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Clinical Feasibility of Biofunctionalized Magnetic Nanoparticles for Detecting Multiple Cardiac Biomarkers in Emergency Chest Pain Patients

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Background: The rapid diagnosis of acute myocardial infarction (AMI) is a clinical and operational priority in emergency departments. Serial serum levels of cardiac biomarkers play a crucial role in the evaluation of patients presenting with acute chest pain, so that an accurate and rapidly responsive assay of cardiac biomarkers is vital for emergency departments.

Methods: Immunomagnetic reduction (IMR) has been developed for rapid and on-site assays with a small sample volume. IMR kits for three biomarkers [myoglobin, creatine kinase-MB (CK-MB), and troponin-I] have been developed by MagQu Co., Ltd., Taiwan (US patent: US20190072563A1). In this study, we examined correlations between IMR signals and biomarker concentrations. The measurement threshold of the IMR kits, dynamic ranges, interference tests in vitro, and reagent stability were tested. Clinical cases were included with serial IMR measurements to determine the time course and peak of IMR-measured cardiac biomarkers after AMI.

Results: The correlations between IMR signals and biomarker concentrations fitted well to logistic functions. The measurement thresholds of the IMR kits (1.03×10^{-8} ng/mL for myoglobin, 1.46×10^{-6} ng/mL for CK-MB, and 0.08 ng/mL for troponin-I) were much lower than the levels detected in the patients with AMI. There was no significant interference in vitro. The peak times of IMR-detected myoglobin, CK-MB, and troponin-I after AMI were 8.2 hours, 24.4 hours, and 24.7 hours, respectively.

Conclusions: IMR is an accurate and sensitive on-site rapid assay for multiple cardiac biomarkers in vitro, and may play a role in the early diagnosis of AMI. Clinical trials are needed.

Key Words: Acute coronary syndrome • Acute myocardial infarction • Cardiac biomarkers • Immunomagnetic reduction • Magnetic nanoparticles • Troponin-I

INTRODUCTION

Coronary artery disease (CAD) has been a leading cause of death worldwide for decades, and over 7 million people die from it annually.[1] Acute myocardial infarction (AMI) is an acute manifestation of CAD triggered by fissuring, erosion, or rupture of atheromatous plaques in coronary arterial walls, resulting in total or critical obstruction of coronary arteries. AMI is a major cause of cardiovascular mortality and is associated with the development of heart failure and millions of hospitalizations annually.[2] Besides the administration of dual anti-platelet agents and an anticoagulant, AMI usually

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prompts early revascularization and the implementation of evidence-based medications to reduce infarct size and subsequent complications.[3-7] Therefore, the rapid and early diagnosis of AMI is a clinical and operational priority at the emergency department (ED).

An elevation in cardiac biomarkers, especially cardiac troponins, is the hallmark for the clinical diagnosis of AMI according to the universal definition of myocardial infarction.[8] Measuring serum levels of cardiac biomarkers using enzyme-linked immunosorbent assay (ELISA) kits currently used at the ED usually takes 30 to 60 minutes. A faster and more sensitive kit to measure serum levels of cardiac biomarkers would help in the rapid and early diagnosis of AMI at the ED.

Immunomagnetic reduction (IMR) has been developed as a rapid and sensitive assay needing a small sample volume.[9-11] The reagent is a solution of homogeneously dispersed magnetic nanoparticles, which are coated with hydrophilic surfactants and bioprobes. When magnetic nanoparticles bind with the bioprobes on the outermost shell and become larger or clustered, the concentration can be measured quantitatively.[12,13] There are two main reasons for the high specificity of IMR. First, IMR detects the magnetic signal instead of the optical signal. Second, the suppression of non-specific binding between the molecules and magnetic nanoparticles. With its ultra-high sensitivity and specificity, IMR is a promising candidate to achieve an accurate *in vitro* diagnosis, and may be suitable for an early-stage diagnosis, disease monitoring and follow-up in different clinical scenarios.[14-23] In this study, IMR kits for three biomarkers [myoglobin, creatine kinase-MB (CK-MB), and troponin-I] developed and prepared by MagQu Co., Ltd., Taiwan (US patent: US20190072563A1) were tested. We examined correlations between IMR signals and biomarker concentrations, their low threshold for detection and dynamic ranges, and interference tests in vitro. Clinical cases were included with serial IMR measurements to determine the time course and peak of IMR-detected cardiac biomarkers after AMI.

METHODS

As previously described, the reagent used in IMR is a solution in which there are homogeneously dispersed magnetic nanoparticles coated with hydrophilic surfactants and bioprobes. Under an external magnetic field, the magnetic nanoparticles in the solution oscillate via magnetic interactions. Thus, reagent exposed to an external magnetic field shows a magnetic property called mixed-frequency ac magnetic susceptibility χ_{ac}. Via bioprobes such as antibodies on the outermost shell, magnetic nanoparticles can associate with and magnetically label bio-molecules (e.g. antigens) which can then be detected. Due to association, magnetic nanoparticles become clustered and thus larger, and the response of these larger magnetic nanoparticles to an external magnetic field is much less than that of original individual smaller magnetic nanoparticles. Thus, the χ_{ac} of the reagent is reduced, and this is why the method is referred to as immunomagnetic reduction. In principle, when greater amounts of the bio-molecules that are to be detected are mixed with a reagent, more magnetic nanoparticles become clustered. A larger reduction in χ_{ac} can then be detected.

Magnetic reagents

Three kinds of antibodies (Table 1) were immobilized onto dextran-coated Fe_3O_4 magnetic particles, respectively, as shown in Figure 1A. The reagent with anti-myoglobin was denoted as MyG reagent, the particle diameter of which was 50.9 ± 13.44 nm. The reagent with anti-CK-MB was denoted as CK-MB reagent, the

Table 1. The antigens and antibodies used as follows

Antibody	Notation	Model
Human myoglobin	MyG	ProSpec, pro-565
Human creatine kinase MB full length protein	CK-MB	Abcam, ab109675
Recombinant human cardiac troponin I	Tn-I	ProSpec, pro-324
Anti-myoglobin antibody [4E2]	Anti-MyG	Abcam, ab8343
Anti-creatine kinase MB [CK1] antibody	Anti-CK-MB	Abcam, ab404
Anti-cardiac troponin I antibody [EP1106Y]	Anti-Tn-I	Abcam, ab52862

particle diameter of which was 56.3 ± 14.29 nm. The reagent with anti-Tn-I was denoted as Tn-I reagent, the particle diameter of which was 51.4 ± 13.96 nm, as shown in Figure 1B. The magnetic concentrations of the MyG and CK-MB reagents were 5.0 mg-Fe/mL, while the magnetic concentration of the Tn-I reagent was 6.5 mg-Fe/mL. The magnetic reagents were stored at 4 °C.

IMR reader

XacPro-E (MagQu Co., Ltd.) was used as a reader to detect the IMR signals of the reagents caused by the association among magnetic particles and the antigens that were to be detected.

Measurement of IMR signal

We diluted the antigen solution with pH-7.2 phosphate-buffered saline, took one of these diluted antigen solutions for IMR measurement, and left the others at 4 °C. The reagent was moved from 4 °C to room temperature and kept at room temperature for 5 minutes. We used Vortex (Mixer) to shake both the reagent and antigen solution for 15 seconds, respectively, and mixed 40 μL of reagent and 60 μL of antigen solution in a glass tube. The rest of the reagent and antigen solution was stored at 4 °C. We used Vortex (Mixer) to shake the mixture of the reagent and antigen solution for 15 seconds, then moved the mixture to XacPro-E for measurement of the IMR signal. Two experiments were conducted for each concentration of antigen solution. We followed the above steps for each test. After each test, the mixture of the reagent and antigen solution was disposed. The analytic properties of the IMR assays were investigated, including low-detection limit, hook effect, assay linearity, dynamic range, reagent stability, and interference test (Table 2).

Clinical study

We have conducted a prospective clinical trial since

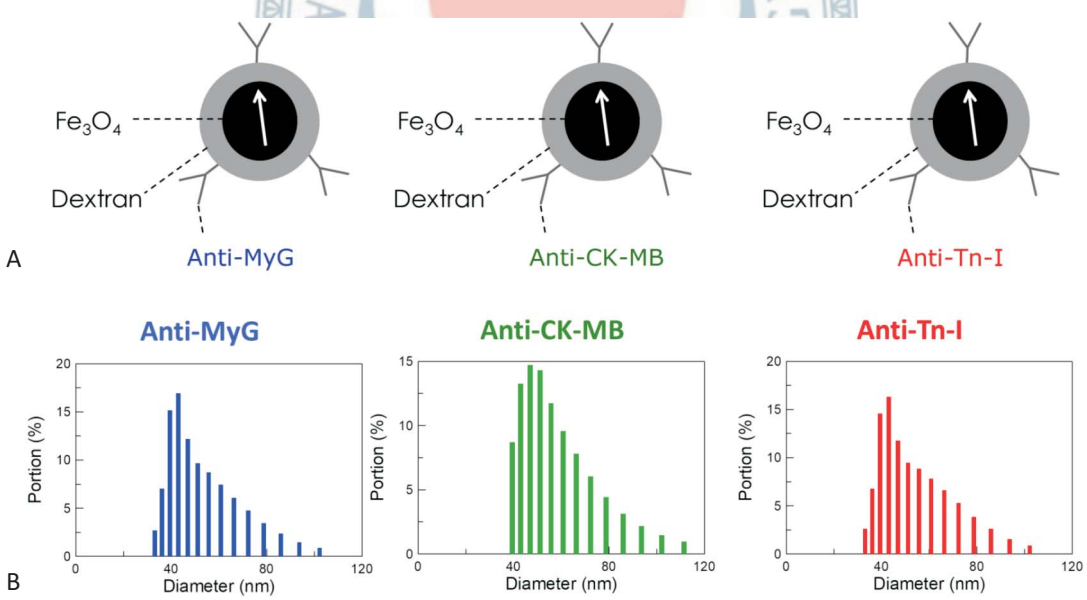

Figure 1. *(A) Antibodies immobilized onto dextran coated Fe_3O_4 magnetic particles. (B) The particle diameters of different biomarker reagents. CK-MB, creatine kinase-MB; Tn-I , troponin I.*

Table 2. Experiment designs

Items	Synthesis of reagent*	Standard curve#	Interference test†	Stability test	Human serum assay
Myoglobin	Done	Done	Done	275 days	Done
Troponin I	Done	Done	Done	141 days	Done
CK-MB	Done	Done	Done	151 days	Done

* Including analysis of particle size. # Including characterizations of low-detection limit, linearity, and dynamic range. † Incldluing the investigations of interfering effects of hemoglobin (Hb), bilirubin (C-BL), triglyceride (TG), and myoglobin (MyG)/troponin I (Tn-I)/ creatine kinase-MB (CK-MB).

2014 to test the diagnostic accuracy of AMI with IMR assays. This study was approved by the Institutional Review Board (IRB) of Far Eastern Memorial Hospital (IRB. No. 103002-N; ClinicalTrials.gov. ID: NCT02226523). All study subjects or their primary caregivers provided informed consent prior to participation in this investigation. All experiments were performed in accordance with relevant guidelines and regulations.

To determine the time course of cardiac biomarkers detected by IMR after AMI, we collected clinical data and IMR-measured concentrations of myoglobin, CK-MB, and troponin-I every 6 hours after the onset of AMI in sixparticipants receiving primary coronary interventions. The subjects were asked to provide a 10-ml non-fasting venous blood sample. The blood samples were centrifuged (3,000 g for 10 minutes at 4 °C) within 1 hour of the draw, and the serum was aliquoted into cryotubes and stored at -80 °C. Colleagues were blind to all samples in the laboratory.

RESULTS

IMR on myoglobin

Myoglobin solutions with concentrations of 0, 1, 10, 100, and 1000 ng/mL were prepared for IMR measurement. The 0-ng/ml solution was used as the negative control test. The IMR signal as a function of myoglobin concentration ϕ_{MyG} was investigated. The results are plotted in Figure 2A. The data points were fitted to the logistic function:

$$\text{IMR (\%)} = \frac{A-B}{1+\left(\frac{\phi_{MyG}}{\phi_o}\right)^{\gamma}}+B$$, with A, B, ϕ_o, and γ being the fitting parameters.

They were found to be A = 0.89, B = 2.96, ϕ_o = 8544.8, and γ = 0.167.

The measurement threshold is usually defined as the concentration with an IMR signal higher than the noise level by three standard deviations for IMR signals at low concentrations. The standard deviation for IMR at low concentrations, such as 0.1-ng/mL myoglobin solution, was found to be 0.007%. Thus, the measurement threshold of the IMR signal was (0.89 + 3 × 0.007)% = 0.91%. According to the logistic function, the low-detection limit for assaying myoglobin using IMR was 1.03×10^{-8} ng/mL, which is lower than the threshold for detecting myocardial infarction by nine orders of magnitude.

Using the logistic function, the detected IMR signals were converted to myoglobin concentrations and denoted as $\phi_{MyG\text{-}IMR}$. The relationship between $\phi_{MyG\text{-}IMR}$ and ϕ_{MyG} is plotted in Figure 2D. $\phi_{MyG\text{-}IMR}$ was proportional to ϕ_{MyG} with a slope of 1.02. The requirement for determining the range of linearity in terms of myoglobin concentration was that the slope was between 0.9 and 1.1. Hence, the dynamic range of assaying myoglobin using IMR was from 1.03×10^{-8} ng/mL to 1000 ng/mL.

For interference tests, materials including hemoglobin (Hb) (H7379-1G, Sigma-Aldrich), conjugated bilirubin (C-BL) (14370-250MG, Sigma), triglyceride (TG) (T2449-10ML, Sigma), Tn-I (pro-324, ProSpec), and CK-MB (ab 109675, Abcam), were mixed with MyG solutions, respectively. The concentrations of the interference materials used in this study are listed in supplementary material. The IMR (%) for each sample with interference material was compared with that of pure MyG solution. T-tests showed that the p values for the IMR signals between MyG solutions with interference materials and pure MyG solution were all above 0.05. This implied that there was no significant effect on IMR signals from these interference materials.

IMR on CK-MB

CK-MB solutions with concentrations of 0, 0.1, 1, 5, 50, 500, 1000, and 5000 ng/mL were prepared for IMR measurement. The 0-ng/mL solution was used as the negative control test. The IMR signal as a function of CK-MB concentration $\phi_{CK\text{-}MB}$ was investigated. The results are plotted in Figure 2B. The data points were fitted to the logistic function:

$$\text{IMR (\%)} = \frac{A-B}{1+\left(\frac{\phi_{CK-MB}}{\phi_o}\right)^{\gamma}}+B$$, with A, B, ϕ_o, and γ being the fitting parameters.

They were found to be A = 0.73, B = 5.50, ϕ_o = 2003527, and γ = 0.169. The coefficient of determination R^2 was 0.998.

The measurement threshold is usually defined as

the concentration with an IMR signal higher than the noise level by three standard deviations for IMR signals at low concentrations. The standard deviation for IMR at low concentrations, such as 0.1-ng/ml CK-MB solution, was found to be 0.014%. Thus, the measurement threshold of the IMR signal was (0.73 + 3 × 0.014)% = 0.77%. According to the logistic function, the low-detection limit for assaying CK-MB using IMR was1.46×10^{-6} ng/mL, which is lower than the threshold for detecting myocardial infarction by seven orders of magnitude.

Using the logistic function, the detected IMR signals were converted to CK-MB concentrations, denoted as $\phi_{CK\text{-}MB\text{-}IMR}$. The relationship between $\phi_{CK\text{-}MB\text{-}IMR}$ and $\phi_{CK\text{-}MB}$ is plotted in Figure 2E. $\phi_{CK\text{-}MB\text{-}IMR}$ was proportional to $\phi_{CK\text{-}MB}$ with a slope of 1.09. The requirement for determining the range of linearity in terms of myoglobin concentration was that the slope was between 0.9 and 1.1. Hence, the dynamic range of assaying myoglobin using IMR was from 1.46×10^{-6} ng/mL to 5000 ng/mL.

For interference tests, materials including Hb (H7379-1G, Sigma-Aldrich), C-BL (14370-250MG, Sigma), TG (T2449-10ML, Sigma), Tn-I (pro-324, ProSpec), and MyG (pro-565, ProSpec) were mixed with CK-MB solutions, respectively. The concentrations of the interference materials used in this study are listed in supplementary material. The IMR (%) for each sample with interference material was compared with that of pure CK-MB solution. T-tests showed that the p values for the IMR signals between CK-MB solutions with interference materials and pure CK-MB solution were all above 0.05. This implied that there was no significant effect on IMR signals from these interference materials.

IMR on troponin-I

Tn-I solutions with concentrations of 0 to 1000 ng/mL were prepared for IMR measurement. The 0-ng/mL solution was used as the negative control test. The IMR signal as a function of Tn-I concentration $\phi_{Tn\text{-}I}$ was investigated. The results are plotted in Figure 2C. The data

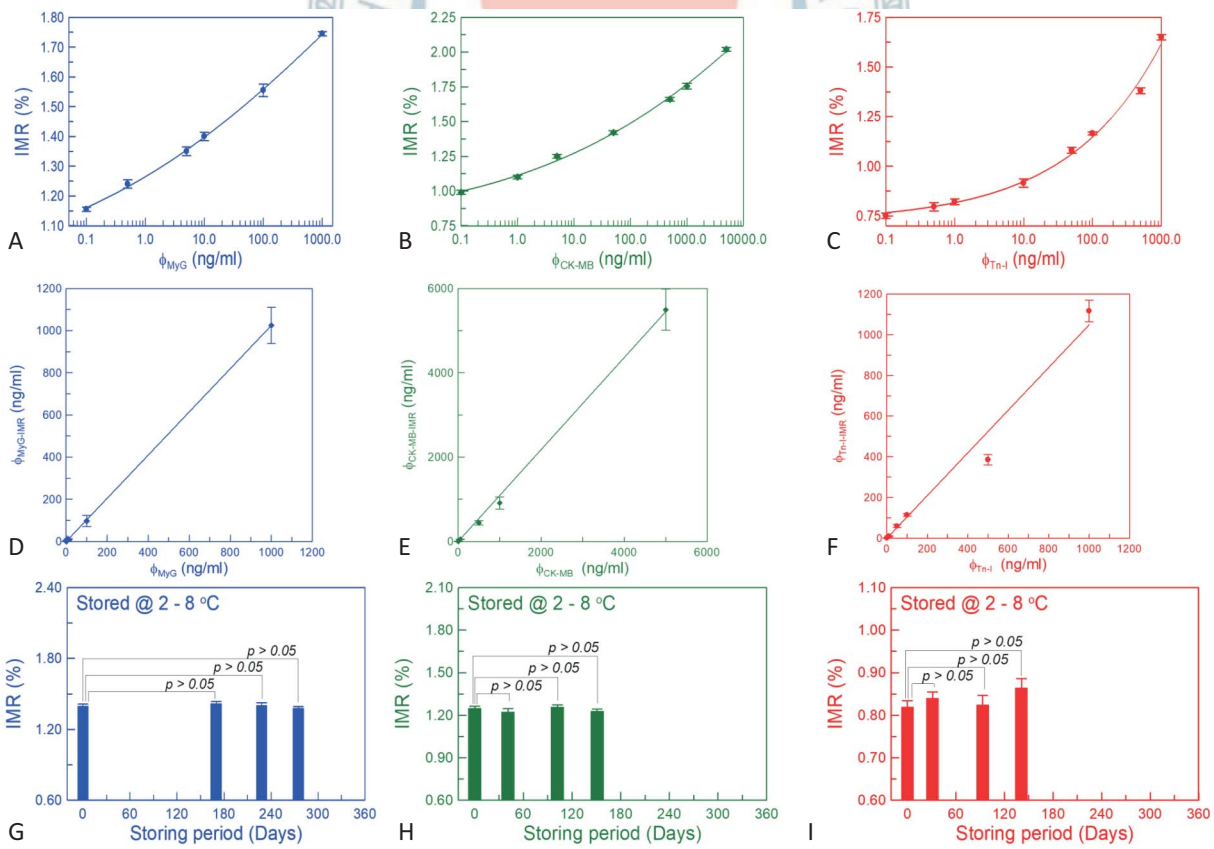

Figure 2. *(A-C) The immunomagnetic reduction (IMR) signals as a function of biomarker concentrations. (D-F) The relationship between IMR-detected biomarker levels and respective biomarker concentrations. (G-I) Reagent stability tests.*

points were fitted to the logistic function:

$$\text{IMR (\%)} = \frac{A-B}{1+\left(\frac{\phi_{Tn-I}}{\phi_o}\right)^{\gamma}} + B$$, with A, B, ϕ_o, and γ being the fitting parameters.

They were found to be A = 0.72, B = 135.88, $\phi_o = 5.11 \times 10^9$, and $\gamma = 0.423$. The coefficient of determination R^2 was 0.993.

The measurement threshold is usually defined as the concentration with an IMR signal higher than the noise level by three standard deviations for IMR signals at low concentrations. The standard deviation for IMR at low concentrations, such as 0.1-ng/mL Tn-I solution, was found to be 0.014%. Thus, the threshold of the IMR signal was (0.72 + 3 × 0.014)% = 0.762%. According to the logistic function, the measurement threshold for assaying Tn-I using IMR was 0.08 ng/ml, which is much lower than the threshold for detecting myocardial infarction.

Using the logistic function, the detected IMR signals were converted to Tn-I concentrations, denoted as $\phi_{Tn\text{-}I\text{-}IMR}$. The relationship between $\phi_{Tn\text{-}I\text{-}IMR}$ and $\phi_{Tn\text{-}I}$ is plotted in Figure 2F. $\phi_{Tn\text{-}I\text{-}IMR}$ was proportional to $\phi_{Tn\text{-}I}$ with a slope of 1.05. The coefficient of determination R^2 is 0.983. The requirement for determining the range of the linearity in terms of Tn-I concentration was that the slope was between 0.9 and 1.1. Hence, the dynamic range of assaying Tn-I using IMR was from 0.08 ng/mL to 1000 ng/mL.

For interference tests, materials including Hb (H7379-1G, Sigma-Aldrich), C-BL (14370-250MG, Sigma), TG (T2449-10ML, Sigma), CK-MB (ab109675, Abcam), and MyG (pro-565, ProSpec) were mixed with Tn-I solutions, respectively. The concentrations of the interference materials used in this study are listed in supplementary material. The IMR (%) for each sample with interference material was compared with that of pure Tn-I solution. T-tests showed that the p values for the IMR signals between Tn-I solutions with interference materials and pure Tn-I solution were all above 0.05. This implied that there was no significant effect on IMR signals from these interference materials.

Reagent stability

The MyG reagent was stored at 2-8 °C. During storage, a portion of the MyG reagent was used for IMR measurement of 10-ng/mL MyG solution. Thus, the IMR signal of 10-ng/mL MyG solution using MyG reagent as a function of storage periods at 2-8 °C could be explored. The results are shown in Figure 2G. The IMR signals detected with various storage periods, the longest of which was 275 days, were not significantly different from that on the first day.

The CK-MB reagent was stored at 2-8 °C. During storage, a portion of the CK-MB reagent was used for IMR measurements of 5-ng/mL CK-MB solution. Thus, the IMR signal of 5-ng/mL CK-MB solution using CK-MB reagent as a function of storage periods at 2-8 °C could be explored. The results are shown in Figure 2H. The IMR signals detected with various storage periods, the longest of which was 151 days, were not significantly different from that on the first day.

The Tn-I reagent was stored at 2-8 °C. During the storage, a portion of the Tn-I reagent was used for IMR measurements of 1-ng/mL Tn-I solution. Thus, the IMR signal of 1-ng/mL Tn-I solution using Tn-I reagent as a function of storage periods at 2-8 °C could be explored. The results are shown in Figure 2I. The IMR signals detected with various storage periods, the longest of which was 141 days, were not significantly different from that on the first day.

The results of interference and stability tests are listed in Table 3 and 4.

Clinical study

Clinical and IMR data were collected from six participants presenting with acute STEMI and receiving primary percutaneous coronary interventions (PCIs). The demographic and clinical information is summarized in Table 5. The mean age was 59 years, and four of them were male. Fitting curves of IMR-measured cardiac biomarker concentrations as a function of time were obtained. The peak times of IMR-measured myoglobin, CK-MB, and troponin-I were 8.2 hours, 24.4 hours, and 24.7 hours, respectively, as shown in Figure 3.

DISCUSSION

In this study, we tested IMR assays for cardiac biomarkers including myoglobin, CK-MB, and cardiac tro-

Table 3. Interference tests

Sample No.	Composition	IMR signal	p value
(1) Myoglobin			
1	10-ng/ml MyG	(1.40 ± 0.014) %	-
2	10-ng/ml MyG with 600-ppm Hb	(1.42 ± 0.007) %	0.078
3	10-ng/ml MyG with 600-ppm C-BL	(1.38 ± 0.021) %	0.150
4	10-ng/ml MyG with 2000-ppm TG	(1.42 ± 0.021) %	0.150
5	10-ng/ml MyG with 50-ng/ml Tn-I	(1.41 ± 0.014) %	0.276
6	10-ng/ml MyG with 100-ng/ml CK-MB	(1.40 ± 0.014) %	0.500
(2) CK-MB			
1	5-ng/ml CK-MB	(1.25 ± 0.014) %	-
2	5-ng/ml CK-MB with 600-ppm Hb	(1.25 ± 0.021) %	0.404
3	5-ng/ml CK-MB with 600-ppm C-BL	(1.22 ± 0.014) %	0.084
4	5-ng/ml CK-MB with 2000-ppm TG	(1.27 ± 0.014) %	0.146
5	5-ng/ml CK-MB with 50-ng/ml Tn-I	(1.26 ± 0.021) %	0.404
6	5-ng/ml CK-MB with 150-ng/ml MyG	(1.24 ± 0.014) %	0.276
(3) Troponin I			
1	1-ng/ml Tn-I	(0.82 ± 0.014) %	-
2	1-ng/ml Tn-I with 600-ppm Hb	(0.84 ± 0.014) %	0.146
3	1-ng/ml Tn-I with 600-ppm C-BL	(0.82 ± 0.014) %	0.500
4	1-ng/ml Tn-I with 2000-ppm TG	(0.86 ± 0.021) %	0.096
5	1-ng/ml Tn-I with 100-ng/ml CK-MB	(0.84 ± 0.014) %	0.146
6	1-ng/ml Tn-I with 150-ng/ml MyG	(0.83 ± 0.014) %	0.276

C-BL, conjugated bilirubin; CK-MB, creatine kinase-MB; Hb, hemoglobin; TG, triglyceride; Tn-I, troponin I.

Table 4. Stability tests

Storing period (day)	IMR signal	p value
(1) Myoglobin		
0	(1.40 ± 0.014) %	-
170	(1.42 ± 0.014) %	0.146
228	(1.41 ± 0.021) %	0.246
275	(1.38 ± 0.014) %	0.146
(2) CK-MB		
0	(1.40 ± 0.014) %	-
170	(1.42 ± 0.014) %	0.146
228	(1.41 ± 0.021) %	0.246
275	(1.38 ± 0.014) %	0.146
(3) Troponin I		
0	(0.82 ± 0.014) %	-
31	(0.84 ± 0.014) %	0.146
93	(0.83 ± 0.021) %	0.404
141	(0.87 ± 0.021) %	0.150

ponin-I, and determined the correlations and conversion functions between IMR signals and biomarker concentrations. The IMR assays were then tested for measurement thresholds, dynamic ranges, interference tests, and reagent stability. Low detection limit, good linearity and wide range of detection without hook effect were noted in the tested ranges in the in vitro experiments. In this experimental platform, it took less than 1 hour for each test. All of these factors suggested that IMR could be a reliable on-site assay for a rapid test of cardiac biomarkers in clinical scenarios such as the evaluation of acute chest pain at the ED.

According to findings on electrocardiography (ECG), mainly ST-segment elevation or not, AMI is classified into ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI). STEMI, diagnosed by the presentation of acute chest pain and ST-segment elevation on ECG (or new left bundle branch block), usually represents an acutely obstructed epicardial coronary artery and transmural myocardial infarction. Once diagnosed or suspected, STEMI prompts immediate cardiac catheterization for emergency revascularization to re-open the obstructed coronary artery, and cardiac biomarkers are no involved in the diagnosis or decision making of suspicious STEMI. On the other hand, the presentation of NSTEMI usually comprises chest pain and various or even no ECG changes, and the diagnosis often depends on serial measurements of serum cardiac biomarkers to detect a rising or declining trend.[24]

Table 5. The clinical characteristics

Case	Age	Sex	Onset time to ER (Hr)	ER to cath (Hr)	DM	HT	HL	Smoking	BMI (m^2)	TCHO (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Initial Tn-T (ng/ml)	Glu (mg/dL)	Cre (mg/dL)	Peak CK (U/L)	Peak MB (U/L)	Cath to Peak time (Hr)	TIMI score	IRA	Lesions	Cardiogenic shock	LVEF (%)	Hosp. days
1	43	M	0.82	2.55	Yes	Yes	Yes	Yes	25.71	227	94	33	182	8190	121	0.77	11977	602	2.62	4	LAD	LAD	Yes	39	8
2	62	F	3.23	0.38	No	Yes	Yes	Yes	23.19	304	310	37	229	2701	0	0.79	1535	172	4.53	3	RCA	RCA, LAD, LCX	Yes	63	6
3	63	M	0.50	0.60	No	Yes	Yes	Yes	27.55	166	94	39	120	1362	121	1.13	1078	103	4.53	2	LAD	LAD	No	68	7
4	67	F	1.13	2.42	Yes	Yes	Yes	No	18.49	164	34	82	83	4163	266	0.52	2385	143	3.75	3	LAD	RCA, LAD, LCX	No	46	6
5	63	M	0.48	2.32	Yes	Yes	Yes	Yes	22.77	157	59	47	100	578	91	0.78	2699	125	6.62	5	LAD	LAD	No	68	5
6	57	M	2.78	0.92	No	No	No	Yes	20.94	192	61	45	144	1404	120	0.72	1317	104	11.72	2	LAD	LAD, LCX	No	62	6

AF, atrial fibrillation; BMI, body mass index; CK, creatine kinase; cre, creatinine; DM, diabetes mellitus; ER, emergency room; Glu, glucose; HL, hyperlipidemia; HT, hypertension; IABP, intraaortic balloon pumping; IRA, infarct-related artery; LAD, left anterior descending artery; LCX, left circumflex artery; LVEF, left ventricular ejection fraction; MB, creatine kinase-MB; RCA, right coronary artery; TCHO, total cholesterol; TG, triglyceride; VF; ventricular arrhythmia; VT, ventricular tachycardia.

Immunomagnetic reduction has been developed as a rapid and sensitive assay needing only a small amount of test sample with ultra-high sensitivity and specificity in assaying proteins, viruses, and chemicals. There are two main reasons for the high specificity of IMR. The first is that IMR detects the magnetic signal instead of the optical signal. Thus, color interference, which usually occurs due to the existence of hemoglobin, bilirubin, or lipids in serum and can induce significant variations in optical signals, is not an issue in IMR. The second is the suppression of non-specific binding between the molecules and magnetic nanoparticles. Once the molecule is associated with the antibody immobilized on a magnetic nanoparticle, it is acted on by a centrifugal force because the magnetic nanoparticle is oscillating under IMR measurement. The centrifugal force becomes stronger as the oscillation frequency increases. Whenever the centrifugal force is stronger than the binding force between the non-targeted molecules and antibody at higher oscillating frequencies, the association between these molecules and antibody is broken. The binding force between the targeted molecule and antibody is stronger than that of non-targeted molecules. Therefore, the centrifugal force can be adjusted to be between the binding forces of targeted molecules and non-targeted molecules. Subsequently, non-specific binding is eliminated and high specificity is achieved. Therefore, with its ultra-high sensitivity and specificity, IMR is a promising candidate to make an accurate in vitro diagnosis, and may be suitable to help make an early-stage diagnosis, disease monitoring and follow-up in different clinical scenarios.

In summary, IMR exhibits several unique factors as a detection tool. First, unbound bio-molecules that are not of interest and magnetic nanoparticles do not need to be removed and they can remain in the reagent. Therefore, the assay process of IMR is simple. Second, because only one kind of bioprobe is used, the time for measurement can be shortened. Third, IMR is a direct and homogeneous assay, which usually shows high reliability and sensitivity. Fourth, because the amount of reduction in χ_{ac} can be accurately measured to correspond to the concentration of the bio-molecules to be detected, the concentration of the bio-molecules can thus be measured quantitatively. Fifth, the IMR assay needs a very small amount of tested sample (< 1 mL), takes a shorter

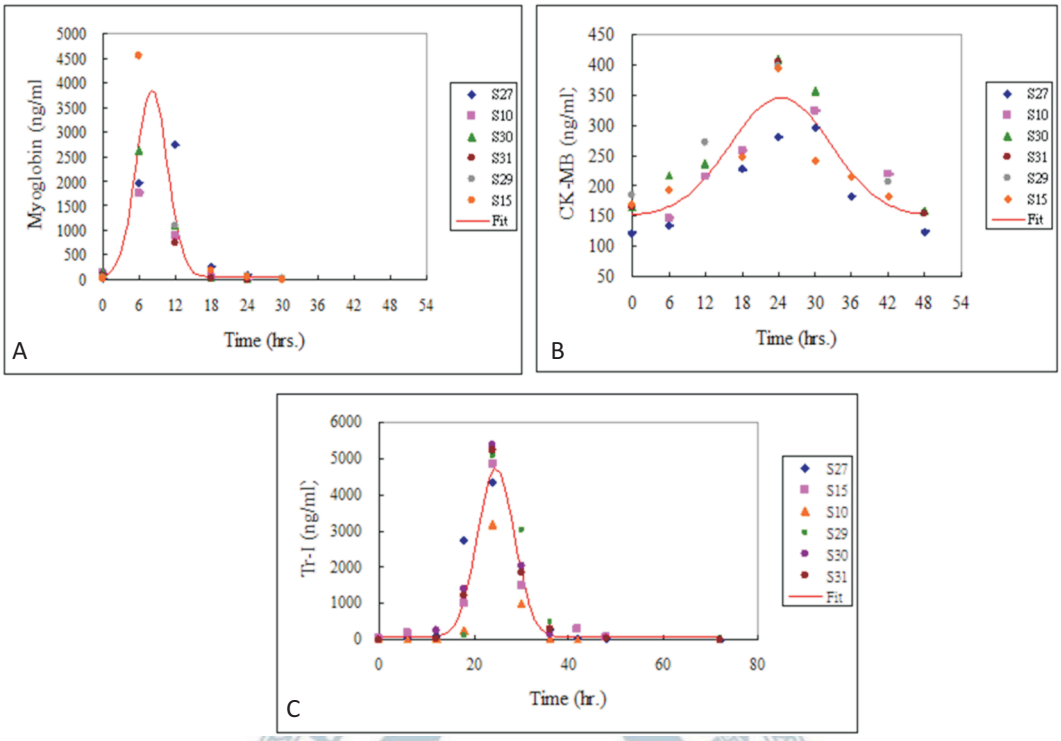

Figure 3. *The time courses and peak times of immunomagnetic reduction (IMR)-detected cardiac biomarker levels after acute myocardial infarction (AMI).*

time than the traditional method, requires less skill to perform, and can be done on-site. Therefore, it can serve as a rapid assay of cardiac biomarkers for the evaluation of acute chest pain at the ED. Ultrahigh sensitivity for tested biomarkers and a substantially shorter total measurement time in multi-channel platforms can be expected in the future. Our positive results should encourage the development of a clinical scale system, and we believe that IMR may serve as a powerful tool for clinical high-throughput screening.

Our preliminary clinical study demonstrated the fitting curve of cardiac biomarkers as a function of time after AMI. Myoglobin, as a fast-phase biomarker, peaked early at 8.2 hours, and CK-MB peaked at 24.4 hours. A concentration ratio of the two biomarkers (CK-MB/MyG) was calculated, and its curve as a function of time was plotted in Figure 4. We found that the CK-MB/MyG ratio remained < 2 in the early phase after AMI and increased at the later phase. The time course of CK-MB/MyG ratio can help estimate the onset time of AMI when the clinical presentation is ambiguous.

This study has several limitations. First, the interference tests involved hemoglobin, conjugated bilirubin, triglyceride, and cardiac biomarkers, but other potentially interfering substances exist in human blood. This should be tested with blood samples in future studies. Second, the fitting curves and peak hours of IMR-detected cardiac biomarker concentrations were obtained from a preliminary clinical study including six participants presenting with STEMI and undergoing primary PCIs, which are known to have an “early-peaking” effect on serial cardiac biomarker levels after AMI. Whether the IMR-measured CK-MB/MyG ratio can be used to evaluate the onset-time of NSTEMI or delayed presentation of STEMI without primary PCI is unknown and needs further investigations. In addition, cardiac troponin testing has been the standard of practice for the diagnosis of AMI, early rule-out, risk stratification, and outcome assessment in patients presenting with ACS and non-ACS myocardial injury. High-sensitivity cTn is used in a healthy (normal) reference population for determining the 99th percentile. However, we did not assay these three biomarkers in a normal population in this pilot methodology study, and further evidence of the effectiveness of the IMR tests may have been provided had we done so. Further validation is needed.

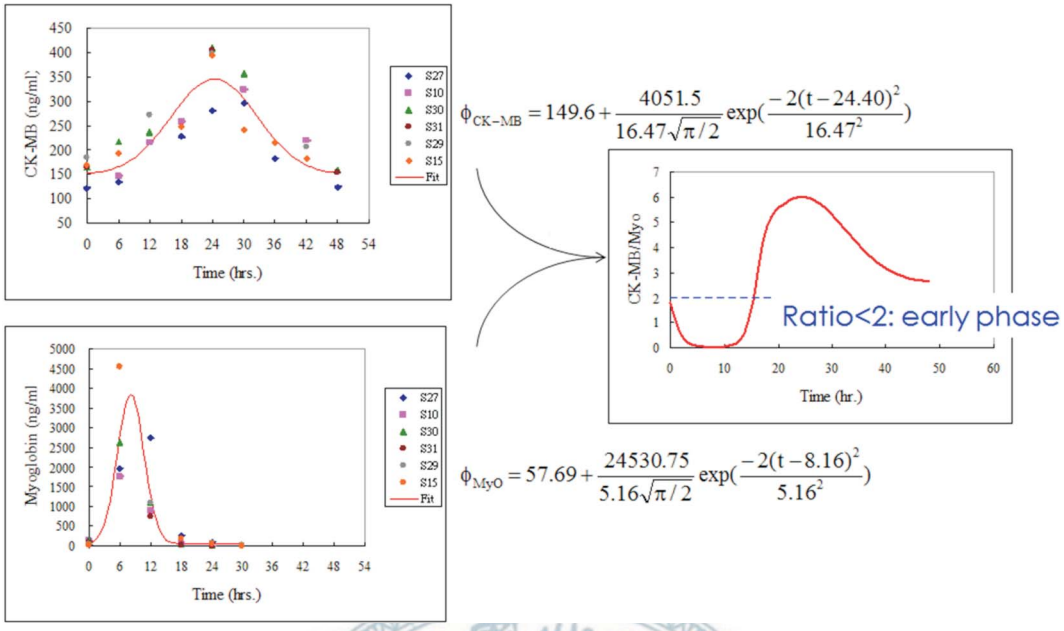

Figure 4. *The time course of creatine kinase-MB (CK-MB)/MyG ratio can help estimate the onset time of acute myocardial infarction (AMI).*

CONCLUSIONS

IMR assays for myoglobin, CK-MB, and cardiac troponin-I were developed and tested for measurement thresholds, dynamic ranges, interference materials, and reagent stability. The time course and peak of IMR-measured cardiac biomarkers after AMI were determined in clinical cases. IMR is an accurate, rapid and sensitive on-site assay for cardiac biomarkers and may help in diagnosing and evaluating suspicious myocardial infarction at the ED. Validation by further clinical trials is needed.

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CONFLICT OF INTEREST

All the authors declare no conflict of interest.

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