Native T1 in Discrimination of Acute and Convalescent Stages in Patients With Clinical Diagnosis of Myocarditis

A Proposed Diagnostic Algorithm Using CMR

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CME Objective for This Article: At the end of this activity the reader should be able to: 1) clarify the state-of art of CMR in clinically suspected myocarditis; 2) recognize the strengths and weaknesses of current Lake Louise criteria for the diagnosis of myocarditis; and 3) discuss the added value of the new proposed protocol based on T1 mapping for the diagnosis of myocarditis.

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PET/CT Imaging of Integrin $\alpha v\beta 3$ Expression in Human Carotid Atherosclerosis

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OBJECTIVES The goal of this study was to evaluate the feasibility of [18F]Galacto-RGD positron emission tomography (PET)/computed tomography (CT) imaging of $\alpha v\beta 3$ expression in human carotid plaques.

BACKGROUND The integrin $\alpha v\beta 3$ is expressed by macrophages and angiogenic endothelial cells in atherosclerotic lesions and thus is a marker of plaque inflammation and, potentially, of plaque vulnerability. [18F]Galacto-RGD is a PET tracer binding specifically to $\alpha v\beta 3$. Therefore, [18F]Galacto-RGD PET/CT imaging of $\alpha v\beta 3$ expression in human carotid plaques might provide a novel noninvasive biomarker of plaque vulnerability.

METHODS [18F]Galacto-RGD PET/CT imaging was performed in 10 patients with high-grade carotid artery stenosis scheduled for carotid endarterectomy. Tracer uptake was measured in the stenotic areas of the carotid arteries, as well as on the contralateral side, and was corrected for blood pool activity, measured in the distal common carotid artery (target-to-background [TB] ratio). TB ratio was correlated with immunohistochemistry of $\alpha v\beta 3$ expression (LM609), macrophage density (CD68), and microvessel density (CD31) of the surgical specimen. In addition, ex vivo autoradiography of the surgical specimen with [18F]Galacto-RGD and competition experiments with an unlabeled $\alpha v\beta 3$-specific RGD peptide were performed.

RESULTS [18F]Galacto-RGD PET/CT showed significantly higher TB ratios in stenotic areas compared with nonstenotic areas ($p = 0.01$). TB ratios correlated significantly with $\alpha v\beta 3$ expression ($R = 0.787$, $p = 0.026$) and intensity of ex vivo autoradiography ($R = 0.733$, $p = 0.038$). Binding to atherosclerotic plaques was efficiently blocked in ex vivo competition experiments. A weak-to-moderate correlation was found with macrophage density ($R = 0.367$, $p = 0.299$) and microvessel density ($R = 0.479$, $p = 0.176$), which did not reach statistical significance.

CONCLUSIONS [18F]Galacto-RGD PET/CT shows specific tracer accumulation in human atherosclerotic carotid plaques, which correlates with $\alpha v\beta 3$ expression. Based on these initial data, larger prospective studies are now warranted to evaluate the potential of molecular imaging of $\alpha v\beta 3$ expression for assessment of plaque inflammation in patients. (J Am Coll Cardiol Img 2014;7:178–87) © 2014 by the American College of Cardiology Foundation.
Stroke is a leading cause of long-term disability and the fourth leading cause of death in the United States (1). Atherosclerosis is the major reason for most clinical cardiovascular events such as stroke, and inflammation is an important feature of atherosclerotic plaque progression and vulnerability (2,3). More recently, intraplaque angiogenesis has also been implicated in rapid plaque growth and plaque rupture, which are of special relevance in carotid atherosclerotic lesions (4,5). Noninvasive imaging of inflammation and angiogenesis within atherosclerotic lesions may therefore be useful to predict future risk of plaque rupture and allow monitoring of antiatherosclerotic therapies, such as with magnetic resonance imaging (MRI) (6,7). However, despite the vast improvements in plaque imaging with MRI, radiotracer approaches might still provide useful additional information because they are more focused on quantifying specific biological processes. Moreover, MRI signals are much harder to quantify compared with radiotracer techniques such as positron emission tomography (PET). Thus, PET and MRI can provide synergistic information with respect to plaque imaging, and combining the data from each technique might even further improve our knowledge of plaque biology and vulnerability (8). This information may be of special relevance for use in the now clinically available hybrid PET/MRI scanners (9).

Multiple experimental and human studies have provided evidence that PET with the use of fluorine-18-fluorodeoxyglucose ([18F]FDG), a glucose analogue that is taken up by macrophages, could provide an index of inflammation in atherosclerotic lesions (10–12). In atherosclerotic lesions, both macrophages and activated endothelial cells can express high levels of the αvβ3 integrin, a cell surface glycoprotein receptor (13–16). Therefore, αvβ3 expression potentially is a combined marker of both inflammation and angiogenesis in atherosclerotic lesions. Imaging of αvβ3 integrin expression might thus be useful as a noninvasive in vivo surrogate parameter of plaque vulnerability (17,18). [18F]Galacto-RGD is a peptide tracer for PET imaging with highly specific binding to the αvβ3 integrin (19). Recently, specific uptake of the αvβ3-targeted tracers [68Ga]DOTA-RGD and [18F]Galacto-RGD has been shown in atherosclerotic plaques in the aorta of hypercholesterolemic mice (20,21). Moreover, [18F]Galacto-RGD uptake in these plaques could be reduced by diet intervention, demonstrating the potential of αvβ3 imaging for evaluation of therapy response (22). Clinically, [18F]Galacto-RGD PET has been successfully validated for imaging the level of αvβ3 expression in tumors (23–25). However, most tumor lesions in patients were substantially larger than atherosclerotic lesions, and the spatial resolution of clinical PET and PET/computed tomography (CT) imaging is limited (26). Thus, the potential of imaging αvβ3 expression in atherosclerotic lesions with [18F]Galacto-RGD PET in the clinical setting remains unknown.

In the present study, we evaluated the general technical feasibility of PET/CT imaging of αvβ3 expression in patients with carotid stenosis who were scheduled for endarterectomy. We correlated the in vivo uptake of [18F]Galacto-RGD in carotid plaques with histopathological data and in vitro autoradiography of the surgery specimen.

**METHODS**

**Patients.** Informed written consent was obtained from all patients. The ethics committee of our university approved the study protocol. Ten patients with high-grade carotid artery stenosis scheduled for carotid endarterectomy were examined within 2 weeks before the operation with [18F]Galacto-RGD PET/CT (mean age 68.5 ± 6.6 years; range 55 to 79 years). Inclusion criteria were carotid artery stenosis >70% in asymptomatic patients and carotid artery stenosis >50% in symptomatic patients. Further inclusion criteria were age >40 years and the ability to provide written and informed consent. Exclusion criteria were pregnancy, lactation period, and impaired renal function (serum creatinine level >1.2 mg/dl). All patients underwent MR angiography or CT angiography and clinical examination, including Doppler ultrasound of the carotid arteries, before operation.

**Radiopharmaceuticals.** Synthesis of the precursor and subsequent [18F]-labeling of Galacto-RGD was conducted as described previously (27).

**[18F]Galacto-RGD PET/CT imaging.** Imaging was performed with a Biograph Sensation 16 PET/CT (Siemens Medical Solution, Forchheim, Germany). Ninety minutes after injection of [18F]Galacto-RGD (188 ± 19 MBq), an emission scan was performed in 3-dimensional mode in the cranio-caudal direction from the floor of the mouth to the upper mediastinum (3-dimensional mode; 1 bed position, 15-min acquisition time) (Online Appendix).

**Image analysis.** The corrected emissions scans were calibrated to standardized uptake values (SUV).
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Results were expressed as percentage of uptake over blood pool (TB ratio in %).

Thromboendarterectomy and tissue sampling. The carotid plaque was removed by standard carotid thromboendarterectomy, excising the intimal part of the artery together with all plaque components. Tissue samples were immediately separated into segments of 3 to 4 mm, embedded in optimum cutting temperature compound (Tissue-Tek; Sakura, Zoeterwoude, the Netherlands), snap-frozen in liquid nitrogen, and stored at \(-70^\circ C\) until further analysis.

Autoradiography. In vitro binding of \( ^{18} \text{F} \)-galacto-RGD to the carotid plaques was studied by digital autoradiography of carotid thromboendarterectomy specimen tissue sections incubated with \( ^{18} \text{F} \)-galacto-RGD in the presence or absence of an excessive amount of competing tracer, as described earlier (28) (Online Appendix).

Histological analyses. Tissues samples embedded in optimum cutting temperature compound were sectioned by using a cryostat (Leica Microsystems, Wetzlar, Germany) and placed on electrically charged glass slides. Histological features of carotid plaques were studied in tissue sections stained with hematoxylin/eosin and elastin van Gieson to assess plaque structure and morphology, elastin, and collagen content. Stained samples were analyzed by using light microscopy. Macrophages, endothelial cells, smooth muscle cells, and integrin \( \alpha v \beta 3 \) were detected by immunostaining with the use of the following primary antibodies: anti-CD31 (clone JC70A, dilution 1:40; Dako, Hamburg, Germany), anti-CD68 (clone KP1, dilution 1:2,000; Dako), anti–smooth muscle actin (clone HHF35; dilution 1:200; Dako), and biotinylated goat anti–mouse anti-\( \alpha v \beta 3 \) (Chemicon, Schwalbach, Germany).

After primary antibody incubation, visualization was performed using either an APAAP ChemMate peroxidase/diaminobenzidine ChemMate Detection Kit (Dako). Biotinylated antibody against \( \alpha v \beta 3 \) was detected by a streptavidin–horseradish peroxidase–complex (Jackson Immunoresearch Laboratories, West Grove, Pennsylvania) and visualized with diaminobenzidine (Sigma-Aldrich, Munich, Germany). Unspecific primary immunoglobulin G antibodies were used as negative controls. Two experienced investigators blinded to the source of each specimen analyzed all images.

Statistical analysis. Signal intensities determined for the different regions are expressed as mean \( \pm \) SEM or in box-and-whisker plots. Differences between the different subgroups were evaluated by using a Mann–Whitney \( U \) test. For linear regression analysis, Spearman rank correlation coefficient \( r \) and the \( p \) value derived from a 2-tailed Student \( t \) distribution were computed. Univariate correlations were calculated using the Pearson correlation method. Statistical significance was assigned for \( p < 0.05 \). Computations were performed using MedCalc (MedCalc Software, Mariakerke, Belgium).

RESULTS

\( ^{18} \text{F} \)-Galacto-RGD PET/CT of atherosclerotic plaques. There were 4 sites of the carotid bifurcation or ICA without stenosis (grade 0), 4 with low-grade stenosis (grade 1), 2 with moderate stenosis (grade 2), 9 with severe stenosis (grade 3), and 1 near total occlusion (grade 4) as classified by using ultrasound and MR angiography.

In vivo PET/CT imaging demonstrated focal \( ^{18} \text{F} \)-Galacto-RGD uptake (>120% TB ratio) in 5 patients that co-localized with atherosclerotic lesions as seen on CT imaging (Fig. 1). Mean uptake of \( ^{18} \text{F} \)-Galacto-RGD was significantly higher in areas of the CCA or ICA with medium- or high-grade stenosis compared with areas with none/low-grade stenosis (\( p = 0.04 \)). Consistent with this finding, mean uptake of \( ^{18} \text{F} \)-Galacto-RGD was significantly higher in areas of the CCA or ICA with a flow velocity >150 cm/s as determined by Doppler ultrasound (Fig. 2).
However, in the group with medium- or high-grade stenosis (or higher flow velocity), there was a wide distribution of tracer uptake, also with no or little uptake in some stenotic areas.

**Correlation to histology.** As shown in Figure 3, histological correlates of $^{[18F]}$Galacto-RGD uptake were studied in serial tissue sections of the thromboendarterectomy specimen. CD31 as a panendothelial marker demonstrated a strong positive signal in the endothelial lining of the plaques and on the plaque neovasculature. CD68 as a marker for macrophages demonstrated macrophage infiltration...
in the plaques to a variable extent. Immunohistochemical staining for \( \alpha v \beta 3 \) demonstrated \( \alpha v \beta 3 \) expression on endothelial cells of intraplaque neovasculature, as well as macrophages. \(^{18}\text{F}\)Galacto-RGD uptake (TB ratio) correlated significantly with \( \alpha v \beta 3 \) expression \((R = 0.787, p = 0.026)\). There was a weak-to-moderate correlation with macrophage infiltration \((R = 0.367, p = 0.299)\), intraplaque neovasculature \((R = 0.479, p = 0.176)\), and total plaque cellularity \((R = 0.488, p = 0.168)\) that did not reach statistical significance \((R = 0.311, p = 0.380)\) or collagen content \((R = 0.026, p = 0.941)\). Due to the limited quality of our fresh frozen specimens, no systematic analysis of plaque composition was feasible.

**Binding of \(^{18}\text{F}\)Galacto-RGD to atherosclerotic plaques in vitro.** Binding of \(^{18}\text{F}\)Galacto-RGD in atherosclerotic plaques was studied by using ex vivo autoradiography of the thromboendarterectomy specimen and compared with in vivo tracer uptake and immunohistochemistry. Specificity of \(^{18}\text{F}\) Galacto-RGD binding in the atherosclerotic plaques was verified in a displacement study \((\text{Figs. 3B and 3C})\). \(^{18}\text{F}\)Galacto-RGD was found to bind to the atherosclerotic lesions to a variable extent \((\text{mean PSL/mm}^2 = 80.0 \pm 26.2)\), and the binding was efficiently and significantly reduced in the presence of an inhibitor, an unlabeled cyclic \( \alpha v \beta 3 \)-specific RGD peptide \((\text{mean PSL/mm}^2 = 10.4 \pm 3.1)\). The ratio of specific to nonspecific binding was approximately 8-fold \((7.9 \pm 2.0)\). In vitro \(^{18}\text{F}\) Galacto-RGD binding to the plaques correlated strongly and significantly with the score of \( \alpha v \beta 3 \) expression in the corresponding slices of the specimen \((R = 0.913, p = 0.010)\). Moreover, in vitro \(^{18}\text{F}\)Galacto-RGD binding to the plaques also correlated significantly with in vivo \(^{18}\text{F}\)Galacto-RGD uptake as measured by using PET/CT imaging \((R = 0.733, p = 0.038)\). These data suggest that in vivo uptake of \(^{18}\text{F}\)Galacto-RGD in PET/CT is specific and correlated with \( \alpha v \beta 3 \) expression in atherosclerotic lesions.

**Uptake of \(^{18}\text{F}\)Galacto-RGD in symptomatic and asymptomatic lesions.** According to the presence of patient symptoms probably caused by carotid stenosis, lesions were classified as either symptomatic \((n = 5)\) or asymptomatic \((n = 11)\). There was no
significant difference in $[^{18}F]$Galacto-RGD uptake in PET/CT imaging in symptomatic (mean TB ratio 21.3 ± 16.7%) or asymptomatic (mean TB ratio 17.8 ± 15.5%; $p = 0.70$) lesions (Fig. 5).

**DISCUSSION**

In this study, we demonstrate for the first time the feasibility of $[^{18}F]$Galacto-RGD PET/CT imaging to show focal increases in $\alpha_v\beta_3$ integrin expression in atherosclerotic plaques in patients with high-grade carotid stenosis. $[^{18}F]$Galacto-RGD uptake significantly correlated with intraplaque $\alpha_v\beta_3$ expression and autoradiography and could be specifically blocked in in vitro competition experiments. On the basis of these initial data, future studies on PET/CT imaging of $\alpha_v\beta_3$ integrin expression are now warranted to further evaluate its role in patients as a potential biomarker for inflammatory activity of atherosclerotic lesions.

$[^{18}F]$Galacto-RGD uptake in atherosclerotic plaques. $[^{18}F]$Galacto-RGD is a tracer that is based on the cyclic pentapeptide cyclo(-ARG-Gly-Asp-D-Phe-Val-) developed by Heckmann and Kessler (29). It shows high affinity and selectivity for $\alpha_v\beta_3$ integrin in vitro as well as receptor-type specific accumulation in vivo in $\alpha_v\beta_3$ integrin–positive tumors in mouse models and humans (18,23,27). Studies have indicated sufficient signal intensity for visualizing increased $\alpha_v\beta_3$ integrin expression in benign lesions such as infarcted myocardium (30,31). However, up to now, it was unknown whether $\alpha_v\beta_3$ expression in human carotid plaques is sufficient for imaging with $[^{18}F]$Galacto-RGD PET. In this study, we found that focal $[^{18}F]$Galacto-RGD uptake can be visualized in atherosclerotic lesions in patients by using PET/CT imaging. As expected, the mean uptake of $[^{18}F]$Galacto-RGD in atherosclerotic lesions was several folds lower than reported for $\alpha_v\beta_3$ integrin–expressing tumors (32–34). This can easily be explained by the small volume even of relatively large atherosclerotic plaques compared with most tumor lesions, which usually were $>20$ mm in diameter in the reported studies. Because the spatial

**Figure 4. Correlation of Tracer Uptake and Immunohistochemistry**

Linear regression analysis of $[^{18}F]$Galacto-RGD uptake in PET/CT measured as target-to-background (TB) ratio and (A) staining intensity of $\alpha_v\beta_3$ expression, (C) endothelial cells (CD31), and (D) macrophages (CD68). (B) Shows the correlation of in vivo tracer uptake with the intensity of autoradiography in vitro. Note that there is a significant correlation of in vivo tracer uptake and $\alpha_v\beta_3$ expression, as well as tracer uptake in vivo and in vitro autoradiography. Abbreviations as in Figure 1.
resolution of PET is limited, the so-called partial volume effect artificially reduces the measured uptake in lesions smaller than approximately 20 to 25 mm (35). However, despite the small size of atherosclerotic lesions, our results suggest that visualization of \( \alpha \beta \) integrin expression in human plaques is generally possible with PET/CT imaging by using \( \alpha \beta \)–specific tracers. It has to be noted, however, that in our small patient population, the correlation was relatively loose, so up to now it has not been clear to what extent tracer uptake and \( \alpha \beta \) expression correlate linearly. This is also somewhat related to the relatively low signal achieved. To improve uptake and TB ratios, tracer optimization by multimerization has been shown to increase signal intensity from \( \alpha \beta \) integrin–expressing tissues (36). Moreover, sophisticated algorithms for partial volume correction might improve the results from PET and PET/CT imaging (37).

**Immunohistochemical correlate of \( [18F] \)Galacto-RGD uptake.** Histological and immunohistochemical analysis demonstrated that uptake of \( [18F] \)Galacto-RGD significantly correlated with \( \alpha \beta \) expression in the plaques, which was mainly expressed on macrophages as well as neovascularure. \( [18F] \)Galacto-RGD uptake did not show a correlation with intraplaque elastin, collagen, or cellularity. A weak-to-moderate correlation was found with macrophage density and neovascularization, which did not reach statistical significance. The low number of samples in the study might, in part, have caused this, as various preclinical studies have shown a correlation of \( \alpha \beta \) integrin–targeted RGD peptides with intraplaque macrophage density and/or neovascularization (20,38). However, mice models often show no substantial intraplaque neovascularization, limiting direct comparability with human atherosclerotic lesions (39–41). In preclinical MRI studies, enhancement of atherosclerotic vessel wall by \( \alpha \beta \) integrin–targeted nanoparticles was mainly explained by the presence of angiogenic vessels around the intima (42). However, MRI nanoparticles remain mainly intravascular due to their larger size compared with small peptide PET tracers; thus, they do not target intraplaque macrophages, limiting direct comparability to our results.

**In vitro autoradiography.** In vitro autoradiography of the thromboendarterectomy specimen with \( [18F] \) Galacto-RGD was performed because the specificity of tracer uptake can be demonstrated with blocking studies by pre-incubating with excess of unlabeled target specific ligands. Moreover, the

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**Figure 5. Correlation of Tracer Uptake and Patient Symptoms**

Acute ipsilateral cortical ischemia in a patient with intense \( [18F] \)Galacto-RGD uptake in a vulnerable carotid plaque. (A) shows the plaque of the patient with the high-grade stenosis of the left ICA in Figure 1 in a T2-weighted axial MRI slice (arrow, open tip). The MRI-PET image fusion (B), which is zoomed in on the stenotic left ICA seen in A, shows the tracer uptake in the plaque, which corresponds to the pattern of tracer uptake in autoradiography in Figure 3B (arrow, open tip; lumen, asterisk). This patient had an acute small ischemia as demonstrated by diffusion-weighted MRI (in the ipsilateral left parietal cortex [arrow; C, b1000 image; D, apparent diffusion coefficient map], proving that the plaque was vulnerable. However, when analyzing all patients for symptomatic or asymptomatic stenosis, there was no significant difference notable between groups (E). Abbreviations as in Figures 1 and 3.
pattern of tracer distribution can be compared with immunohistochemistry.

Indeed, $^{[18\text{F}]}$Galacto-RGD uptake could efficiently and significantly be blocked by an unlabeled $\alpha\beta 3$-specific cyclic RGD peptide in vitro. Moreover, in vivo uptake of $^{[18\text{F}]}$Galacto-RGD measured by using PET/CT correlated significantly with in vitro autoradiography, which again correlated strongly and significantly with $\alpha\beta 3$ expression in the respective slices used for autoradiography. The more moderate correlation of in vivo $^{[18\text{F}]}$Galacto-RGD uptake and $\alpha\beta 3$ expression compared with in vitro autoradiography and $\alpha\beta 3$ expression might be explained by factors additionally influencing measured tracer uptake in PET/CT imaging in patients (e.g., lesion size, partial volume effects, tracer access to the intraplaque structures) (43).

The data strongly suggest that in vivo uptake of $^{[18\text{F}]}$Galacto-RGD measured by using PET/CT is specific and allows for at least semi-quantitative noninvasive assessment of the level of $\alpha\beta 3$ expression in atherosclerotic lesions.

**Comparison to other imaging modalities.** PET with $^{[18\text{F}]}$FDG is currently the best-characterized radiotracer approach for imaging plaque inflammation (44). Compared with $^{[18\text{F}]}$FDG PET, TB ratios in our study were in the range or only slightly lower compared with reported data (11,12). Moreover, a recent preclinical study compared $^{[18\text{F}]}$Galacto-RGD uptake with $^3\text{H}$-DG, an in vitro analogue of $^{[18\text{F}]}$FDG, and uptake ratios between plaque and normal vessel wall were comparable for both tracers (20). Compared with $^{[18\text{F}]}$FDG, which is only taken up by intraplaque macrophages, $^{[18\text{F}]}$Galacto-RGD targets both macrophages and intraplaque neovasculature, which have both been implicated in progression and rupture of atherosclerotic lesions. Moreover, $\alpha\beta 3$ integrin may be directly involved in the degradation of the protective fibrous cap of atherosclerotic lesions because it has been identified as a binding moiety that localizes protease activity, particularly matrix metalloproteinase 2, to the cell surface (45,46). Therefore, $^{[18\text{F}]}$Galacto-RGD PET/CT visualizes several important features of plaque instability simultaneously, which might potentially be advantageous compared with $^{[18\text{F}]}$FDG (47).

In terms of imaging of $\alpha\beta 3$ expression with MRI, the higher resolution of MRI combined with excellent soft tissue contrast is an advantage compared with PET (42,48). However, in MRI, larger absolute amounts of probes usually have to be used compared with PET. Thus, adverse effects of most PET tracers are limited or not present at all. This facilitates translation of results of PET tracers into the clinical setting, as was successfully demonstrated for $^{[18\text{F}]}$Galacto-RGD in this study.

**Study limitations.** Because it was uncertain whether a reliable PET signal could be achieved in patients with atherosclerotic lesions, the present study was deliberately intended as a feasibility study with a limited number of patients. However, even in this small sample, a significant correlation of $^{[18\text{F}]}$Galacto-RGD uptake and $\alpha\beta 3$ expression as well as autoradiography could be demonstrated. It is beyond the scope of this study to clarify in detail the ultimate clinical value of PET/CT imaging of $\alpha\beta 3$ expression for identifying patients with vulnerable plaques. The value of such imaging must be addressed by future larger, prospective studies.

Moreover, due to the unavailability of $\alpha\beta 3$ antibody for formalin-fixed and paraffin-embedded samples, we were forced to use fresh frozen tissue for immunohistochemical analyses, which generally (especially regarding atherosclerotic tissue) result in markedly lower histological quality. Thus, assessment of plaque stability according to histopathology was not possible due to the limited quality of the specimen, which is an important topic to be clarified in future studies. However, macrophage content and neovascularization could be assessed, which are also considered to be crucial factors for plaque vulnerability.

**Conclusions**

We demonstrated that $^{[18\text{F}]}$Galacto-RGD PET/CT specifically visualizes elevated integrin $\alpha\beta 3$ expression in human carotid plaques. On the basis of these promising preliminary results, further prospective studies must now be conducted to define the clinical value of PET/CT or even PET/MR imaging of $\alpha\beta 3$ expression for assessment of plaque vulnerability and patient prognosis concerning development of symptomatic stenosis.

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REFERENCES


Key Words: biomarker  carotid stenosis  imaging  molecular imaging  plaque.

APPENDIX
For an expanded Methods section, please see the online version of this article.
Native T1 Mapping in Transthyretin Amyloidosis

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OBJECTIVES The aims of the study were to explore the ability of native myocardial T1 mapping by cardiac magnetic resonance to: 1) detect cardiac involvement in patients with transthyretin amyloidosis (ATTR amyloidosis); 2) track the cardiac amyloid burden; and 3) detect early disease.

BACKGROUND ATTR amyloidosis is an underdiagnosed cause of heart failure, with no truly quantitative test. In cardiac immunoglobulin light-chain amyloidosis (AL amyloidosis), T1 has high diagnostic accuracy and tracks disease. Here, the diagnostic role of native T1 mapping in the other key type of cardiac amyloid, ATTR amyloidosis, is assessed.

METHODS A total of 3 groups were studied: ATTR amyloid patients (n = 85; 70 males, age 73 ± 10 years); healthy individuals with transthyretin mutations in whom standard cardiac investigations were normal (n = 8; 3 males, age 47 ± 6 years); and AL amyloid patients (n = 79; 55 males, age 62 ± 10 years). These were compared with 52 healthy volunteers and 46 patients with hypertrophic cardiomyopathy (HCM). All underwent T1 mapping (shortened modified look-locker inversion recovery); ATTR patients and mutation carriers also underwent cardiac 3,3-diphosphono-1,2-propanodicarboxylicacid (DPD) scintigraphy.

RESULTS T1 was elevated in ATTR patients compared with HCM and normal subjects (1,097 ± 43 ms vs. 1,026 ± 64 ms vs. 967 ± 34 ms, respectively; both p < 0.0001). In established cardiac ATTR amyloidosis, T1 elevation was not as high as in AL amyloidosis (AL 1,130 ± 68 ms; p = 0.01). Diagnostic performance was similar for AL and ATTR amyloid (vs. HCM: AL area under the curve 0.84 [95% confidence interval: 0.76 to 0.92]; ATTR area under the curve 0.85 [95% confidence interval: 0.77 to 0.92]; p < 0.0001). T1 tracked cardiac amyloid burden as determined semiquantitatively by DPD scintigraphy (p < 0.0001). T1 was not elevated in mutation carriers (952 ± 35 ms) but was in isolated DPD grade 1 (n = 9, 1,037 ± 60 ms; p = 0.001).

CONCLUSIONS Native myocardial T1 mapping detects cardiac ATTR amyloid with similar diagnostic performance and disease tracking to AL amyloid, but with lower maximal T1 elevation, and appears to be an early disease marker.
Native T1 in Discrimination of Acute and Convalescent Stages in Patients With Clinical Diagnosis of Myocarditis
A Proposed Diagnostic Algorithm Using CMR

ABSTRACT

OBJECTIVES This study investigated whether T1 mapping by cardiac magnetic resonance (CMR) reflects the clinical evolution of disease in myocarditis and supports its diagnosis independently of the disease stages.

BACKGROUND Acute viral myocarditis is characterized by a range of intracellular changes due to viral replication and extracellular spill of debris within days of viral infection. Convalescence may be characterized by a chronic low-grade inflammation leading to ventricular remodelling, but also a complete resolution of myocardial changes.

METHODS Patients with clinical diagnosis of viral myocarditis (N = 165) underwent routine clinical CMR protocol (1.5- and 3.0-T) for assessment of cardiac function and structure, and tissue characterization with T2-weighted imaging and late gadolinium enhancement. T1 mapping was obtained in a mid-ventricular short-axis slice before and >20 min after administration of 0.2 mmol/kg of gadobutrol.

RESULTS Compared with control subjects (n = 40), T1 indexes were increased in patients with myocarditis. Patients with acute symptoms (n = 61) had higher values of T1 indexes compared with patients in clinical convalescence (n = 67). Native T1 is an independent discriminator between health and disease, as well as a discriminator between acute and convalescent stage of the disease. Native T1 was superior to T2-weighted imaging and late gadolinium enhancement with high diagnostic accuracy and positive and negative predictive values. Using pre-defined cutoff values for normal ranges, we demonstrated that acute myocarditis can be independently identified by native T1 of >5 SD above the mean of normal range, whereas convalescence is best defined by either abnormal native T1 (>2 SD) or presence of late gadolinium enhancement. We prospectively tested a new diagnostic algorithm in an independent dataset of patients with clinical diagnosis of myocarditis and achieved similar diagnostic performance.

CONCLUSIONS The new diagnostic algorithm using native T1 can reliably discriminate between health and disease and determine the clinical disease stage in patients with a clinical diagnosis of myocarditis. (J Am Coll Cardiol Img 2015;8:37–46) © 2015 by the American College of Cardiology Foundation.

Myocarditis is an important cause of cardiac morbidity and mortality (1-6). Characterized by a wide spectrum of myocardial changes and nonspecific clinical presentation, cardiac disease can be challenging to detect, and clinical management pathways can be uncertain. Myocardial tissue changes include intracellular and interstitial edema and inflammatory infiltration within days of viral infection, followed by chronic low-grade inflammation, myocardial remodeling, and fibrosis but also complete resolution, marking the stages of acute disease and clinical convalescence, respectively (1,2). Whether accurate diagnosis improves the clinical course or facilitates treatment remains unclear.

Tissue characterization by cardiac magnetic resonance (CMR) is increasingly used when myocarditis is suspected clinically. Current diagnostic recommendations on the basis of Lake Louise criteria (LLC) rely on a combination of T1-weighted (relative enhancement ratio), T2-weighted imaging (edema ratio >1.9, regional increase in T2 signal intensity [SI]), and late gadolinium enhancement (LGE) imaging, whereby the positive findings of 2 of 3 imaging techniques confirm the diagnosis (7). These recommendations are on the basis of the concept that either the inflammatory involvement of the myocardium (edema, hyperemia, and capillary leakage) and/or myocardial necrosis/replacement fibrosis can be visualized and a suspected diagnosis confirmed (6-12) (Figure 1).
However, in the absence of positive findings, the LLC do not allow a reliable exclusion of disease (7,9,13). Furthermore, current means of differentiation between acute and convalescent myocarditis rely on detection of inflammatory components mainly by increased T2 signal and evidence of normalization after clinical resolution on a follow-up study (9,14-19). Patients commonly undergo CMR imaging outside of their acute period and without an opportunity for a serial follow-up. Recent evidence suggests that novel approaches on the basis of T1 mapping provide an improved detection of myocardial edema in acute myocarditis (20,21) and subclinical low-grade myocardial inflammation (22) compared with conventional T2 imaging and of diffuse myocardial fibrosis in ventricular remodeling (23) compared with LGE. We examined whether myocardial T1 mapping may potentially discriminate between health and disease, as well as determine the stage of disease (i.e., between acute myocardial inflammation and convalescent disease).

**METHODS**

Consecutive patients presenting with a clinical diagnosis of viral myocarditis were recruited to this study. Clinical diagnosis of viral myocarditis was established in all cases by the presence of cardiac symptoms in the course of a recent flu-like illness, documented electrocardiogram changes, rise in inflammatory marker and/or troponin levels, and exclusion of significant coronary artery disease (4,5). Characteristics were recorded for all patients, including age, sex, body mass index, and presence of traditional cardiovascular risk factors. Symptoms at presentation, systolic/diastolic blood pressure, troponin leak, and C-reactive protein levels were recorded. Endomyocardial biopsy was not routinely used for confirmation of disease (3,17,19). Patients with various stages of disease presented for CMR imaging; we classified the condition of those presenting with active symptoms and increased levels of serological markers early in the course of disease (typically via inpatient CMR referrals, within a week after the onset of symptoms) as acute myocarditis (n = 61). Patients who were no longer symptomatic and had serological marker levels within the normal range represented the group of convalescent myocarditis (n = 67; commonly as outpatient CMR referrals from district peripheral hospitals). There was no patient overlap between the 2 groups. An additional 37 patients with the active symptomatic stage of disease underwent 2 serial CMR scans and a follow-up study after clinical resolution and served as a comparison cohort for the studied groups of independent patients. An independent cohort of 52 patients fulfilling the above inclusion criteria (acute myocarditis, n = 24; convalescent myocarditis, n = 28) were sourced from the International T1 Multicentre CMR Study for prospective validation of the diagnostic algorithm (King’s College London, United Kingdom, n = 23; St. Vincent’s University Hospital, Sydney, Australia, n = 29). Forty healthy subjects with no clinical or serological evidence of systemic inflammation or permanent use of anti-inflammatory medication (e.g., aspirin, nonsteroidal anti-inflammatory drugs, corticosteroids, antihistamines) and normal findings in CMR imaging served as controls.

**FIGURE 1 Acute Myocarditis**

Pink arrows indicate the presence of late gadolinium enhancement (LGE) or increased T2 signal. Pericardial effusion (yellow arrows) in Patient #3. ER = edema ratio.
Exclusion criteria for all subjects were the generally accepted contraindications to CMR (e.g., implantable devices, cerebral aneurysm clips, cochlear implants, severe claustrophobia) or a history of renal disease with a current estimated glomerular filtration rate <30 ml/min/1.73 m². The study protocol was reviewed and approved by institutional ethics committees, and written informed consent was obtained from all participants.

**CARDIAC MAGNETIC RESONANCE.** All subjects underwent a routine clinical protocol for volumes and mass and tissue characterization with T2-weighted imaging and LGE using 1.5- or 3.0-T CMR imaging scanners equipped with advanced cardiac package and multitransmit technology (Achieva, Philips Healthcare, Best, the Netherlands) (12). T1 mapping was performed in a single mid-ventricular short-axis slice before contrast administration and scar imaging, respectively (22–24). All routine CMR analyses were performed on commercially available software (Circle CVI 42, Calgary, Canada). T1 measurements and indexes were obtained as reported previously (22–25). Details of CMR sequence parameters and image post-processing are included in the Online Appendix.

**STATISTICAL ANALYSIS.** Statistical analysis was performed using SPSS software (version 21.0, SPSS, Chicago, Illinois). Normality of distributions was tested with the Kolmogorov-Smirnov statistic. Categorical data are expressed as percentages and

### TABLE 1 CMR Findings in the Study Population

<table>
<thead>
<tr>
<th>Functional parameters</th>
<th>Controls (n = 40)</th>
<th>Acute Myocarditis (n = 61)</th>
<th>Convalescent Myocarditis (n = 67)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV-EDV index, ml/m²</td>
<td>74 ± 12</td>
<td>94 ± 35*</td>
<td>86 ± 25</td>
<td>0.001</td>
</tr>
<tr>
<td>LV-ESV index, ml/m²</td>
<td>30 ± 8</td>
<td>51 ± 33*</td>
<td>41 ± 25</td>
<td>0.001</td>
</tr>
<tr>
<td>LV mass index, ml/m²</td>
<td>50 ± 14</td>
<td>70 ± 21*</td>
<td>60 ± 16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>61 ± 5</td>
<td>49 ± 15*</td>
<td>55 ± 11*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RV ejection fraction, %</td>
<td>57 ± 8</td>
<td>53 ± 13</td>
<td>57 ± 10</td>
<td>0.12</td>
</tr>
<tr>
<td>Tissue characterization</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pericardial effusion</td>
<td>0 (0)</td>
<td>17 (28)</td>
<td>9 (13)</td>
<td>0.001</td>
</tr>
<tr>
<td>T2 edema ratio</td>
<td>1.3 (1.1–1.6)</td>
<td>2.3 (1.5–3.5)*</td>
<td>1.4 (1.1–2.3)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Increased T2 SI</td>
<td>0 (0)</td>
<td>38 (62)*</td>
<td>8 (12)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Myocardium LGE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>0 (0)</td>
<td>51 (84)</td>
<td>59 (88)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nonischemic pattern</td>
<td>0 (0)</td>
<td>51 (84)</td>
<td>59 (88)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pericardial enhancement</td>
<td>0 (0)</td>
<td>18 (29)*</td>
<td>5 (7)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T1 mapping</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native T1, ms</td>
<td>940 ± 20</td>
<td>1,064 ± 37*</td>
<td>995 ± 19*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1.5-T</td>
<td>1,045 ± 23</td>
<td>1,189 ± 52*</td>
<td>1,099 ± 22*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Post-contrast T1, ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5-T</td>
<td>422 ± 68</td>
<td>373 ± 42*</td>
<td>383 ± 43*</td>
<td>0.03</td>
</tr>
<tr>
<td>3.0-T</td>
<td>422 ± 68</td>
<td>397 ± 62</td>
<td>426 ± 73</td>
<td>0.06</td>
</tr>
<tr>
<td>Lambda, %</td>
<td>42 ± 4</td>
<td>50 ± 7*</td>
<td>46 ± 9</td>
<td>0.005</td>
</tr>
<tr>
<td>1.5-T</td>
<td>44 ± 5</td>
<td>53 ± 8*</td>
<td>45 ± 8*</td>
<td>0.002</td>
</tr>
<tr>
<td>3.0-T</td>
<td>44 ± 5</td>
<td>53 ± 8*</td>
<td>45 ± 8*</td>
<td>0.002</td>
</tr>
<tr>
<td>Abnormal native T1, n (%)</td>
<td>0 (0)</td>
<td>60 (98)*</td>
<td>47 (76)*</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n (%), or median (range). *Significant differences between patients with myocarditis compared with control subjects. †Between the disease groups.

**FIGURE 2** Comparison of Independent Cohorts for Native T1 Values

Native T1 values were increased in both acute and convalescent myocarditis at both field strengths compared with controls. Post-hoc tests revealed significantly higher native T1 in acute versus convalescent myocardium.
continuous variables as mean ± SD or median (interquartile range), as appropriate. For comparison of 2 and more than 2 normally distributed variables, Student t test, 1-way analysis of variance (with Bonferroni post-hoc test) for continuous variables and chi-square test for categorical variables were used, as appropriate. Correlations were assessed using Pearson correlation coefficient for normally distributed variables and Spearman correlation coefficient for nonparametric data. Associations were explored by single and multivariate linear regressions. Binary logistic regression analyses were used to test for discrimination between the presence or absence of myocarditis, as well as between acute disease and chronic convalescence. Cutoff values for separate field strengths were defined on the basis of the previously derived normal ranges for native T1 as 2 SD higher than the mean (1.5-T: 950 ± 21 ms; 3.0-T: 1,052 ± 23 ms; i.e., 992 ms at 1.5-T and 1,098 ms at 3.0-T, respectively) (26). All tests were 2 tailed and a p value of <0.05 was considered significant.

RESULTS

Patients with acute myocarditis underwent CMR study within a median of 5 days (range 7 days), whereas patients in the convalescent group presented on average 6 months (range 2 months) after onset of the symptoms. Detailed clinical characteristics of the study cohort are included in the Online Appendix.

Compared with control patients, patients with acute myocarditis had significantly raised cardiac volumes and mass (p < 0.01 for all) (Table 1). Global systolic function was significantly reduced in both patient groups (p < 0.01). Groups were similar compared between field strengths (1.5-T vs. 3.0-T: control patients n = 18 vs. n = 22; acute myocarditis n = 23 vs. n = 38; convalescent myocarditis n = 33 vs. n = 34; p > 0.05).

In the acute myocarditis group, patients exhibited visually detectable increases in T2 signal and had a high T2 edema ratio (Table 1). Myocardial LGE in the epicardial and mid-myocardial nonischemic pattern of distribution was present in 84% of patients with acute myocarditis and 88% of patients with convalescent myocarditis, respectively (p = 0.36). None of the patients showed ischemic-type LGE. A total of 22 patients, 10 in the acute stage and 12 in the convalescent stage showed no LGE or increased T2 SI.

Native T1 values were increased in patients at both field strengths (F = 66 and F = 119 for 1.5-T and 3.0-T, respectively; p < 0.001) (Table 1). Native T1 was significantly higher in acute myocarditis compared with convalescent myocarditis at both field strengths (p < 0.001) (Figure 2). Compared with controls, lambda was significantly higher in acute myocarditis (p < 0.01). In convalescent myocarditis, lambda was not significantly different than that in controls. Native T1 values were significantly higher in patients with LGE and increased T2 SI (p < 0.001 for both) in acute myocarditis. In convalescent

![Figure 3: Reconstructed Time Course of Native T1 Values as a Function of Time Since Onset of Symptoms](image)

Comparison of independent patients in study cohort with a cohort of patients with serial scans. The fitting lines are approximate and for illustration only.

**TABLE 2 Diagnostic Accuracy of CMR Parameters Using a Single or Combined Approach: Acute Myocarditis**

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Diagnostic Accuracy, %</th>
<th>PPV, %</th>
<th>NPV, %</th>
<th>AUC</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single approach</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased T2 signal</td>
<td>56</td>
<td>94</td>
<td>70</td>
<td>95</td>
<td>55</td>
<td>0.751</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LGE</td>
<td>72</td>
<td>100</td>
<td>86</td>
<td>100</td>
<td>79</td>
<td>0.892</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Native T1</td>
<td>98</td>
<td>100</td>
<td>99</td>
<td>100</td>
<td>99</td>
<td>1.000</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Combined approach</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>T2 and LGE</td>
<td>54</td>
<td>100</td>
<td>68</td>
<td>100</td>
<td>48</td>
<td>0.742</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Native T1 and LGE</td>
<td>73</td>
<td>100</td>
<td>87</td>
<td>100</td>
<td>79</td>
<td>0.894</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Native T1 and T2 and LGE</td>
<td>54</td>
<td>100</td>
<td>68</td>
<td>100</td>
<td>48</td>
<td>0.742</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Diagnostic accuracy of CMR parameters for discrimination between control subjects and acute myocarditis using native T1 as a categorical variable, on the basis of a predefined cutoff value (>2 SD higher than the mean in the healthy subjects: 992 ms at 1.5-T and 1,098 ms at 3.0-T, respectively) (27). The diagnostic accuracy of several combinations of tissue criteria (2 or 3 criteria positive): LGE = T2-weighted imaging versus LGE = T2-weighted imaging + native T1 for all myocarditis and acute and convalescent myocarditis separately. AUC = area under the curve; other abbreviations as in Table 1.
myocarditis, native T1 values were increased in the areas of LGE (native T1; neighboring LGE-negative segments vs. LGE-positive segments, mean difference: 1.5-T \(82\pm 19\) ms, \(p<0.01\); 3.0-T \(102\pm 18\) ms, \(p<0.01\)).

Details for the independent testing cohorts of patients are included in the Online Appendix. Thirty-seven patients presenting for CMR at the onset of symptoms (median 3 days, range 7 days) underwent a follow-up study (median 6 months, range 163 days). Of these, 19 patients showed increased T2 SI at initial presentation, whereas 22 had present LGE. Compared with patients with acute myocarditis, native T1 values were significantly lower in the follow-up scan but still higher compared with those of control patients (Online Appendix).

**DISCRIMINATION BETWEEN HEALTH AND DISEASE AND STAGES OF DISEASE.** Binary logistic regression revealed that native T1 is an independent discriminator between control subjects and patients with myocarditis, as well as between patients with acute myocarditis and convalescent myocarditis at both field strengths (Online Appendix). Tables 2 and 3 summarize the comparative diagnostic accuracy of CMR parameters for discrimination between control subjects and convalescent myocarditis using native T1 as a categorical variable, on the basis of a predefined cutoff value (\(\geq 2\) SD higher than the mean in the healthy subjects): 1,000 ms at 1.5-T and 1,106 ms at 3.0-T, respectively (27). The diagnostic accuracy of several combinations of tissue criteria (2 or 3 criteria positive): LGE + T2-weighted imaging versus LGE + T2-weighted imaging + native T1 for all myocarditis and acute and convalescent myocarditis separately.

AUC = area under the curve; other abbreviations as in Table 1.

**FIGURE 4** Diagnostic Accuracy of Discrimination Between Health and Disease Using Native T1, T2-Weighted, and LGE Imaging

(A) All patients with myocarditis versus controls. (B) Acute myocarditis versus controls. (C) Convalescent myocarditis versus controls. Native T1 was marginally better than LGE in discrimination between health and all patients with myocarditis (A). Rigorous separation between active disease and clinical convalescence revealed an insightful phenotypical signature by considerably higher native T1 values in acute disease (B), contrasting the much lower or even "normalized" values in convalescence (C). Abbreviation as in Figure 1.
CMR using a single or combined approach (native T1, T2, LGE) to detect acute myocarditis or convalescent myocarditis, respectively. To simplify the comparisons with T2 and LGE being categorical values (present/absent), which are field strength independent, native T1 was also transformed into a binary categorical variable (normal/abnormal) on the basis of the predefined cutoff value of 2 SD higher than the mean of the normal range per given field strength (26). Native T1 was able to discriminate between health and disease in all patients with a clinical diagnosis of myocarditis (Figure 4A), as well as its separate disease stages (Figures 4B and 4C) with high negative predictive value (NPV). In the acute setting, abnormal native T1 alone resulted in the highest diagnostic accuracy. In fact, diagnostic accuracy of native T1 was reduced when it was used in a combined approach requiring both positive LGE and abnormal native T1. On the contrary, in the convalescent stage, a native T1 and/or LGE approach held the highest diagnostic accuracy. The addition of increased T2 SI to any of the combined approaches did not improve the diagnostic accuracy.

**NOVEL DIAGNOSTIC ALGORITHM AND PROSPECTIVE TESTING IN AN INDEPENDENT COHORT.** We developed a novel diagnostic algorithm (native T1 of >5 SD for acute myocarditis, and native T1 >2 SD or LGE for convalescent stage, higher than the mean of the normal range per given field strength) (26) (Figure 5). This algorithm resulted in diagnostic accuracy of 96% for acute myocarditis (sensitivity 96% and specificity 100%; positive predictive value [PPV] 100%; NPV 97%) with 4% misclassification due to 1 patient not achieving native T1 of >5 SD. In convalescent myocarditis, diagnostic accuracy was 97% (sensitivity 93% and specificity 100%; PPV 100%; NPV 94%) with 3% misclassification due to 2 patients not achieving native T1 of >2 SD or having a positive LGE. Prospective testing of this algorithm in an independent cohort of patients fulfilling identical inclusion criteria for groups of disease (n = 52; acute myocarditis, n = 24, convalescent myocarditis, n = 28, control subjects, n = 30) achieved similar diagnostic performance.

**DISCUSSION**

We demonstrated that native T1 values are significantly increased in patients with a clinical diagnosis of myocarditis. We further showed that native T1 values were higher in acute myocarditis compared with convalescent myocarditis. Our findings revealed a steady decline in native T1 values from acute disease to chronic convalescence. We also demonstrated that native T1 is an independent discriminator between health and disease, as well as a discriminator between acute and convalescent stages of the disease. Acute myocarditis can be independently identified by native T1 of >5 SD higher than the mean of the normal range, whereas convalescence is best defined by either abnormal native T1 (>2 SD) or presence of LGE.

The commonly nonspecific clinical symptoms and limited time window of laboratory markers pose a significant difficulty in early identification of patients, and the symptomatic phase of disease can be consumed in the collateral diagnostic workup. Patients with acute myocarditis frequently travel along the standardized management pathways of acute coronary syndrome (27), whereby a finding of unobstructed coronary arteries provides an indication to myocarditis. CMR is thus commonly ordered late in the diagnostic cascade (28,29). Increasingly, CMR imaging contributes early diagnostic clarity, previously not thought possible without a formal tissue diagnosis (6). Even though LLC provide a diagnostic pathway, the individual components of the criteria suffer from technical limitations. Early changes observed during acute myocarditis, such as expansion of intracellular compartments due to viral
replication, are difficult to detect with LGE, which specifically targets the extracellular space. Similarly, appreciation of increased T2 SI relies on the regional differences. Edema ratio is on the basis of relative comparison of signal to skeletal muscle, which can be also affected, thus resulting in a pseudo-normalized value. Relative enhancement is technically limited by low reproducibility and susceptibility to artifacts (12). Contribution of relative enhancement to diagnostic accuracy of detecting myocarditis on the basis of 2 relatively small studies (9,10) was subsequently shown to be of little relevance; in a prospective study applying LLC, the combination of T2 and/or LGE resulted in a higher diagnostic accuracy and higher NPV in comparison with an “any 2 of 3” approach (13).

As such, the Society for Cardiovascular Magnetic Resonance recommendations classify relative enhancement ratio as “optional” in the myocarditis imaging protocol (30); therefore, it was not used in the present study.

Our findings support previous reports showing that imaging readouts reflect the phenotypical expression of the complex pathophysiology and underlying myocardial inflammation following viral infection (1–6,17–19). Acute myocarditis is classically defined by widespread intracellular changes related to viral replication, and an extracellular spill of debris within days of viral infection, followed by an acute inflammatory response and autoimmune reactions. Convalescence or healing is characterized by contained disease with regional scarring or a chronic syndrome of prolonged low-grade inflammation, both of which can result in myocardial remodeling, leading to heart failure. Commonly, however, there is a complete resolution of changes (1,2). Consistent with previous findings, LGE findings are similarly prevalent at the acute presentation and convalescence (Figure 5), and increased T2 signal is more common in patients with acute myocarditis (8,9,18–20). We further demonstrated that native and post-contrast T1 myocardial values, as well as the hybrid derivatives, were increased in patients with acute myocarditis compared with control subjects at both field strengths. Our findings confirm previous studies using T1 mapping in myocardial inflammation showing the higher diagnostic performance of native T1 compared with conventional LGE and T2-weighted imaging in patients with clinical diagnosis of myocarditis (19–21) (Figure 4A).

We further showed that by a rigorous separation between the stages of active inflammation and convalescence, native T1 can provide an insightful phenotypical signature by considerably higher values in acute disease (Figure 4B), contrasting the much lower or even “normalized” values in convalescence (Figure 4C). Native T1 is also the only T1 mapping index that can differentiate between health and disease independently of the disease stage. Both findings suggest that native T1 approximates the myocardial pathology in the presence of active myocarditis and subsequent low-grade inflammatory involvement (1,2,20–22).

Owing to these new insights, we provide a new concept whereby native T1 serves as a diagnostic clinical application for confirmation/exclusion of myocarditis independently of the stage of disease. We propose that acute myocarditis can be best differentiated by the “markedly” increased native T1 values (>5 SD higher than normal range), which can be separated from the “abnormal” native T1 (>2 SD higher than normal range) (Figure 5).

The findings of the present study allow us to indicate possible pathophysiological routes in the chronic disease. A finding of an abnormal native T1 is congruent with persisting low-grade inflammation and ventricular remodeling. Next, a finding of normal T1 and positive LGE suggests that the initially diffuse and widespread disease process of inflammation has turned into a contained process marked by myocardial scarring. This pathway is reflected in the lower diagnostic accuracy on the basis of abnormal native T1 alone in convalescent myocarditis (88%) (Tables 2 and 3); this is improved by using a combined approach with LGE (94%). Finally, a complete resolution of myocardial changes may be reflected in the misclassification rate of 6%. A combination of tools that target intracellular, interstitial, and regional disease in a complementary algorithm using abnormal native T1 and/or LGE may be able to follow the pathophysiological complexity and characterize the phenotype of the convalescent myocardium.

Results of the present study suggest that native T1 mapping may offer a novel approach to detect and grade myocardial inflammation, as well as potentially allow the detection of patients at risk of developing heart failure. A quantifiable measure of acute injury, which is distinctively different in the convalescent stage of disease, may be useful to determine the disease stage when residual myocardial injury can be objectively assessed and the ensuing prognosis potentially anticipated. So far, a few studies have attempted to clarify whether and what treatment might reduce the severity of changes, as well as potentially reduce the rate of remodeling as a sequela to the viral myocarditis, with mixed results (1–4). Improved characterization of the stages of disease may help to support initiation of treatments that target the underlying pathophysiology.
STUDY LIMITATIONS. Even though the majority of the patients in the original cohort study underwent CMR at a single time point, the separation between the acute and convalescent group was tested against an independent sample of patients with serial scans. A higher rate of positive troponin may reflect a referral bias for clinical CMR but also an inadvertent inclusion of more severe cases. The novel diagnostic algorithm has been prospectively tested in an independent, predominantly external dataset, which was acquired using identical imaging parameters and setups, allowing the use of the unified normal ranges of native T1 values. Currently, particular setups of available T1 mapping methods are site and vendor specific, and accuracy and precision of T1 measurements may vary between CMR systems and sequences; therefore, for application of this diagnostic algorithm, it is necessary for sites to have established their own local normal ranges. Cross correlations with biopsy tissue samples would lend further support to our findings.

CONCLUSIONS

We demonstrated that native T1 values can reliably discriminate between health and disease, as well as provide insight into the stage of disease in patients with a clinical diagnosis of myocarditis. Native T1 values may emerge as a novel approach to monitor inflammatory myocardial injuries as a dynamic index of disease activity and progression.

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REFERENCES


KEY WORDS cardiac magnetic resonance, myocarditis, native T1, T1 mapping

APPENDIX For a supplemental Methods section as well as tables, please see the online version of this article.

Go to http://cme.jaccjournals.org to take the CME quiz for this article.
Two types of amyloidosis typically infiltrate the ventricular myocardium: immunoglobulin light-chain (AL, or primary systemic) type and transthyretin (ATTR) type. ATTR encompasses senile systemic amyloidosis, in which wild-type transthyretin (TTR) is deposited as amyloid, and hereditary forms, in which genetically variant forms of TTR are implicated. Some TTR variants affect mainly the heart (familial amyloid cardiomyopathy); others mainly the peripheral and autonomic nervous systems in addition to the heart (familial amyloid polyneuropathy). Cardiac ATTR amyloidosis is a progressive and often fatal disorder that may be greatly underdiagnosed and is certainly an underappreciated cause of heart failure in the elderly and specific ethnic populations. ATTR amyloid deposits are present in 8% to 16% of hearts at autopsy in those over age 80 years (1). One particular TTR variant, V122I, which confers susceptibility to amyloid cardiomyopathy, has a population prevalence of 3% to 4% in Afro-Caribbeans (2) and a 10% frequency among individuals of this ethnicity who present with heart failure (3).

Diagnosing cardiac ATTR amyloidosis is often challenging. A suggestive constellation of electrocardiography (ECG), echocardiography, and biomarker findings are found mainly in advanced disease, but interpretation may be confounded by common comorbidities such as left ventricular hypertrophy (LVH), diabetes, diastolic dysfunction, and renal disease (4–6). The challenging diagnosis and lack of validated quantitative investigations to monitor the course of the disease pose unique problems for ATTR amyloidosis are emerging (7).

New imaging modalities are, however, showing promise. Technetium-labeled bone scintigraphy tracers, notably 3,3-diphosphono-1,2-propanodicarboxylic acid (DPD), localize strikingly to hearts infiltrated by ATTR amyloid (8), whereas cardiac magnetic resonance (CMR) late gadolinium enhancement (LGE) produces a characteristic appearance (9). However, neither is truly quantitative, and both have limitations (10).

Recently, native myocardial T1 mapping has been shown in cardiac AL amyloid to track disease (11). Here, we assess this test in patients with ATTR amyloidosis. We hypothesized that the native myocardial T1 would be elevated in this disease, that elevation would correlate with other disease markers (e.g., intensity of DPD uptake), and that T1 elevation would be an early marker of disease.

**METHODS**

**Amyloidosis patients.** Subjects were recruited at the National Amyloidosis Centre, Royal Free Hospital, London, United Kingdom, from 2010 to 2013. A total of 172 individuals were categorized into 3 groups.

**ATTR AMYLOID PATIENTS.** Eighty-five consecutive, consenting patients with cardiac ATTR amyloidosis (70 male; age 73 ± 10 years) were recruited. The presence of cardiac amyloid was defined by presence of ATTR amyloid in a myocardial biopsy or positive DPD scintigraphy. A total of 82% (n = 70) had histological proof of ATTR amyloidosis by Congo red and immunohistochemical staining of myocardial (n = 30, 35%) or other tissues (n = 40, 47%). All patients underwent sequencing of exons 2, 3, and 4 of the TTR gene. No consensus criteria exist for definite cardiac involvement in ATTR amyloid patients, and in this study, definite cardiac ATTR amyloid was defined as: 1) cardiac biopsy showing ATTR amyloid; 2) noncardiac biopsy showing ATTR amyloid in association with LV/right ventricular (RV) thickening in the absence of other explanatory causes; 3) intense DPD uptake in heart (grade 2 or 3 as defined by Perugini et al. [8]) in the absence of a plasma cell dyscrasia; or 4) noncardiac biopsy showing presence of ATTR amyloid and LGE consistent with cardiac amyloid. In practice,
all had apparent LVH. Possible cardiac involvement was defined by minimal cardiac DPD uptake (grade 1 as defined by Perugini et al. [8]) in the absence of LVH; in practice, none of these patients had LVH.

**TTR GENE CARRIERS.** There were 8 TTR gene carriers (n = 8; 3 male, age 47 ± 6 years), defined as individuals with pathogenic TTR gene mutation but no evidence of disease (no cardiac uptake on technetium-99m [99mTc]-DPD scintigraphy and normal echocardiography, CMR, N-terminal pro-brain natriuretic peptide [NT-proBNP], and troponin T). This group constituted the noncardiac involvement group.

**AL AMYLOID PATIENTS.** There were 79 patients with systemic AL amyloid (55 male; age 62 ± 10 years), as proven with biopsies from the myocardium (n = 6, 8%) or other tissues (n = 73, 92%). Cardiac categorization was based on international consensus criteria (12) but with an additional “possible involvement” category. Categorization was defined as: 1) definite cardiac involvement: LV wall thickness of >12 mm in the absence of any other known cause or RV free wall thickening coexisting with LV thickening in the absence of systemic or pulmonary hypertension; 2) possible cardiac involvement: LV wall thickening in the presence of hypertension; RV thickening in the presence of pulmonary hypertension or normal wall thickness with diastolic dysfunction and raised serum biomarkers; and 3) no suspected involvement: normal wall thickness with normal serum biomarkers.

These 3 groups were compared with 46 patients with hypertrophic cardiomyopathy (HCM) and 52 healthy volunteers.

**HCM patients.** There were 46 patients with HCM (n = 46; age 50 ± 13 years, 34 male) fulfilling diagnostic criteria (13). A total of 72% of patients had an asymmetrical septal hypertrophy pattern (the remainder had apical predominant hypertrophy); 60% had LV outflow tract obstruction; and 76% were found to have LGE in a variety of locations, such as at the RV insertion points or the LV apex.

**Healthy volunteers.** A total of 52 healthy volunteers (n = 52; age 46 ± 15 years, 17 male) were recruited through advertising in hospitals, universities, and general practitioner surgeries. All had no history or symptoms of cardiovascular disease or diabetes mellitus, and all had normal 12-lead ECG and normal CMR scan. No patient was on cardioactive medication, except for 4 patients on statins for primary prevention.

All patients and healthy controls underwent 12-lead ECG. Cardiac amyloid patients/carriers additionally underwent assays of cardiac biomarkers (NT-proBNP and troponin T), echocardiography, and a 6-min walk test when health and patient choice permitted (e.g., test was not undertaken when prohibited by arthritis, postural hypotension, or neuropathy). The ATTR group also underwent DPD scintigraphy. The baseline characteristics of all patients are provided in Table 1. All ethics were approved by the UCL/UCLH Joint Committees on the Ethics of Human Research Committee.

**Exclusion criteria.** All patients with contraindications to CMR (i.e., glomerular filtration rate <30 ml/min, incompatible devices) were excluded from the study.

**CMR protocol.** All subjects underwent standard CMR on a 1.5-T clinical scanner (Avanto, Siemens Healthcare, Erlangen, Germany). A standard volume and LGE study was performed (14). The contrast agent was 0.1 mmol/kg of gadolinium-based contrast (gadoterate meglumine [Dotarem, Guerbet SA, Paris, France]) with 5-min delay, and the LGE sequence was either a standard fast low-angle single shot inversion recovery or true fast imaging with steady state free precession sequence, with a phase sensitive inversion recovery sequence or magnitude reconstruction. For native T1 mapping, basal and midventricular short-axis and 4-chamber long-axis views were acquired using the shortened modified look-locker inversion recovery (ShMOLLI) sequence after regional shimming, as previously described (Fig. 1) (15).

**99mTc-DPD scintigraphy.** Patients were scanned using 2 GE Medical Systems (Fairfield, Connecticut) hybrid single photon emission computed tomography (SPECT) computed tomography (CT) gamma cameras (Infinia Hawkeye 4 and Discovery 670) following administration of 700 MBq of intravenously-injected 99mTc-DPD. The 3-h (delayed) whole body planar images were acquired, followed by SPECT of the heart with a low-dose, noncontrast CT scan. Gated/nongated cardiac SPECT reconstruction and SPECT-CT image fusion was performed on the GE Xeleris workstation. Cardiac retention of 99mTc-DPD was visually scored as:

- Grade 0: no visible myocardial uptake in both the delayed planar or cardiac SPECT-CT scan;
- Grade 1: cardiac uptake on SPECT-CT only or cardiac uptake of less intensity than the accompanying normal bone distribution;
- Grade 2: moderate cardiac uptake with some attenuation of bone signal; and
- Grade 3: strong cardiac uptake with little or no bone uptake.
CMR image analysis. All CMR images and maps were analyzed offline. For T1 measurements, the basal ventricular short-axis or the 4-chamber ShMOLLI image was manually contoured approximately 2 pixels in (to minimize partial volume effects) from the endocardium and epicardium, and the average T1 value was calculated (Fig. 1). This was drawn without review of the LGE images. The LGE images were visually analyzed for the presence or absence of enhancement, blinded to T1 mapping results. The presence of LGE was classified as circumferential in the subendocardium; diffuse circumferential (extend into the epicardial layer); and abnormal contrast handling on T1 scout with no discernible LGE.

Statistical analysis. Statistical analysis was performed using IBM SPSS Statistics version 19 (IBM, Somers, New York). All continuous variables were normally distributed (Shapiro-Wilk) other than NT-proBNP and troponin T, which were therefore log-transformed for bivariate testing; these are presented as mean ± SD, with nontransformed NT-proBNP presented as median and interquartile range. Comparisons between groups were performed by 1-way analysis of variance with post-hoc Bonferroni correction. The chi-square test or Fisher exact test was used to compare discrete data as appropriate. Receiver-operating characteristic (ROC) curve analysis was performed to define the diagnostic accuracy of native T1. Correlation between continuous variables was assessed using Pearson’s r correlation coefficient. Statistical significance was defined as p < 0.05.

RESULTS
A total of 85 patients with ATTR amyloid, 8 TTR mutation carriers and 79 patients with AL amyloid were enrolled. These were compared with 52 healthy volunteers and 46 patients with HCM. Baseline characteristics are shown in Table 1. Amyloid patients had the following comorbidities:
treated hypertension (22% ATTR, 15% AL); diabetes (12% ATTR, 3% AL); and angiographically-confirmed coronary artery disease (13% ATTR, 9% AL). The echocardiogram was performed within 1 day of the CMR. The time gap between the DPD and CMR was 26 ± 36 days. Thirty-five ATTR amyloid patients were familial (V122I [n = 18], T60A [n = 6], V30M [n = 2], and E54G [n = 2], and all others unique: D38Y, G47V, E89K, I84S, I107F, L12P, and S77Y); 50 had senile systemic amyloidosis (SSA). Of the 8 gene carriers, 5 had TTR V30M and 3 had T60A. Compared with definite cardiac AL patients, definite ATTR amyloid patients had a higher LV mass index (133 ± 27 vs. 101 ± 25) and reduced ejection fraction (53 ± 15% vs. 61 ± 11%). The PR interval and QRS were longer in ATTR (PR 209 ± 54 ms vs. 185 ± 38 ms, QRS 116 ± 28 ms vs. 106 ± 23 ms; both p < 0.05). The NT-proBNP and troponin T biomarker concentrations were similar.

**T1 diagnostic accuracy.** The ROC curve analysis was performed for the discrimination of possible or definite cardiac amyloid from the meaningful combined differentials of HCM, systemic amyloid without detected cardiac involvement, or ATTR mutation-positive patients without evidence of cardiac amyloid. Using ROC analysis, ATTR and AL amyloid patients with possible or definite cardiac involvement had an area under the receiver-operating characteristic curve (AUC) of 0.85 (95% confidence interval [CI]: 0.79 to 0.92).

Example cut-off values to diagnose cardiac amyloid (high specificity) are 1,048 ms, 1,065 ms, and 1,090 ms. These values have 80%, 85%, and 90% specificity and 83%, 74%, and 56% sensitivity, respectively. Example cut-off values to rule out cardiac amyloid (high sensitivity) are 954 ms, 968 ms, and 1,012 ms. These values have 99%, 98%, and 95% sensitivity and 17%, 30%, and 58% specificity, respectively. Native T1 was similarly accurate for AL and ATTR (AUC: 0.84, 95% CI: 0.76 to 0.91 and AUC 0.85, 95% CI: 0.77 to 0.92, respectively) (Fig. 4).

**T1 and DPD/LGE findings.** T1 increased with increasing cardiac amyloid burden, as assessed by bone scintigraphy (p < 0.0001 for trend) (Fig. 5). T1 was not elevated in mutation carriers (952 ± 35 ms)
but was elevated in the 9 patients with isolated
DPD grade 1 (1,037 ± 60 ms, p = 0.001), all of
which had no amyloid-like LGE (but 1 had inferior
myocardial infarction and 1 had RV LGE).

T1 and cardiac function, biomarkers, ECG,
and 6-min walk test. Correlations were broadly similar for AL
and ATTR disease (Table 2). T1 correlated with
indexes of systolic and diastolic function, indexed
LV mass, and known prognostic biomarkers both
in ATTR and AL amyloid patients. In ATTR
patients, T1 correlated with indexed left atrial area,
6-min walk test performance, and PR and QRS
duration on ECG, whereas in AL patients, T1
correlated with indexed stroke volume, ECG, limb
lead mean voltage, and E deceleration time
(Table 2).

DISCUSSION

In this, the largest ever CMR study in patients with
amyloidosis, we found that native myocardial T1
mapping has a high diagnostic accuracy for cardiac
amyloid for both AL and ATTR when compared
against HCM, a relevant clinical differential diag-
nosis. Furthermore, T1 tracks cardiac amyloid bur-
den in both diseases, and is more sensitive for
detecting early disease in gene mutation carriers than
LGE imaging. In both amyloid types, T1 tracks
markers of systolic and diastolic function, mass,
and prognostic markers. In ATTR amyloid, T1 addi-
tionally correlates with ECG PR and QRS duration
and indexed left atrial area, whereas in AL type, it
correlates with reductions in limb lead voltages. T1
also has functional associations with a reduction in
6-min walk test in ATTR amyloidosis. Interestingly
and perhaps unexpectedly (16), T1 elevation was
lower in ATTR compared with AL type.

Amyloidosis is considered the exemplar of an
interstitial disease, as the quantity of amyloid in the
extracellular space amounts to kilograms overall in

Figure 3. Native T1 in Healthy Volunteers, Mutation Carriers, HCM,
Definite AL, and Definite ATTR

Mean native myocardial T1 ± 2 SE in healthy control subjects, gene carriers,
patients with definite AL cardiac amyloidosis, and patients with definite ATTR
cardiac amyloidosis. Abbreviations as in Figure 2.

Figure 4. ROC Curve for Native T1

Receiver operating characteristic (ROC) curve for the discrimination of possible or definite cardiac amyloid by native myocardial T1 from the
clinically significant differentials of HCM. Systemic amyloid without detected cardiac involvement or ATTR mutation-positive patients without
apparent cardiac amyloid for: (A) cardiac amyloid, type unspecified (AL and ATTR); (B) AL amyloid alone; or (C) ATTR amyloid alone.

AUC = area under the curve; CI = confidence interval; other abbreviations as in Figure 2.
some patients and is able to constitute the majority of the heart by weight at times (17).

Our earlier work in AL amyloidosis demonstrated that measurement of myocardial T1 times using ShMOLLI had high diagnostic accuracy (against aortic stenosis) and tracks disease burden (11). Here, this work is extended to ATTR, and the diagnostic accuracy was tested against hypertrophic cardiomyopathy, a relevant clinical differential. Although T1 was raised in the ATTR amyloid, it was not as high as in AL type, a surprising finding given that ventricular wall thickness is greater in ATTR amyloid (16). T1 mapping measures a composite tissue signal from both cells and the interstitium. The extent and/or distribution of amyloid, plus how it interacts with water or changes in the myocyte signal, could all be implicated in causing the T1 difference. Therefore, the less raised T1 value found in patients with ATTR amyloidosis could be due to (amongst others), a lower amyloid burden, less hydration of the amyloid, less collagen associated with amyloid, or differential effects on the intracellular signal. Finally, AL may have additional processes occurring, such as edema from possible light chain toxicity (18,19). Further work is needed.

Many differences in the biology of ATTR and AL cardiac amyloidosis have been described but are not understood. This study shows specific differences between AL and ATTR in their correlations with other parameters; for example, the positive correlations in ATTR amyloid of T1 with left atrial area, PR, and QRS duration, and the negative correlations in AL amyloid of T1 with mean QRS voltage in the limb leads. These findings support the concept that ATTR amyloid may be a more purely infiltrative disease, whereas AL may have a dual pathology with contributions from interstitial expansion and cell death.

Native myocardial T1 yielded high diagnostic accuracy in ATTR and AL amyloidosis against the common clinical differential diagnosis of HCM. These findings combine a number of key advantages of the mapping technique: the absence of need for contrast, a single breath-hold per T1 map, simple analysis, and the potential for measurement of whole-heart T1. Other investigators have proposed bone scintigraphy using the DPD tracer as the noninvasive “gold standard” for diagnosis of cardiac ATTR amyloidosis (20). However, this is semiquantitative and is scored in 4 grades based on visual estimation; although the radiation dose is low, serial follow-up is problematic for gene mutation carriers. Native T1 showed high concordance with DPD scintigraphy, and T1 was measured on a continuous scale, which is a possible advantage. DPD scanning dichotomizes mutation carriers into DPD negative or DPD grade 1. T1 was exactly concordant with this dichotomy, with T1 elevation and DPD grade 1 patients having no other abnormalities (no hypertrophy, no biomarker

**Table 2. Correlations Between T1 and Cardiac Function, Biomarkers, ECG, and 6-Min Walk Test in ATTR and AL Patients**

<table>
<thead>
<tr>
<th></th>
<th>ATTR Patients</th>
<th>AL Patients</th>
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<tbody>
<tr>
<td>LV structure by CMR</td>
<td></td>
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</tr>
<tr>
<td>LV mass, g/m²</td>
<td>0.62*</td>
<td>0.44*</td>
</tr>
<tr>
<td>LA area, cm²/m²</td>
<td>0.31*</td>
<td>0.058</td>
</tr>
<tr>
<td>LV systolic function by CMR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEF, %</td>
<td>−0.22*</td>
<td>−0.36*</td>
</tr>
<tr>
<td>SV, ml/m²</td>
<td>−0.151</td>
<td>−0.28*</td>
</tr>
<tr>
<td>LV diastolic function by echocardiogram</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E/E</td>
<td>0.32*</td>
<td>0.47*</td>
</tr>
<tr>
<td>E-deceleration time, ms</td>
<td>−0.106</td>
<td>0.41*</td>
</tr>
<tr>
<td>6-min walking test</td>
<td>−0.32</td>
<td>−0.147</td>
</tr>
<tr>
<td>Biomarkers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT-proBNP, pmol/l</td>
<td>0.57*</td>
<td>0.56*</td>
</tr>
<tr>
<td>Troponin T, pmol/l</td>
<td>0.57*</td>
<td>0.32*</td>
</tr>
<tr>
<td>ECG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR, ms</td>
<td>0.32*</td>
<td>0.073</td>
</tr>
<tr>
<td>QRS, ms</td>
<td>0.26*</td>
<td>0.077</td>
</tr>
<tr>
<td>ECG limb lead mean voltage, mm</td>
<td>−0.151</td>
<td>−0.48*</td>
</tr>
</tbody>
</table>

Values are Pearson’s r correlation coefficient. *p < 0.01. *p < 0.05. ECG = electrocardiogram; other abbreviations as in Table 1.
Native myocardial T1 mapping detects cardiac ATTR amyloid and has similar performance for diagnosis and tracking disease in both ATTR and AL amyloidosis. The lower T1 elevation in ATTR amyloid, and the specific differences between AL and ATTR correlations with other cardiac findings, may support a concept of ATTR amyloid as a more purely infiltrative disease, whereas the AL type may have a dual pathology with both interstitial expansion and cell death.

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REFERENCES

Key Words: amyloidosis ■ cardiac magnetic resonance ■ T1 mapping ■ transthyretin.
Echocardiographic Correlates of Acute Heart Failure, Cardiogenic Shock, and In-Hospital Mortality in Tako-Tsubo Cardiomyopathy

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OBJECTIVES  The purpose of this study was to determine clinical and echocardiographic correlates of acute heart failure, cardiogenic shock and in-hospital mortality in a large cohort of tako-tsubo cardiomyopathy (TTC) patients.

BACKGROUND  Despite good long-term prognosis, life-threatening complications due to hemodynamic instability can occur early in TTC patients.

METHODS  The study population consisted of 227 patients (66.2 ± 12.2 years of age; females, 90.3%) enrolled in the Tako-tsubo Italian Network, undergoing transthoracic two-dimensional echocardiography on admission and at short-term follow-up (4.3 [4 to 6] weeks). Patients were divided into two groups according to the presence or absence of major adverse events, a composite of acute heart failure, cardiogenic shock, and in-hospital mortality.

RESULTS  Major adverse events occurred in 59 patients (25.9%). The variables for elderly patients ≥75 years of age (42.4% vs. 23.8%; p = 0.011): left ventricular (LV) ejection fraction (35.1 ± 5.9% vs. 38.4 ± 4.6%, p < 0.001), wall motion score index (1.9 ± 0.2 vs. 1.7 ± 0.2, p < 0.001), E/e’ ratio (13.5 ± 4.3 vs. 9.9 ± 3.3 [where E/e’ is ratio of mitral E peak velocity and averaged e’ velocity], p < 0.001), LV outflow tract obstruction (23.7 vs. 8.9%, p = 0.006), pulmonary artery systolic pressure (47.4 ± 12.3 mm Hg vs. 38.0 ± 9.2 mm Hg; p < 0.001), right ventricular involvement (28.8 vs. 9.5%; p < 0.001), and reversible moderate-to-severe mitral regurgitation (49.1 vs. 11.9%; p < 0.001), were significantly different between groups and were associated with adverse events. At multivariate analysis, LV ejection fraction (HR: 0.92; 95% CI: 0.89 to 0.95; p < 0.001), E/e’ ratio (HR: 1.13; 95% CI: 1.02 to 1.24; p = 0.011), reversible moderate to severe mitral regurgitation (HR: 3.25; 95% CI: 1.16 to 9.10; p = 0.025), and age ≥75 years (HR: 2.81; 95% CI: 1.05 to 7.52; p = 0.039) were independent correlates of major adverse events.

CONCLUSIONS  Echocardiographic parameters provide additional information compared to other variables routinely used in clinical practice to identify patients at higher risk of hemodynamic deterioration and poor in-hospital outcome, allowing prompt institution of appropriate pharmacological treatment and adequate mechanical support.  (J Am Coll Cardiol Img 2014;7:119–29) © 2014 by the American College of Cardiology Foundation
Tako-tsubo cardiomyopathy (TTC) is typically characterized by transient left ventricular (LV) systolic dysfunction with morphological features of apical ballooning, although other variant forms (e.g., midventricular ballooning) have also been described (1–3). It occurs most often in post-menopausal women and is usually triggered by emotional or physical stress, with complete recovery of LV systolic function within a few days or weeks (4,5). Despite its favorable long-term prognosis and very low mortality, TTC is not considered a benign condition, because of the occurrence of life-threatening complications during the acute phase, related to hemodynamic instability (e.g., acute heart failure, cardiogenic shock) in a substantial proportion of patients (6–8). Owing to its widespread use in critical care settings, echocardiography has become the noninvasive imaging modality of choice for assessing TTC (2,9).

However, the combination of clinical, electrocardiographic (ECG), laboratory, and echocardiographic measures routinely used in clinical practice for TTC patients experiencing major adverse events due to hemodynamic instability have not yet been well described. The aim of this study was to identify the clinical and echocardiographic determinants of major adverse events, a composite of acute heart failure, cardiogenic shock, and in-hospital mortality, in a large cohort of TTC patients.

**METHODS**

**Study population.** The study population consisted of 227 patients enrolled in the Tako-Tsubo Italian Network, undergoing comprehensive transthoracic 2-dimensional echocardiography on admission and at short-term follow-up (4.3 [4 to 6] weeks) (8,10). The diagnosis of TTC was based on the following Mayo Clinic criteria (5):

- Transient akinesia or dyskinesia of LV apical and/or midventricular segments;
- No angiographic evidence of ≥50% coronary artery stenosis, or plaque rupture, or intracoronary thrombus formation;
- New ECG abnormalities (dynamic ST-T changes or T-wave inversion);
- Absence of intracranial bleeding, pheochromocytoma, and myocarditis.

Patients with a poor acoustic window (suboptimal visualization of endocardial borders) were excluded. All participants provided informed written consent, and the study was approved by the local ethics committee.

**Data collection.** Clinical variables were recorded on a standardized form that included information on patient demographics (sex, age, heart rate, systolic and diastolic blood pressure), signs and symptoms at presentation, medical history, trigger events, ECG ST-segment changes and presence of prolonged QTc interval on admission, and clinical observations during hospitalization (including major cardiac events). Emotional or physical triggers were identified as previously described (8). Venous blood was collected every 3 hours to measure troponin I concentration in the acute phase, and collection continued until a peak value was observed. All patients underwent coronary angiography and left ventriculography within 24 hours of symptom onset.

**Definition of major adverse events due to hemodynamic instability.** Major adverse events were defined as a composite of acute heart failure, cardiogenic shock, and in-hospital mortality. In particular:

- Acute heart failure was defined as the presence of pulmonary edema, dyspnea, and/or oxygen desaturation requiring drug therapy and/or mechanical support;
- Cardiogenic shock was defined as systolic blood pressure <90 mm Hg with signs of tissue hypoperfusion requiring inotropic agents and/or fluid therapy;

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• In-hospital mortality was defined as cardiac death or death from any cause.

**Echocardiography.** All echocardiographic examinations were performed within 6 hours of hospital admission, before coronary angiography, and were repeated at 5 ± 1.6 (range 4 to 6) weeks after symptom onset. A commercially available cardiac ultrasonography system with a 2.5 to 4.5-MHz phased-array transducer with second harmonic capability was used for complete 2-dimensional Doppler echocardiography. All examinations were performed by observers blinded to clinical data. LV regional wall motion abnormalities and presence of typical circular systolic dysfunction with involvement of ≥4 coronary territories were evaluated by visual assessment of multiple apical and short-axis views, as previously described (9). All echocardiographic images were digitally recorded and reviewed by 2 expert readers (R.C. and F.R.). Three cardiac cycles from the apical 4- and 2-chamber views and the parasternal short-axis view at the level of the mitral valve and papillary muscles were stored in cine-loop format for off-line analysis. LV ejection fraction (EF) was calculated using biplane Simpson’s rule from the apical 4- and 2-chamber views (11). Left atrial (LA) volume was assessed by the biplane area-length method and indexed to body surface area (11). Right ventricular (RV) wall motion was evaluated by visual assessment for the detection of RV involvement (12). The echo transducer was adjusted to the level of the RV chamber to achieve optimal visualization of RV size and RV endocardial borders. Tricuspid annular plane systolic excursion (TAPSE) was calculated as previously described (13). LV diastolic function was evaluated according to American Society of Echocardiography recommendations (14). Early (e') diastolic tissue Doppler velocities were measured at the septal and lateral corners of the mitral annulus, and the mean between the two values was calculated. The ratio of mitral E peak velocity and averaged e' velocity (E/e') was calculated. Mitral regurgitation (MR) was quantified from color Doppler imaging and semiquantitatively graded as absent, minimal (within normal limits), mild, moderate, or severe, using standardized criteria (15). Moderate MR was confirmed by vena contracta measurement (3 to 7 mm) (16). Reversible significant MR was defined as reversible moderate to severe MR detected during the first echocardiographic evaluation that disappeared at follow-up examination. LV outflow tract obstruction (LVOTO) was detected by continuous wave Doppler. Using the modified Bernoulli equation, a cut-off value of 25 mm Hg for dynamic intraventricular pressure gradient was considered significant LVOTO (17). With continuous wave Doppler echocardiography, peak tricuspid regurgitant velocity recorded from any view was used to determine pulmonary artery systolic pressure (sPAP) with the simplified Bernoulli equation \[ \text{sPAP} = 4 \times \text{peak velocity}^2 + \text{mean right atrial pressure} \]; mean right atrial pressure was estimated as previously described (18).

**Statistical analysis.** Statistical analyses included descriptive statistics (frequency and percentage of categorical variables, mean ± SD of continuous normally distributed variables, and median values and an interquartile range of continuous non-normally distributed variables). The normal distribution of continuous variables was verified with the Kolmogorov–Smirnov goodness-of-fit test. Continuous variables were then compared by using an independent sample Student t test or Mann–Whitney U test, and categorical variables were compared with chi-square statistics or a Fisher exact test, when appropriate. The associations of selected variables with major adverse events were assessed with Cox proportional hazard models using univariate and stepwise multivariate procedures. A significance of 0.05 was required for a variable to be included in the multivariate model, whereas 0.1 was the cut-off value for exclusion. Hazard ratios (HR) with 95% confidence intervals (CIs) were estimated. The following covariates were analyzed: age ≥75 years, heart rate, chest pain with dyspnea, brain natriuretic peptide (BNP), LVEF, E/e' ratio, sPAP, moderate to severe MR, RV involvement, and LVOTO. The Hosmer–Lemeshow statistic was used to assess the goodness-of-fit of the logistic regression model. A p value <0.05 was considered statistically significant. In addition, to investigate the incremental prognostic value of echocardiographic correlates of major adverse events compared with clinical, we conducted interactive stepwise procedures with ECG and laboratory findings. Therefore, clinical data (including age, sex, presenting symptoms, blood pressure, heart rate) were first analyzed and the global chi-square value was calculated. Subsequent steps were created after adding ECG changes at admission, laboratory findings (troponin I, CK-MB, and BNP peak) and independent echocardiographic correlates of major adverse events (moderate to severe MR, E/e' ratio, and EF). The incremental prognostic value of the added variables was determined by comparison of the global chi-square value calculated at each step. The chi-square of the model was calculated from the log likelihood ratio. A statistically significant increase in global chi-square
after the addition of further variables was considered the incremental prognostic value. Statistical analysis was performed using SPSS version 20.0 statistical software (SPSS Inc., Chicago, Illinois).

**RESULTS**

Demographic and clinical characteristics. The study population included 227 patients (66.2 ± 12.2 years of age; females, 90.3%). Demographic, clinical, and ECG characteristics of the overall population are shown in Table 1.

Patients with and without major adverse events were compared. There was a striking prevalence of females (91.5% vs. 89.9%, respectively; \( p = 0.80 \)) in menopause (85.1% vs. 88.7%, respectively; \( p = 0.47 \)) in both groups. A significantly higher proportion of elderly patients ≥75 years of age (42.4% vs. 23.8%, respectively; \( p = 0.011 \)) was observed between those with major adverse events. Chest pain with dyspnea (25.4% vs. 8.9%, respectively; \( p = 0.003 \)) and increased heart rate (88.8 ± 20.5 beats/min vs. 83.4 ± 14.1 beats/min, respectively; \( p = 0.031 \)) were significantly more frequent among patients with major adverse events. No significant differences were found between groups in ECG presentation, LV morphology, prevalence of triggers, and common cardiovascular risk factors. Conversely, the involvement of ≥4 coronary territories was more frequent (89%) and prevalent in patients with hemodynamic instability (57 [96.6%] vs. 147 [87.5%] patients, respectively; \( p = 0.047 \)). Length of hospital stay was significantly longer in patients with major adverse events (9 [5.0 to 12.2] vs. 6 [5 to 9] days, respectively; \( p = 0.002 \)).

Major adverse events: acute heart failure, cardiogenic shock, and in-hospital mortality. Overall complications are listed in Table 2. Acute heart failure was the most common major complication (n = 45; 19.6%). Among the patients with acute heart failure, 5 patients had cardiogenic shock, 1 patient had cardiogenic shock and stroke, 3 patients had ventricular tachycardia/fibrillation, 1 patient had ventricular tachycardia/fibrillation and atrial fibrillation, 2 patients had atrial fibrillation alone, and 1 patient had apical thrombosis. Among patients with cardiogenic shock (n = 18; 7.8%), 3 had active cancer, 1 had stroke and preexisting atrial fibrillation, and another one had new-onset atrial fibrillation. Thirteen patients with cardiogenic shock (5.7%) were treated with positive inotropic drugs or vaso-pressors or both (dobutamine at an infusion rate of 2 to 20 \( \mu g/kg/min \); norepinephrine at an infusion rate of 0.2 to 1.0 \( \mu g/kg/min \); or levosimendan at an

| Table 1. Clinical and Demographic Characteristics of the Study Population |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                             | Overall Population (n = 227) | Patients With Major Complications (n = 59) | Patients Without Major Complications (n = 168) | \( p \) Value |
| Age, yrs                    | 66.2 ± 12.2                  | 67.5 ± 14.5                  | 65.8 ± 11.4                  | 0.372                      |
| Age ≥ 75 yrs                | 65 (28.6)                    | 25 (42.4)                    | 40 (23.8)                    | 0.011                      |
| Female                      | 205 (90.3)                   | 54 (91.5)                    | 151 (89.9)                   | 0.804                      |
| Body surface area, m²       | 1.6 ± 0.1                    | 1.6 ± 0.1                    | 1.6 ± 0.1                    | 0.054                      |
| Medical history             |                             |                             |                             |                            |
| Hypertension                | 137 (60.4)                   | 33 (55.9)                    | 104 (61.9)                   | 0.442                      |
| Hypercholesterolemia        | 88 (38.8)                    | 20 (33.9)                    | 68 (40.5)                    | 0.438                      |
| Diabetes mellitus           | 25 (11.0)                    | 10 (16.9)                    | 15 (8.9)                     | 0.096                      |
| Smoking                     | 47 (20.7)                    | 15 (25.4)                    | 32 (19.0)                    | 0.351                      |
| Menopause                   | 180 (82.8)                   | 46 (85.1)                    | 134 (88.7)                   | 0.477                      |
| Coronary artery disease     | 19 (8.4)                     | 4 (6.8)                      | 15 (8.9)                     | 0.787                      |
| Active cancer*              | 16 (8.0)                     | 6 (11.3)                     | 10 (6.8)                     | 0.376                      |
| Chronic obstructive pulmonary disease* | 19 (9.5)                   | 6 (11.3)                     | 13 (8.9)                     | 0.593                      |
| Presenting features         |                             |                             |                             |                            |
| Chest pain                  | 161 (70.9)                   | 27 (45.8)                    | 134 (79.8)                   | <0.001                     |
| Chest pain and dyspnea      | 30 (13.2)                    | 15 (25.4)                    | 15 (8.9)                     | 0.003                      |
| Dyspnea                     | 26 (11.5)                    | 11 (18.6)                    | 15 (8.9)                     | 0.057                      |

Continued on the next page
infusion rate of 0.1 μg/kg/min). Despite treatment, 3 patients died during intensive care unit stay. Intra-aortic balloon pumping was used in 6 of 18 patients with cardiogenic shock admitted to facilities where implantation of the device could be performed. Of note, 10 patients with cardiogenic shock had reversible moderate to severe MR. Death occurred in 6 patients (2.6%). Cardiac death was related to cardiogenic shock in 3 patients and to acute heart failure in 1 patient. Noncardiac death was associated with malignancy in the remaining 2 patients. Among 11 patients with ventricular tachycardia/fibrillation, only 1 had torsade de pointes, and 9 had prolonged QTc intervals (p = 0.012).

### Echocardiographic findings on admission

Patients with major adverse events had significantly higher indexed LV end-diastolic volume (p = 0.002) and LV end-systolic volume (p < 0.001), lower EF (p < 0.001), higher wall motion score index (WMSI) (p < 0.001) and larger LA volumes (p < 0.001) than patients without major adverse events (Table 3). Regarding diastolic function, all mitral inflow-derived parameters were significantly different between groups, as follows: E peak velocity (p < 0.001), E/A ratio (p < 0.001), E-wave deceleration time (p = 0.011), and e’ peak velocity (p = 0.007). In the overall population, LVOTO was detected in 29 patients (only 2 received inotropic agents) and was

### Table 1. Continued

<table>
<thead>
<tr>
<th></th>
<th>Overall Population (n = 227)</th>
<th>Patients With Major Complications (n = 59)</th>
<th>Patients Without Major Complications (n = 168)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other symptoms</td>
<td>10 (4.4)</td>
<td>6 (10.2)</td>
<td>4 (2.4)</td>
<td>0.021</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>131.1 ± 25.6</td>
<td>129.6 ± 32.6</td>
<td>131.6 ± 23.1</td>
<td>0.606</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>77.4 ± 12.8</td>
<td>76.7 ± 14.1</td>
<td>77.7 ± 12.4</td>
<td>0.619</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>84.8 ± 16.1</td>
<td>88.8 ± 20.5</td>
<td>83.4 ± 14.1</td>
<td>0.031</td>
</tr>
<tr>
<td>Presence of identifiable trigger events</td>
<td>187 (82.4)</td>
<td>48 (81.4)</td>
<td>139 (82.7)</td>
<td>0.843</td>
</tr>
<tr>
<td>Emotional trigger</td>
<td>133 (58.6)</td>
<td>31 (52.5)</td>
<td>102 (60.7)</td>
<td>0.286</td>
</tr>
<tr>
<td>Physical trigger</td>
<td>54 (23.8)</td>
<td>17 (28.8)</td>
<td>37 (22.0)</td>
<td>0.190</td>
</tr>
<tr>
<td>Serum assays</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Troponin I, ng/ml</td>
<td>0.9 (0.3–2.0)</td>
<td>0.7 (0.3–1.6)</td>
<td>0.9 (0.3–2.2)</td>
<td>0.691</td>
</tr>
<tr>
<td>CK-MB, ng/ml</td>
<td>19.7 (8.2–35)</td>
<td>18.5 (8.1–31)</td>
<td>20 (8.2–37.9)</td>
<td>0.401</td>
</tr>
<tr>
<td>BNP, pg/ml</td>
<td>806.8 ± 181.3</td>
<td>856.0 ± 207.0</td>
<td>790.0 ± 169.4</td>
<td>0.047</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>12.9 ± 1.5</td>
<td>12.8 ± 1.5</td>
<td>12.9 ± 1.5</td>
<td>0.596</td>
</tr>
<tr>
<td>GFR, ml/min</td>
<td>70.2 ± 21.5</td>
<td>66.4 ± 25.2</td>
<td>71.6 ± 19.5</td>
<td>0.118</td>
</tr>
<tr>
<td>CRP, mg/dl</td>
<td>12.6 (6.5–26.4)</td>
<td>13 (6.1–27.5)</td>
<td>12.5 (6.5–26.2)</td>
<td>0.938</td>
</tr>
<tr>
<td>ECG features at admission</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolated STE</td>
<td>72 (31.7)</td>
<td>15 (25.4)</td>
<td>57 (33.9)</td>
<td>0.258</td>
</tr>
<tr>
<td>STE with TWI</td>
<td>72 (31.7)</td>
<td>21 (35.6)</td>
<td>51 (30.4)</td>
<td>0.516</td>
</tr>
<tr>
<td>Non-STE</td>
<td>53 (23.3)</td>
<td>15 (25.4)</td>
<td>38 (22.6)</td>
<td>0.721</td>
</tr>
<tr>
<td>Isolated TWI</td>
<td>30 (13.2)</td>
<td>8 (13.6)</td>
<td>22 (13.1)</td>
<td>1.000</td>
</tr>
<tr>
<td>Prolonged QTc-interval</td>
<td>99 (43.6)</td>
<td>23 (39.0)</td>
<td>76 (45.2)</td>
<td>0.447</td>
</tr>
<tr>
<td>Left ventricular morphology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apical ballooning</td>
<td>175 (77.1)</td>
<td>42 (71.2)</td>
<td>133 (79.2)</td>
<td>0.213</td>
</tr>
<tr>
<td>Midventricular ballooning</td>
<td>41 (18.1)</td>
<td>12 (20.3)</td>
<td>29 (17.3)</td>
<td>0.694</td>
</tr>
<tr>
<td>Basal ballooning</td>
<td>11 (4.8)</td>
<td>5 (8.5)</td>
<td>6 (3.6)</td>
<td>0.159</td>
</tr>
<tr>
<td>Involvement of ≥4 coronary territories</td>
<td>204 (89.9)</td>
<td>57 (96.6)</td>
<td>147 (87.5)</td>
<td>0.047</td>
</tr>
<tr>
<td>Length of hospitalization, days</td>
<td>7 (5–10)</td>
<td>9 (5–12.2)</td>
<td>6 (5–9)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Continuous normally distributed variables are mean ± SD. Categorical variables are n (%). Continuous non-normally distributed variables are median (interquartile range). *Data were available for 199 patients (53 with major complications). (Data were available for 157 patients (40 with major complications). **BNP = brain natriuretic peptide; CK-MB = creatine kinase-MB; CRP = C-reactive protein; ECG = electrocardiography; GFR = glomerular filtration rate; STE = ST-segment elevation; TWI = T-wave inversion.
more common in patients with major adverse events (p = 0.006). Reversible moderate to severe MR was detected in 49 patients and was prevalent in patients with major adverse events (49.1% vs. 11.9%, respectively, p < 0.001). Acute heart failure and cardiogenic shock occurred in 28 of 49 patients (57%). Patients with moderate to severe MR had lower EF (median [IQR] = 38% [31 to 40] vs. 40% [36 to 42], respectively) than patients without significant MR. Apical ballooning (40 of 49 patients; 81%) was prevalent in this subgroup. Seventeen patients had concomitant LVOTO and systolic anterior motion (34.6%). RV involvement (28.8% vs. 9.5%, respectively; p = 0.001) was more frequent among patients with major adverse events. Indexes of LV filling pressure, such as E/e’ ratio (p < 0.001) and sPAP (p < 0.001), were significantly higher, suggesting a more compromised hemodynamic status in the group with major adverse events.

**Echocardiographic findings at short-term follow-up.** At short-term follow-up, all echocardiographic measures examined were not significantly different between groups, except for LA volume (p < 0.001), E peak velocity (p = 0.002), E/A ratio (p = 0.001), e’ peak velocity (p < 0.001), E/e’ ratio (p < 0.001), and sPAP (p = 0.027), which remained higher in patients who experienced major adverse events (Table 4).

**Table 2. Overall Complications**

<table>
<thead>
<tr>
<th>Major complications</th>
<th>Overall Population (n = 227)</th>
<th>Patients With Major Complications (n = 59)</th>
<th>Patients Without Major Complications (n = 168)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute heart failure</td>
<td>45 (19.6)</td>
<td>45 (77.9)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cardiogenic shock</td>
<td>18 (7.8)</td>
<td>10 (16.9)</td>
<td>8 (4.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cardiac death</td>
<td>4 (1.7)</td>
<td>3 (5.1)</td>
<td>1 (0.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Other cardiac events</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventricular tachycardia/fibrillation</td>
<td>11 (4.8)*</td>
<td>5 (8.5)</td>
<td>6 (3.6)</td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td>3 (1.3)</td>
<td>2 (3.4)</td>
<td>1 (0.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apical thrombosis</td>
<td>3 (1.3)</td>
<td>1 (1.6)</td>
<td>2 (1.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Supraventricular tachycardia</td>
<td>1 (0.4)</td>
<td>1 (1.6)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>15 (6.6)</td>
<td>10 (16.9)</td>
<td>5 (3.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Syncope</td>
<td>1 (0.4)</td>
<td>0 (0)</td>
<td>1 (0.6)</td>
<td></td>
</tr>
</tbody>
</table>

Values are n (%). *9 patients had prolonged QTc intervals (p = 0.012).
Independent clinical and echocardiographic correlates of major adverse events. At univariate analysis, age ≥75 years, heart rate, LVOTO, RV involvement, chest pain with dyspnea, LVEF, E/e’ ratio, sPAP, and presence of moderate to severe MR at admission were significantly associated with the occurrence of major adverse events (Table 5). At multivariate analysis, LVEF, E/e’ ratio, reversible moderate to
severe MR and age ≥75 years were independent correlates of the composite outcome (Table 5). When considering only 2 of 3 components of the composite outcome, that is, cardiogenic shock and death, heart rate (HR: 1.028, 95% CI: 1.004 to 1.052, p = 0.022), LVEF (HR: 0.872, 95% CI: 0.819 to 0.928, p < 0.001), and reversible moderate to severe MR (HR: 4.498, 95% CI: 1.366 to 14.811, p = 0.013) were identified as independent predictors. The Hosmer-Lemeshow statistic was not significant (p = 0.45), indicating a good model fit.

**Incremental prognostic value of independent echocardiographic correlates of major adverse events.** Sequential models for the prediction of major adverse events were used to assess the incremental contribution of echocardiographic correlates of major adverse events compared with clinical, ECG, and laboratory findings. For major adverse events, the clinical model (chi-square: 40.7, p < 0.001) was slightly but not significantly improved by the addition of ECG (chi-square: 46.2, p = 0.24) and laboratory data (chi-square: 50.9, p = 0.19), respectively. A major and significant improvement was obtained only by the addition of echocardiographic findings (chi-square: 88.7, p < 0.001) (Fig. 1).

**DISCUSSION**

This study addressed the role of echocardiography compared with those of other clinical, ECG, and laboratory measures routinely used in daily practice in the early evaluation of patients with TTC. The main findings can be summarized as follows: 1) in a large series of TTC patients, approximately 25% developed pulmonary edema or cardiogenic shock during the acute phase; 2) several echocardiographic findings, such as LVEF, WMSI, E/e ratio, LVOTO, sPAP, and RV involvement and reversible moderate to severe MR, were associated with such adverse events; and 3) LVEF, reversible moderate to severe MR, E/e ratio, and age ≥75 years were independent correlates of acute heart failure, cardiogenic shock, and in-hospital mortality. In addition, the prevalence of some peculiar echocardiographic findings previously reported in small cohorts of TTC patients have been documented in larger series.

**Clinical relevance of systolic and diastolic echocardiographic parameters.** Most patients with TTC are usually paucisymptomatic and have a favorable in-hospital course. However, adverse events due to hemodynamic instability (e.g., acute heart failure or cardiogenic shock) may occur in up to one-fourth of patients, even during the first hours after clinical onset.

In our study, higher WMSI and lower LVEF were associated with pulmonary edema and cardiogenic shock, confirming the fact that marked LV systolic dysfunction contributes to the development of hemodynamic instability (7). Increased sympathetic stimulation seems to play a central role in the occurrence of systolic impairment related to myocardial stunning in TTC patients, through a variety of pathophysiological mechanisms, including myocyte calcium overload, oxidative stress, and microvascular dysfunction (4,19,20). Lower peak levels of cardiac enzymes are observed in TTC than in acute coronary syndrome, despite more pronounced myocardial dysfunction. The slight elevation of troponin and CK-MB levels seems to be associated with myocardial stunning rather than necrosis, as abnormal troponin levels do not necessarily indicate true myocardial damage. The potential role of ECG abnormalities in predicting adverse events in TTC has been previously investigated (21). In the study by Takashio et al. (21), the magnitude and extent of ST-segment elevation with ECG were found to be independent predictors of in-hospital adverse events. However, those findings were not confirmed by other authors (22). Although ST-segment elevation is the most common ECG presentation, it is detected in roughly 50% of patients, especially in Western countries (23). In clinical practice, a marked disproportion between troponin peak levels and the magnitude of ECG changes compared with the extent of regional myocardial dysfunction is frequently observed. Our results demonstrate that LVEF is an independent correlate of acute heart failure and is superior to...
troponin and ECG in identifying patients at risk of hemodynamic instability. Echocardiography seems to better mirror the amount of dysfuncitoning myocardium than ECG alterations and serum parameters, confirming the importance of multi-parametric LV assessment in TTC patients.

TTC patients may show volume and pressure overload secondary not only to acute systolic but also to diastolic dysfunction. Interestingly, advanced age and female sex that typically prevail in TTC were found to be associated with LV diastolic stiffening coupled with a stiff vascular system and endothelial dysfunction (24). Basal hyperkinesis and apical LV wall stress induce intraventricular diastolic and systolic pressure gradients and are believed to be the primary mechanisms responsible for increased LV filling pressure in this peculiar cardiomyopathy. In TTC patients, elevated BNP levels were shown to correlate with the severity of LV diastolic dysfunction independent of troponin peak levels (25). In our study as well as in other series, E/e’ ratio was elevated (26).

A relationship between decreased LV untwisting (a regional diastolic index) and increased E/e’ ratio (a global diastolic index) has been reported, demonstrating that diastolic function is impaired even in the early phase of TTC (27). E/e’ ratio is considered a good predictor of LV filling pressure, and it has already been proven useful in predicting outcome in different cardiovascular diseases, also showing an incremental prognostic value compared to BNP (28). Our study demonstrates for the first time that E/e’ ratio is a strong determinant of acute heart failure and in-hospital mortality in patients with TTC. This parameter should therefore be assessed early and systematically in order to identify patients at higher risk of hemodynamic instability and to guide appropriate management. In addition, the improvement in E/e’ ratio and LV systolic function at follow-up may be considered a useful indicator of LV function recovery. Heart failure can also be precipitated by elevated pulmonary artery pressure, usually related to reduced LA compliance (advanced LV diastolic dysfunction in elderly patients) or LA volume overload (significant MR) (28). Of note, in our study, the echocardiographic parameters of both systolic and diastolic function, including E/e’ ratio, were significantly more compromised in patients with adverse events than in those without. At short-term assessment, despite comparable recovery of systolic function, some parameters of diastolic function (namely, E/A ratio, E/e’ ratio, and LA volume) remained significantly outside of the normal range in patients who experienced adverse events. As a possible explanation for this finding, it may be hypothesized that pre-existing diastolic dysfunction may have been unmasked following the resolution of the acute phase, suggesting that TTC patients with impaired diastolic function are more prone to develop acute heart failure.

Reversible mitral regurgitation, acute heart failure, and cardiogenic shock. Several aspects regarding the impact of reversible significant MR on the clinical picture of TTC patients are still debated. First, the true incidence remains to be clearly defined. In our study population, reversible significant (moderate to severe) MR during the acute phase was observed in 21% of TTC patients. This finding is consistent with the prevalence (range 19% to 25%) reported in the literature (29–31). Second, the cause of acute MR remains to be fully elucidated. Two distinct underlying mechanisms seem to be associated with the development of MR in TTC: 1) the coexistence of systolic anterior motion and LVOTO; and 2) tethering of the mitral valve leaflets (31). In our study, only a minority of patients with significant MR showed concomitant LVOTO and systolic anterior motion (7%), confirming the hypothesis that different underlying mechanisms may induce dynamic functional MR. Finally, equivocal data for the association of MR with ballooning pattern, systolic function, and prognosis have been reported so far. In our study, patients with significant MR had a striking prevalence of apical ballooning pattern and, if compared with patients without MR, had a lower EF. These findings lead us to hypothesize that significant MR may be associated with more extensive myocardial dysfunction. It is unquestionable that in a context of stunning myocardium typical of TTC, the left ventricle can compensate for the development of an acute MR by increasing preload and emptying, as expected in a normal ventricle (32). LV volume overload results in increased myocardial tension that, in a vicious circle, leads to systolic dysfunction and reduced stroke volume (33). Ejection of the regurgitant volume into the left atrium before aortic valve opening further reduces forward effective cardiac output, predisposing to cardiogenic shock. In addition, LA pressure rises abruptly, especially in the presence of normal or reduced LA compliance, often leading to pulmonary congestion and acute heart failure. In our study, 18 patients developed cardiogenic shock and among these 10 (55%) had moderate to severe MR. The prevalence of cardiogenic shock in TTC was 15% in the study by Tsuchuhashi et al. (1), but even rarer in other series (34). Song et al. (35) reported the occurrence of cardiogenic shock in 16 of 50 (32%) TTC patients. Patients with cardiogenic shock had a significantly higher
Echocardiography in Tako-Tsubo Cardiomyopathy

Catecholamines, and the occurrence of stunned among sympathetic activation, elevated circulating acute phase of TTC. In addition, to date, the link world setting, where measurement of catecholamine levels were not reported. However, been affected by the tethering phenomenon. Second, good(9), visual assessment of wall motion may have ability in evaluating LV wall motion contraction were ability in evaluating LV wall motion contraction were 10.8%. LVOTO should be systematically ruled out LVOTO has been reported in 25% of patients in a small series of 32 TTC patients (17). In our larger population, the prevalence of LVOTO was 12.8%. LVOTO should be systematically ruled out by echocardiography in order to implement an appropriate therapeutic strategy. In patients with LVOTO and markedly impaired LV systolic function, β-blockade and intra-aortic balloon counterpulsation should be the preferred treatment options compared to inotropic agents to prevent the development of significant intraventricular gradients and subsequent hemodynamic deterioration (36).

Study limitations. Several issues should be considered when interpreting these data. First, only patients with good acoustic windows were evaluated. Although our intraobserver and interobserver variability in evaluating LV wall motion contraction were good (9), visual assessment of wall motion may have been affected by the tethering phenomenon. Second, catecholamine levels were not reported. However, our observation reflects clinical practice in a real-world setting, where measurement of catecholamine levels is not systematically performed in the acute phase of TTC. In addition, to date, the link among sympathetic activation, elevated circulating catecholamines, and the occurrence of stunned myocardium in TTC remains hypothetical (37). It would have been of interest to compare the association of echocardiographic and cardiac magnetic resonance (CMR) parameters in TTC patients with and without complications. In a prospective study, Eitel et al. (38) reported robust data for CMR in a large cohort of TTC patients enrolled from 7 tertiary care centers in Europe and North America. However, their series included only 1 patient with cardiogenic shock, probably because CMR is difficult to perform in patients with hemodynamic instability. Finally, CMR is not largely available and not systematically used, especially in the early evaluation of patients with acute coronary syndrome or similar conditions.

Conclusions

Echocardiographic parameters provide additional information compared to other variables routinely used in clinical practice in identifying patients at higher risk of hemodynamic deterioration and poor in-hospital outcome. Our study demonstrates for the first time that LVEF, reversible moderate to severe MR, E/e’ ratio, and age ≥75 years are independent correlates of major adverse events (i.e., acute heart failure, cardiogenic shock, and in-hospital mortality) in a large cohort of TTC patients. Comprehensive seriated echocardiographic examinations should be systematically performed in patients with TTC to monitor systolic and diastolic LV function recovery. Special attention should be paid to patients with significant MR and intraventricular obstruction to promptly institute appropriate pharmacological treatment and adequate mechanical support.

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Key Words: acute heart failure | cardiogenic shock | echocardiography | stress cardiomyopathy | tako-tsubo cardiomyopathy.
Carotid Intima-Media Thickness and Plaque in Cardiovascular Risk Assessment

Tasneem Z. Naqvi, MD, MMM,* Ming-Sum Lee, MD, PhD

ABSTRACT

Carotid intima-media thickness (CIMT) has been shown to predict cardiovascular (CV) risk in multiple large studies. Careful evaluation of CIMT studies reveals discrepancies in the comprehensiveness with which CIMT is assessed—the number of carotid segments evaluated (common carotid artery [CCA], internal carotid artery [ICA], or the carotid bulb), the type of measurements made (mean or maximum of single measurements, mean of the mean, or mean of the maximum for multiple measurements), the number of imaging angles used, whether plaques were included in the intima-media thickness (IMT) measurement, the report of adjusted or unadjusted models, risk association versus risk prediction, and the arbitrary cutoff points for CIMT and for plaque to predict risk. Measuring the far wall of the CCA was shown to be the least variable method for assessing IMT. However, meta-analyses suggest that CCA-IMT alone only minimally improves predictive power beyond traditional risk factors, whereas inclusion of the carotid bulb and ICA-IMT improves prediction of both cardiac risk and stroke risk. Carotid plaque appears to be a more powerful predictor of CV risk compared with CIMT alone. Quantitative measures of plaques such as plaque number, plaque thickness, plaque area, and 3-dimensional assessment of plaque volume appear to be progressively more sensitive in predicting CV risk than mere assessment of plaque presence. Limited data show that plaque characteristics including plaque vascularity may improve CV disease risk stratification further. IMT measurement at the CCA, carotid bulb, and ICA that allows inclusion of plaque in the IMT measurement or CCA-IMT measurement along with plaque assessment in all carotid segments is emerging as the focus of carotid artery ultrasound imaging for CV risk prediction. (J Am Coll Cardiol Img 2014;7:1025–38) © 2014 by the American College of Cardiology Foundation.

Recent American Heart Association/American College of Cardiology guidelines designated carotid intima-media thickness (CIMT) along with coronary artery calcium (CAC) score a class IIa recommendation for cardiovascular (CV) risk assessment in asymptomatic adults at intermediate risk of cardiovascular disease (CVD) (1). In addition, consensus documents from the national societies (2) and from the American Society of Echocardiography have simplified intima-media thickness (IMT) and plaque assessment methodology (3). Dedicated ultrasound systems for IMT assessment now incorporate IMT datasets from large clinical studies, which allows generation of CIMT percentile values for individual patients. These recent developments have made IMT and plaque assessment a useful method for CVD risk reclassification in clinical practice (4). Nonetheless, most insurers consider CIMT and plaque assessment as investigational and the data to be insufficient and contradictory to justify reimbursement of CIMT for CV risk assessment (5). This is similar to assessment for CAC, which is considered investigational by insurers (6). Along the same lines the recent US national guidelines recommend against performing CIMT in routine
CIMT is measured between the intimal-luminal and the medial-adventitial interfaces of the carotid artery wall represented as a double-line density on an ultrasound image (Figure 1). The accuracy of the common carotid artery (CCA) far wall IMT measurement was validated against histological specimens (8) as representing the true biological thickness of the vessel wall, whereas the near-wall IMT measurement was shown to have a systematic measurement error because of the echogenicity of the adventitial layer masking the adventitial-medial boundary (9,10) as well as being affected by gain settings (10). The annual changes in IMT are small, and the differences between 25th and 75th percentiles are <1 mm, and, therefore, a high degree of precision is required in CIMT measurement. With sonographer training and strict adherence to quality control of IMT scanning protocol including the angles at which CIMT measurements are made, CIMT offers good interscan and interobserver reproducibility with the sensitivity of detecting atherosclerotic changes. Other differences in imaging protocol include the phase of the cardiac cycle (end-systole vs. end-diastole) when CIMT is measured also differs (23). The phase of the cardiac cycle (end-systole vs. end-diastole) when CIMT is measured also differs among studies. Because of systolic lumen diameter expansion that leads to thinning of CIMT during systole, CIMT values obtained from end-systole are lower than those obtained in end-diastole (24).

The development of automated edge-tracking software, which obviated the need to perform manual measurements, further improved the reproducibility of CIMT measurements (12).

CIMT MEASUREMENT

The carotid artery includes 4 segments, beginning with the CCA. This gives rise to the carotid bulb from which arise the external carotid artery and the internal carotid artery (ICA) (Figure 2). Large clinical studies that measured CIMT to determine its value in predicting incident CVD are listed in Table 1. These CIMT studies varied in the comprehensiveness with which CIMT was assessed. Some imaged only 1 side of the neck, whereas others imaged bilaterally (Table 1). Some included imaging of a single segment (13); others imaged multiple segments (14–16); some studies imaged the far wall of multiple segments (17), whereas others imaged both near and far walls (4,18,19). Far wall measurements of the CCA alone have been favored because the CCA is perpendicular to the ultrasound beam, easily assessable, and reproducible (8–10), whereas the carotid bulb and ICA lie at an oblique angle and are more difficult to image (12). Studies also differed in the type of IMT measurements made (mean or maximum for single measurements, mean of the mean, or mean of the maximum for multiple measurements), varying definition of plaque, whether plaques were included in the IMT measurements, and the different arbitrary cutoff points for CIMT to predict risk. Because of the focal nature of the atherosclerotic process (Figures 2 and 3A), IMT measurements at a site can be very different from those taken at another site (14); hence, measuring CIMT from a single site can lower the sensitivity of detecting atherosclerotic changes.
Framingham risk score (FRS) have been largely negative. In the MESA (Multi-Ethnic Study of Atherosclerosis), CCA-IMT did not predict either coronary artery disease or stroke risk after adjusting for the FRS (area under the curve of 0.78 for risk factors plus CIMT vs. 0.77 for risk factors alone in both models) (30). Another study found the area under the curve of 0.69 for CIMT and FRS versus 0.66 for FRS (31). The CAPS showed that even though CIMT was significant and independently predictive of CV events, when added to the FRS and the European cardiovascular disease risk assessment model systemic coronary risk evaluation (SCORE) models, it did not consistently improve the risk classification of individuals (27). A review of the CIMT studies by Simon et al. (32) shows that in some studies, CIMT added little to the coronary heart disease (CHD) prediction by risk factors, as judged by c-statistic and receiver-operating characteristic curve analysis and that the CHD prediction by CIMT was inferior to that by carotid plaque. Meta-analyses of CIMT studies have also yielded contradictory results. The first meta-analysis that included major clinical studies with CIMT assessment of single or multiple carotid segments showed that for every 0.1-mm increase in CIMT, the future risk of myocardial infarction (MI) increases by 10% to 15% (33). A second meta-analysis that evaluated CCA-IMT alone and excluded CCA or bulb IMT or plaques in 45,828 patients from 14 population-based studies showed that the addition of CIMT does not add clinically meaningful information to the standard prediction modalities (34,35). The Net Reclassification Index (NRI) with the addition of CCA-IMT was only 0.8% for the overall cohort and 3.6% for those at intermediate risk. The NRI examines the net effect of adding a biomarker to the risk-prediction model, and the clinical NRI is the NRI in intermediate-risk patients only. This meta-analysis (34) limited to the evaluation of predictive value of CCA IMT alone was the basis for the recent recommendation of downgrading of CIMT test by the 2013 ACC/AHA prevention guidelines (7). Both meta-analyses (33,34) noted a significant variability in the CIMT methodologies and in reporting of mean or maximal CIMT of single or multiple segments, making it difficult to compare studies or to combine the results from different studies. The findings of this meta-analysis are in contrast to the findings of the ARIC study, which found no significant difference in CHD risk prediction when CCA-IMT alone was added to plaque and traditional risk factors (TRFs) versus the mean combined IMT of all carotid segments added to plaque and TRFs (36). These differences can partly be explained by the fact that in the ARIC study, ICA IMT was only measurable in 43% of study subjects (17).
<table>
<thead>
<tr>
<th>Study</th>
<th>Sample Size, Age of Subjects, yrs</th>
<th>Follow-Up</th>
<th>Carotid Ultrasound Parameters</th>
<th>Plaque</th>
<th>Endpoints</th>
<th>CIMT, RR (95% CI)</th>
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</thead>
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<tr>
<td>KIH (25)</td>
<td>1,257 (0) 42-60 yrs</td>
<td>1 month to 2.5 yrs</td>
<td>CCA-IMT, mean of maximal IMT, near and far wall, bilateral</td>
<td>Focal calcified plaque not included</td>
<td>AMI, stroke, CV death, all-cause mortality</td>
<td>CIMT increment, 0.1 mm; RR: 2.14 (1.08–4.26)</td>
</tr>
<tr>
<td>OHS (16)</td>
<td>5,020 (60) 72.6 ± 5.5 yrs</td>
<td>5 days to 12 yrs (median, 11 yrs)</td>
<td>CCA and ICA-IMT, mean of maximal IMT, near and far wall, bilateral</td>
<td>Plaque included</td>
<td>MI, stroke, CV death, all-cause mortality</td>
<td>Highest tertile: RR: 1.84 (1.54–2.20)</td>
</tr>
<tr>
<td>ARIC (17)</td>
<td>12,841 (57) 45-64 yrs</td>
<td>Mean follow-up, 15.1 yrs</td>
<td>Mean far wall IMT at 6 sites (CCA, bulb, ICA, bilateral)</td>
<td>Plaque included</td>
<td>MI, CV death</td>
<td>IMT ≥1.0 mm: women: HR: 5.07 (3.08–8.36); men: 1.85 (1.28–2.69)</td>
</tr>
<tr>
<td>CAPS (95)</td>
<td>5,056 (51) 19-90 yrs</td>
<td>Mean follow-up, 4.2 yrs</td>
<td>Mean far wall IMT bilaterally at CCA, carotid bifurcation, ICA bulb</td>
<td>Not specified</td>
<td>MI, stroke, death</td>
<td>RR for 1 SD: RR 1.17 (1.08–1.26) for CIMT; RR 1.14 (0.55–1.24), for carotid bulb-IMT; RR 1.09 (1.01–1.18) for ICA-IMT.</td>
</tr>
<tr>
<td>MDCS (28)</td>
<td>5,163 (59) 46-68 yrs</td>
<td>Median, 7 yrs</td>
<td>Mean far wall right distal CCA</td>
<td>Plaque included</td>
<td>MI, CV death</td>
<td>RR for highest tertile: 1.50 (0.81–2.59)</td>
</tr>
<tr>
<td>Rotterdam Study (15)</td>
<td>6,389 (61.9) 69.3 ± 5.5 yrs</td>
<td>Mean, 7 yrs</td>
<td>Average of maximal CCA-IMT of near and far wall, bilateral</td>
<td>Not specified</td>
<td>MI</td>
<td>HR: 1.95 (1.19-3.19)</td>
</tr>
<tr>
<td>LILAC (96)</td>
<td>298 (60) Mean, 79.6 yrs</td>
<td>Mean, 1,152 days</td>
<td>Average of CCA bilaterally, near and far wall</td>
<td>Not specified</td>
<td>MI</td>
<td>All-cause mortality</td>
</tr>
<tr>
<td>Three-City Study (52)</td>
<td>5,895 (62.9) 65-85 yrs</td>
<td>Median, 5.4 yrs</td>
<td>Mean CCA-IMT bilaterally, near and far wall</td>
<td>Plaque excluded</td>
<td>MI, angina, CV death, revascularization</td>
<td>HR for fifth quintile: 0.8 (0.5-1.2)</td>
</tr>
<tr>
<td>IMPROVE (69)</td>
<td>3,703 (52) Median, 64.4 yrs</td>
<td>Mean, 36.2 months</td>
<td>Maximal and mean CCA, ICA, bifurcation, bilateral</td>
<td>Plaque included</td>
<td>MI, SCD, angina, stroke, TIA, heart failure, revascularization</td>
<td>HR for 1 SD increase: mean CCA-IMT: 1.33 (1.18-1.50); mean bifurcation IMT: 1.28 (1.12-1.47); mean ICA-IMT: 1.34 (1.18-1.51)</td>
</tr>
<tr>
<td>MESA (30)</td>
<td>6,814 (33.3) 45-84 yrs</td>
<td>Median, 7.6 yrs</td>
<td>Mean of maximal right CCA-IMT, far wall</td>
<td>Plaque excluded</td>
<td>MI, revascularization, SCD, CV death</td>
<td>RR: 1.17 (0.95-1.45)</td>
</tr>
<tr>
<td>The Edinburgh Artery Study (13)</td>
<td>1,007 (51.7) Mean, 69.4 yrs</td>
<td>12 yrs</td>
<td>Maximal far wall CCA-IMT, bilateral</td>
<td>Not specified</td>
<td>MI, stroke, angina, calcification</td>
<td>IMT &gt;0.9 mm, OR: 1.59 (1.07-2.37)</td>
</tr>
<tr>
<td>Framingham Offspring Study (84)</td>
<td>2,965 (55.3) 58–10 yrs</td>
<td>Average, 7.2 yrs</td>
<td>Mean CCA-IMT, or maximal CCA-IMT, maximal ICA-IMT, bilateral</td>
<td>Plaque excluded</td>
<td>MI, angina, CV death, stroke, restenosis, heart failure</td>
<td>HR for 1 SD mean CCA-IMT: 1.13 (1.02-1.24), HR for 1 SD mean maximal CCA-IMT: 1.21 (1.13-1.29); HR for 1 SD maximal ICA-IMT: 1.21 (1.13-1.29)</td>
</tr>
<tr>
<td>Charlottesville study (42)</td>
<td>727 (45) 16-85 yrs</td>
<td>Mean, 4.78 yrs</td>
<td>Mean CCA-IMT, bifurcation, ICA-IMT, near and far wall, bilateral</td>
<td>Plaque included</td>
<td>MI, revascularization, stroke, TIA</td>
<td>OR for highest quintile of carotid bulb IMT: 5.8 (1.3-26.6)</td>
</tr>
<tr>
<td>FATE (97)</td>
<td>1,574 (0) 49.4 ± 9.9 yrs</td>
<td>Mean, 7.2 yrs</td>
<td>Right CCA-IMT</td>
<td>Plaque excluded</td>
<td>CV death, revascularization, MI, angina, stroke</td>
<td>RR: 1.45 (1.15-1.83)</td>
</tr>
<tr>
<td>OSACA (98)</td>
<td>574 (45.2) 65.3 ± 9.5 yrs</td>
<td>Mean, 2.6 yrs</td>
<td>Mean maximal CCA-IMT, bifurcation, ICA-IMT, near and far wall, bilateral</td>
<td>Plaque included</td>
<td>MI, CABG, angioplasty, PAD, stroke</td>
<td>For 1 SD increase, RR: 1.57 (1.11-2.20)</td>
</tr>
<tr>
<td>Tromso Study (54)</td>
<td>6,226 (44) 25-84 yrs</td>
<td>6 yrs</td>
<td>Mean of near and far wall right CCA-IMT, and far wall of the bulb</td>
<td>Plaque included</td>
<td>MI</td>
<td>Highest IMT quartile, 1.73 (0.98-3.06) in men and 2.86 (0.07-7.65) in women</td>
</tr>
<tr>
<td>CCC (99)</td>
<td>2,190 (55) ≥35 yrs</td>
<td>Median, 10.5 yrs</td>
<td>Maximal CCA-IMT, far wall, bilateral</td>
<td>Plaque excluded</td>
<td>MI, CV death, PCI, CABG</td>
<td>RR: 1 SD, 1.38 (1.12-1.70)</td>
</tr>
<tr>
<td>APSIS (100)</td>
<td>558 (33) 60 ± 7 yrs</td>
<td>Median, 3.0 yrs</td>
<td>Maximal left CCA-IMT, far wall</td>
<td>Not specified</td>
<td>CV death, MI, revascularization</td>
<td>IMT ≥0.26 mm; RR: 0.78 (0.36-1.70) for CV death or MI; RR: 1.07 (0.56-2.04) for revascularization</td>
</tr>
<tr>
<td>Cournot et al. (101)</td>
<td>2,561 (38.2) 51.6 ± 10.5 yrs</td>
<td>2-10 yrs</td>
<td>CCA-IMT, ICA-IMT bilaterally</td>
<td>Plaque excluded</td>
<td>CV death, MI, angina</td>
<td>IMT ≥0.63 mm; RR: 2.26 (1.35-3.79)</td>
</tr>
</tbody>
</table>

AMI = acute myocardial infarction; APSIS = the Angina Prognosis Study in Stockholm; BF = bifurcation; CABG = coronary artery bypass graft; CCA = common carotid artery; CCC = Chin-Shan Community Cardiovascular Cohort Study; CI = confidence interval; CIMT = carotid intima-media thickness; CV = cardiovascular; ICA = internal carotid artery; FATE = Firefighters and Their Endothelium study; IMT = intima-media thickness; CV = cardiovascular; HR = hazard ratio; MDCS = Malmo Die and Cancer Study; MI = myocardial infarction; NOMAS = Northern Manhattan Study; OR = odds ratio; OSACA = Osaka Follow-Up Study for Carotid Atherosclerosis; PCI = percutaneous coronary intervention; PAD = peripheral artery disease; RR = relative risk; SCD = sudden cardiac death; TIA = transient ischemic attack.
ability to image ICA-IMT due to an improvement in ultrasound imaging techniques and pixel resolution, found that ICA-IMT is associated with higher relative risk of incident CVD compared with CCA-IMT (37).

Studies have found greater prediction of stroke by CCA-IMT (30), whereas ICA-IMT appears to predict atherosclerotic cardiac events better (16). In the MESA study (38), age-, race-, and sex-adjusted risk of stroke per SD increase was 2.5 for CIMT versus 0.4 for CAC after multivariable adjustment of risk factors including age, race, sex, ethnicity, smoking, diabetes, blood pressure, low-density lipoprotein, total cholesterol, and use of lipid-lowering medication. The influence of blood pressure on CCA-IMT was indirectly observed in the RADIANCE (Rating Atherosclerotic Disease by Imaging with A New CEPP inhibitor) study in which torcetrapib treatment was associated with an increase in blood pressure and a nearly significant increase in mean CCA-IMT during study period ($p = 0.06$), without a net yearly rate of change in the maximal IMT of 12 carotid segments (21), suggesting that CCA-IMT is more affected by blood pressure than by atherosclerosis. Besides intimal thickening, CIMT represents smooth muscle hypertrophy and/or hyperplasia, which may be induced by pressure overload and/or age-related sclerosis (39-41). CIMT is a measurement of the combined thickness of the intima and the media of the carotid vessel wall. This combined thickness is chosen because current ultrasound instruments with the standard transducers have insufficient axial resolution to discriminate between the intimal and the medial layers that comprise 20% and 80% of IMT, respectively (8-10).

**QUANTIFYING CIMT**

The definition of an abnormal IMT also differs between studies. Some use a definition of an IMT greater than the 75th percentile (3,42). Others define an IMT that is $\geq 1$ SD above the mean or IMT at the upper quartile or IMT at the upper tertile, or an absolute IMT value of $\geq 0.9$ mm or $\geq 1$ mm (13,17). An American Society of Echocardiography consensus statement recommends the use of CIMT greater than the 75th percentile for age, ethnicity, and sex as being abnormal (3).

**CAROTID PLAQUE.** Perhaps the most important difference in the methodology between studies is how plaque is defined and how the plaque data are analyzed. The transition from an increased CIMT to plaque is arbitrarily defined, and it is debated whether the transition from increased carotid IMT to carotid plaque formation is a continuous process (45) or whether carotid IMT and plaques are separate phenotypes (40). Plaque definition used in some studies may represent development (43). Not all CIMT studies include plaque in the CIMT measurements (Table 1). Some studies specifically exclude plaque and selectively measure CIMT in a plaque-free region (44). Others include plaque when measuring CIMT (17). Figure 3A shows a patient with a focal nonobstructive carotid plaque with acoustic shadowing at the far wall of the carotid bulb. In this case, the CCA-IMT is thin and normal. If CIMT is measured in the plaque-free CCA, the CIMT value would be in the normal range, and this patient’s CV risk as predicted by CIMT would be misclassified as being low. This is in contrast to the patient in Figure 3B, who has a focal long plaque but also has thickening of the CIMT in the plaque-free area. In this patient, the CIMT value would be abnormal. Importantly, near-wall IMT in all segments appears thicker than far-wall IMT in this patient, but near-wall IMT was often not measured or reported in several studies that may have led to underestimation of CVD risk.

CIMT studies have varied widely in how plaques are defined and how the plaque data are analyzed. The transition from an increased CIMT to plaque is arbitrarily defined, and it is debated whether the transition from increased carotid IMT to carotid plaque formation is a continuous process (45) or whether carotid IMT and plaques are separate phenotypes (40). Plaque definition used in some studies may represent
Some studies define plaque as a focal thickening of the intima-media >1 mm, protruding into the lumen, which is at least twice as thick as the IMT on either side (46). Other studies define plaques as carotid IMT >1.2 mm (47). Yet others subjectively define plaques as present or absent (48). The European Mannheim consensus defined plaque as a focal thickening that encroaches into the lumen by 0.5 mm or by 50% of the surrounding IMT or where IMT is >1.5 mm (49). Other common criteria for plaque identification are shadowing in wall texture, roughness, and inconsistency in the visualization of structural boundaries along with bright echogenicity (44).

In studies in which plaques are taken into consideration, the way in which plaques are analyzed differs. Table 2 lists studies in which carotid plaque was evaluated as a prognostic predictor of CV events. In some studies, plaque assessment is qualitative, in which the presence or absence of plaques was recorded and analyzed categorically as either “yes” or “no” (50). Others rely on visual assessment of plaque size and burden, classifying plaque burden as none, mild, moderate, or severe (51). Other studies are more quantitative and include detailed analysis of plaque burden, in which the number of plaques (52), plaque thickness (53), and plaque area (54–56) are assessed. Some studies show that “plaque phenotypes” such as plaque irregularity (57), and plaque calcification (44) add to CV risk prediction. Figure 3 illustrates the variability in plaque size and appearance. Figure 3C shows a patient with multiple plaques in the carotid bulb, and Figure 3D shows a patient with a large calcified layered plaque along the carotid vessel wall. Thus, plaques differ in their morphology and composition, and simply categorizing plaque as “yes” and “no” clearly fails to capture plaque complexity and its implications for CV risk.

New technology may aid in the ultrasound characterization of complex plaques. Computerized algorithms based on gray-scale pixel analysis have been developed for texture analysis of plaques. Pixel-distribution analysis further provides a quantitative method for assessing plaque composition (58). These tools have been validated against tissue characteristics of endarterectomy specimens (58,59) as well as clinical endpoints. Echolucent carotid plaques and plaques with surface irregularity are associated with a higher risk of future ischemic stroke and a low level of high-density lipoprotein cholesterol level (60). Furthermore, plaque lucency is more reproducible than plaque thickness measurement (61). Three-dimensional measurement of plaque volume (62) and vessel volume (63) has shown promise in determining regression of atherosclerosis and is being tested for CV risk prediction (64). Plaque vascularity, which relates to activity of atherosclerosis, can be assessed with the use of ultrasound contrast agents, but its use has not been tested in a prognostic setting (65). Celi et al. (66) demonstrated that quantitative evaluation of vasa vasorum on pathology correlated with qualitative assessment of plaque vascularity by ultrasound. Additionally, increased plaque vascularity by ultrasound correlated well with B-mode echolucency, which is a sign of a vulnerable plaque.

**Predictive Value of IMT Versus Plaque in Population-Based Studies in Predicting Future MI.** Given that plaque formation is a manifestation of atherosclerosis, it is not surprising that the presence of plaques predicts future CV events. A meta-analysis of 11 population-based studies including 54,336 patients showed that carotid plaque, when compared with CIMT (inclusive of CCA, bulb, and/or ICA depending on the study), had a significantly higher diagnostic accuracy for the prediction of future MI (67). After adjusting for Framingham risk factors, the relative diagnostic odds ratio comparing plaques and CIMT assessment was 1.35, suggesting that plaque assessment was 35% better than CIMT in predicting future cardiac events. The specificity of event prediction was also higher with carotid plaque. The 10-year event rates of MI after negative results were lower with carotid plaques compared with a normal CIMT.

These studies suggest that varying results among studies are likely related to methodology and that CCA-IMT measurement at sites not containing plaque, versus IMT measurement in the carotid bulb and ICA, inclusive of plaque, if present, represent 2 separate phenotypes. Measurement of carotid plaque alone was more predictive of CV events than either IMT phenotype in a meta-analysis (67,68). In addition, assessment of CIMT at multiple angles evaluates the asymmetrical nature of atherosclerosis better than measurement at a single angle only.

Analyses of individual studies also suggest that plaque is more effective than CIMT in predicting future CV events. Mean CIMT of all segments when added to TRFs and plaque significantly increased CHD risk prediction in men but not in women in the ARIC study (50). The ARIC study (Table 1) included 13,145 healthy subjects (7,463 women) between 45 and 64 years of age at the time of the baseline study visit (50). Over a mean follow-up of 15.2 years, there were 1,822 CV events that included MI, death, and revascularization. The model that performed the best included TRFs plus CIMT plus plaque. When the TRFs plus CIMT plus plaque model was compared with the TRFs-only model, the NRI was 9.9% in the overall sample (8.9% in men and 9.8% in women) and
<table>
<thead>
<tr>
<th>Study (Ref. #)</th>
<th>Sample Size, No. (% Women)</th>
<th>Age of Subjects, yrs</th>
<th>Follow-Up</th>
<th>Definition of Plaque</th>
<th>Endpoints</th>
<th>Plaque RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tromso Study (54)</td>
<td>6,226 (44)</td>
<td>25-84</td>
<td>6 yrs</td>
<td>Localized protrusion of the vessel wall into the lumen</td>
<td>MI</td>
<td>Highest plaque area tertile, RR: 1.56 (1.04-2.36) in men and RR: 3.95 (2.16-7.19) in women</td>
</tr>
<tr>
<td>APSIS (100)</td>
<td>558 (33)</td>
<td>60 ± 7</td>
<td>Median, 3.0 yrs</td>
<td>Distinct area with IMT more than twice that of neighboring sites</td>
<td>CV death, MI</td>
<td>Presence of plaque, RR: 1.83 (0.96-3.51)</td>
</tr>
<tr>
<td>KHD (25)</td>
<td>1,288 (0)</td>
<td>42-60</td>
<td>1 month to 2.5 yrs</td>
<td>Area with mineralization or focal protrusion into the lumen, measured at the carotid bulb</td>
<td>MI</td>
<td>Small plaque, RR: 4.15 (1.51-11.47), large plaque, 6.71 (1.33-33.91)</td>
</tr>
<tr>
<td>Rotterdam Study (15)</td>
<td>6,389 (61.9)</td>
<td>69.3 ± 9.2</td>
<td>7-10 yrs</td>
<td>Focal widening relative to adjacent segments with protrusion into the lumen</td>
<td>MI</td>
<td>HR for severe plaque: 1.83 (1.27-2.62)</td>
</tr>
<tr>
<td>ARIC (50)</td>
<td>13,145 (57)</td>
<td>54.0 ± 5.8</td>
<td>Mean, 15.1 yrs</td>
<td>Plaque defined as meeting 2 of 3 criteria: 1) CIMT &gt;1.5 mm; 2) protrusion into the lumen; and 3) abnormal wall texture</td>
<td>MI, CV death, revascularization</td>
<td>HR varied depending on risk factors. Model with plaque and CIMT improved area under the curve from 0.742 to 0.755</td>
</tr>
<tr>
<td>MDCS (28)</td>
<td>5,163 (59)</td>
<td>46-68</td>
<td>Median, 7 yrs</td>
<td>Focal IMT &gt; 1.2 mm</td>
<td>MI, CV death</td>
<td>RR for presence of plaque: 1.81 (1.14-2.87)</td>
</tr>
<tr>
<td>Cournot et al. (101)</td>
<td>2,561 (38.2)</td>
<td>51.6 ± 10.5</td>
<td>2-10 yrs</td>
<td>Focal protrusion into the vessel lumen</td>
<td>MI, angina, CV death</td>
<td>RR for the presence of plaque 2.81 (1.84-4.29)</td>
</tr>
<tr>
<td>KIHD (25)</td>
<td>1,288 (0)</td>
<td>33 yrs</td>
<td>1 month to 2.5 yrs</td>
<td>Area with mineralization or focal protrusion into the lumen, measured at the carotid bulb</td>
<td>MI</td>
<td>RR: 1.38 (1.14-1.67); RR for mortality: 1.23 (1.04-1.44)</td>
</tr>
<tr>
<td>Framingham Offspring Study (84)</td>
<td>2,965 (55.3)</td>
<td>58 ± 10</td>
<td>Average, 7.2 yrs</td>
<td>Wall thickening ≥50% compared with surrounding vessel wall</td>
<td>MCA plaque, MI, CV death</td>
<td>Presence of plaque, RR: 1.19 (1.49-2.47)</td>
</tr>
<tr>
<td>Prati et al. (57)</td>
<td>1,348 (53)</td>
<td>18-99</td>
<td>Average, 12 yrs</td>
<td>Wall thickening ≥50% compared with surrounding vessel wall</td>
<td>MI, angina, CV death</td>
<td>RR: 1.16 (1.03-1.31)</td>
</tr>
<tr>
<td>Sestri et al. (102)</td>
<td>403 (0)</td>
<td>77.7 ± 3.6</td>
<td>48 months</td>
<td>Focal widening with protrusion into the lumen</td>
<td>CV death</td>
<td>Event incidence for group with high total plaque score: 50-75 yrs, 2.9% (1.1-7.5); &gt;75 yrs, 9.6% (5.6-17.0)</td>
</tr>
<tr>
<td>Xie et al. (103)</td>
<td>3,258 (59)</td>
<td>38-79</td>
<td>5 yrs</td>
<td>Focal structure encroaching into the lumen with maximal thickness &gt;1.5 mm</td>
<td>MI, CVA</td>
<td>CCA plaque, RR: 1.90 (1.15-3.13); BIF plaque, RR: 1.26 (0.86-1.83)</td>
</tr>
<tr>
<td>CAFES-CAVE (88)</td>
<td>10,000 (30.5)</td>
<td>53.2 ± 6.3</td>
<td>10 yrs</td>
<td>Class I, normal; class II, IMT &gt;1 mm; class III, plaque defined as IMT 1 mm with irregular increased echogenicity; class IV, stenotic plaque with stenosis &gt;50%</td>
<td>MI, CV death, revascularization</td>
<td>Event rate by class: class I, 0.1%; class II, 8.6%; class III, 39.28%; class IV, 81.5%</td>
</tr>
<tr>
<td>CAFES-CAVE (88)</td>
<td>6,562 (52.6)</td>
<td>61.1 ± 10.2</td>
<td>Mean, 7.8 yrs</td>
<td>MI, angina, CV death, revascularization, stroke, death after stroke</td>
<td>MI, CV death, angina</td>
<td>Plaque &gt;0%, HR: 1.67 (1.33-2.10); plaque 25%, HR: 1.67 (1.30-2.13)</td>
</tr>
</tbody>
</table>

CAFES-CAVE = carotid-femoral morphology and cardiovascular events; CHD = coronary heart disease; CVA = cerebrovascular accident; other abbreviations as in Table 1.
21.7% in the intermediate-risk groups (16.4% in men and 25.4% in women). None of the subjects were reclassified from the high-risk group to the low-risk group or vice versa. In this study, the ICA IMT could not be measured in more than one-half of the cases, and plaques were classified categorically as “yes” or “no.” Despite these limitations, this study showed that when plaque data are added to any level of IMT (at less than the 25th percentile, 25th to 75th percentiles, or higher than the 75th percentile), there was an added improvement in risk CHD prediction in both men and women. It should be noted that, in this study, plaque data were obtained from the near and far wall of all carotid segments, whereas IMT data were obtained only from the far wall of all segments and was inclusive of plaque in the far wall. In this study, adding plaque and CIRM data best improved risk prediction in men and women in the intermediate-risk group; however, plaque presence had a more profound effect on improving risk prediction in women than in men. It may be that CIMT (of the far wall of all carotid segments) in men had already included plaques, which are more prevalent in men, whereas additional assessment of plaque in the near wall in women, who might have small plaque burden overall, improved assessment of atherosclerosis that might not have been represented by CIMT of the far wall alone.

The Three-City Study evaluated older individuals 65 to 85 years of age over a median follow-up period of 5.4 years. Among 5,895 adults with no history of coronary artery disease, the presence of carotid plaque, but not CCA-IMT measured at a plaque-free site, was found to be an independent predictor of a first cardiac event (52). On multivariate analysis, carotid plaque at 1 site was associated with a hazard ratio (HR) of 1.5 (95% confidence interval: 1.0 to 2.2), and the presence of plaques at ≥ 2 sites was associated with an HR of 2.2 (95% confidence interval: 1.6 to 3.1). Adding carotid plaques to conventional risk factors significantly improved carotid risk prediction, with an NRI of 13.7%. This study highlights the methodological issue of differentiation of CIMT at plaque-free sites versus IMT inclusive of plaque.

The Tromso Study evaluated total plaque area in 6,226 individuals 25 to 84 years of age with no history of MI. Plaque burden was separated into tertiles. After a 6-year follow-up, MI occurred in 6.6% of men and 3.0% of women. Men in the highest tertile of plaque had a 56% higher risk of MI compared with those with no plaque. For women, those in the highest tertile had a 3.9-fold higher risk. This study also analyzed carotid IMT and found that IMT did have predictive power, but when carotid bulb IMT was excluded from the analyses, IMT did not predict MI in either sex (54).

Because plaque develops in the carotid bulb, presumably the loss of predictive power was due to the exclusion of plaque from the analysis. Extending the analysis to 10 years, the Tromso group found that plaque, but not IMT, was predictive of first-ever ischemic stroke. The multivariable-adjusted HR in the highest quartile of plaque area versus no plaque was 1.73 (p = 0.004) in men and 1.62 (p = 0.03) in women. There was no difference in stroke risk across quartiles of IMT in multivariate analysis (55).

The investigators for the Three-City Study showed that plaque but not CIMT added to CVD risk prediction over TRFs (52), and the HRs for incident CVD events increased as the number of sites with plaque increased. Similar findings were observed in the IMPROVE (Carotid Intima Media Thickness [IMT] and IMT-Progression as Predictors of Vascular Events in a High Risk European Population) study (69), where the mean of maximum measurements of all carotid ICA IMT segments performed significantly better than CCA far-wall mean IMT in reclassification of coronary or CV events in models adjusted for risk factors. In this study, the presence of at least 1 plaque, defined as maximal IMT >1.5 mm, performed significantly better than mean IMT only when the latter was measured in plaque-free areas; otherwise, the predictive value of the plaque presence alone was always significantly worse. This is likely because, in this study, mean or maximal IMT was inclusive of plaque in all segments and was representative of combined CIMT and plaque. These studies suggest that carotid plaque has predictive power for incident atherosclerotic heart disease. Plaques tend to form at the carotid bulb and at the ICA. This may explain why IMT measurements from these segments are good predictors of CV events, whereas IMT measurements from the CCA alone or IMT measurements that specifically exclude plaque are less predictive of atherosclerotic cardiac disease. Because increased CCA-IMT is associated with an increased stroke risk, combined assessment of FRS, IMT, and plaque may enhance prediction of total CVD (70).

**ASSOCIATION OF CIMT AND PLAQUE WITH RISK FACTORS**

Another clue to the link between CIMT and plaque in CVD risk prediction comes from their association with risk factors. The British Regional Heart Study, in which CCA far-wall IMT was measured along with bulb IMT and plaque, found that CCA-IMT and bulb IMT were correlated with each other but showed differing patterns of association with risk factors and prevalent atherosclerotic disease (71). CCA-IMT was strongly
associated with risk factors for stroke and with prevalent stroke, whereas bulb IMT and plaque were more directly associated with ischemic heart disease risk factors and prevalent ischemic heart disease. IMT is strongly influenced by genetic determinants, but plaque appears to be determined by common CHD risk factors such as age, sex, hypertension, diabetes mellitus, hypercholesterolemia, amount of nicotine consumed, factor VIII, and von Willebrand factor but not genetic inheritance (72). Plaques also correlate with other measures of atherosclerotic vascular disease, such as aortic stiffness, whereas no such association was found for CIMT (73). In particular, echogenic plaques are associated with increased arterial stiffness (74). In multivariable linear regression, traditional coronary risk factors explain only 15% to 17% of IMT, as assessed by the R² statistic (75) but account for 52% of the carotid total plaque area (76).

Besides association with TRFs and a predictive role for CVD risk, an important question in the development of a new biomarker is its clinical utility (i.e., does the novel risk marker change predicted risk sufficiently to change recommended therapy). Single-center studies suggest such imaging results lead to changes in physician prescribing pattern (77), although larger studies are needed in this area. Whether the use of CIMT and plaque assessment will improve clinical outcomes in a randomized clinical trial remains to be tested and may never be performed, given the costs involved. Cost-effectiveness analyses have suggested justification of the additional costs of testing with CIMT and plaque assessment and treatment (78).

3-DIMENSIONAL PLAQUE ASSESSMENT FOR CORONARY ARTERY DISEASE AND PLAQUE PROGRESSION AND REGRESSION. The advent of 3-dimensional (3D) ultrasound allows more accurate quantification of plaque burden. Plaques are outlined on cross-sectional images, and plaque areas are summed up to obtain the total plaque burden (64) (Figure 4). An advantage of performing 3D measurements is the large dynamic scale range of plaque volume or plaque area that enables the assessment of progression or regression of disease in individual subjects. With CIMT measurements, most measurements fall in the submillimeter range. The annual change of CIMT value is roughly 0.01 to 0.04 mm per year in health (26) and disease (79,80), which is lower than the current-generation ultrasound pixel resolution of 0.1 to 0.2 mm, making it very difficult to follow CIMT change in individual subjects in the short term. A meta-analysis that included 16 studies with 36,984 participants in whom CIMT was assessed at least twice showed that even though the CIMT value itself at both time points was associated with future CV risk, there was no association between the progression of CIMT and future CV risk (81). Compared with CIMT, total plaque area has a dynamic range of 5 to 500 mm², ~2 orders of magnitude higher than the range for CIMT measurements (82). The average change in total plaque area is 10 mm² per year, so progression or regression of disease can be easily measured in months (56), allowing assessment of the effect of therapy with a short follow-up. As an example, using 3D plaque volume assessment, 1 group has been able to show large effects of therapy on atherosclerosis within 3 months with a sample size of only ~20 patients per group (62). Carotid vessel wall volume, as assessed using carotid 3D ultrasound, is another parameter that has a good dynamic range. This was used successfully in a dietary intervention trial to evaluate the effect of different diets on atherosclerosis (63). 3D plaque volume was also shown to have a high negative predictive value to

![Figure 4: Carotid Plaque Burden Quantitation](image-url)
exclude significant coronary artery disease compared with 2-dimensional assessment of plaque by indicating its potential role as a clinical screening tool to help identify patients who are at low risk of significant coronary artery disease (83).

**3D PLAQUE CHARACTERISTICS.** Apart from improving quantification of plaque burden, contemporary studies have also started to focus on better characterization of plaque morphology. A plaque scoring system was developed that incorporates stenosis degree, plaque surface irregularity, echolucency, and plaque texture. Individuals in the San Daniele study who had high plaque scores, which correspond to plaques that are stenotic, with high echogeneity, complex heterogeneous echocardiographic pattern, and irregular plaque contours, had a higher risk of the development of CV events (57). This plaque score was shown to significantly increase the predictive power of using TRFs alone.

**NRI OF CAC VERSUS IMT VERSUS PLAQUE IN CVD RISK RECLASSIFICATION**

Studies that have compared CIMT and CAC scores head to head in asymptomatic individuals have in general found that IMT and plaque assessment is more sensitive to detect atherosclerosis than the CAC score (4,64). Unlike the CAC score cutoff values of <100, 100 to 400, and >400 (64), the abnormal IMT value is not as defined. IMT is a normal structure, whereas CAC is pathological. Varying measures have been used to define IMT from single or multiple sites from the near or far wall including, maximal IMT and mean IMT. IMT cutoff that is 75th percentile for age, race, and sex is the most widely used definition, although quintiles, SD, and upper and lower quartiles or tertiles are also used. Hence, what is an abnormal IMT is more difficult to define than the CAC score. In a recent study, Polak et al. (84) evaluated the 2,965-member Framingham Offspring Study cohort for an average follow-up of 7.2 years. The NRI increased significantly after the addition of IMT of the ICA (7.6%) but not IMT of the CCA (0.0%). Because plaques form in the ICA, this was analyzed specifically and the presence of plaque, which was defined as IMT of the ICA >1.5 mm, was associated with an NRI of 7.3%. The analysis of the MESA study data by Polak et al. (85) also showed an NRI of 7% for the mean of maximum measurements of ICA IMT added to TRFs.

IMT and CAC do not appear comparable for risk prediction. The annual NRI and clinical NRI of plaque and CIMT combined with the FRS were 9.9% and 21.7%, respectively, in the ARIC study. CAC studies, on the other hand, have shown NRIs of 21.7% and 30.6% at CAC cutoff points of 100 and 400, respectively (86). CV event rates of 1.43% per year occurred during prospective follow-up of patients who had a CAC score of >100 in the MESA (38), in which adjustment for TRFs showed that each 1-SD increase in log-transformed CAC was associated with an HR of CV events of 2.1, whereas for CIMT, it was 1.3; however, CIMT predicted stroke risk modestly better in this study (HR: 2.1 for CIMT vs. 1.2 for CAC). Plaque was not included in this CIMT analysis. A more comparable HR for incident CVD was found in the Pittsburgh Field Center of the Cardiovascular Health Study for CIMT (HR: 2.3) and CAC score (HR: 2.1) in subjects with a mean age of 80 years (87). This suggests that the mere presence of 1 or more 1.5-mm plaque does not comparably represent atherosclerotic burden as a CAC score of 100 or 400. A much larger plaque burden as a nonhemodynamically obstructive plaque or an obstructive plaque was associated with CV event rates of 39% and 81%, respectively, at 10 years (88). These event rates are comparable to those predicted by CAC scores of 400 in the MESA (38) and St. Francis Heart Study (89). IMT and plaque assessment as used at present is a 2-dimensional technique, whereas CAC is a 3D technique. 3D ultrasound assessment of plaque area or volume was found to correlate more strongly with CAC score (chi-square: 450) than CIMT (chi-square: 24) and would be more reliable in CV risk prediction (64).

**FOLLOW-UP IMT MEASUREMENT**

The theoretical axial resolution of an ultrasound system varies from 0.08 to 0.11 mm for 12-MHz and 7-MHz transducer frequency, respectively. A small change in IMT cannot be measured in individuals (on the order of 0.01 to 0.1 mm) in clinically meaningful timeframes, especially taking into consideration reader error and patient factors leading to variability. Follow-up measurement of IMT is only recommended in large research studies in which standardized IMT protocols including multiple angles, anatomic landmarks, and automated edge detection software technology are used to assess IMT progression or regression on serial measurements in a large dataset. Duplicate measurement of IMT at baseline and follow-up reduces this error.

**UTILITY OF IMT IN YOUTHS**

CIMT provides a measurable reliable marker of the atherosclerotic disease process in the young, a group in whom vascular events will not occur for decades and in whom plaque formation or calcification has not occurred (90). Mean CCA-IMT, bulb, and ICA-IMT were
RELATIVE MERITS/DEMERITS OF CIMT/PLAQUE MEASUREMENT VERSUS CAC IN ASSESSING CV RISK IN ASYMPTOMATIC INDIVIDUALS. One of the limitations of ultrasound is image quality, which depends highly on the sonographer's ability to provide a comprehensive scan using appropriate standardized angles, and the like. CAC measurement, on the other hand, is fairly automated and easy to perform. Patient body habitus can similarly have a greater impact on ultrasound images relative to other imaging modalities. Finally, because CIMT measurements are exacting, small changes (which may occur with minor changes in the angle of imaging) can have an impact on the measured value and hence the interpretation of the test. The advantages of ultrasound scanning are several: there are no major side effects to the test (minimal heating of tissue is possible), no radiation is involved, scans can be done on portable devices, and the overall acquisition time is fast, which offers the possibility of higher throughput, lower cost, and relative safety.

ASSESSMENT OF IMT AND PLAQUE IN CLINICAL PRACTICE

Previous studies required offline measurements of IMT using calipers or edge detection software in a core laboratory, making IMT measurement cumbersome and time-consuming. Recent advances including the use of dedicated IMT ultrasound systems (92) and simplification of IMT protocols have made IMT measurement practical (3). Measurement of CCA-IMT has become automated and is standardized and reproducible if a careful protocol is followed. Our own experience shows good interobserver agreement in IMT and plaque assessment when nonsonographer physician residents are trained in IMT acquisition on more recent generation ultrasound systems that perform online edge detection of IMT (93). On the other hand, assessment of plaque burden is more qualitative at present, and reproducibility of carotid plaque quantification has not been well studied. Use of anatomic landmarks, similar angles at baseline and follow up as well as development of technology that tracks imaging angle and annotates it on the screen may be used to more precisely assess IMT progression or regression on serial measurements (93).

FUTURE DEVELOPMENT

It appears that plaque detection by ultrasound imaging may not require the intensive training that is required for measurement of CIMT and that plaque screening may be more easily accomplished in the outpatient setting. Plaque reproducibility needs to be defined in large multicenter studies. Abnormal cutoff values for plaque presence and size or volume adjusted for age, race, and sex and plaque measurement variability need to be defined. Effect of plaque characteristics on outcome is not well defined in large studies, although preliminary data suggest the utility of contrast imaging in assessing plaque neovascularization (94). Considerable research is needed to illuminate the conditions and/or cohorts where one methodology may be superior to the other. Current methodologies are relatively silent on plaque vulnerability and triggering conditions, limiting attainable improvements in risk classification.

CONCLUSIONS

The controversy surrounding the usefulness of CIMT measurement in risk stratification appears to result from the lack of uniform methodology used in CIMT studies. Measurements of IMT at the carotid bulb and at the ICA are more useful than CCA-IMT, both for risk classification and risk prediction, likely because intimal thickening and plaques form at the bulb and at the ICA. Assessment of plaque burden is a better measure of atherosclerosis and CV risk than is a simple assessment of the presence or absence of plaques. Combined CIMT and plaque assessment appear better than either measure alone. 3D plaque volume correlates with CAC score. In the future, plaque progression and regression assessed by 3D ultrasound may serve as a powerful tool to evaluate the effect of CV therapy.

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**KEY WORDS** cardiovascular risk, carotid artery, intima-media thickness, plaque, ultrasound
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