9337); 94.4% (85/90) of the data were within the conformance limit. In addition, in the analysis of 52 patients with confirmed AMI, the clinical specificity of the quantum dot fluorescence immunoassay in peripheral blood was the same as that in venous blood samples (89.5%:89.5%). Finally, the area under the receiver operating characteristic (ROC) curve of the peripheral blood quantum dot fluorescence immunoassay was 0.9352, the 95% confidence interval (CI) was 0.8829 to 0.9876, the cut-off value was 1.598, and the sensitivity was 82.69%, which was not significantly different from the venous blood method (P value =0.089).

Conclusions: Rapid detection of hs-cTnI by quantum dot fluorescence immunoassay in peripheral blood is feasible. It has a high correlation and consistency with the venous blood method, as well as a high clinical diagnostic value for AMI and is more convenient and easier to detect.

Keywords: Peripheral blood; quantum dot; high-sensitivity troponin I (hs-cTnI); acute myocardial infarction (AMI)

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Introduction

Cardiovascular disease (CVD) is the main cause of premature death and disability worldwide, with an increasing incidence (1,2). Acute myocardial infarction (AMI) is the most common clinical form of coronary syndrome, and non-diagnostic AMI may lead to increasing human mortality worldwide (3,4). Therefore, rapid monitoring and follow-up of AMI symptoms in patients is important for clinical diagnosis, which will greatly reduce the risk of further life-threatening problems (5). In general, the occurring AMI affects the increase in cardiac biomarker levels in the blood. Cardiac troponin is an outstanding biomarker for AMI, which is of great value for the monitoring and diagnosis of AMI (6). Therefore, the development of an easy-to-use quantitative troponin method is highly desirable for rapid diagnosis of AMI disease and follow-up of treatment process

Current methods of detecting cTnI include electrochemiluminescence and enzyme-linked immunofluorescence, which require to collect venous blood samples from the patient's arm (7,8). The time it takes between a blood sample and the reporting of test findings is a significant impediment to making quick decisions. The time spent transferring blood to a central laboratory and then centrifuging it to create a plasma sample is a substantial component of the turnaround time in this procedure. Point-of-care cardiac troponin tests that use peripheral blood and have quick turnaround times may help to speed up decision making.

In recent years, peripheral blood testing has attracted more and more attention. For instance, Tomimuro *et al.* developed a μTADs device combined with Bret's sensor protein to rapidly detect antibodies in human peripheral blood samples (9). Sarangadharan *et al.* have developed a hand-held biosensor system to rapidly screen for brain natriuretic peptide (BNP) from a single drop of whole blood (10). There are few reports on the detection of peripheral blood troponin. This study used quantum dot immunofluorescence technology combined with bedside POCT instrument to detect troponin in peripheral blood.

Quantum dots are characterized by high fluorescence efficiency, good stability, high sensitivity and rapid quantification (11,12). Quantum dots is applied in many biomedical filed for its unique optoelectronic properties as a novel option (13,14). Quantum dots are used as superior luminescence tags for their broad excitation spectrum, narrow emission spectrum and large Stokes shift (>100 nm). Such optoelectronic properties are important in multiplexed applications as unique light source, since it could excite multiple quantum dots with different sizes at the same time (15). Additionally, quantum dots demonstrate higher molar adsorption coefficients and quantum yield compared with organic fluorophore. Therefore, fluorescent NPs are almost 20-fold brighter and thousand-fold more enduring compared with traditional organic dyes (16). Such exceptional optical properties illustrate that quantum dots are one of the important photoluminescent probes which could be applied in many analytical experiments.

In this research, we evaluated a novel technique for the rapid testing of high-sensitivity cTnI (hs-cTnI) in peripheral blood by quantum dot fluorescence immunoassay and explored the clinical application of peripheral blood hs-cTnI. This technology has important value in pre-hospital emergency care, early AMI screening and the construction of national chest pain centers. We present the following article in accordance with the STARD reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-436/rc).

Methods

Patients

A total of 90 patients with chest pain admitted to Wuxi Second People's Hospital of Nanjing Medical University between June 2019 and January 2021 were enrolled. Of them, 52 were diagnosed with AMI based on the 2017 ESC STEMI Guidelines (17). Laboratory evidence of AMI was defined as cTn value >99th percentile of the upper reference limit (URL) at least once. The clinical manifestations were consistent with those of myocardial ischemia. Patients with moderate to severe liver and kidney insufficiency, tumor or other chronic diseases were not included. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Wuxi Second People's Hospital of Nanjing Medical University (No. 2020-Y-19) and informed consent was taken from all the patients.

Materials and Instruments

The instrument is Nanjing Vazyme dry fluorescence immunoassay analyzer AFS-1000. The reagent is the matching troponin test card and the diluent used to detect peripheral blood. The venous blood is lithium heparin

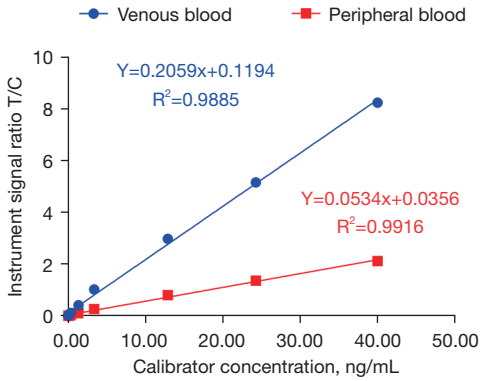

Figure 1 Standard curves of hs-cTnI in peripheral and venous blood samples. hs-cTnI, high-sensitivity troponin I.

anticoagulated whole blood, and the peripheral blood is the patient's fingertip blood.

Study methods

At present, venous blood troponin detection is routinely used in clinical practice. We compared the peripheral blood troponin results with the venous blood troponin results. A 30 µL peripheral blood sample was collected from each patient's ring finger and added to 90 µL of diluent. After the sample was mixed, take 80 µL from the dilution to detect hs-cTnI using Vazyme dry fluorescence immunoassay analyzer AFS-1000. A 3–5 mL venous blood sample was collected from the patient's vein and was placed into a lithium heparin anticoagulant tube. Taken 80 µL from lithium heparin anticoagulation tube to detect hs-cTnI using Vazyme dry fluorescence immunoassay analyzer AFS-1000. The peripheral and venous blood samples were collected at the same time and comprised the two study groups. The diagnostic criteria for AMI include a history of ischemic chest pain, myocardial ischemic necrosis ECG dynamic changes, and serum myocardial dynamic changes biochemical markers (mainly hs-cTn). The results of hs-cTn are based on venous blood quantum dot immunofluorescence detection.

Statistical analysis

GraphPad Prism8 and SPSS24 were used for statistical analysis. Measurement data were expressed as $\bar{x}\pm s$, *t*-test was used to compare the differences between the two groups, and linear correlation was used for correlation analysis. A quantum dot fluorescence immunoassay was used to establish the standard curves for clinical detection of hs-cTnI in peripheral and to verify the test's precision and linear range.

We constructed a receiver operating characteristic (ROC) curve to analyze the diagnostic efficacy of peripheral blood quantum dot immunofluorescence hs-cTnI in AMI, and then compared the area under the ROC curve (AUC) with the corresponding value of venous blood hs-cTnI Compare. Calculate the sensitivity, specificity, correct rate, positive predictive value and negative predictive value of peripheral blood hs-cTn for diagnosing AMI. The value of the area under the ROC curve is between 0.5 and 1.0. When AUC is greater than 0.5, the closer the AUC is to 1.0, the better the diagnostic effect. Bland-Altman analysis use SPSS24 to assess the agreement of the two methods. All hypothesis tests were two-tailed. P<0.05 was considered statistically significant

Results

Establishment of rapid detection of hs-cTnI in peripheral blood by quantum dot fluorescence immunoassay

Establishment of test standard curve

Using 9 concentration gradients of hs-cTnI as the X-axis and the signal ratio T/C of the dry fluorescence immunoassay analyzer as the Y-axis (n=3), the standard curves of hs-cTnI detection in venous blood and peripheral blood by quantum dot fluorescence immunoassay were established (*Figure 1*).

Precision verification

The levels of hs-cTnI (n=3) of fixed batches at low (0.03 ng/mL), medium (1.33 ng/mL), and high (24.26 ng/mL) concentration gradients were measured using the above established standard curves of venous blood and peripheral blood (*Figure 2A-2C*). The intra-batch precision of venous blood and peripheral blood was calculated (*Table 1*), and the coefficient of variation (CV) of peripheral blood method was <15% (*Table 1*).

Linear range verification

Nine concentration gradients of hs-cTnI were detected. Linear regression was performed using the X-axis as the standard concentration and the Y-axis as the measured concentration (n=3). The venous blood and peripheral blood methods were linear in 0.02–40 ng/mL (*Figure 3*, *Table 1*).

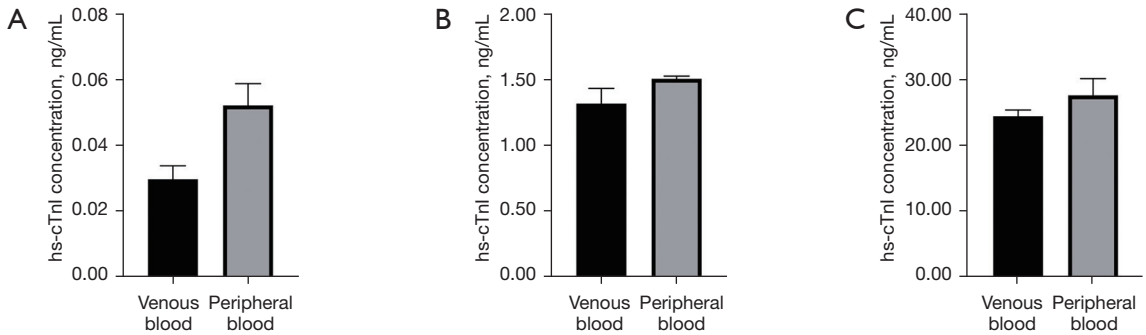

Figure 2 (A-C) Detection results of hs-cTnI at low, medium and high concentrations in venous blood and peripheral blood. hs-cTnI, high-sensitivity troponin I.

Table 1 Analytical performance of hs-cTnI detected in venous blood and peripheral blood

Analytical performance	Venous blood	Peripheral blood
Linear range	0.02–40 ng/mL	0.05–40 ng/mL
Limit of detection	≈0.02 ng/mL	≈0.05 ng/mL
In-batch precision (low)	13.3%	12.7%
In-batch precision (medium)	8.6%	1.3%
In-batch precision (high)	3.6%	9.2%

hs-cTnI, high-sensitivity troponin I.

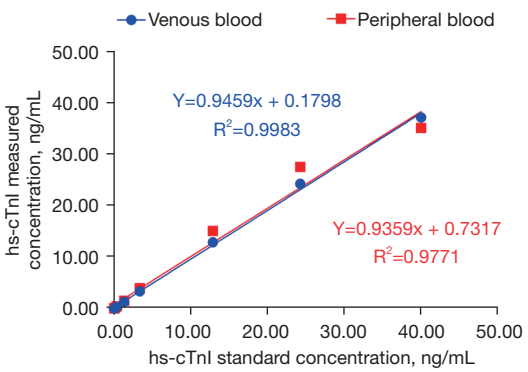

Figure 3 Linear regression between hs-cTnI standard concentration and measured concentration in venous blood and peripheral blood. hs-cTnI, high-sensitivity troponin I.

Minimum detection limit verification

hs-cTnI concentrations of 0.02, 0.03, 0.04, 0.05 and 0.06 ng/mL were detected by quantum dot fluorescence immunoassay in peripheral blood, and the lowest detection limit was about 0.05 ng/mL (*Table 1*).

Correlation analysis of hs-cTnI detection results by two methods

The detection result of hs-cTnI in peripheral blood was the X-axis, and the detection result of hs-cTnI in whole venous blood was the Y-axis. The regression equation between them was Y=1.026x+0.521, and the correlation coefficient was R^2=0.9337 (*Figure 4A*). There were 39 cases with hs-cTnI <0.5 ng/mL. The regression equation was Y=0.655x+0.015, and the correlation coefficient R^2=0.8011. All were linearly correlated (P<0.05) (*Figure 4B*). With hs-cTnI >0.5 ng/mL as the positive limit, there were 51 cases in total. The regression equation was Y=1.005x+1.249, and the correlation coefficient R^2=0.9099 (*Figure 4C*).

Distribution of hs-cTnI in peripheral blood and venous blood and Bland-Altman analysis

The hs-cTnI test results of the two methods ranged from 0.01 to 66.34 ng/mL, and *t*-test revealed P=0.7462, showing no statistically significant difference (*Figure 5A*). The mean value of the hs-cTnI test results of the two methods was the X-axis, and the difference was the Y-axis: 94.4% (85/90) of the data were within the consistency limit (*Figure 5B*).

Evaluation of clinical diagnostic efficacy

Using hs-cTnI 0.5 ng/mL as the positive threshold, we predicted the diagnosis of AMI and compared the predicted results with the final clinical diagnosis. Clinical sensitivity (94.5%:87.3%), specificity (89.5%:89.5%), accuracy (90%:87.8%), positive predictive value (90.4%:91.8%), negative predictive value (87.2%:82.3%) of hs-cTnI

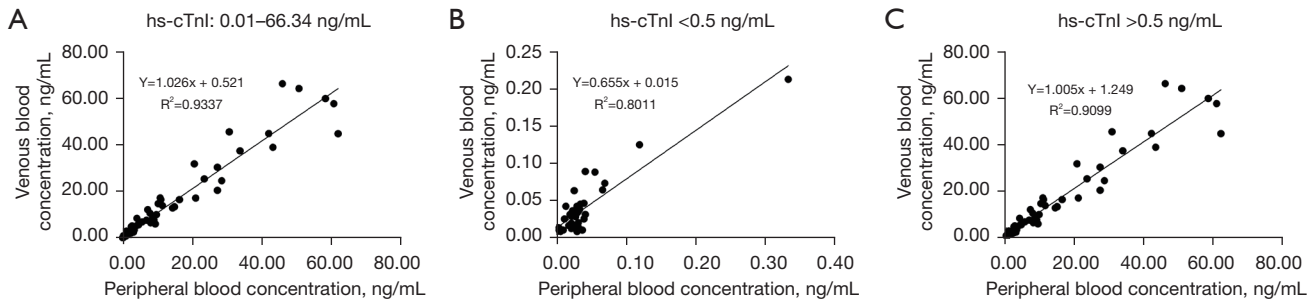

Figure 4 Correlation analysis of hs-cTnI test results between venous blood and peripheral blood. hs-cTnI, high-sensitivity troponin I.

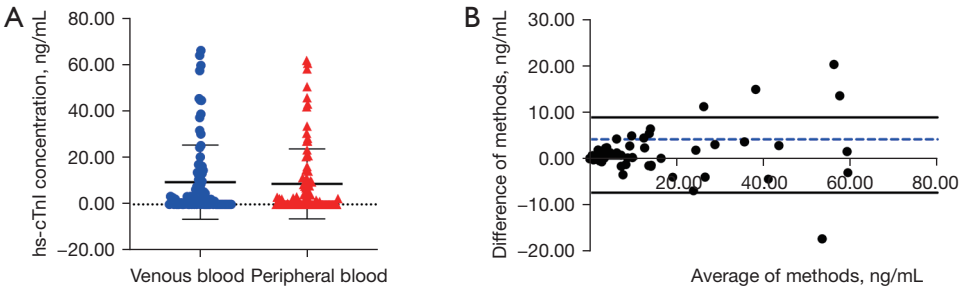

Figure 5 Distribution of hs-cTnI results in venous blood and peripheral blood hs-cTnI in 90 cases, and Bland-Altman analysis of hs-cTnI results in venous blood and peripheral blood. (A) Distribution of venous blood and peripheral blood and (B) Bland-Altman analysis.

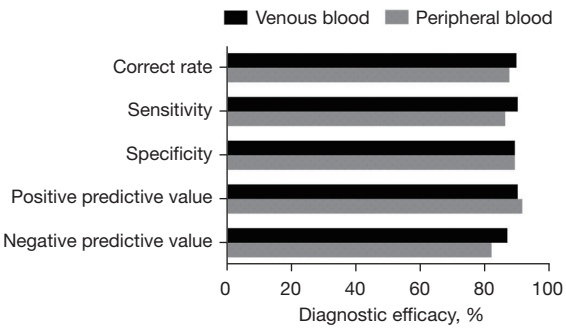

Figure 6 Comparison of diagnostic efficacy of hs-cTnI in venous blood and peripheral blood. hs-cTnI, high-sensitivity troponin I.

detected in venous blood and peripheral blood are shown in *Figure 6*.

Diagnostic value in patients with AMI

The receiver operating characteristic curves of AMI diagnosis and prediction were drawn. The area under the curve for hs-cTnI was 0.9431 [95% confidence interval (CI), 0.8918 to 0.9944] for venous blood and 0.9352 (95% CI, 0.8829 to 0.9876) for peripheral blood. The cut-off value of hs-cTnI in venous blood for the diagnosis of AMI was 1.598, and the sensitivity was 82.69%. The diagnostic accuracy was high, and the difference was not statistically significant (P>0.05) (*Figure 7*).

Discussion

The level of troponin in the blood has important value in predicting infarct size, evaluating the thrombolytic effect and identifying unstable angina pectoris (18-20). When cardiomyocytes undergo necrosis due to ischemia and hypoxia, as in AMI, cTnI is initially released in a free state, but with progression of the injury, cTnI in the bound state is continuously released as a result of the cellular destruction. Therefore, the blood cTnI concentration shows a bimodal change, and the diagnostic time window can be as long as several weeks, which has important clinical significance for the rapid diagnosis of AMI.

We attempted to establish a new method for the rapid detection of hs-cTnI using a quantum dot fluorescence immunoassay in peripheral blood and evaluated it as a good substitute for the detection of hs-cTnI in whole venous blood.

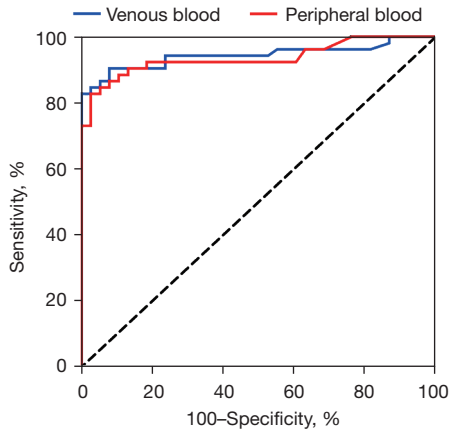

Figure 7 ROC curves of diagnosis and prediction of AMI. ROC, receiver operating characteristic curve; AMI, acute myocardial infarction.

Quantum dots are semiconductor nanoparticles with a radius that is smaller than or close to the radius of the Bohr exciton, but generally, they are 1–10 nm. Quantum dots are used as fluorescent probes, generating fluorescence signals through excitation of the dots, which can be measured by a device as quantitative data. Their advantages include wide excitation wavelength range, narrow emission wavelength, adjustable fluorescence size, high sensitivity, good optical stability, long fluorescence life, large Stokes displacement, and high quantum fluorescence efficiency (21,22). Quantum dots overcome the disadvantages of other markers, such as short chemiluminescence, precise environmental requirements, poor reproducibility, poor stability (e.g., fluorescent dyes), inactivation of enzymes and low sensitivity (23,24). Quantum dots fluorescence immunoassay combines the advantages of immunoassay technology and chromatography, making it simple, rapid and highly specific for rapid diagnosis of AMI.

The results showed that detection of hs-cTnI in peripheral blood by the quantum dot fluorescence immunoassay met the basic requirements of clinical detection with good analytical performance. It detected hs-cTnI in peripheral blood in a certain linear concentration range, and at a low concentration. Secondly, there was a significant correlation between the hs-cTnI detection results for peripheral blood and those for venous blood, with good comparability and consistency. For the clinical diagnosis of AMI, the specificity was the same and the diagnostic accuracy was high. Therefore, quantum dot fluorescence immunoassay of peripheral blood can be used for rapid detection of hs-cTnI and thus the clinical diagnosis and treatment of AMI patients. Our study reveals the potential of a simple and miniaturized peripheral blood device for bedside analysis. The key point of this technology is the sensitivity and accuracy of peripheral blood detection. In terms of sensitivity, it can be further optimized by developing highly specific antibody raw materials and more suitable process formulations; the values of other manufacturers are compared and verified with the clinical symptoms of patients.

Further optimization of the detection of hs-cTnI by quantum dot fluorescence immunoassay in peripheral blood for clinical application is a new direction that can be realized in the future. With continuous research and development of the peripheral blood hs-cTnI detection kit, it will certainly assist in the rapid diagnosis and differential diagnosis of AMI in clinical departments and primary hospitals and meet the requirement of convenience.

Conclusions

Rapid detection of hs-cTnI in peripheral blood by quantum dot fluorescence immunoassay was successfully established as feasible. The quality indexes met the requirements of clinical detection, and the results were highly correlated and consistent with the results for venous blood, providing a good prospect for application in clinical settings.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-436/rc

Data Sharing Statement: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-436/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-436/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all

aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013), and was approved by the Ethics Committee of Wuxi Second People's Hospital of Nanjing Medical University (No. 2020-Y-19) and informed consent was taken from all the patients.

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