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Development and Validation of a New Method to Diagnose Apical Hypertrophic Cardiomyopathy By Gated Single-Photon Emission Computed Tomography Myocardial Perfusion Imaging

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Development and validation of a new method to diagnose apical hypertrophic cardiomyopathy by gated SPECT MPI

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Abstract

Aims: The aim of this study is to develop and validate a new method to diagnose apical hypertrophic cardiomyopathy (AHCM) by the integral quantitative analysis of myocardial perfusion and wall thickening from gated single-photon emission computed tomography (SPECT) myocardial perfusion imaging (MPI).

Methods and results: Twenty-two consecutive patients**,** who showed T wave inversion≥3mm in precordial leads and sinus rhythm in electrocardiogram, were enrolled. All the patients underwent cardiac magnetic resonance (CMR), gated rest SPECT MPI and echocardiography. According to CMR diagnostic results, 13 patients were categorized as in the AHCM group and the remaining 9 patients were categorized as in the non-AHCM group. Operators who were blinded to the CMR diagnosis independently performed the diagnosis by gated SPECT MPI. The regions of interest (ROIs) inside the apical hotspots on the perfusion polar map were drawn and the mean values of wall thickening in the drawn ROIs were calculated. Using MRI diagnosis as the gold standard, AHCM was diagnosed based on receiver operating characteristic (ROC) analysis of the mean wall thickening in the apical perfusion hotspot. The area under curve (AUC), sensitivity, specificity, and accuracy of our method were 0.97, 100%, 89%, and 95%, respectively.

Conclusions: Our new method has high sensitivity, specificity, and accuracy against CMR diagnosis. It has great promise to become a clinical tool in the diagnosis of AHCM.

Introduction

Echocardiography is commonly used to screen apical hypertrophic cardiomyopathy (AHCM). A spade-like appearance of the left ventricular (LV) cavity and a wall thickness ≥15 mm of the LV apex are considered the diagnostic hallmark of AHCM [1, 2]. However, a transthoracic echocardiogram may be difficult to diagnose AHCM because of the suboptimal acoustic windows. The multiplane transesophageal echocardiogram allows high-resolution imaging of the LV apex [3], but it causes discomfort to patients. Cardiac magnetic resonance imaging (CMR) has recently become the gold standard to diagnose AHCM, because it can acquire high-quality and reliable 3D images of the heart throughout the entire cardiac cycle [4-6]. However, the high medical cost, long scanning time and claustrophobia limit its wide clinical use.

Due to low resolution and inaccuracy to measure the thickness of hypertrophic LV myocardium, the number of studies using myocardial perfusion imaging (MPI) to diagnose AHCM is very limited. Visual analysis based on increased apical tracer uptake and a characteristic solar map pattern on single photon emission computed tomography (SPECT) MPI has been used to diagnose AHCM [7, 8]. However, certain AHCM patients didn't show this solar polar pattern [8]. Accordingly, these methods have limitations in the accuracy and reproducibility.

It has been demonstrated that fractional wall thickening is inversely correlated with myocardial wall thickness [9]. Gated SPECT MPI can quantitatively assess LV functional parameters with high reproducibility and repeatability and thus has the promise to diagnose AHCM [10-12]. The aim of this study is to develop and validate a new method to diagnose AHCM by the integral quantitative analysis of myocardial perfusion and wall thickening from gated SPECT MPI.

Methods

Patient enrollment

A total of twenty-two consecutive patients were enrolled in this study at Nanjing Medical University Hospital from September 2011 to October 2017. Inclusion criteria were: electrocardiogram (ECG) showed T wave inversion≥3mm in precordial leads and sinus rhythm. Exclusion criteria were: any of the coronary artery stenosis ≥ 50% by coronary angiography (CAG) or CT angiography (CTA), pericardial disease, congenital heart disease, hypertensive heart disease and congestive heart failure. All patients underwent CMR, gated rest SPECT MPI and echocardiography.

The study was approved by Institutional Ethical Committee of the First Affiliated Hospital of Nanjing Medical University, and all patients gave written informed consent.

CMR to diagnose AHCM

CMR scans were performed using a 3.0T scanner (Magnetom Trio; Siemens, Erlangen, Germany) with surface coils and electrocardiographic gated triggering. The MR cines of two-chamber view, three-chamber view and four-chamber view were acquired using retrospective ECG gating steady-state free precession (SSFP) sequence. LV short-axis images were acquired from apex to base to cover the entire LV volume. Repeated breath-holds were required in order to create adequate images. The main sequence parameters of CMR cine included: TR, 61.5 ms; TE, 1.5 ms; slice thickness, 8 mm; slice gap, 0; flip angle (FA), 50°; parallel acquisition technique factor, 2 and bandwidth, 930 Hz/PX. The temporal resolution was 25 frames per RR interval.

The diagnostic criteria in this study were the maximal wall thickness on the LV short-axis CMR image at the apex ≥12mm and the ratio of the maximal wall thickness on the LV short-axis CMR image at the apex to that of the base ≥1.3 [13].

SPECT MPI to diagnose AHCM

The nuclear cardiology specialists who were blinded to MRI diagnosis processed the MPI images and performed independent diagnosis. The gated rest SPECT MPI was performed around 60

minutes post injection of 20-30 mCi of Tc-99m sestamibi. SPECT planar images were acquired on a dual-headed camera (Philips Medical Systems, Milpitas, CA, USA) using a standard resting protocol. The imaging parameters were 20% energy window around 140KeV, 180^{o} orbit, 32 steps with 25 seconds per step, 8-bin gating and 64 projections per gate. The total acquisition time was 14 minutes for each patient. Image reconstruction and reorientation were completed with Emory Reconstruction Toolbox (ERToolbox; Atlanta, GA). SPECT images were reconstructed by ordered subset expectation maximization (OSEM) with 3 iterations and 10 subsets, and then filtered by a Butterworth low-pass filter with a cutoff frequency of 0.4 cycles/cm and an order of 10.

The resulting short-axis images, as illustrated in Figure 1a, were input into Emory Cardiac Toolbox (ECToolbox; Atlanta, GA) to measure myocardial perfusion, mechanical dyssynchrony and wall thickening [8-10]. Both LV myocardial perfusion and wall thickening were reported as polar maps, as demonstrated in Figure 1b and 1c, respectively. A region of interest (ROI) drawing software tool was developed for the operators to manually specify the myocardial perfusion hotspot within the LV apical region on the perfusion polar map, as illustrated in Figure 1d. The ROI shape could be irregular. Multiple ROIs might be drawn for a single patient if multiple hotspots were present. Once drawn, the mean value of wall thickening in the ROI was calculated for the same region on the corresponding wall thickening polar map, as demonstrated in Figure 1e. When multiple ROIs were drawn, only the ROI with the smallest mean wall thickening was used for statistical analysis.

Echocardiography to diagnose AHCM

Echocardiography was performed in a semi-recumbent position and different degrees of left lateral decubitus for each patient using the Vivid E9 ultrasound system (GE Health Care, Wauwatosa, WI, USA). The interventricular septal and posterior wall thickness were measured from the parasternal long-axis view at end diastole. Maximal apical wall thickness was measured from the apical 4-chamber and apical 2-chamber and apical short-axis view at end diastole. The transducer was placed as low and as lateral as possible from the LV apical impulse in order to

cover the maximal cavity section of the LV. Measurements were obtained from the apical endocardium to the visceral pericardium. The diagnostic criteria by echocardiography for AHCM was the same as the diagnostic criteria by CMR, i.e., the maximal wall thickness on the LV short-axis at the apical level ≥12mm and ratio of the maximal wall thickness on the LV short-axis at the apical level to that at the basal level≥1.3.

Statistical analysis

All statistical analyses were performed with SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Normal distributed continuous variables were expressed as mean ± SD. Using CMR as the gold standard, the receiver operating characteristic (ROC) analysis was applied into wall thickening measurements from gated SPECT MPI to diagnose AHCM. The Kappa value was calculated to observe the agreement. $P<0.05$ was considered to be statistically significant.

Results

Patient characteristics

All the 22 patients were divided into AHCM and non-AHCM groups according to the CMR results. Table 1 shows the baseline characteristics of these two groups. The age was 58.50±12.21 years and body mass index (BMI) was 24.79±2.24. For the general clinical tests, 12 patients had hypertension, 1 had diabetes, 6 had typical angina, 7 had atypical angina, and 3 had palpitation. 18 patients had T-waves inversion in lead V2-6, 9 had T-waves inversion in lead V3-4, 17 had ST segment depression, and 2 had ST segment elevation. For the LV mechanical dyssynchrony parameters by SPECT MPI, phase standard deviation (PSD) and phase bandwidth (PBW) were 10.32±3.49^{o} and 38.96±12.18^{o}, respectively. All these general variables did not show a significant differences between the AHCM and non-AHCM groups (all $P>0.05$).

Table 2 shows the LV myocardial viability, mechanical dyssynchrony, and wall thickening in ROIs between the AHCM and non-AHCM groups. There were no significant differences for myocardial perfusion, PSD, and PBW in ROIs between the two groups (all $P>0.05$). However, the wall thickening in the AHCM group was significantly smaller than that in the non-AHCM group ($P<0.01$).

Diagnosis of apical hypertrophic cardiomyopathy

Using MRI diagnosis as the gold standard, a ROC curve, shown in Figure 2, was generated for the mean values of the wall thickening in the perfusion hotspots. The cut-off value, AUC, sensitivity, specificity, and accuracy by SPECT MPI were 0.68, 0.97, 100%, 89%, and 95%, respectively. Only one patient who was in the non-AHCM group by CMR diagnosis was diagnosed by MPI to have ACHM, achieving a kappa value of 0.90 (95% confidence interval [CI]: 0.72 to 1.09).

The consistency between echocardiography and MRI was moderate (Kappa value, 0.538; 95% CI, 0.184-0.892). The sensitivity, specificity, and accuracy by echocardiography were 77%, 78%, and 77%, respectively.

Discussion

To our knowledge, this is the first study to diagnose AHCM using the integral quantitative analysis of myocardial perfusion and wall thickening from gated SPECT MPI. The main finding is that our new method has excellent accuracy.

Existing methods to diagnose AHCM by SPECT MPI

A significant number of AHCM patients have inversion T wave in electrocardiogram and the symptoms of coronary artery disease (CAD), and thus are referred to SPECT MPI. Due to low resolution and inaccuracy to measure the thickness of hypertrophic LV myocardium, the number of studies using MPI to diagnose AHCM is very limited. Cianciulli et al revealed that an

appearance of a significant increased apical tracer uptake, a spade-like LV deformity, and the solar polar map on SPECT MPI was considered AHCM [7]. Using echocardiography as the reference standard, they reported that the sensitivity and specificity of AHCM diagnosis by SPECT MPI were 75% and 100%. Ward et al [8] found that patients with AHCM had a characteristic solar polar map pattern caused by increased apical counts on rest MPI and a relative apical ischemia on the stress MPI. However, these two methods failed to explicitly explain why certain AHCM patients didn't show the solar polar pattern [4]. As a result, the diagnosis methods based on visual analysis of SPECT MPI have limitations in the accuracy and reproducibility.

Our new method to diagnose AHCM by gated SPECT MPI

Dong et al [9] demonstrated that weak wall motion with decreased thickening capability indicated regional hypertrophic myocardium [14-17], so fractional wall thickening was inversely correlated with myocardial wall thickness. Mechanical interference between myocardial cells, increased connective tissue content, and decreased active force generation might be three reasons to explain the inverse correlation between fractional wall thickening and wall thickness [9]. Therefore, besides showing hotspots on perfusion polar map due to increased counts [7, 8], AHCM patients show decreased wall thickening inside the apical segment on the wall thickening polar map. Accordingly, the integral quantitative analysis on myocardial perfusion and wall thickening from widely practiced gated SPECT MPI has great promise to provide a clinical tool to improve the diagnosis of AHCM.

Our new method has good accuracy. Using CMR as the gold standard, the sensitivity, specificity, and accuracy by our method were 100%, 89%, and 95%, respectively. Only one patient who was in the non-AHCM group by CMR was diagnosed to have AHCM by SPECT MPI. As shown in Figure 3, the decreased wall thickening is present in the perfusion hotspot ROI.

In addition, several points may be noted: 1) Multiple perfusion hotspots may be present in the

apical region of perfusion polar map for a single patient. Because regional wall thickening is inversely correlated with myocardial wall thickness and hypertrophic myocardial wall is located at the region with decreased wall thickening, only the ROI with the smallest mean wall thickening for each patient was input into the ROC analysis. 2) An interactive tool was provided to specify the ROIs of apical perfusion hotspot. Only ≥90% overall maximum perfusion level was considered the perfusion hotspot. It might be suggested that the ROIs be automatically extracted; however, in this pilot study, the interactive ROI specification provided significant flexibility. A new fully automatic tool may be developed in the following studies.

We excluded the patients with any of the coronary artery stenosis≥50% by coronary angiography (CAG) or CT angiography (CTA), so stress perfusion studies were not needed to differentiate patients with AHCM showing ischemic changes in ECG from those with ischemic heart disease.

Clinical implications

A previous study [7] demonstrated that AHCM is easily diagnosed as CAD by mistake due to the similar symptoms such as marked ECG abnormalities and with or without chest pain, so the suspected AHCM patients are usually referred to radionuclide scans. Our method by widely practiced SPECT MPI may improve the cost-effectiveness. Most importantly, compared with existing methods, our method has good accuracy and reproducibility.

Our method adds important new clinical values of cardiac functions to the diagnosis and prognosis of myocardial hypertrophy. While this study was targeted to apical hypertrophy, our method based on functional parameters from gated SPECT MPI may be generalized to detect myocardial hypertrophy at different locations and due to different reasons. LV hypertrophy may occur at only one to all LV segments [18] and myocardium may also thicken in normal individuals due to high blood pressure or prolonged athletic training. A recent study found that the apical morphological features of subjects with ECG abnormality were significantly different from normal subjects though

the absolute thickness of apical wall was below the current diagnostic criteria of AHCM [17]. Since gated SPECT MPI is widely performed and can assess myocardial viability, mechanical dyssynchrony, and wall motion and thickening, in a single scan, our method may improve risk stratification for mild to severe myocardial hypertrophy as well.

Study limitation

The technical accuracy of the new method was tested in a relatively small sample size because AHCM is relatively rare, it has a relatively benign prognosis in terms of cardiovascular mortality and all the enrolled patients in this study required both CMR and gated rest SPECT MPI. A prospective study in a larger population is needed to validate clinical usefulness of this method.

Conclusions

A new method based on the integral quantitative analysis of myocardial perfusion and wall thickening from gated SPECT MPI has been developed and validated to diagnose AHCM. It has high diagnostic sensitivity (100%), specificity (89%), and accuracy (95%) against CMR. The method has great promise to become a clinical tool in the diagnosis of AHCM.

Conflict of interest

All the authors have no conflicts of interest to disclose.

Funding

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Table 1. Baseline characteristics of all the enrolled patients.

Variables	All(n=22)	AHCM (n=13)	Non-AHCM (n=9)	*P* value
Age(years)	58.50±12.21	60.31±12.85	55.89±11.45	0.418
Male (n, %)	16	10	6	0.442
BMI	24.79±2.24	24.35±2.37	25.44±1.97	0.270
Hypertension	12	8	4	0.429
Diabetes	1	1	0	0.219
Typical angina	6	3	3	0.595
Atypical angina	7	4	3	0.899
Palpitation	3	1	2	0.329
T wave inversion	6.05±2.38	6.46±2.37	5.44±2.40	0.336
Inverted T wave in V2-V6	18	12	6	0.125
Inverted T wave in V3-V4	9	5	4	0.779
ST segment depression	17	10	7	0.784
ST segment elevation	2	1	1	0.962
PSD (degree)	10.32±3.49	10.77±2.44	9.66±4.72	0.474
PBW(degree)	38.96±12.18	40.46±8.66	36.78±16.37	0.499

Abbreviations: BMI, body mass index; PSD, phase standard deviation; PBW, phase bandwidth.

Table 2. Myocardial viability, dyssynchrony, and wall thickening in ROIs

Variables	All(n=22)	AHCM (n=13)	Non-AHCM (n=9)	P value
Perfusion in ROI	0.9646±0.01	0.9648±0.01	0.9644±0.01	0.92
PSD in ROI	4.18±3.60	4.74±3.53	3.38±3.76	0.40
PBW in ROI	16.00±8.60	18.46±9.33	12.44±6.25	0.11
Wall thickening in ROI	0.66±0.15	0.56±0.08	0.79±0.11	<0.01

Abbreviations: ROI, region of interest; PSD, phase standard deviation; PBW, phase bandwidth.

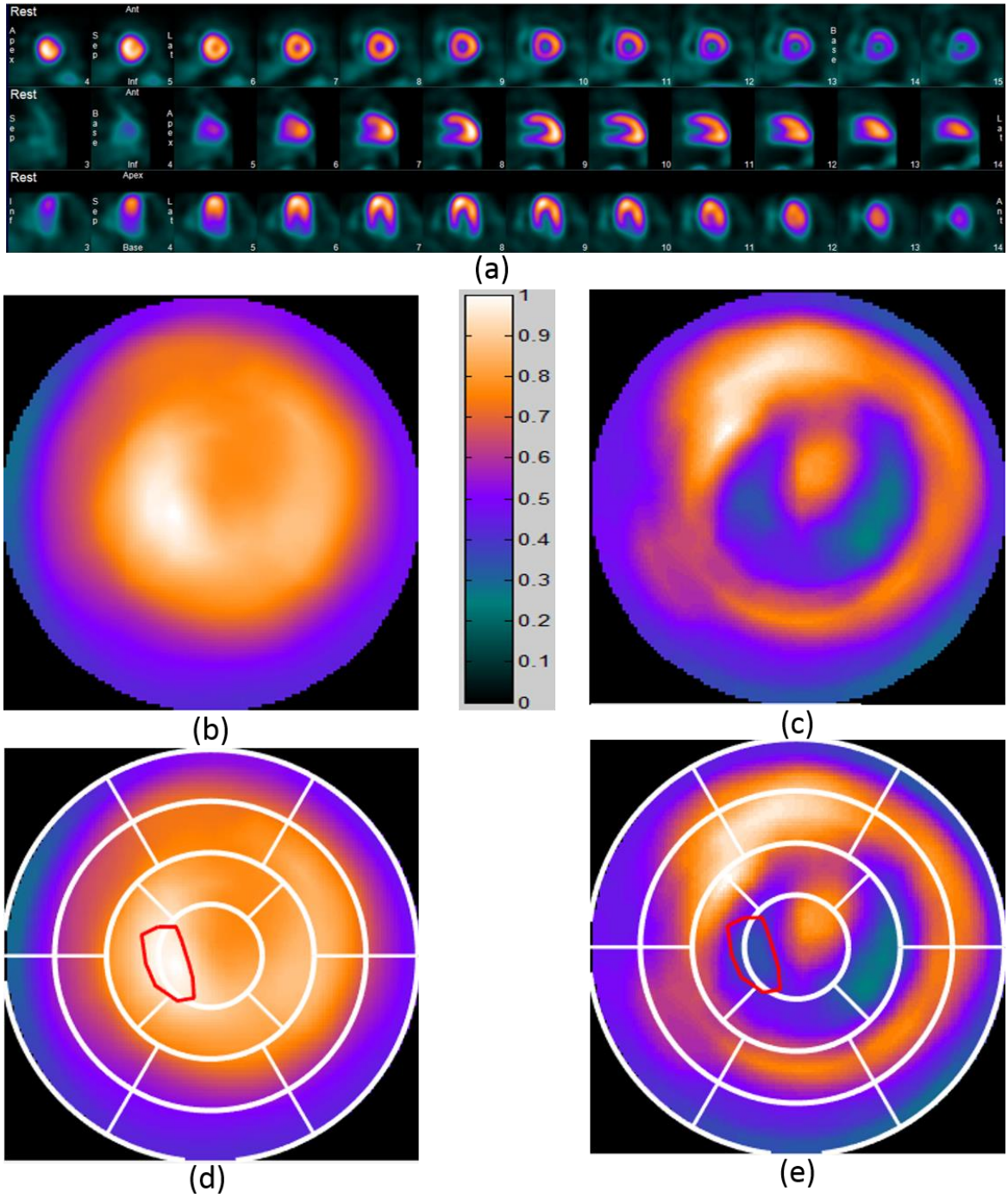

Figure 1. Image processing of the perfusion and wall thickening polar maps generated from gated SPECT MPI. (a) Left-ventricular short-axis and long-axis images. (b) Perfusion polar map. (c) Wall thickening polar map. The color scale is shown between (b) and (c) to differentiate the quantification level. (d) Region of interest (ROI) in the hotspot drawn in the apical region on the perfusion polar map. (e) ROI on the corresponding wall thickening polar map. During the detection of apical hypertrophic myocardium, the operator manually drew the ROI in perfusion hotspot as illustrated in (d) and the mean value of wall thickening was automatically calculated in the entire ROI as illustrated in (e).

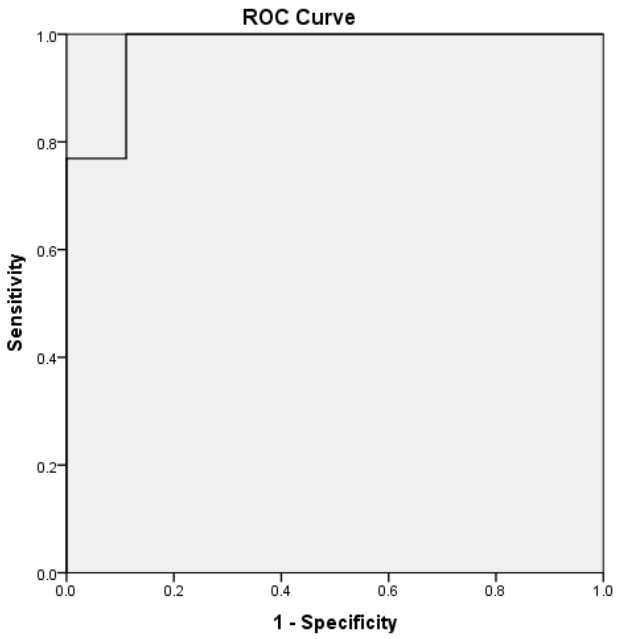

Figure 2. Receiver operating characteristic (ROC) analysis to diagnose apical hypertrophic cardiomyopathy by gated SPECT MPI. CMR was used as the gold standard. The area under curve (AUC), sensitivity, specificity, and accuracy of our method were 0.97, 100%, 89%, and 95%, respectively. ROC, receiver operating characteristic; SPECT, single-photon emission computed tomography; MPI, myocardial perfusion imaging; CMR, cardiac magnetic resonance imaging.

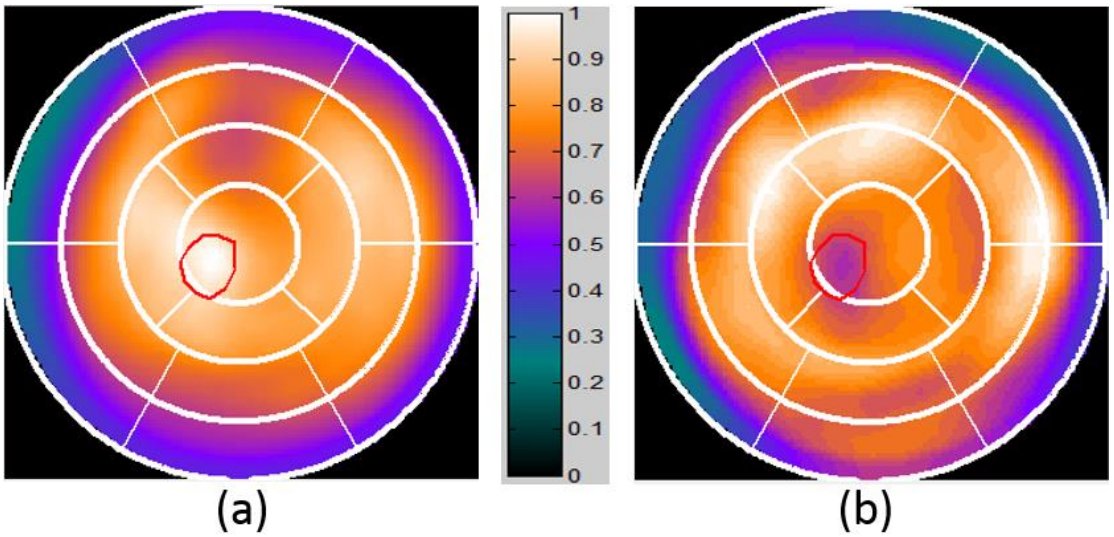

Figure 3. A patient who was in the non-AHCM group by CMR diagnosis was diagnosed to have AHCM by gated SPECT MPI. (a) Region of interest (ROI) in the hotspot drawn in the apical region on the SPECT perfusion polar map. (b) ROI on the corresponding wall thickening polar map. The deceased wall thickening can be observed in the ROI.

We sincerely appreciate the comments from the reviewers and editor. Our point-by-point responses are as follows.

Reviewer #1:

1. Have not mentioned about stress studies at all in the revised manuscript though said it was done in response

Re:

1) Our method requires only rest gated SPECT MPI. It has high sensitivity (100%), specificity (89%), and accuracy (95%) against CMR diagnosis. Accordingly, stress imaging is unnecessary in our study. Nevertheless, our future study may test whether stress SPECT MPI can improve the accuracy.
2) This is a retrospective study. All the enrolled patients received rest MPI but not all patients received both stress and rest MPI. In our last revision, we mentioned stress studies in our response to comments, but didn't mention in our manuscript. More importantly, our method using rest-only MPI has high accuracy.
3) Our current method using rest-only gated MPI may reduce the radiation dosage and increase the clinical applicability in the prospective study. It has great promise to become a clinical tool in the diagnosis of AHCM.

2. Authors have mentioned that they did two days protocol with large dose in rest studies as patients were with large body habitus, though their patients' cohort was less than 25 (24.79)

Re: A two-day imaging protocol is commonly performed in our center. Higher doses of the tracer are injected (20 to 30 mCi) both at rest and at peak stress in order to optimize myocardial count rate. Although Chinese patients do not have as large body habitus as western patients, Chinese doctors prefer 2-day protocol.

3. They have not responded to query no.7 "Integral quantitative analysis has not been described in the manuscript"

Re: We appreciate that the reviewer marked this point again. However,

1) The Query 7 in the last revision: 7. What about SPECT MPI perfusion finding - any defect in apical area would result in decreased counts in that area (secondary to myocardial infarction or stress induced apical defects). It would defeat the purpose of study.

   Re: In our study, myocardial infarction was excluded by CAG or CTCA. Although many of these patients underwent both rest and stress MPI, we only used rest MPI images, so it will not defeat our purpose.
2) Regarding the integral quantitative analysis, it is explained as follows in our method: A region of interest (ROI) drawing software tool was developed for the operators to manually specify the myocardial perfusion hotspot within the LV

apical region on the perfusion polar map, as illustrated in Figure 1d. The ROI shape could be irregular. Multiple ROIs might be drawn for a single patient if multiple hotspots were present. Once drawn, the mean value of wall thickening in the ROI was calculated for the same region on the corresponding wall thickening polar map, as demonstrated in Figure 1e. When multiple ROIs were drawn, only the ROI with the smallest mean wall thickening was used for statistical analysis

4. Query 10 has not been addressed and rewritten as such without making sense. The statement is either from Dong et al (9) or from 13-15). Need to correct. Similarly for query no. 11

Re: There was no query 10 or 11. After double check, we guess that this reviewer refers to Query 8.

The Query 8 in the last revision: 8. Reference no 13-15 have been quoted in the statement of Dong et al (ref 9) without their relevance, need to re-write the sentence.

Re: The sequence of reference has been corrected and the related modifications have been pasted below:

Dong et al [9] demonstrated that weak wall motion with decreased thickening capability indicated regional hypertrophic myocardium [13-15], so fractional wall thickening was inversely correlated with myocardial wall thickness.

The following is the last revision:

We sincerely appreciate the comments from the reviewers and editor. We have revised the manuscript accordingly.

Reviewer Comments:

Reviewer #1:
1. Echocardiography is still the best modality to pick up AHCM and suboptimal acoustic window may be an issue in only few patients. The patients in this cohort must have undergone echocardiography (reference standard as mentioned in manuscript also), inclusion of findings of echo in the manuscript would make manuscript better.

Re: All patients underwent echocardiography and we have added the method and the result of echocardiography in the manuscript (Page 5-7, the related modifications are pasted below). It is worth pointing out that the reproducibility of echocardiography is not very good.

Echocardiography to diagnose AHCM
Echocardiography was performed in a semi-recumbent position and different degrees of left lateral decubitus for each patient using the Vivid E9 ultrasound system (GE Health Care, Wauwatosa, WI, USA). The interventricular septal and posterior wall thickness were measured from the parasternal long-axis view at end diastole. Maximal apical wall thickness was measured from the apical 4-chamber and apical 2-chamber and apical short-axis view at end diastole. The transducer was placed as low and as lateral as possible from the LV apical impulse in order to cover the maximal cavity section of the left ventricle. Measurements were obtained from the apical endocardium to the visceral pericardial. The diagnostic criteria by echocardiography for AHCM was the same as the diagnostic criteria by CMR, i.e., the maximal wall thickness on the LV short-axis at the apical level ≥12mm and ratio of the maximal wall thickness on the LV short-axis at the apical level to that at the basal level≥1.3.

The consistency between echocardiography and MRI was moderate (Kappa value, 0.538; 95% CI, 0.184-0.892). The sensitivity, specificity, and accuracy by echocardiography were 77%, 78%, and 77%, respectively.

2. Patients in old age group might be having the coronary artery disease which may be discernible on stress MPI and that was not done here. That aspect needs

explanation, as authors have mentioned that the patients with CAD were excluded by angiography or CTCA. This itself exclude the need to do stress MPI. In these conditions: is there indication of doing MPI studies with radiation dose being delivered to patients and information is easily available with echo and CMR

Re: In China, CAG or CTCA is more commonly used than MPI to diagnose CAD. So most of the patients with T wave inversion had undergone angiography and CTCA before MPI. A few of them were scheduled to do both rest and stress MPI. If the CAD was excluded, the patient would be scheduled to do echo or CMR. In order to fulfill our study, all the patients enrolled in our study underwent rest MPI, echocardiography and CMR, and all of them signed informed consent.

3. Authors have mentioned about LV dyssynchrony showing global PSD and PBW values, however if feasible, it would have been better to have apical dyssynchrony values (regional) which might be different/higher in AHCM than non-AHCM due to hypertrophy rather than writing the global dyssynchrony values

Re: We investigated the global dyssynchrony in both groups and expected to see if there was a statistically significant difference between AHCM and Non-AHCM patients, so reported the global PSD/PBW values. According to the reviewer's suggestion, we have added Table 2 in the manuscript, including myocardial viability, dyssynchrony, and wall thickening parameters in the ROIs. There were no significant differences for myocardial perfusion, PSD, and PBW in ROIs between the two groups (all P>0.05). However, the wall thickening in the AHCM group was significantly smaller than that in the non-AHCM group (P<0.01).

Table 2. Myocardial viability, dyssynchrony, and wall thickening in ROI

Variables	All(n=22)	AHCM (n=13)	Non-AHCM (n=9)	*P* value
Perfusion in ROI	0.9646±0.01	0.9648±0.01	0.9644±0.01	0.92
PSD in ROI	4.18±3.60	4.74±3.53	3.38±3.76	0.40
PBW in ROI	16.00±8.60	18.46±9.33	12.44±6.25	0.11
Wall thickening in ROI	0.66±0.15	0.56±0.08	0.79±0.11	<0.01

Abbreviations: ROI, region of interest; PSD, phase standard deviation; PBW, phase bandwidth.

4. Only rest gated MPI study was performed and dose of tracer seems to on higher side, needs explanation

Re: There are three basic protocols with Tc 99m-labeled tracers: (1) a single-day study, in which myocardial blood flow is interrogated at rest and at peak stress, or in the reverse order, as long as the first injected dose is low (8 to 10 mCi) and the second injected dose is high (22 to 30 mCi); (2) a 2-day study, (commonly performed in patients with large body habitus) in which higher doses of the tracer are injected (20 to 30 mCi) both at rest and at peak stress in order to optimize myocardial count rate; and (3) a dual-isotope technique, which combines injection of thallium at rest followed by injection of a Tc 99m tracer at peak stress. In our hospital, we use a 2-day study, so the dose of tracer is higher than 1-day study.

5. How the ROI drawing software tool has been developed and integral quantitative analysis (a table with quantitative values is desirable) has not been described in the manuscript so it can be better understood by readers also.

Re: The software tool was developed by MATLAB and the related modification is pasted below. In addition, we added Table 2 in the manuscript which shows the quantitative analysis of myocardial perfusion, PSD, PBW, and wall thickening in ROIs.

A region of interest (ROI) drawing software tool was developed for the operators to manually specify the myocardial perfusion hotspot within the LV apex region on the perfusion polar map, as illustrated in Figure 1d. The ROI shape could be irregular. Multiple ROIs might be drawn for a single patient if multiple hotspots were present. Once drawn, the mean value of wall thickening in the ROI was calculated for the same region on the corresponding wall thickening polar map, as demonstrated in Figure 1e. When multiple ROIs were drawn, only the ROI with the smallest mean wall thickening was used for statistical analysis.

6. Age may be written with years in the manuscript.

Re: Age has been written with years (page 6).

7. What about SPECT MPI perfusion finding - any defect in apical area would result in decreased counts in that area (secondary to myocardial infarction or stress induced apical defects). It would defeat the purpose of study

Re: In our study, myocardial infarction was excluded by CAG or CTCA. Although many of these patients underwent both rest and stress MPI, we only used rest MPI images, so it will not deteat our purpose.

8. Reference no 13-15 have been quoted in the statement of Dong et al (ref 9) without their relevance, need to re-write the sentence.

Re: The sequence of reference has been corrected and the related modifications have been pasted below:
Dong et al [9] demonstrated that weak wall motion with decreased thickening capability indicated regional hypertrophic myocardium [13-15], so fractional wall thickening was inversely correlated with myocardial wall thickness.

9. Mean values for wall thickening in hot spot areas (in quantitative terms) have not been provided, which might have been better for understanding of readers.

Re: We have added the Table 2 in the manuscript to show the mean values for wall thickening in hot spot areas.

Reviewer #2:

1. Is T-wave inversion > 3mm adequate? Usually ~1 cm inversion seen in apical hypertrophic cardiomyopathy needs explanation and referencing for use of these cutoffs.

Re: The commonly used electrocardiogram finding in apical hypertrophic cardiomyopathy is giant negative T waves (GNT) (≥1 mV or 10 mm) in the left precordial leads. These GNT waves often change over time, or even disappear, as part of the natural history of AHCM. In our study, three patients had giant negative T waves (10 mm deep in leads V3-V6), whereas ten had lesser degrees of T-wave inversion (range, 3-9 mm).

2. Earlier in the introduction, it is noted that 15 mm is the characteristic wall thickness noted in apical hypertrophic cardiomyopathy , but diagnostic criteria notes that any thickness > 12 mm is considered apical HCM. This needs explanation and backing up with references, else it seriously effects the validity of the work.

Re: A wall thickness of ≥15 mm is considered the diagnostic hallmark of AHCM by some researchers. However, because the apex is the thinnest part of the LV, many studies suggested that apical wall thickness ≥12 mm but less than 15 mm, or the ratio of apical maximal thickness to basal posterior wall ≥1.3, represents early stage of AHCM, we added a reference about the diagnostic criteria of AHCM (reference 13 in the manuscript).

3. In my opinion there should a bit more detail provided about how potential false positive causes of reduced myocardial wall thickening may effect results and how the study deals with them

Re: Echocardiography and cardiac MRI pay more attention to the anatomical index, however, SPECT pays attention to the functional indicators. The reduced apical myocardial wall thickening may be an earlier signal for LV hypertrophy than the absolute wall thickness, especially for mild hypertrophy, which will remind the doctors to follow-up the patient with caution in order to diagnose AHCM as early as possible.

Points 1 & 2 in my opinion can potentially question the works validity/potential clinical applicability & potential patient selection thus need to be beyond doubt
Re: Please refer to our answers and the section *clinical implication* in our manuscript.