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ORIGINAL RESEARCH PAPER

Magnetic particle-based chemiluminescence immunoassay for serum human heart-type fatty acid binding protein measurement

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Abstract
Objectives Human heart-type fatty acid binding protein (HFABP) is a biomarker for diagnosis, risk assessment, and prognosis of acute myocardial infarction, and we aimed to establish an immunoassay for HFABP quantitation.
Methods Human HFABP monoclonal antibodies (mAbs) were developed, evaluated by enzyme-linked immunosorbent assay, and a chemiluminescence enzyme immunoassay (CLEIA) generated. Analytical performance of the CLEIA was evaluated by measuring serum HFABP.
Results The prokaryotically expressed rHFABP was purified and four anti-HFABP mAbs with superior detection performance were obtained after immunizing BALB/c mice. MAbs 2B8 and 6B3 were selected as respective capture and detection antibodies for HFABP measurement by CLEIA (detection range, 0.01–128 µg/L). Results using the CLEIA showed excellent correlation (r, 0.9622) and the correlation coefficient was 0.9809 ($P<0.05$) by the Tukey test statistical analysis with those of latex-enhanced immunoturbidimetry in hospitals.
Conclusion Our mAbs and CLEIA for HFABP detection represent new diagnostic tools for measurement of human serum HFABP.

Keywords Chemiluminescence enzyme immunoassay · Double sandwich enzyme-linked immunosorbent assay · Heart-type fatty acid binding protein · Monoclonal antibody

Jiao Zheng and Yilan Qiu make equal contribution to this paper.

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Introduction

Human heart fatty acid binding protein (HFABP) is a low molecular-weight protein (approximately 15 kDa) composed of 132 amino acids (Furuhashi et al. 2008). HFABP is mainly found in cardiomyocytes, and HFABP levels are very low in serum or

plasma from healthy humans (Xu et al. 2018; Colli et al. 2007). During acute myocardial infarction (AMI), cardiomyocytes are damaged and HFABP can be quickly released into the extracellular space; it can be detected at 1–3 h, reaches a peak at 6–8 h, and returns to normal at 12–24 h (Xu et al. 2008; Rezar et al. 2020). These characteristics contribute to more comprehensive diagnosis and treatment of AMI, making HFABP a potentially promising biomarker for AMI detection (Kakoti et al. 2013).

Clinically, HFABP detection methods include the latex turbidimetric immunoassay (Kim et al. 2010), enzyme-linked immunosorbent assay (ELISA) (Lichtenauer et al. 2017), colloidal gold immunochromatography analysis (Watanabe et al. 2001), and an organic photoelectrochemical method (Shi et al. 2022), among others. However, the accurately detection of serum HFABP remains critical and challenging for the diagnostic value of HFABP in serum (only ~ 6.2 μg/L) (Otaki et al. 2017). These methods are usually limited by expensive instruments and detection reagents or insufficient sensitivity. Therefore, it is still highly desirable to develop simply, rapidly and high-performance methods for detection of serum HFABP. Our developed relatively low-cost monoclonal antibodies and preliminarily established detection methods could match to domestic instruments which could reduce dependence on imports and had more advantages in promotion in China.

At present, commonly used indicators for clinical diagnosis of AMI include myoglobin, troponin, and creatine kinase and its isoenzymes (Ye et al. 2018). However, these indicators only increase after a few hours of myocardial necrosis, whereas HFABP is extremely sensitive for early diagnosis of AMI, in the first 6 h after myocardial injury (Goel et al. 2020), and can even be detected within 1 h for ruling out AMI in low-risk early presenters. The sensitivity and specificity of HFABP are better than those of cardiac troponin I (cTnI) and creatine kinase isoenzymes (CK-MB) (Tarighi et al. 2017; Kabekkodu et al. 2016; Pyati et al. 2015), but its accuracy rate gradually decreases after 6 h. Therefore, a combination of HFABP and other markers, such as cTnI which is a routine diagnosing marker for AMI and maintains the contractile and diastolic ability of the heart, can effectively improve the accuracy of clinical diagnosis (Guan et al. 2022; Topf et al. 2021). In addition, HFABP is also related to other clinical parameters in patients with AMI, such as leukocytosis, and that may be caused by a complex cascade of inflammatory in the setting of AMI (Schernthaner et al. 2017). Therefore, HFABP can serve as a better biochemical indicator for early diagnosis, risk assessment, and prognosis judgment in AMI.

In recent years, the development of new, rapid methods to detect crucial biomarkers has become a focus of immunodiagnostics (Mihailescu et al. 2015). In this context, the purpose of the present research was to develop monoclonal antibodies (mAbs) with superior performance against HFABP, and use them to establish a magnetic particle-based chemiluminescence enzyme immunoassay (CLEIA) for accurate, rapid, and low-cost HFABP quantification. To evaluate the prospects for potential application of the developed monoclonal antibodies and CLEIA, serum HFABP levels determined by CLEIA were compared with clinical values detected by latex-enhanced immunoturbidimetry in the hospital.

Materials and methods

Materials

The HFABP amplification plasmid and expression plasmid, competent *Escherichia coli* (TOP10F`, ER2566), and the myeloma cell line SP2/0 were maintained in our laboratory. Human cardiomyocytes were donated by the Second Xiangya Hospital of Central South University. Endonuclease was purchased from TAKARA. Fetal bovine serum was purchased from ExCell Bio. BALB/c mice (6–8 weeks-old) were purchased from an experimental animal company in Hunan Province. RPMI 1640 medium was purchased from Gibco. Hypoxanthine-thymidine, hypoxanthine-aminopterin-thymidine, complete and incomplete Freund's adjuvant, and polyethylene glycol (PEG) were from Sigma-Aldrich. Ammonium sulfate, BCA protein assay kit, Ni-chelating and Protein A/G affinity chromatographic columns were obtained from Sangon Biotech. Magnosphere™ MS300/Tosyl beads were from JSR Life Sciences. Horseradish peroxidase (HRP), alkaline phosphatase (AP), Sulfo-NHS, and EDC were from Thermo Fisher. All chemicals used were molecular biology grade or higher and purchased from Sangon Biotech.

Human serum samples and ethics approval

Clinical serum samples were donated by the First Affiliated Hospital of Hunan Normal University (Hunan Provincial People's Hospital), and stored at -80℃ until use. Ethical approval was obtained from Hunan Normal University. Animal experiments were approved by the Ethics Committee Board of Hunan Normal University, and performed according to the guidelines of the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

Expression and purification of recombinant HFABP (rHFABP).

The HFABP coding sequence was amplified from human cardiomyocytes, and sequenced to verify its authenticity. The recombinant plasmid, pTO-T7-HFABP, was transformed into *E. coli* strain ER2566, and recombinant his-tag fusion HFABP was expressed after induction with 1 mmol/L isopropyl-β-D-thiogalactoside (IPTG). Cells expressing HFABP protein were harvested and lysed by sonication. Supernatants were purified by saturated ammonium sulfate precipitation, Ni-affinity chromatography, and molecular sieve chromatography.

Preparation and purification of mAbs

The rHFABP and Freund's adjuvant were fully emulsified at a 1:1 ratio and used to immunize BALB/c mice (6–8 weeks-old) by multi-point subcutaneous injection at a dose of 100 µg per mouse and at 2 week intervals. Myeloma cells (strain SP2/0) were fused with spleen cells from immunized mice using PEG, and the resulting hybridoma cells secreting antibodies against HFABP screened by indirect ELISA (Liao et al. 2021). After four rounds of subclone screening using the limiting dilution method, hybridoma cell lines that could stably secrete anti-HFABP mAbs were stored in liquid nitrogen.

Each BALB/c mouse was inoculated with 1×10^6 hybridoma cells to produce ascites, 1 week after sensitization by intraperitoneal injection with 0.5 ml mineral oil. Solution containing mAb against rHFABP from ascites was purified by successive 50% saturated ammonium sulfate precipitation and Protein A/G affinity chromatography. Purified antibodies were dialyzed against phosphate buffer (pH 7.45) at 4 °C for 12 h. The concentration of rHFABP and antibodies was determined by BCA protein assay kit.

Indirect ELISA

Each well of a 96-well microplate was coated with 100 µL purified rHFABP (10 µg/mL) at 4℃ overnight, and then blocked with 200 µL TBST buffer (0.2 mol/L Tris–HCl, 0.9% NaCl, 0.05% Tween-20, pH7.4) containing 0.25% casein, at 37℃ for 2 h. Then, 100 µL of sample was added to each well of the prepared microplate and incubated at 37℃ for 60 min, followed by washing three times with TBST using an automatic plate washer. Diluted HRP-conjugated anti-mouse IgG antibody solution (100 µL per well) was added to the plate and incubated at 37℃ for 30 min, plates washed three times with TBST, and a TMB enzymatic reaction conducted using a reagent kit, according to the manufacturer's instructions. Absorbance at 450/630 nm was measured and recorded using a microplate reader (Biotek Elx800).

Double antibody sandwich ELISA (DAS-ELISA)

Detection mAb (5 mg) was labeled with activated HRP, the antibody precipitate dissolved in 1 mL 50% glycerol, and HRP-labeled antibody stored at -20ºC. Each well of a 96-well microplate was coated with 100 µL capture mAb (10 µg/mL) at 4 °C overnight. Then, 200 µL TBST buffer containing 0.25% casein was added to each well to block the remaining reactive sites, and incubated at 37℃ for 2 h. After washing the plates three times with TBST buffer, 100 µL of sample was added per well and plates incubated at 37 °C for 30 min, followed by washing three times with TBST buffer. HRP-conjugated antibody solution (100 µL) was added to each well and incubated at 37℃ for 30 min, plates washed three times with TBST, and the enzymatic chromogenic reaction visualized using TMB substrate, with absorbance recorded at 450/630 nm, as described above.

Development of a CLEIA

Magnetic beads-conjugated monoclonal antibodies and AP-labeled monoclonal antibodies were generated as previously reported by our research team (Liao et al. 2021). Magnetic beads-conjugated mAbs were used as capture antibodies, and AP-labeled

mAbs were used as detection antibodies. Detection and capture antibodies were paired with one another and the best antibody pairing for detection obtained by screening.

Standard HFABP assay kits used in hospitals (based on latex-enhanced immunoturbidimetry) (Ref, H-FABP 7080; Lot, H-FABP 191101) were used as standard, and diluted with dilution buffer (50 mmol/L Tris, 2% hydrolyzed casein, 5% BSA, 2% Lactose, 0.2% EDTA, pH 7.4). Calibrator concentrations were 0, 0.2, 2, 10, 50, and 100 µg/L (as declared by the manufacturer) and calibrators and samples were measured using a RangeCL-1200i Chemiluminescence Immunoanalyzer (Yuan Jing Biotechnology Co. Ltd., Changsha, Hunan, China). Sample (10 µL) and magnetic beads-conjugated antibody (30 µL, 0.05 mg/mL) were added into reaction tubes and incubated at 37 °C for 5 min, then washed three times. Then, 50 µL AP-labeled antibody (diluted at 1:500 or higher) was added and incubated at 37 °C for 5 min, then washed three times, followed by addition of 200 µL chemiluminescence substrate solution (Lot, 20,200,518; Yuan jing Biotech) and reading of total relative luminous unit (RLU) values in 10 s. All measurements of HFABP calibrators and samples were conducted in triplicate. Logistic four-parameter fitting was conducted using Zecen software on the RangeCL Immunoanalyzer, based on HFABP calibrator concentrations and corresponding calculated mean RLU read values.

Western blot

Serum HFABP (42.85 µg/L) which was collected from visiting patient with suspected AMI in the Hunan Provincial People's Hospital, was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and then electrophoretically transferred to polyvinylidene fluoride membrane. After blocking, mAbs were incubated with the membranes (dilution, 1:2000). After washing, membranes were further incubated with HRP-conjugated secondary antibody (goat anti-mouse IgG, abs20001, Absin Bioscience Co. Ltd., China; dilution, 1:5000). ECL reagent (130,231, Monad Biotech. Co. Ltd., China) was used to detect immune complexes and chemiluminescence signals imaged using a Tanon 5500 system (Tanon Co. Ltd., Shanghai, China).

Measurement of serum HFABP by CLEIA

Human serum samples (n = 106) for HFABP measurement were collected from local hospital within 5 days. Human serum samples were respectively collected from 73 visiting patient with suspected AMI in department of cardiology, and 33 healthy individuals for physical examination in the same period in the Hunan Provincial People's Hospital. Clinical HFABP concentration values were simultaneously obtained using the Heart-type fatty acid-binding protein assay kit (latex-enhanced immunoturbidimetry). Aliquots (10 µL) of serum samples were used for each HFABP test, and then the CLEIA procedure performed using the methods described above. HFABP serum samples were diluted to > 100 µg/L and 0.05 mg/mL magnetic beads-conjugated 2B8 was used as capture antibody, with 6B3-AP (dilution, 1:2,000) as detection antibody. Then, serum HFABP values were calculated based on calibration curves generated using HFABP standards (0, 0.2, 2, 10, 50, and 100 µg/L).

Data analyses.

Calculation of mean and standard deviation (SD) values, analysis of linear regression and correlation, and Tukey test were all carried out using GraphPad PRISM 6 software. The closer the value of r is to + 1, the stronger the linear relationshipis (correlation) between two variables. Two methods were considered to be in good agreement and statistically significant for $P < 0.05$. CLEIA calibration curves were plotted using Zecen software on the RangeCL immunoanalyzer.

Results

Prokaryotic expression and purification of rHFABP

Total RNA was extracted from human cardiomyocytes and reverse transcribed into cDNA, which was used as a template to amplify the HFABP coding sequence by PCR (Fig. 1A). The HFABP coding sequence was cloned into an expression vector, and then transformed into *E. coli* strain ER2566. Recombinant his-tag fusion HFABP was expressed after induction with IPTG (Fig. 1B and 1C). Supernatants of bacterial lysates expressing rHFABP were purified by saturated ammonium sulfate precipitation,

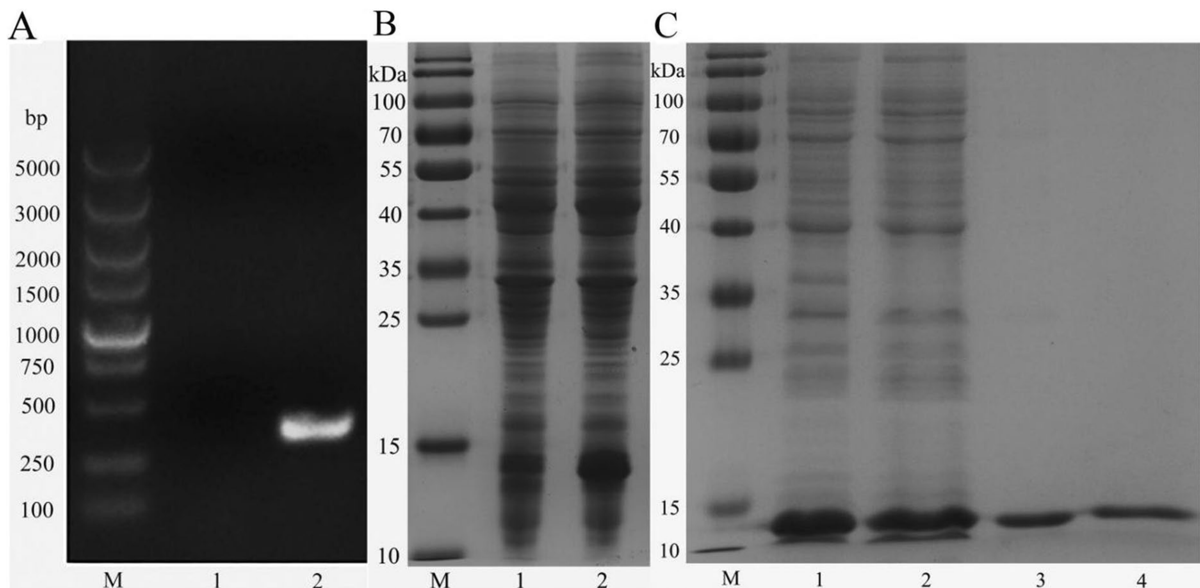

Fig. 1 Prokaryotic expression of rHFABP and protein purification and identification. **A** Amplification of the HFABP coding sequence by PCR (lane M, DNA relative molecular weight markers; lane 1, negative control; lane 2, HFABP coding sequence). **B** rHFABP expression (lane M, protein relative molecular weight markers; lanes 1 and 2, bacterial lysate without or with IPTG induction, respectively. **C** rHFABP purification (lane M, protein relative molecular weight markers; lane 1: total proteins in the supernatant; lane 2, protein purified using 60% saturated ammonium sulfate; lane 3, protein purified using Ni-IDA resin; lane 4: protein purified by molecular sieve chromatography (TSK-GEL G3000 SW)

Ni-affinity chromatography, and molecular sieve chromatography.

Development of mAbs against rHFABP

Four cell lines stably secreting monoclonal antibody (mAb) against HFABP were screened by the hybridoma technique and the four developed mAbs (2B8, 10D5, 6B3, and 7E7) were purified. Anti-HFABP mAb subtypes were determined by indirect ELISA with prepared mAb solution (1 mg/mL). As shown in Fig. 2A, the four developed mAbs (2B8, 10D5, 6B3, and 7E7) were identified as IgG1 subtype, and showed good efficient for rHFABP detection, at titers of 1:51,200 or higher in indirect ELISA (Fig. 2B).

Development of CLEIA for HFABP detection

To screen mAb pairs for recombinant HFABP detection, the developed mAbs were investigated with DAS-ELISA. As illustrated in Fig. 3A, the mAb 2B8 and 7E7 were superior as capture antibody and the HRP-conjugated mAb 6B3 and 10D5 were superior as detection antibody. In addition, the monoclonal antibodies simultaneously as capture antibody and detection antibody meant that mAbs should target different epitopes in DAS-ELISA. The superior monoclonal antibody pairing had different epitopes, such as mAb 2B8 and 10D5. Then mAb 7E7 and 6B3 may target the same epitope. Based on the performance of the developed mAbs in DAS-ELISA, a CLEIA was established. MAb 2B8 and 7E7 were selected as magnetic beads-conjugated antibodies and mAb 6B3 and 10D5 as AP-conjugated antibodies.

As illustrated in Fig. 3B, sensitivity was superior for 2B8/6B3-AP to other mAb pairs, was considered to be the most promising for effective detection of rHFABP, and was therefore used for further performance evaluation.

The detection efficacy of AP-conjugated antibodies at a series of dilutions was investigated. As shown in Fig. 3C, mAb 6B3-AP presented detection effect at a dilution ratio of 1:2000 and was, therefore, used at this ratio in subsequent analyses for cost saving.

As shown in Fig. 3D and 3E, mAb 2B8 and 6B3 were confirmed to have good immunoreactivity for recognizing and detecting endogenous HFABP in

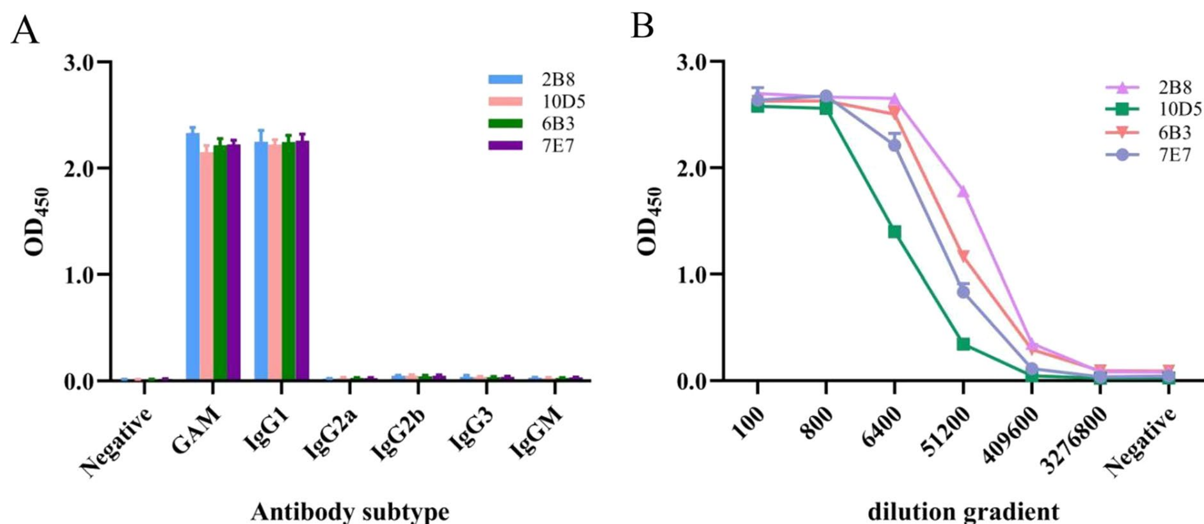

Fig. 2 The evaluation of developed anti-HFABP monoclonal antibodies. **A** Antibody subtypes were determined by indirect ELISA. **B** Developed mAbs titers were determined by indirect ELISA. The four developed mAbs, 2B8, 10D5, 6B3, and 7E7, had a good efficient for rHFABP

serum by western blot and formed an immune complex band at 15 kDa.

Evaluation of the developed CLEIA based on the 2B8/6B3-AP mAb pair

The limit of detection (LoD) is the lowest detectable concentration of analyte distinguishable from null concentration. Analyte signal is generally required to be three to ten times greater than the background signal, which defines the point at which analysis becomes possible. 20 replicates of the blank control (rHFABP 0 μg/L) were measured, and then the mean and SD of RLU read values calculated. LoD was defined as concentration converted from mean RLU plus three SD ($n=20$) of blank control readings. The LoD of the developed CLEIA, calculated according to the calibration curve from the same batch, was 0.01 μg/L.

The rHFABP was diluted to ten gradient concentrations ranging from 0.01 to 0.1 μg/L, and measured using the developed CLEIA. As illustrated in Fig. 4A, the proposed CLEIA exhibited good correlation for measurement of HFABP at low concentrations ($r=0.9912$, $p<0.0001$). Dose–response is an important indicator for quantitative analysis. The rHFABP was diluted to 12 gradient concentrations ranging from 0 to 128 μg/L and detected using the proposed CLEIA. As illustrated in Fig. 4B, the assay showed good measurement linearity ($r=0.9964$, $p<0.0001$).

The locally weighted regression (LWR) method aims to model nonlinearity using several local linear regressions and also can verify other widely used linear approaches, such as linear regression (Zavala-Ortiz et al. 2020). As illustrated in Fig. 4C, the residuals scattered randomly from the zero line which indicated the linear model fit a linear relationship, and can predict concentration linearity.

Coefficient of variation (CV) is defined as the standard variation divided by the mean. Three HFABP calibrators and two serum samples were measured in triplicate each day for 3 days. As shown in Fig. 4D, the precision CV for HFABP measurement was $<7\%$, indicating satisfactory reproducibility of the proposed assay.

HFABP measurement in serum samples using the developed CLEIA

Human serum samples ($n=106$) were measured using the developed CLEIA based on the mAb pair, 2B8/6B3-AP, to evaluate its prospects for clinical application. The serum HFABP values obtained using our assay were compared with clinical values obtained using latex-enhanced immunoturbidimetry at our hospital (Hunan Provincial People's Hospital).

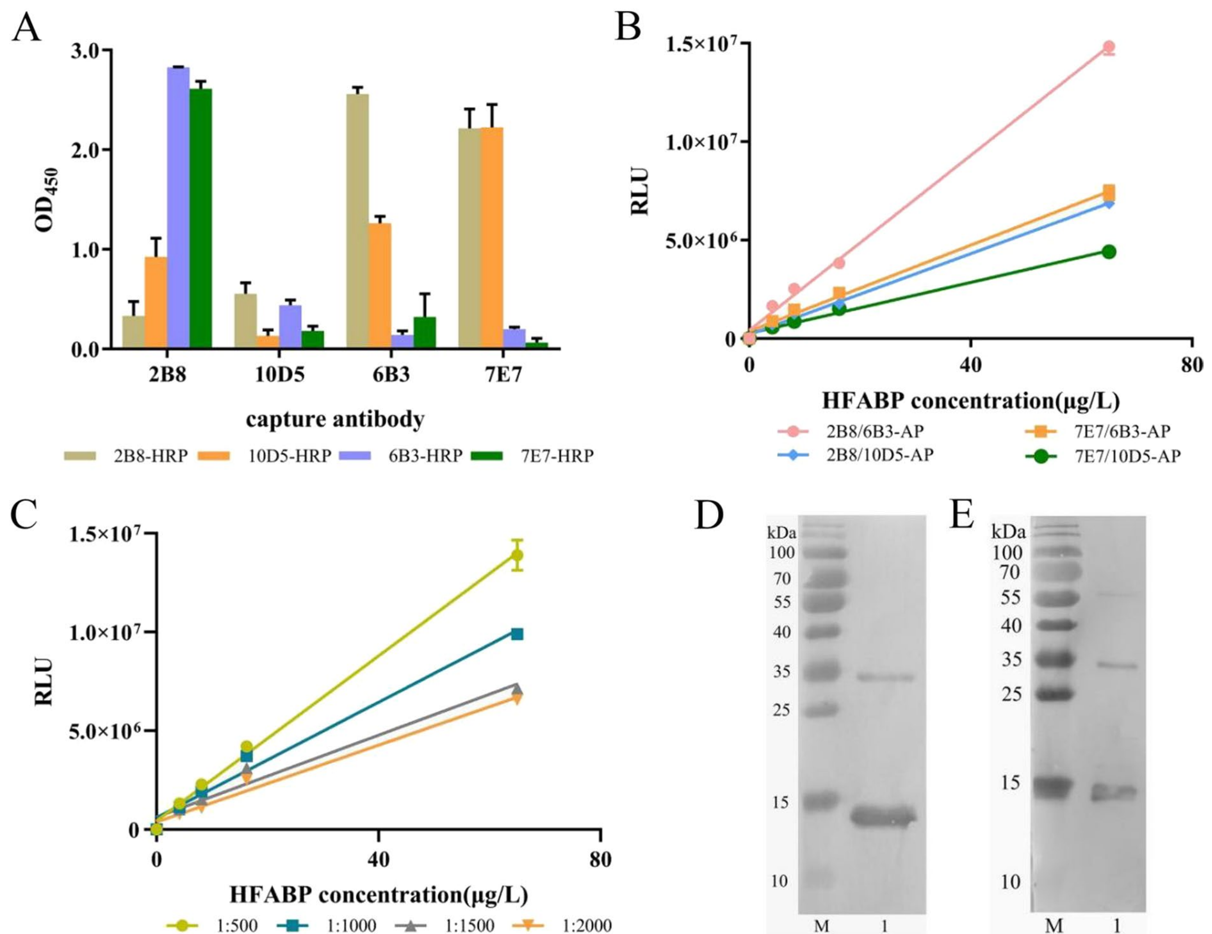

Fig. 3 Detection performances of mAb pairs in CLEIA. **A** Evaluation of developed mAbs by DAS-ELISA. The reactivity of our mAbs against human rHFABP was explored by cross-pairing. The mAb pair, 2B8/6B3-HRP, exhibited excellent sensitivity. **B** Comparison of rHFABP detection efficacy among four mAb pairs. The RLU is relative luminous uint. Magnetic beads-conjugated mAb 2B8 or 7E7 (0.05 mg/mL) was used as capture antibody and AP-conjugated 6B3 or 10D5 (dilution, 1:500) was used as detection antibody. **C** Optimization of the dilution ratio of AP-conjugated antibody. Magnetic beads-conjugated mAb 2B8 (0.05 mg/mL) was used as capture antibody. AP-conjugated 6B3 was used as detection antibody at dilution ratios ranging from 1:500 to 1:2000. **D** and **E** Immunoreactive identification of endogenous HFABP in serum using mAb 2B8 and 6B3 respectively (lane M, protein relative molecular weight markers)

As shown in Fig. 5A and 5B, there was a significant correlation between the two assays in measurement of serum HFABP in the range 0–200 μg/L ($r = 0.9622$, $n = 106$, $p < 0.0001$). These results suggest that our proposed analytical method has outstanding prospects for application in clinical diagnosis.

Many researchers use a Tukey test for statistical analysis of two related paired samples (Zavala-Ortiz et al. 2022). The correlation coefficient, r, is a measure of the strength of the linear relationship (correlation) between two variables. The statistical analysis by Tukey test showed that the correlation coefficient was 0.9809 and P-value < 0.05 which revealed significant correlation between our developed CLEIA and latex-enhanced immunoturbidimetry at our hospital for the whole concentration frame (0–200 μg/L).

Discussion

HFABP potentially has great clinical application value as a biomarker for early diagnosis, risk assessment, and prognosis judgment of cardiovascular disease (Collinson et al. 2017), with high sensitivity and

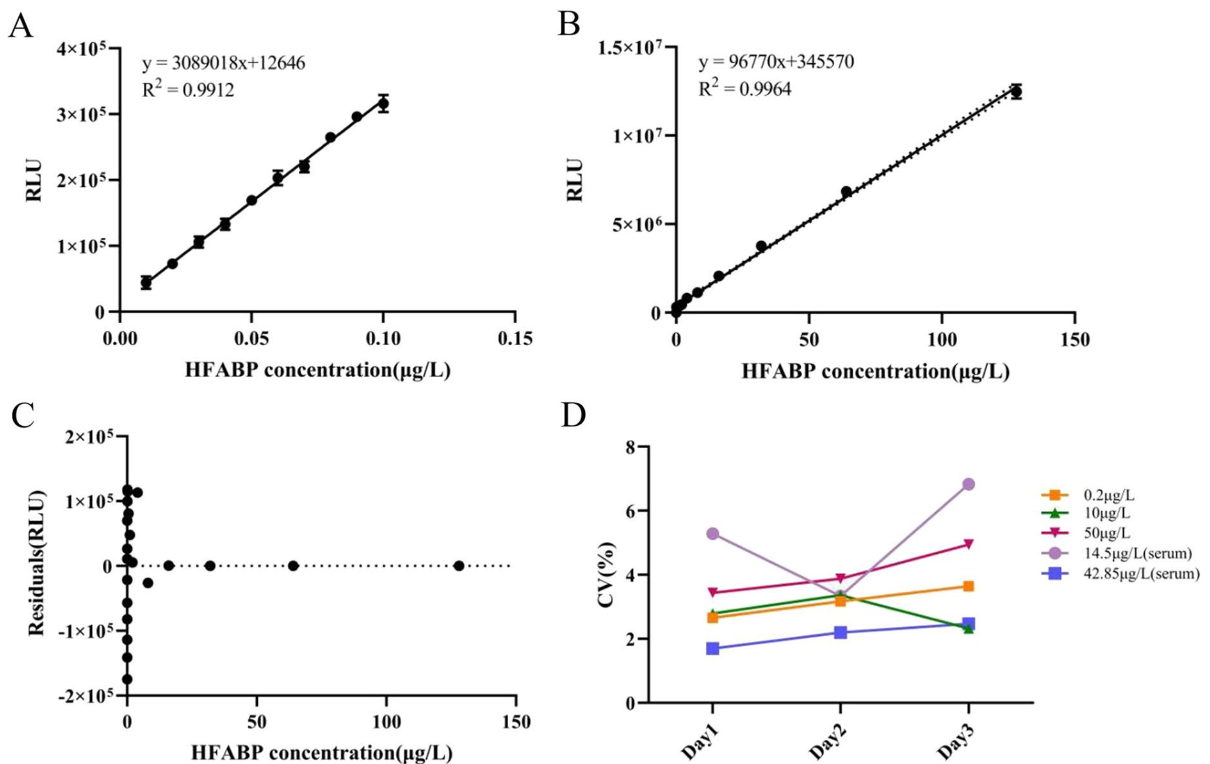

Fig. 4 Sensitivity and linearity analysis of proposed CLEIA. Magnetic particles-conjugated mAb 2B8 (0.05 mg/mL) and AP-labeled mAb 6B3 (diluted as 1:2000) were used to establish the developed CLEIA for HFABP measurement. **A** Ten gradient diluted rHFABP samples (0.01 to 0.1 μg/L) were measured using the developed CLEIA. Linear regression of the RLU reads and assigned rHFABP concentrations was performed. **B** A gradient of rHFABP dilutions was constructed (0, 0.1, 0.2, 0.5, 1, 2, 4, 8, 16, 32, 64, and 128 μg/L) and measured using the developed CLEIA based on the 2B8/6B3-AP mAb pair. **C** The locally weighted regression (LWR) method showed that the residuals scattered randomly from the zero line and had a good linear relationship for the concentration frame (0-128 μg/L). **D** Three rHFABP samples (0.2, 10 ,and 50 μg/L) and two serum samples (14.5 and 42.85 μg/L) were measured three times per day for 3 days using the developed CLEIA. The CV value was calculated based on daily RLU reads for each sample

specificity for the early diagnosis and treatment of AMI and acute coronary syndrome, and is superior to commonly used markers, such as MYO, CK-MB, cTnI, and cTnT (Tarighi et al. 2017; Zeng et al. 2013; Kang et al. 2015). However, in China, the main technology and key raw materials for HFABP detection are essentially completely dependent on imports. Hence, the establishment of anti-HFABP monoclonal antibody production and HFABP rapid quantitative detection technologies, with independent intellectual property rights, is the current direction of development of diagnostic reagents for cardiovascular disease in China.

Monoclonal antibody quality is vital for establishment of analytical methods with high sensitivity and precision. The prokaryotically expressed rHFABP was used to immunize BALB/c mice after purified. Several rounds of immunization and screening were conducted to obtain anti-HFABP mAbs targeting different epitopes with superior detection performance and independent intellectual property rights. The developed CLEIA was calibrated using the same standards as those for the latex-enhanced immunoturbidimetry in a hospital setting, to secure concordance and correlation at established clinical cut-off points. There was a significant correlation through statistical analysis between our CLEIA and the latex-enhanced immunoturbidimetry in hospital for detection of serum HFABP in the concentration frame (0–200 μg/L). In the present study, our proposed CLEIA (HFABP detection range, 0.01–128 μg/L) also had comparable sensitivity to

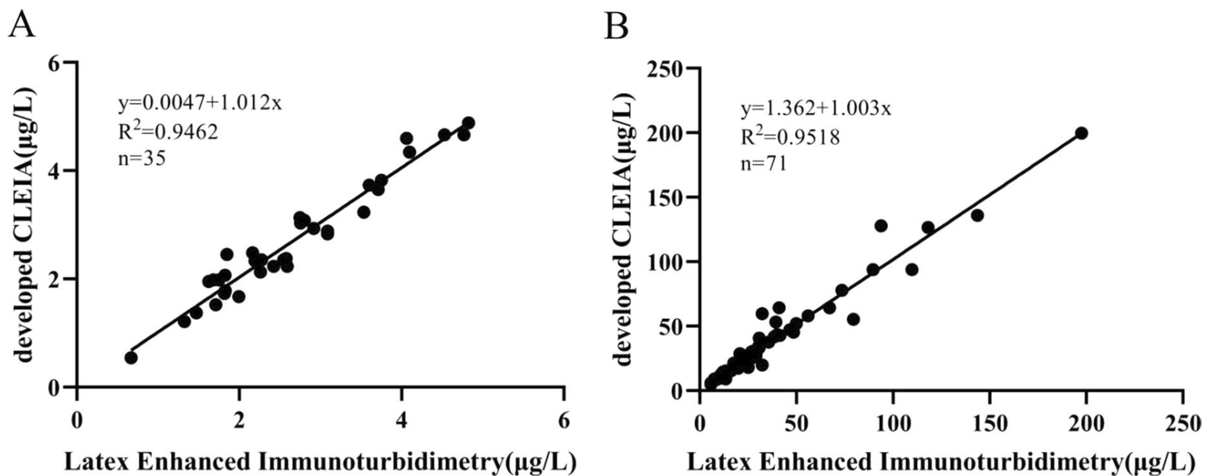

Fig. 5 Correlation analysis of serum HFABP values obtained by two analytical methods. Serum samples (n = 106) were measured by latex-enhanced immunoturbidimetry and using our developed CLEIA based on the mAb pair, 2B8/6B3-AP. Serum HFABP values obtained by these two assays were analyzed by linear regression, Pearson correlation analysis, and Bland-Altman analysis. **A** HFABP concentration, 0 to 5 μg/L. **B** HFABP concentration, 5 to 200 μg/L

the Randox Laboratories immunoturbidimetric assay (0.747–120 μg/L) (Van et al. 2018; Da et al. 2014; Vera et al. 2021). In addition, the developed CLEIA was superior to the integrated microfluidic electrochemiluminescence device for point-of-care testing with a detection range of 0.72–100 μg/L, which needed high-cost chip and mesoporous silica nanoparticles (Zhu et al. 2023). The results of preliminary assessment of correlation and clinical concordance between our assay and the latex-enhanced immunoturbidimetry were quite encouraging. However, analysis of serum samples from a larger population should be completed to draw a more credible conclusion.

The performance of our proposed CLEIA for HFABP detection met the guidelines commonly used by health authorities in China. Our HFABP CLEIA system will be further optimized for clinical use in the near future, with the aim of reducing costs, relative to imported HFABP assays. We are actively preparing to declare the approval of the assay as a medical diagnostic reagent for HFABP with a cooperating local company (Yuan Jing Biotechnology Co. Ltd., Changsha, Hunan, China). We also plan to use our newly-developed mAbs to establish other more simple and efficient immunoassay methods.

Author contributions RSL, JZ and YLQ designed the study. JZ, ZHZ, YW, QLW and RSL drafted and revised the manuscript. JZ, YX, XZ, and MFQ performed the research, collected data and analyzed the data. CX revised the manuscript. All the authors reviewed and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval The authors state that they have followed the principles outlined in the Declaration of Helsinki for all animal experimental investigations.

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