



IRM cardiaque : mise au point d'un logiciel semi-automatique pour l'étude de la trabéculatation du ventricule gauche

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THÈSE

Pour obtenir le grade de

DOCTEUR DE LA COMMUNAUTE UNIVERSITE GRENOBLE ALPES

Spécialité : **BIS – Biotechnologie, instrumentation, signal et imagerie pour la biologie, la médecine et l'environnement**

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Présentée par

Julien FRANDON

Thèse dirigée par **Daniel FAGRET (EDISCE), UJF**, et
codirigée par **Alexis JACQUIER, CHU Marseille, CRMBM**

préparée au sein du **Laboratoire Radiopharmaceutiques
biocliniques et du CRMBM**
dans **l'École Doctorale Ingénierie pour la santé la Cognition et
l'Environnement**.

IRM cardiaque: mise au point d'un logiciel semi-automatique pour l'étude de la trabéculatation du ventricule gauche

Thèse soutenue publiquement le **Jeudi 13 décembre 2018**,
devant le jury composé de :

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Résumé :

La non compaction du ventricule gauche est une pathologie rare, avec une incidence chez l'adulte estimée à 0,014 % (1). Son pronostic est mal connu, avec une symptomatologie variable (insuffisance cardiaque, trouble du rythme cardiaque, épisodes emboliques) pouvant aller jusqu'à la transplantation cardiaque et au décès (2,3).

Il n'existe pas de gold standard diagnostique. Une non compaction est suspectée devant un aspect hypertrabéculé du myocarde avec un épaissement de la couche non compactée et de profonds récessus. Certains auteurs ont proposé des critères 2D basés sur des rapports de longueurs entre couche compactée et non compactée comme Jenni et al en échographie cardiaque (4) ou Petersen et al en IRM (5). Jacquier et al ont proposé une mesure de la masse non compactée totale en utilisant le volume complet de l'acquisition IRM mais avec une segmentation manuelle incluant le sang entre les trabéculations (6).

L'objectif de ce travail est la mise au point d'un logiciel de segmentation semi automatique de la trabeculation en IRM cardiaque pour avoir des mesures plus précises et reproductibles des trabécules en supprimant le sang. Le logiciel a été validé techniquement sur des IRM 1,5 et 3 Tesla de pratique clinique courante. Il permet une segmentation précise et reproductible en 15 minutes de la masse compactée et non compactée du myocarde. Les mesures de masse ont été comparées à des mesures histologiques réelles sur modèle murin, avec une corrélation excellente. Le logiciel a été testé sur une large cohorte de patients sains, permettant de définir les valeurs normales de trabéculations par tranche d'âge et par sexe. Il est en cours de validation sur les patients atteints de non compaction.

Mots clés :

IRM cardiaque, non compaction, hypertrabéculation, logiciel semi automatique

Title :

Cardiac MR: development of a semiautomatic software to study left ventricular trabeculation.

Summary :

Left ventricular non-compaction is a rare cardiomyopathy with an incidence of 0,014 % in adult patients. Its prognosis remains little known. Untreated, it can lead to heart failure, cardioembolic events, tachyarrhythmia, heart transplant and death.

Non compaction is suspected on echocardiography or CMR when a characteristic double-layered aspect of the myocardium with a thick, non-compacted endocardial layer, prominent trabeculations and deep recesses are observed, but there is no gold standard for the diagnosis. Some have proposed 2D criteria based on ratios of lengths between compacted and non compacted layers evaluated by sonography or MRI. Jacquier and al have proposed a quantification of the total amount of LV trabeculation on CMR but with a manual contouring including blood inside the trabeculae.

Our purpose was the development of a semi-automatic software package to calculate the non-compacted mass that suppresses blood from the trabeculae and evaluates the total amount of compacted and non compacted mass. The feasibility of this trabeculation quantification algorithm was illustrated in multicenter, multivendor 1,5 and 3 T MRI. The software allows a precise and reproducible segmentation in about 15 mins. The quantification software was compared with histology, and tested for accuracy and reproducibility in a mouse model of non-compaction. The software was tested in a large

cohort of healthy subjects to provide the range of normal values of trabeculae across age and gender. Clinical validation on patients with non compaction is in progress.

Keywords :

cardiac MR, non compaction, hypertrabeculated, semi automatic software

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Au Jury :

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1 Contexte :

1.1 Généralités sur la trabéculatation cardiaque (7) :

Au cours du développement embryonnaire, deux processus essentiels au bon fonctionnement cardiaque se déroulent successivement: la trabéculatation et la compaction.

Ces processus font partie de la formation du muscle cardiaque, qui se fait en 4 étapes :

- formation du tube cardiaque primitif avec un endocardium unicellulaire.
- invagination de l'endocardium et apparition des cardiomyocytes qui vont former le trabeculum : c'est la phase de trabéculatation. Les cardiomyocytes restent polygonaux. Ce trabeculum forme un réseau spongiforme avec des récessus profonds. Ce trabeculum n'est pas libre et reste relié au futur myocarde. Il communique avec la circulation sanguine libre au sein du tube cardiaque.
- Entre la 5^{ème} et 8^{ème} semaine de développement, les cardiomyocytes trabéculés vont se regrouper sur le versant externe du mur cardiaque : c'est la phase de compaction. Les cardiomyocytes se modifient : ils s'allongent, se polarisent et se chargent en myofibrille pour devenir contractiles. Cette compaction se fait de l'épicarde vers l'endocarde, de la base vers l'apex et du septum vers la face latérale (8). Cette phase de compaction est plus ou moins complète, avec la persistance de quelques trabécules.
- Puis vient le phénomène de maturation cardiaque avec la formation d'un myocarde compacté, multicouche et spiralé.

La phase de trabéculatation initiale permettrait d'augmenter les apports en oxygène et en nutriments en offrant une plus grande surface d'échange avec le sang puisqu'il n'existe pas encore de vascularisation par les artères coronaires. Ces artères se développeront par la suite, au cours de la phase de compaction.

Au cours des phases de trabéculatation et de compaction, de nombreux gènes commandants différents facteurs de croissance et de signalisation intra-cellulaire interviennent. La trabéculatation et la compaction sont liées par de phénomènes complexes et non totalement élucidés. Une réduction significative de la trabéculatation est habituellement associée à des hypoplasies du muscle cardiaque conduisant à la mort in utero. A l'inverse, une hypertrabéculatation est souvent associée à la non compaction cardiaque.

Ces deux phases indépendantes laissent à penser que deux phénomènes sont possibles : soit une hypertrabéculatation du myocarde (épaississement des trabécules), soit une hypertrabéculatation associée à une non compaction du muscle cardiaque (amincissement du muscle cardiaque compacté associé à l'hypertrabéculatation).

1.2 Mise au point sur la non compaction :

Le ventricule gauche se distingue du ventricule droit par l'épaisseur de sa couche musculaire, qui lui permet de pomper le sang contre une circulation à haute résistance. Cette couche musculaire ou myocarde, se compose de 2 sous-couches :

- une couche de cellules compactée, épaisse, qui lui assure sa contractilité. Cette couche forme la masse musculaire compactée.
- une couche de cellules non compactée, fine, formant des trabéculations. Cette couche forme la masse musculaire non compactée.

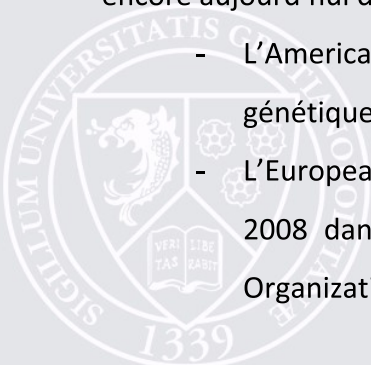
Parfois, ces trabéculations sont très développées et donnent au cœur un aspect « d'éponge » comme cela a été décrit pour la première fois en 1932 par Bellet et Gouley chez un nouveau-né présentant une cardiopathie complexe (9). Il faut attendre 1990 et l'équipe de Chin pour qu'apparaisse le concept de « non compaction du ventricule gauche » (10). Ce diagnostic de « non compaction » est suspecté devant une présentation caractéristique en échographie cardiaque : une fine couche myocardique compactée, une

couche non compactée très épaisse, formant de profonds récessus communiquant avec la cavité ventriculaire. C'est une pathologie rare, qui peut toucher le nourrisson (0,81/100 000), l'enfant (0,12/100 000) (11) et l'adulte (0,014 %) (1). Son pronostic est mal connu, avec une symptomatologie variable (insuffisance cardiaque, trouble du rythme cardiaque, épisodes emboliques) pouvant aller jusqu'à la transplantation cardiaque et au décès (1–3). Oechslin décrit jusqu'à 62% d'insuffisance cardiaque dans sa cohorte suisse de 34 malades atteints de non compaction, en faveur d'une maladie grave (1).

La « non compaction » peut se présenter de manière isolée, ou associée à d'autres cardiopathies comme la cardiomyopathie hypertrophique et la cardiomyopathie dilatée, ainsi qu'à d'autres pathologies neuromusculaires (12–14). Elle peut être sporadique, ou familiale associée à des mutations génétiques reconnues (15,16). L'aspect hypertrabéculé du myocarde peut être congénital, ou acquis au cours de processus pathologiques comme dans l'anémie à cellules de sickle (17), mais aussi au cours de phénomènes physiologiques comme chez le sportif de haut niveau (18). Des aspects hypertrabéculés transitoires chez les femmes enceintes au cours du 3^{ème} mois de la grossesse (19) ou des variations ethniques avec une trabéculatation plus importante dans les populations noires et asiatiques (20,21) ont également été décrits.

Il est donc difficile de savoir si la « non compaction » est une véritable maladie, ou s'il ne s'agit que d'un trait phénotypique partagé par plusieurs processus, pathologiques ou non. C'est la raison pour laquelle, la classification de cette « non compaction » reste un sujet encore aujourd'hui débattu par les sociétés savantes :

- L'American Heart Association la classe en 2006 dans les cardiomyopathies génétiques et la considère donc comme une cardiomyopathie primaire (22).
- L'European Society of Cardiology donne un avis moins tranché, et la classe en 2008 dans les cardiomyopathies non classifiées (23), comme la World Heart Organization's International Classification en 1995 (24).



2 Problématique :

2.1 Problème diagnostique de la non compaction :

Le problème principal de la non compaction réside dans la définition d'un seuil diagnostique, car aucun gold standard n'existe. L'échographie cardiaque a été le premier outil diagnostique et plusieurs critères ont été successivement définis :

- Chin et al en 1990 (10) ont défini un ratio par la mesure 2D de l'épaisseur de la couche myocardique compactée seule (X) par rapport à la longueur des trabéculations + la couche myocardique compactée (Y), au niveau de l'apex cardiaque (en coupe para-sternale petit axe et en vues apicales), en télé-diastole. Le diagnostic de « non compaction » est porté si $X/Y \leq 0,5$.
- Jenni et al en 2001 (4) ont précisé cette formule en introduisant quatre critères (i) un aspect de double couches bien individualisées avec un rapport épaisseur non compactée (NC) sur épaisseur compactée (C) $NC/C \geq 2$ en coupe para-sternale petit axe et en télésystole ; (ii) l'absence d'autre anomalie structurale du myocarde ; (iii) des trabéculations nombreuses et volumineuses avec des récessus inter-trabéculaires profonds ; (iiii) des récessus communiquant avec la cavité ventriculaire en doppler couleur.
- Stollberger et al en 2002 (14) définissent eux 2 critères : (i) plus de 3 trabéculations naissant du mur ventriculaire latérale visible sur une coupe, en position apicale par rapport aux muscles papillaires ; (ii) des récessus intertrabéculaires vascularisés par la cavité ventriculaire en doppler couleur.

Cependant ces critères ont été définis sur des études de très faible puissance statistique et n'ont pas fait leur preuve sur de plus grosses cohortes. En effet, Kohli et al, en reprenant 199 patients hospitalisés pour défaillance ventriculaire gauche, ont montré que 18,5 % des

patients répondaient aux critères diagnostiques de « non compaction » de Chin, 15 % à ceux de Jenni et 12,5 % à ceux de Stollberger. De plus, 5 sujets normaux sur la cohorte de 60 sujets sains utilisée en comparatif, étaient positifs à ces critères, confirmant que les seuils définis étaient trop sensibles (20).

Avec les progrès technologiques et notamment la mise au point des séquences SSFP (steady state free precession) ou en état d'équilibre, le myocarde est désormais explorable de manière plus précise en IRM. Petersen et al ont alors tenté de définir un nouveau seuil diagnostique avec cette modalité. En 2005, sur une étude comparant les IRM cardiaques de 45 volontaires sains, 25 athlètes, 45 patients suivis pour une cardiomyopathie hypertrophique, 14 patients suivis pour une cardiomyopathie dilatée, 17 patients hypertendus, 30 patients avec une sténose aortique serrée et 7 patients porteurs de non compaction (diagnostic clinique + histoire familiale + critères échographiques), un nouveau seuil diagnostique a été défini avec un ratio NC/C $\geq 2,3$ en télédiastole sur des coupes long axes 4 cavités ou 2 cavités (5). Cependant Kawel et al en 2012, en reprenant les IRM de 323 sujets normaux recueillis dans le cadre de la large cohorte de la Multi-Ethnic Study of Atherosclerosis, ont montré que ce seuil de 2,3 était très répandu dans la population générale avec 43 % des patients ayant une zone répondant aux critères (21).

Devant l'échec de la définition d'un ratio de rapport de longueurs, certains auteurs ont choisi d'autres axes de recherche. Captur et al, en 2013 se sont attachés à montrer des différences d'indice de fractalité des trabécules chez des sujets normaux noirs et blancs, et chez des sujets atteints de « non compaction » (25). Il s'agit d'une méthode de quantification de la complexité de la géométrie des trabécules au niveau de la moitié apicale des ventricules. Cette méthode fournit un score sans dimension, difficile à appréhender, faible pour une géométrie simple avec peu de trabécules comme chez le sujet sain blanc (1,235 +/- 0,03), un peu plus élevé chez des sujets hypertrabéculés, comme les sujets noirs (1,253 +/- 0,025) et bien plus élevé chez les sujets atteints de non compaction (1,392 +/- 0,053). En appliquant

cette mesure fractale aux patients de la cohorte MESA, Captur a affiné son score en créant le Fractal max, correspondant au score fractal le plus élevé pour la moitié apicale du VG. Ce score est plus élevé chez les sujets noirs, hispaniques, hypertendus et hypertrophiques (26). Dans cette étude, 2,2% des sujets normaux avait des scores Fractals pathologiques à plus de 1,385, en faveur d'une non compaction, alors que le rapport NC/C pour les mêmes patients était inférieur à 2,3, normal. De plus, il s'agit d'une mesure en 2D qui n'exploite pas l'acquisition IRM 3D de manière optimale et qui ne permet pas d'avoir une idée de la répartition topographique des trabécules. D'autres équipes se sont attachées à étudier la déformation longitudinale, circonférentielle et radiale du myocarde (tissu tracking imaging) en IRM (27) et en échographie (28). Ils ont montré une atteinte précoce des paramètres de déformation du myocarde chez l'adolescent et l'adulte jeune atteints de non compaction. Enfin, Araujo-Filho et al, ont montré des signes précoces de fibrose avec allongement du T1 et augmentation du volume extra cellulaire chez les patients atteints de non compaction sur les séquences T1 mapping(29).

En 2010, Jacquier et al ont exploité l'acquisition volumique de l'IRM en ne s'intéressant plus seulement à un rapport de longueur ou un indice (2D), mais à un rapport de masse compactée et non compactée en utilisant l'acquisition 3D (6). En contournant le myocarde et les trabéculations à main levée, ils ont défini un ratio $NC/C \geq 20\%$ pour établir le diagnostic de non compaction (6). Le principal écueil de cette mesure est la reproductibilité inter et intra observateur avec des coefficients de corrélation mesurés jusqu' à 0,5, en faveur d'un accord seulement modéré entre les mesures (25). Cela est principalement en rapport avec l'aspect non automatisé de la technique, et la grande difficulté à contourner la zone non compactée, comme cela a été décrit par Papavassiliu et al, dans leur étude comparant une méthode de contourage incluant et excluant les trabéculations (30). Le second défaut de la méthode est l'inclusion du volume de sang entre les trabécules dans le calcul de la masse non compactée. Ceci est à l'origine d'une surestimation de la masse non compactée,

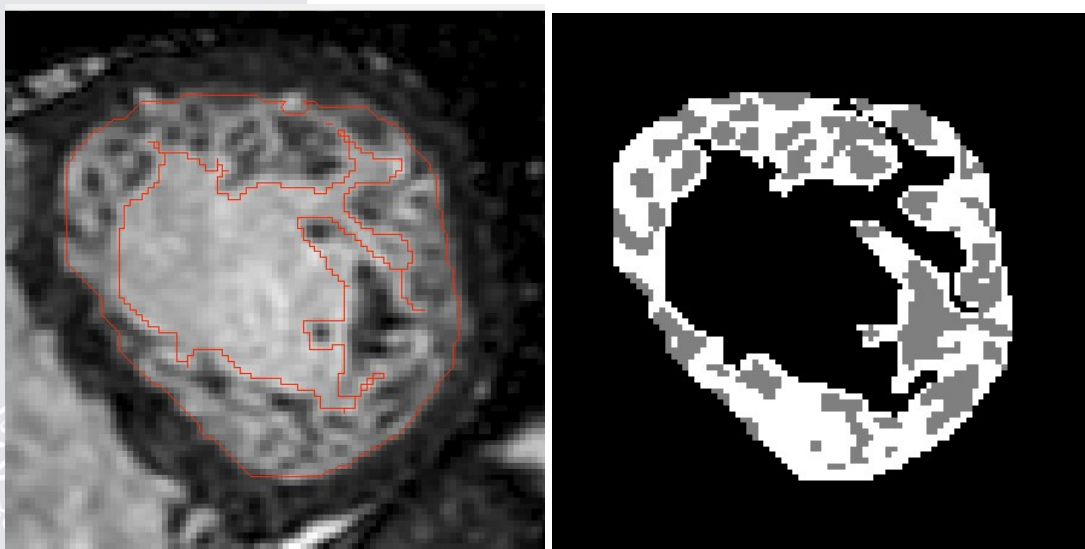
d'autant plus marquée que le réseau tabéculaire est complexe et difficile à contourer, comme c'est le cas dans la non compaction.

2.2 Problème de segmentation des trabéculations :

En effet les trabécules sont difficiles à individualiser au sein de la cavité cardiaque, entourées du sang circulant. Mais leur segmentation est importante car elle modifie significativement les mesures de masse trabéculaire.

Nous avons étudié 15 patients avec un diagnostic de non compaction du ventricule gauche (diagnostic clinique, échographique et IRM) et avons comparé le calcul de la masse non compactée (NC) en incluant et excluant le sang entre les trabécules.

SCMR 2016 : poster P274, Evaluation of trabeculated mass in patient with non compaction: do we need criteria reappraisal?



Exemple de segmentation chez un patient suivi pour non compaction : à gauche contour manuel (en rouge) avec inclusion du sang au sein des trabécules, et à droite segmentation des trabécules en éliminant le sang.

Calcul de la masse non compactée (NC):

NC avec suppression du sang: $35,46 \pm 20,23$ g

NC sans suppression du sang: $52,43 \pm 24,37$ g ($p=.03$)

Sang supprimé: $16,97 \pm 9,56$ mL

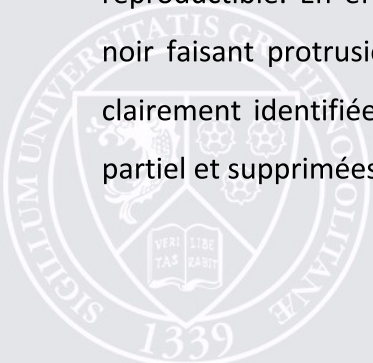
NC/C avec suppression du sang: $24,97 \pm 14,77$ %

NC/C sans suppression du sang: $37,58 \pm 15,68$ % ($p=.74$)

La suppression du sang modifie donc significativement le calcul de la masse non compactée (NC), même sur un petit volume de patients. En revanche, le ratio masse non compactée / masse compactée (NC/C) est diminué de manière non significative.

Grothoff et al se sont attachés à segmenter la masse non compactée en supprimant le sang sur 12 patients atteints de non compaction, 24 sujets contrôles, 10 patients avec une cardiopathie hypertrophique et 11 avec une cardiopathie dilatée (31). Ils ont logiquement trouvé des masses non compactées inférieures à l'étude de Jacquier, mais ont utilisé une méthode de contourage manuelle. Cette méthode manuelle implique une difficulté de contourage des petites trabéculations, et fait questionner sur la reproductibilité de la technique. De plus leur méthode ne détaille pas la technique de segmentation des muscles papillaires et leur inclusion dans la masse trabéculée ou compactée, alors qu'ils peuvent être à l'origine d'erreur de calcul de masse (30,32).

La méthode de segmentation des trabécules doit être stricte et très restrictive pour être reproductible. En effet, les trabécules doivent être clairement définies en IRM: myocarde noir faisant protrusion à l'intérieur de la cavité myocardique blanche. Les structures non clairement identifiées comme des trabécules doivent être considérées comme du volume partiel et supprimées.



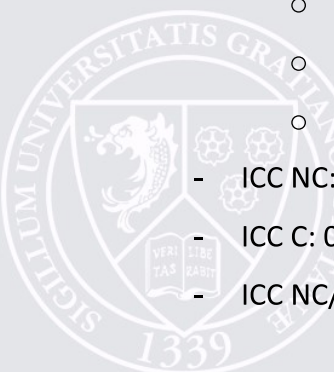
Nous avons effectué des tests de reproductibilité entre différents opérateurs, avant et après formation à la segmentation des trabécules. Nous avons étudié les différences de mesure de masse compacté (C), non compactée (NC) et le ratio NC/C, par calcul du Bland et altman (BA), et calculé le coefficient de corrélation intra classe (ICC). Les segmentations ont été faites par chaque opérateur en aveugle des résultats de l'autre opérateur. 10 patients avec IRM normale ont été segmentés par chaque opérateur avant formation. 10 autres patients avec IRM normale ont été segmentés par chaque opérateur après formation. Un opérateur a segmenté une troisième fois, après formation, pour la reproductibilité intra opérateur. Cette dernière segmentation a été réalisée 10 jours après les précédentes pour éviter les biais de rappel.

Reproductibilité inter observateur avant formation :

- BA:
 - NC: 5,8 g +/- 2,3 g
 - C: 6,4 g +/- 4,1 g
 - NC/C: 0,6 % +/- 3,7 %
- ICC NC: 0,62
- ICC C: 0,97
- ICC NC/C : 0,37

Reproductibilité inter observateur après formation :

- BA:
 - NC: -0,64 g +/- 1,35 g
 - C: -3,61 g +/- 6,1 g
 - NC/C: -0,58 % +/- 1,87 %
- ICC NC: 0,94
- ICC C: 0,98
- ICC NC/C: 0,8



Reproductibilité intra observateur après formation :

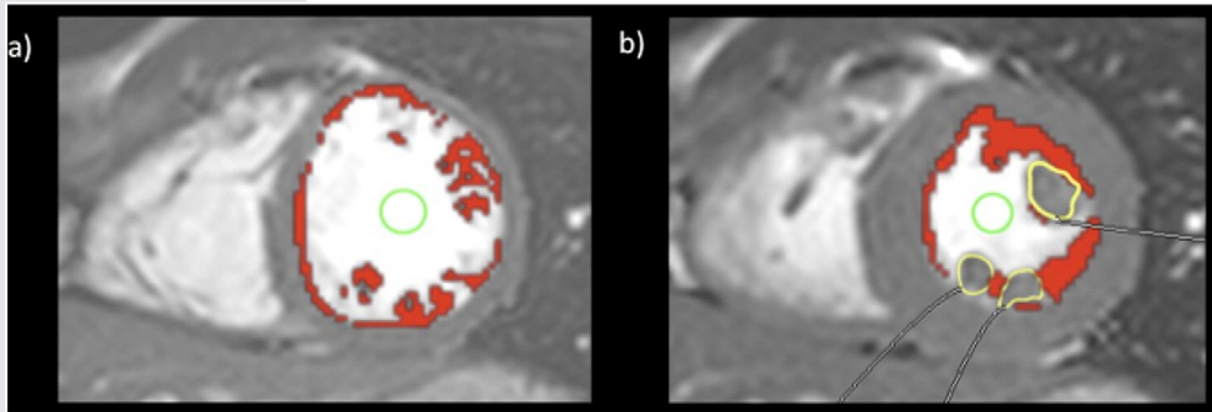
- BA:
 - NC: -0,16 g +/- 0,39 g
 - C: 0,7 g +/- 2 g
 - NC/C: - 0,21 % +/- 0,47 %
- ICC NC: 0,99
- ICC C: 0,98
- ICC NC/C: 0,99

Ces résultats mettent en évidence la difficulté de la segmentation des trabécules à la différence de la segmentation des contours épicaudiques et endocardiques, qui sont reproductibles même sans formation particulière.

Une des solutions pour rendre cette segmentation plus reproductible serait d'avoir un logiciel de segmentation automatique pour limiter les interactions humaines.

Choudhary et al ont essayé d'automatiser la segmentation des trabécules en mettant au point un système de seuillage (33). Leur seuillage est cependant trop sensible, avec beaucoup de volume partiel inclus dans les trabécules et ainsi des masses calculées faussement hautes. Les rapports de masse NC/C étaient à 26 % en moyenne chez les sujets normaux, ce qui est bien au dessus du seuil pathologique de 20 % de Jacquier, qui pourtant incluait le sang dans le calcul de la masse.





Exemple de segmentation avec le logiciel de Choudhary basé sur un système de seuillage unique. Le sang n'est pas totalement supprimé entre les trabécules (en rouge) et beaucoup de volume partiel est inclus dans le calcul de la masse au niveau de la limite endocardique. Ceci explique les résultats élevés pour le calcul de la masse non compactée.

2.3 Objectifs du projet de recherche :

Il apparaît donc important de mettre au point un logiciel de segmentation des trabéculations pour aider le radiologue à segmenter de manière précise la masse compactée et non compactée. Ce logiciel devra permettre une étude 3D de la trabéculations pour avoir un calcul de masse et une répartition de la masse au niveau du ventricule gauche. Un logiciel de segmentation semi automatique semble plus pertinent pour limiter les interactions humaines et ainsi augmenter la reproductibilité.

Ce logiciel devra être compatible avec les différents IRM utilisés en pratiques clinique : 1,5 T et 3T, multivendeurs (Siemens, Philips, GE..). Il devra également être adapté à une utilisation clinique, en permettant de réaliser des segmentations rapides, au cours d'une vacation classique (IRM cardiaque en 20-30 minutes).

Ce logiciel devra être validé sur le plan technique, mais aussi clinique, avec la nécessité d'étudier la trabéculations chez le sujet normal pour créer un atlas normal de la trabéculations

mais aussi chez le sujet malade, pour mieux définir les critères diagnostiques de la non compaction cardiaque, aucun gold standard n'existant à l'heure actuelle.

3 Mise au point et validation technique du logiciel :

3.1 Description du logiciel :

La première étape a consisté à mettre au point le logiciel avec l'aide du Dr Stephanie Bricq et du laboratoire L2ei de Dijon. Le Dr Bricq a programmé le logiciel. Le Dr Frandon a fait le travail de beta testeur avec un travail en boucle vertueuse d'amélioration continue.

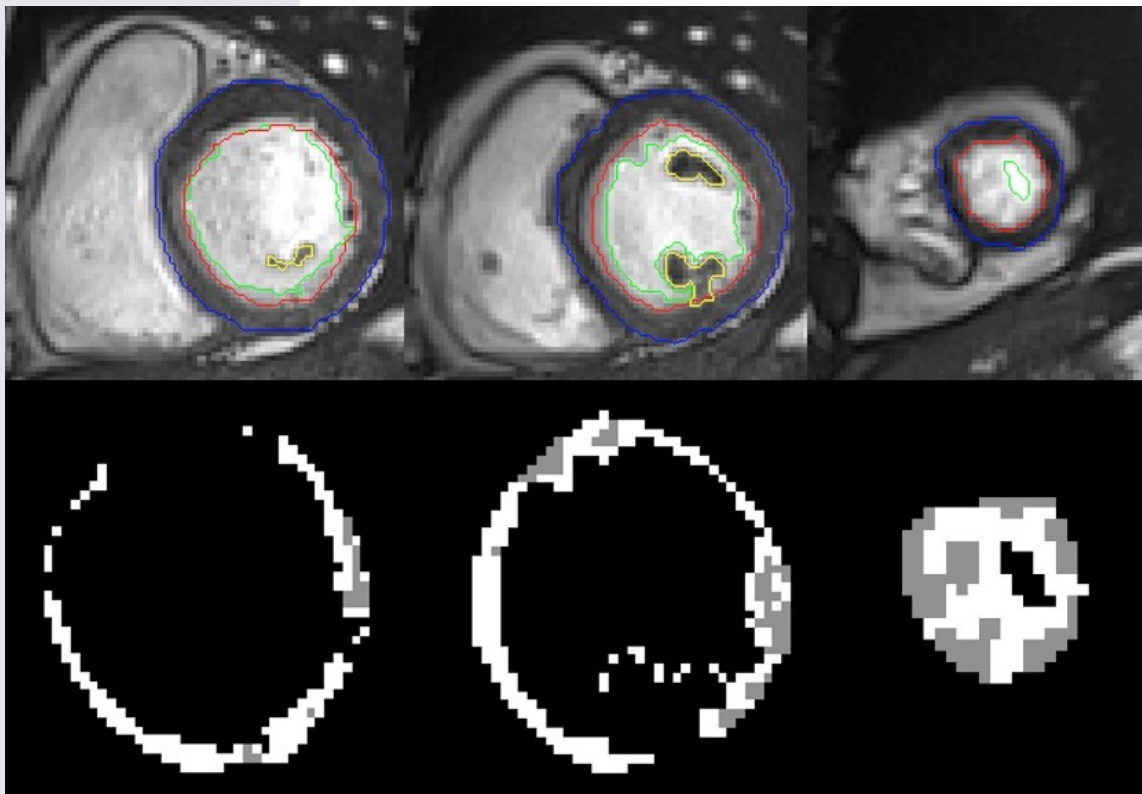
Il s'agit d'un logiciel off line développé sur matlab, téléchargeable sur ordinateur pc ou mac.

A partir de l'acquisition SSFP petit axe, une série d'images en télédiastole, de la base à l'apex, est sélectionnée. Ces images sont chargées dans le logiciel de segmentation semi-automatique. Le logiciel segmente de manière automatique le ventricule gauche après initiation par un clic au centre de la cavité du ventricule gauche :

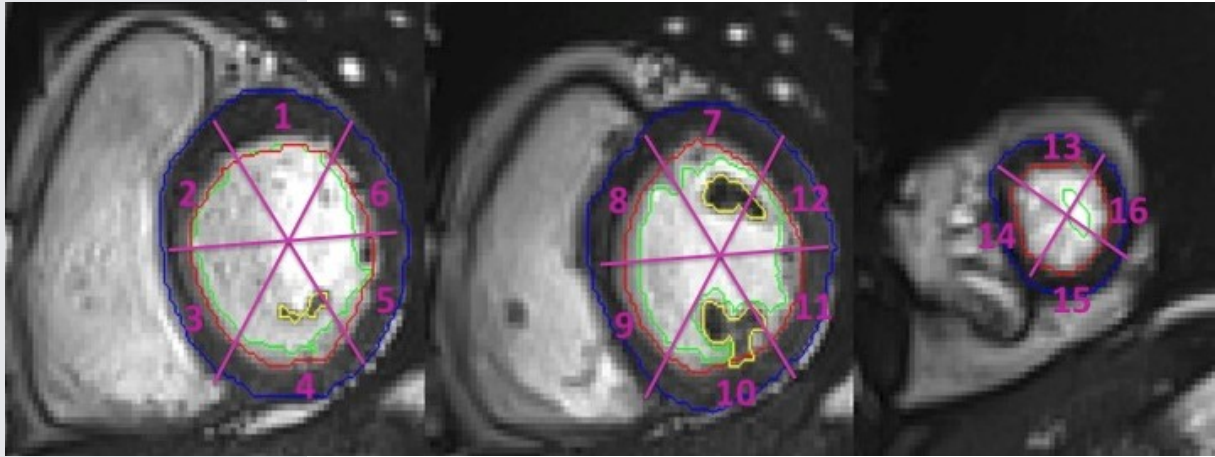
- le contour épicardique du myocarde correspondant à la surface externe du cœur (contour bleu)
- le contour endocardique du myocarde correspondant à la limite interne de la masse compactée (contour rouge)
- le contour des trabéculations correspondant à la surface de la masse non compactée (contour vert)

Chaque contour est modifiable par l'opérateur. Un système de seuillage permet de détecter les muscles papillaires, muscle permettant d'actionner les valves cardiaques et considérés comme de la masse compactée (contour jaune). Lorsque les muscles papillaires ne pouvaient pas être clairement différenciés des trabéculations, ils étaient considérés comme des trabéculations. Un second seuillage permet de supprimer le sang des trabécules pour le calcul de la masse non compactée. Les trabécules sont définies comme du myocarde noir

faisant protrusion à l'intérieur de la cavité myocardique blanche. Les structures non clairement identifiées comme des trabécules étaient supprimées.



Le logiciel permet de segmenter de manière automatique le cœur en 16 segments selon le référentiel de l'American Heart Association (34). Le 17^{ème} segment, le plus apical était extrapolé. Pour le calcul de la masse myocardique, la densité volumique utilisée est de 1,05g/ml. Le logiciel fournit au final un calcul de la masse non compactée et compactée, totale et par segment.



Pour étalonner le logiciel, des patients normaux ont été segmentés, et différents outils d'aide à la segmentation ont été développés, comme le rognage des trabécules et l'érosion du volume partiel, pour avoir un calcul de la masse compactée et non compactée cohérent avec la littérature internationale : compacté $< 90 \text{ g/m}^2$, non compactée $9 \pm 4 \text{ g/m}^2$ (d'après l'étude de Jacquier et al).

Un tutoriel détaillé pour faciliter l'utilisation du logiciel a été réalisé (annexe 1).

3.2 Article :

J Magn Reson Imaging. 2016. doi: 10.1002/jmri.25113.

Semiautomatic detection of myocardial contours in order to investigate normal values of the left ventricular trabeculated mass using MRI.

Bricq S, Frandon J, Bernard M, Guye M, Finas M, Marcadet L, Miquerol L, Kober F, Habib

G5, Fagret D, Jacquier A, Lalande A.

(Article intégral, annexe 2)



L'objectif de cet article était de valider techniquement le logiciel en le testant sur plusieurs machines 1,5 et 3 T, de vendeurs différents, dans des conditions de pratique clinique courante.

Les explorations IRM ont été réalisées dans plusieurs centres sur 5 types de machines : Siemens Magnetom Vision 3 Tesla et Siemens Trio Tim 1,5 Tesla (Dijon), Philips Intera 1,5 Tesla et Siemens Skyra 3 Tesla (Marseille), Philips Achieva 1,5 Tesla (Grenoble).

Le protocole d'acquisition était classique, avec des acquisitions ciné en SSFP en petit axe (temps de répétition entre 3.07 et 3.57 ms, temps d'écho entre 1,17 et 1,79 ms, résolution spatiale entre $0,74 \times 0,74 \text{ mm}^2/\text{pixel}$ et $1,95 \times 1,95 \text{ mm}^2/\text{pixel}$, épaisseur de coupe entre 5 et 10 mm, 6 à 11 séries de vues pour couvrir l'ensemble du VG). Ces acquisitions permettaient le calcul de la fraction d'éjection, la mesure des volumes télésystoliques et télédiastoliques, la masse totale et l'épaisseur segmentaire du myocarde. Une acquisition tardive, 10 min après l'injection de 0,2mmol/kg de gadolinium, était réalisée à la recherche de fibrose myocardique.

Les sujets sains étaient définis par: l'absence d'antécédent cardiovasculaire, l'absence de symptomatologie cardiaque, fraction d'éjection $> 55\%$, volume télédiastolique $< 100 \text{ mL/m}^2$, masse du ventricule gauche $< 90 \text{ g/m}^2$, épaisseur myocardique segmentaire $< 12\text{mm}$, absence de rehaussement tardif.

L'objectif était également d'évaluer la reproductibilité du logiciel pour tester l'apport du semi automatisme sur la procédure de segmentation.

Le calcul de puissance pour évaluer le nombre de sujets nécessaires n'a pas pu être réalisé initialement car, le logiciel n'ayant jamais été testé, il était impossible d'estimer la variation des contours tracés entre observateurs. Cependant, après avoir segmenté les 14 premiers cas, ceux-ci ont été utilisés pour faire le calcul de puissance. Landis et Koch indiquent qu'un test a une concordance pratiquement parfaite lorsque le coefficient de corrélation intraclasse (CCI) est compris entre 0,81 et 1 (35). En prenant le CCI calculé à partir des 14 premiers cas segmentés (CCI = 0,91), le nombre de sujets nécessaire pour obtenir un CCI

significativement $\geq 0,81$ avec un risque α de première espèce égal à 5 % et une puissance de 80 % était de 60. Il a ainsi été choisi d'inclure 60 patients avec 20 patients par tranche d'âge, et parité par tranche d'âge (soit 10 hommes et 10 femmes), pour couvrir un champ de population plus vaste, et avoir un meilleur reflet de la trabéculatation dans la population générale en fonction de l'âge et du sexe.

Les segmentations ont été possibles sur tous les types de machine. La segmentation se faisait en moyenne en 15 minutes : détection automatique des contours en 3 minutes, détection semi automatique des muscles papillaires en 3 minutes, segmentation des trabécules en 8 minutes.

Nos résultats étaient cohérents avec ceux de la littérature : masse non compactée à $6,29 \pm 2,03 \text{ g/m}^2$, avec un rapport NC/C à $10,27 \pm 3,27 \%$. La masse non compactée était plus basse que dans l'étude de Jacquier et al probablement en lien avec la suppression du sang autour des trabécules. Il n'y avait pas de différence significative en fonction de la machine ou de la taille du pixel. Les segments les plus trabéculés se trouvaient à l'apex et sur la paroi latérale. La reproductibilité était bonne allant de 0,8 pour NC à 0,99 pour C.

Pour évaluer la précision de notre segmentation, le logiciel a été testé sur 4 coupes d'IRM de souris avec comparaison aux 4 coupes histologique correspondantes. Bien qu'il s'agisse d'une étude très partielle, les résultats étaient encourageants avec une corrélation à 0,98.



4 Validation histologique des mesures :

4.1 Pourquoi un modèle animal ?

Le premier article a permis de tester le logiciel et de montrer sa bonne reproductibilité pour la mesure de la masse compactée et non compactée. Ce logiciel a montré des résultats concordants et cohérents avec les résultats déjà publiés dans la littérature. Cependant, il fallait démontrer que les mesures réalisées correspondaient bien aux trabécules, et non aux trabécules associées au volume partiel.

Un modèle animal était donc nécessaire pour pouvoir comparer la mesure IRM à la mesure histologique réelle. Ce modèle animal devait présenter un phénotype variable de trabéculations pour vérifier si le logiciel était capable de détecter ces différences.

Par souci de logistique et de disponibilité animale, nous nous sommes tournés vers un modèle murin développé par l'équipe du Dr Lucille Miquerol au CRMBM (projet "mouse cardiovascular development system", n° 01055.02).

4.2 Article :

JCMR : article accepted

Semi-automatic detection of myocardial trabeculation using CMR: correlation with histology and reproducibility in a mouse model of non-compaction.

Julien FRANDON, Stéphanie BRICQ, Zakarya BENTATOU, Laetitia MARCADET, Pierre Antoine BARRAL, Mathieu FINAS, Daniel FAGRET, Frank KOBER, Gilbert HABIB, Monique BERNARD, Alain LALANDE, Lucile MIQUEROL, Alexis JACQUIER

(Article intégral annexe 3)

Un modèle murin de non compaction a été utilisé, basé sur une mutation inductible d'un gène de transcription essentiel au développement précoce du cœur : le gène Nkx 2.5.

Différents types de souris ont été comparés : des souris sauvages (n=5) et des souris mutantes (n=8) avec une mutation soit hétérozygote (n=3), soit homozygote (n=5), induite à différentes phases du développement cardiaque, permettant d'obtenir différents phénotypes de trabéculatation.

Toutes les souris bénéficiaient d'une IRM 11.75 T HD (taille du pixel 0,086x0,086) sur une coupe médio ventriculaire et un sous groupe de 6 souris avaient en plus une acquisition entière du ventricule gauche en petit axe avec une définition standard (taille du pixel 0,172x0,172). La séquence IRM utilisée était du spoiled gradient echo cine.

Toutes les souris étaient sacrifiées, les cœurs récupérés et segmentés en petit axe. Chaque coupe IRM était segmentée avec le logiciel pour le calcul de la masse compactée et non compactée. Chaque coupe histologique était segmentée avec Image J (version 1.49 v, National Institute of Health, Bethesda, USA) selon la même méthode.

L'étude 3D de la trabéculatation du ventricule gauche a montré une répartition homogène de la trabéculatation chez la souris, sans différence significative entre la base, le corps et l'apex du cœur. Une acquisition HD étant très longue (20-30 minutes), elle ne peut donc se réaliser que sur une coupe. Une coupe médioventriculaire a été choisie car elle était, à priori, représentative de la trabéculatation de la souris et offrait plusieurs marqueurs qui permettaient de bien recaler l'image histologique correspondante : muscles papillaires, trabéculations...

La corrélation à l'histologie était excellente. Le logiciel permettait de séparer souris mutantes et sauvages. Les sous-groupes de souris mutantes n'étaient par contre pas différenciables. Le logiciel offre donc la possibilité de réaliser un suivi de la trabéculatation sans sacrifice de la souris.

5 Validation clinique :

5.1 Article sur l’atlas de la trabéculatation normale :

Diagn Interv Imaging. 2018. doi: 10.1016/j.diii.2018.08.014

Distribution of left ventricular trabeculation across age and gender in 140 healthy Caucasian subjects on MR imaging.

Bentatou Z, Finas M, Habert P, Kober F, Guye M, Bricq S, Lalande A, Frandon J, Dacher JN, Dubourg B, Habib G, Caudron J, Normant S, Rapacchi S, Bernard M, Jacquier A.

(Article intégral en annexe 4)

Le premier article sur la validation technique avait étudié la trabéculatation myocardique chez 60 patients normaux. Ces patients avaient été sélectionnés pour évaluer la faisabilité de la segmentation, et la reproductibilité du logiciel. L’article manquait par contre de puissance pour créer un atlas de la trabéculatation normale.

L’objectif de cet article, étudiant une plus large cohorte, était de fournir des valeurs normales de trabéculatation par tranche d’âge et par sexe.

La population se composait de sujets sains, âgés entre 18 et 70 ans (patient de la heart map study, clinical trial NCT01712581, clinicaltrials.gov): 70 hommes et 70 femmes, 28 patients (H=14, F=14) par tranche d’âge (20-29 ans, 30-39 ans, 40-49 ans, 50-59 ans, 60-69 ans). La population était caucasienne. La même méthode de segmentation était utilisée.

La masse compactée moyenne était de $53.9 \pm 11.2\text{g/m}^2$ [26.5-93.4], la masse non compactée moyenne était de $4.9 \pm 2.4\text{g/m}^2$ [1.1-11.4]. Le ratio non compacté/compacté moyen était de $9.2 \pm 4.5\%$ [2.0-29.4]. Les segments les plus trabéculés étaient situés en antéro-apical et latéro-apical avec des ratio à plus de 20 %. Ceci explique qu’une étude monosegmentaire chez le sujet normal soit source de faux positifs.

La masse compactée, non compactée et le volume diminuaient avec l'âge en rapport avec le vieillissement du myocarde. La masse non compactée était dépendante du sexe, du volume télédiastolique et de l'âge. Elle est donc fortement liée au phénomène de précharge, ce qui peut expliquer les hypertrabéculations de la femme enceinte et du sportif.

Le ratio de masse non compactée / compactée était indépendant du sexe, de l'âge et du volume télédiastolique. Il semble être le facteur le plus intéressant à étudier pour s'affranchir des facteurs confondants.

5.2 Travaux préparatoires sur cardiomyopathie dilatée, hypertrophique et non compaction :

La non compaction est parfois considérée comme un phénotype de la cardiomyopathie dilatée (23,24). En effet, les patients avec cardiomyopathie dilatée présentent un aspect hypertrabéculé du ventricule gauche. Amzulescu et al ont montré que l'hypertrabéculation, mesurée en utilisant la méthode de Petersen et de Jacquier, n'était pas liée au pronostic de la cardiomyopathie dilatée (36). Cette constatation est donc plutôt en faveur d'une maladie indépendante, c'est à dire que toute hypertrabéculation n'est pas une non compaction.

Le logiciel a été testé sur une cohorte de 50 patients avec une cardiomyopathie dilatée (CMD), répartis en 5 groupes de 10 patients en fonction de leur fraction d'éjection (50-60%, 40-50%, 30-40%, 20-30%, moins de 20 %).



JFR 2016 : Poster Etude en IRM de la relation entre la fraction d'éjection et la trabéculatation chez les patients avec une CMD

FEVG	50-60 %	40-50 %	30-40 %	20-30 %	10-20 %	p
Volume télédiastolique (mL)	151,78	175,7	222,76	290,23	435,8	<.001
Masse non compactée (g)	14,75	18,33	20,97	22,74	28,1	.069
Masse compactée (g)	121,8	150,94	147,2	180,05	242,6	<.001
Ratio non compactée / compactée (%)	11,99	12,72	13,70	12,34	11,344	.864

Comme chez le sujet normal, la masse non compactée augmente avec l'augmentation du volume et la baisse de la fraction d'éjection. Cependant cette hypertrabéculatation semblait plutôt s'inscrire dans un remodelage, avec un ratio masse non compactée / compactée qui restait stable.

Il est intéressant de noter que la masse non compactée était très supérieure aux valeurs normales retrouvées chez le sujet sain, mais le ratio masse non compactée / compactée restait assez proche et nettement en dessous du 20% décrit par Jacquier et al dans la non compaction.

De la même manière, des sous-phénotypes de cardiomyopathie hypertrophique «hypertrabéculées » ont été décrits (37). Par exemple dans la maladie de Fabry, Kozor et al ont montré une augmentation de la masse trabéculée chez les patients atteints, avec un différentiel plus important que pour la masse compactée par rapport au sujet sain (38).

Nous avons testé le logiciel sur 75 patients avec une cardiomyopathie hypertrophique dans le cadre du travail de thèse du Dr Mathieu FINAS.

Cardiomyopathie hypertrophique, en comparaison à des sujets sains. Médecine humaine et pathologie. 2017. <dumas-01664944>

Quantification of left ventricle trabeculae in Hypertrophied Cardiomyopathy (HCM), in comparison with healthy subjects

M FINAS, Z. BENTATOU, J. FRANDON, P. HABERT, A. JANKOWSKI, S. RAPACCHI, F. KOBER, S. BRICQ, A. LALANDE, M. BERNARD and A. JACQUIER

(Article intégrale en annexe 5)

Le recrutement concernait des patients porteurs d'une cardiomyopathie hypertrophique (CMH), suivis au CHRU Marseille la Timone, et ayant bénéficié dans ce cadre d'une IRM cardiaque durant l'année 2015. Des volontaires sains ont été recrutés prospectivement et appariés aux patients en termes de genre et d'âge (patient de la heart map study, clinical trial NCT01712581, clinicaltrials.gov). Pour chaque sujet, la masse trabéculée (T), la masse compactée (C) et le ratio T/C étaient calculés à l'aide du logiciel.

75 sujets ont été recrutés dans chaque groupe (cardiomyopathie hypertrophique et sujets sains). Une différence significative existait pour T/C entre les sujets sains et les sujets malades (13.4+/-3.1% versus 9.9+/-2.6%, $p<0.001$).

Chez les patients du groupe cardiomyopathie hypertrophique, les segments les plus trabéculés étaient les segments 12, 13 et 16 (segmentation AHA) et les plus hypertrophiés étaient les segments 1, 3 et 9.

Le ratio masse non compactée / masse compactée semblait à nouveau être le plus discriminant et indépendant. L'hypertrabéculatation des patients avec CMH conservait aussi un ratio inférieur au seuil de 20 %.

Le logiciel a également été testé sur les patients non compactés de la série de Jacquier et al, puis ont été comparé aux 60 patients segmentés pour le premier article sur la validation technique.

SCMR 2016 : poster P274, Evaluation of trabeculated mass (NC) in patient with non compaction (LVNC): do we need criteria reappraisal?

Calculation of NC mass in population with LVNC:

NC without blood was $35,46 \pm 20.23$ g

NC freehand was $52,43 \pm 24,37$ g ($p=.03$)

Blood suppressed was $16,97 \pm 9,56$ mL

NC/C without blood was $24,97 \pm 14.77$ %

NC/C freehand was $37,58 \pm 15,68$ % ($p=.74$)

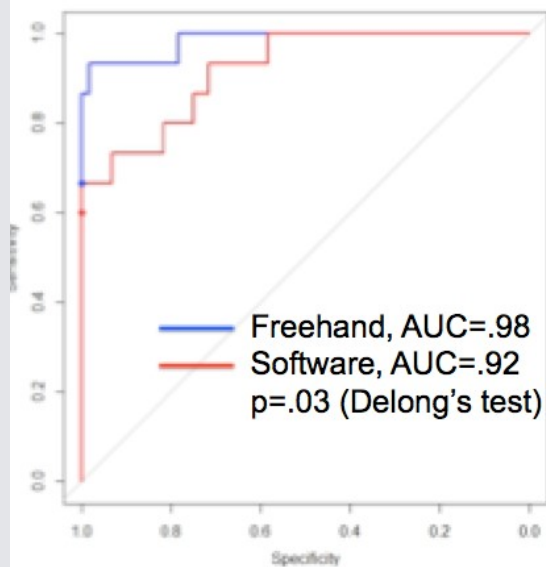
Calculation of NC mass in population control:

NC without blood was 10.57 ± 3.89 g

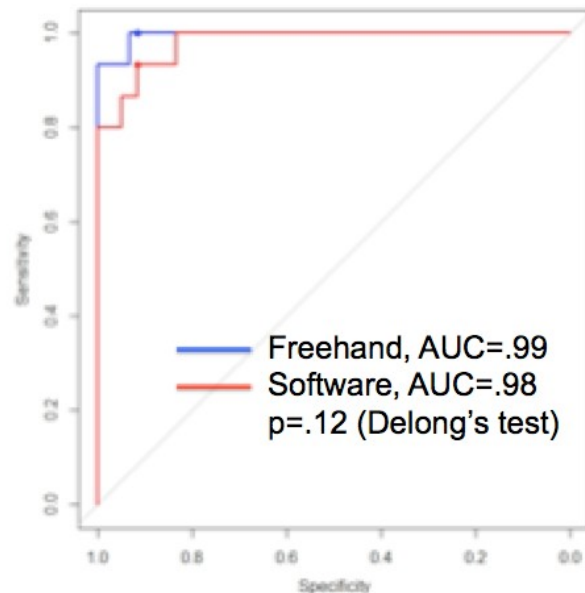
NC/C software was 10.26 ± 3.27 %



NC/C LV mass threshold = 20 %



NC threshold = 15 g



Chez le patient non compacté, le ratio masse non compactée / compactée était bien plus élevé que chez le sujet sain, avec des ratios moyens à plus de 20 %. Cependant, chez certains patients malades, il n'y avait pas d'hypertrabéculatation significative (ratio aux alentours de 10 %).

La suppression du sang faisait chuter significativement le ratio NC/C, avec des différences moins marquées qu'avec la segmentation manuelle. Cela se traduisait sur les courbes ROC, qui présentaient une aire sous la courbe plus importante avec la segmentation manuelle pour les seuils de 20%. Il n'y avait pas de différence significative avec l'évaluation de la masse trabéculaire en prenant un seuil de 15 g de trabécules.



6 Perspectives :

Les perspectives de ce travail sont immenses et concernent plusieurs domaines : technique, exploration animale et clinique.

Pour le moment, il s'agit d'un logiciel off line à télécharger sur son ordinateur. L'équipe de Stéphanie BRICQ et du Le2i de Dijon, travaille sur une amélioration du logiciel pour le rendre plus ergonomique, plus intuitif et plus souple. L'objectif est d'obtenir une version gratuite, disponible en ligne, pour que chaque centre puisse l'utiliser en pratique clinique courante. Le principal défaut du logiciel reste son coté semi automatique, à l'origine d'un certain temps de segmentation et d'erreurs possibles de mesure, pouvant faire diminuer la reproductibilité. Le développement actuel de l'intelligence artificielle avec notamment les techniques de « machine learning » et de « deep learning » permettent d'espérer à court terme la mise au point d'un logiciel totalement automatique qui permettra d'augmenter la reproductibilité des mesures et diminuer le temps de segmentation.

Le logiciel a montré la possibilité de mesurer de manière fiable et précise la trabéculatation des souris en IRM. Ceci permet d'envisager un monitoring non invasif de la trabéculatation au cours du développement de la souris et de différencier les souris sauvages et mutantes sans les sacrifier. Des études animales à long terme sur ces souris pourraient être réalisées, en corrélant les signes cliniques (insuffisance cardiaque, trouble du rythme) à l'hypertrabéculatation et sa distribution. Les effets post nataux de certaines mutations inductibles pourraient également être étudiés afin d'évaluer si l'hypertrabéculatation peut être acquise.

Des valeurs normales de trabéculatation chez le sujet normal par tranche d'âge et par sexe ont été définies, cependant ces valeurs ne sont valables que pour une population caucasienne.

Kawel et al ont montré qu'en étudiant la cohorte multi-ethnique de patients de la MESA study américaine (Multi-Ethnic Study of Atherosclerosis composée de 6000 hommes et femmes), les populations noire et asiatique étaient plus trabéculées que les autres (21). Il serait intéressant de tester le logiciel sur cette population pour vérifier si des différences réelles de la masse trabéculaire existent, ou si la suppression du sang permet de les gommer.

Les différentes études cliniques menées sur patients normaux ou avec une cardiomyopathie dilatée / hypertrophique / non compactée, ont montré l'importance d'utiliser le ratio masse non compactée / compactée pour s'affranchir du remodelage cardiaque et des effets de pré charge. Par son étude 3D du myocarde, le logiciel permet d'évaluer la distribution des trabécules en proposant des résultats par segment AHA. Cette distribution est également intéressante à étudier, pour rechercher une différence de profil de trabéculatation entre les patients non compactés et les patients atteints d'autres cardiomyopathies. Des corrélations existent peut-être entre distribution de la trabéculatation, importance de cette trabéculatation et signes cliniques. La médecine devenant de plus en plus prédictive, le logiciel pourrait ainsi, dans un avenir proche, permettre de connaître l'évolution de la non compaction, et ainsi l'évolution pronostique de la maladie.

La possibilité de supprimer le sang permet de diminuer drastiquement le volume de trabécules et ainsi limiter les défauts de sensibilité des différents tests diagnostiques liés aux faux positifs. Des études sur des cohortes plus larges de patients non compactés sont nécessaires pour affiner les seuils diagnostiques. Il est nécessaire de s'orienter vers un test spécifique et non sensible car la probabilité pré-test pour cette pathologie est très faible (prévalence à 0,014 % (16)).

Le logiciel offre une approche quantitative précise de la trabéculatation. Captur et al proposent une approche qualitative avec un indice de fractalité, reflet de la complexité des trabécules. D'autres auteurs ont étudié le T1 mapping et la déformation segmentaire du

myocarde. Il apparaît indispensable de comparer ces différentes approches pour apporter de nouvelles solutions.

L'ensemble de ces travaux permettrait de nous aider à mieux comprendre la non compaction, et de vérifier s'il s'agit d'une véritable maladie en lien avec l'hypertrabéculatation. Un logiciel précis, fiable, rapide et gratuit sera un atout majeur pour aider les cliniciens à prendre en charge et suivre les patients atteints de non compaction.



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ANNEXES



Annexe 1 : tutoriel de formation pour utilisation du logiciel



NCProcessing segmentation protocol

NCProcessing requires end-diastolic short axis cine images, covering the entire left ventricle (LV) from basal to apical slice. To load images, use the “Open” menu, then select “Open Multiple images” to load a stack of images, “Open image” to load one slice or “Open Contour” to load a previously saved contour file. If the images are displayed from apex to base, choose “Inversion Base -> Apex” from the right toolbar to inverse the display order (Figure 1).

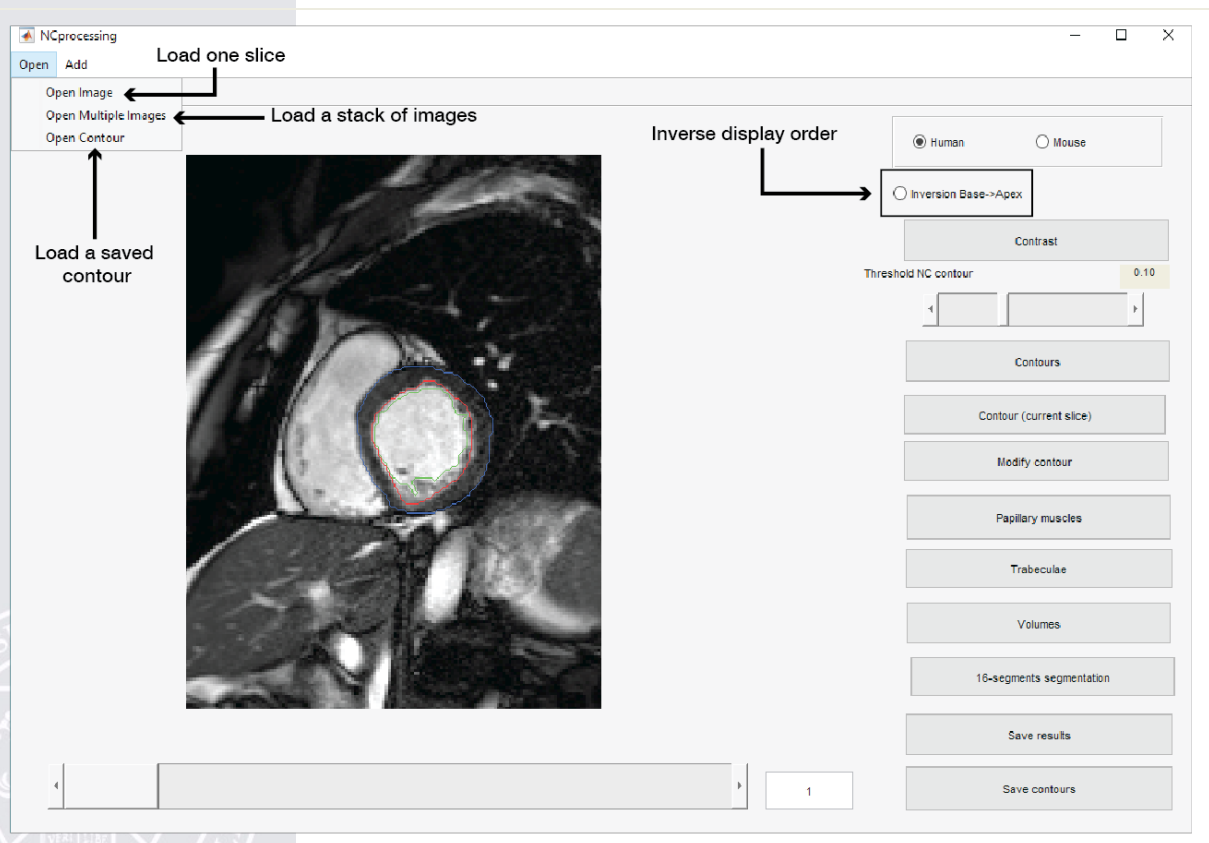


Figure 1: Loading of the images to analyse

PS: The most basal slice is defined as the one that still showed at least 50% of the LV myocardium circumference.

Use the « Add » menu to select a slice from the explorer to add it to the analysed stack of images. To adjust the intensity distribution in the images, click the « Contrast » button. From the displayed popup window, adjust the intensity levels by modifying the values in “Low level” and “High level” (Figure 2 A), or using the sliding red and blue bars of the “Intensity Distribution” at the bottom of the window (Figure 2 B).



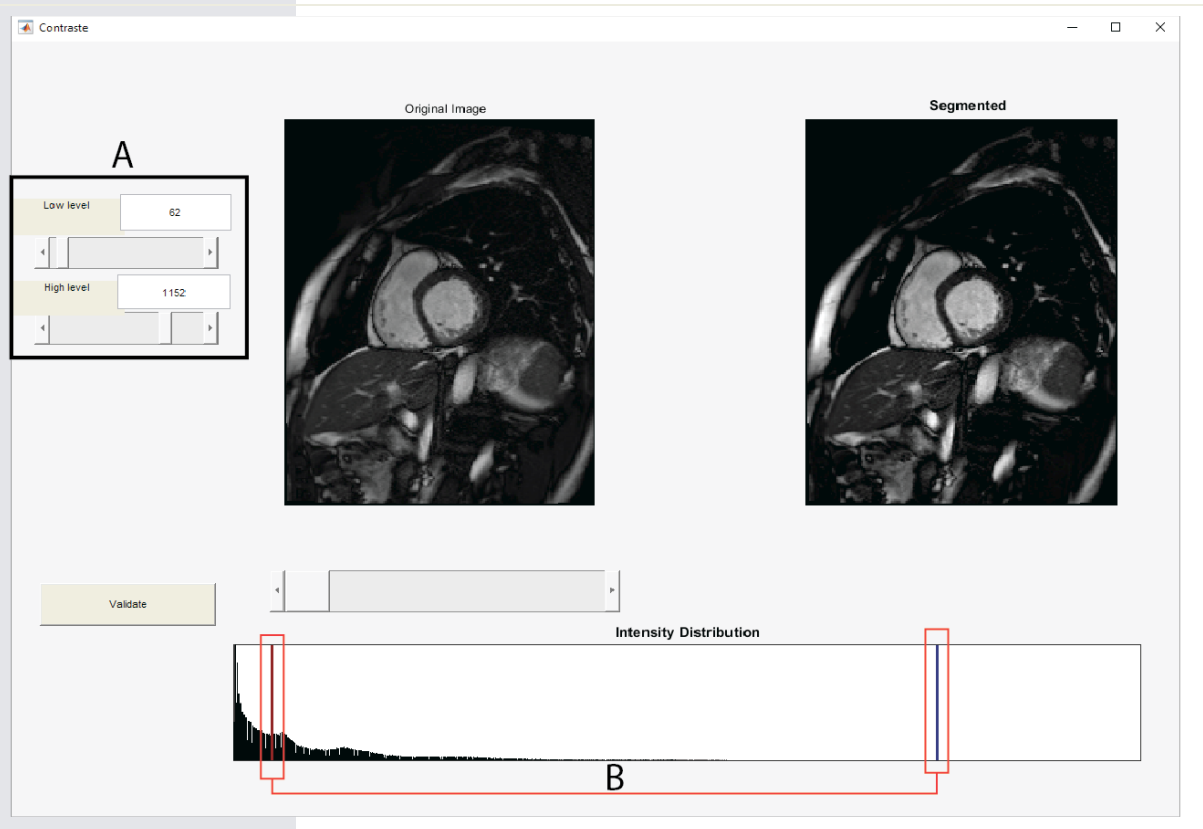


Figure 2: Images contrast modification

Step 1: contour detection

For this step, it's important to start from the basal slice for the tool to properly detect the LV contours. Use the "Contours" tool from the right toolbar (figure 3 A) to automatically segment the epicardium, the endocardium and the trabeculations of all images. To get the contours of one slice only, use "Contour (current slice)" button (Figure 3 B). When using one of those two buttons, a popup window appears asking to click in the centre of the LV (Figure

3 C). Do so and wait for the tool to finish processing. The segmentation may take 3 to 4 minutes, depending on how many images are being segmented and the performance of your hardware.

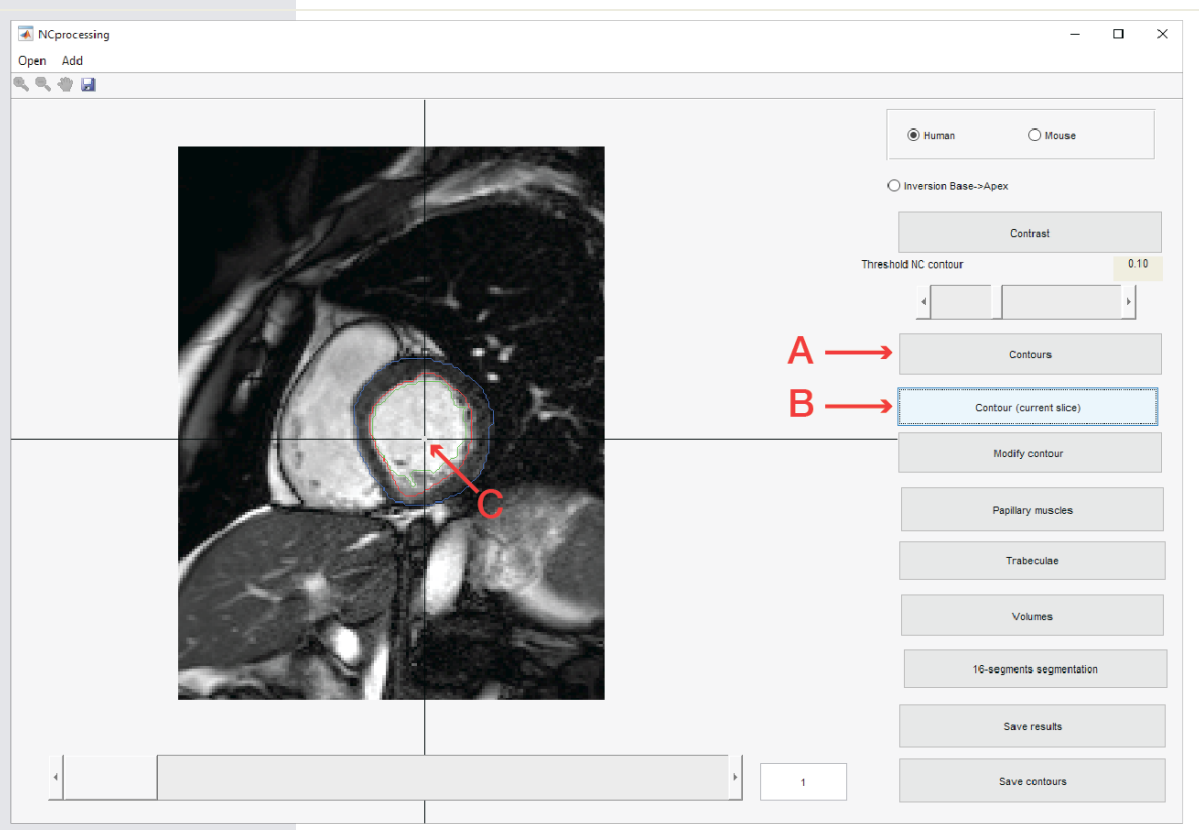


Figure 3: Automatic segmentation of the LV

One can manually modify, if needed, the contours segmented previously using the “Modify contour” tool, for a more accurate segmentation of the epicardium, endocardium and the trabeculations. From the displayed window, choose a contour to modify (Figure 4 A) and use one of the following actions:

- “New contour” to start over and draw your own contour manually (Figure 4 B).
- “Existing contour” to move, add or remove the vertices of the automatically segmented contour (Figure 4 C).

Use the “a” keyboard shortcut to add a vertex to the contour (Hold “a” and click where you want to add a vertex on the contour). To delete a vertex, right click on it and choose “delete vertex”.

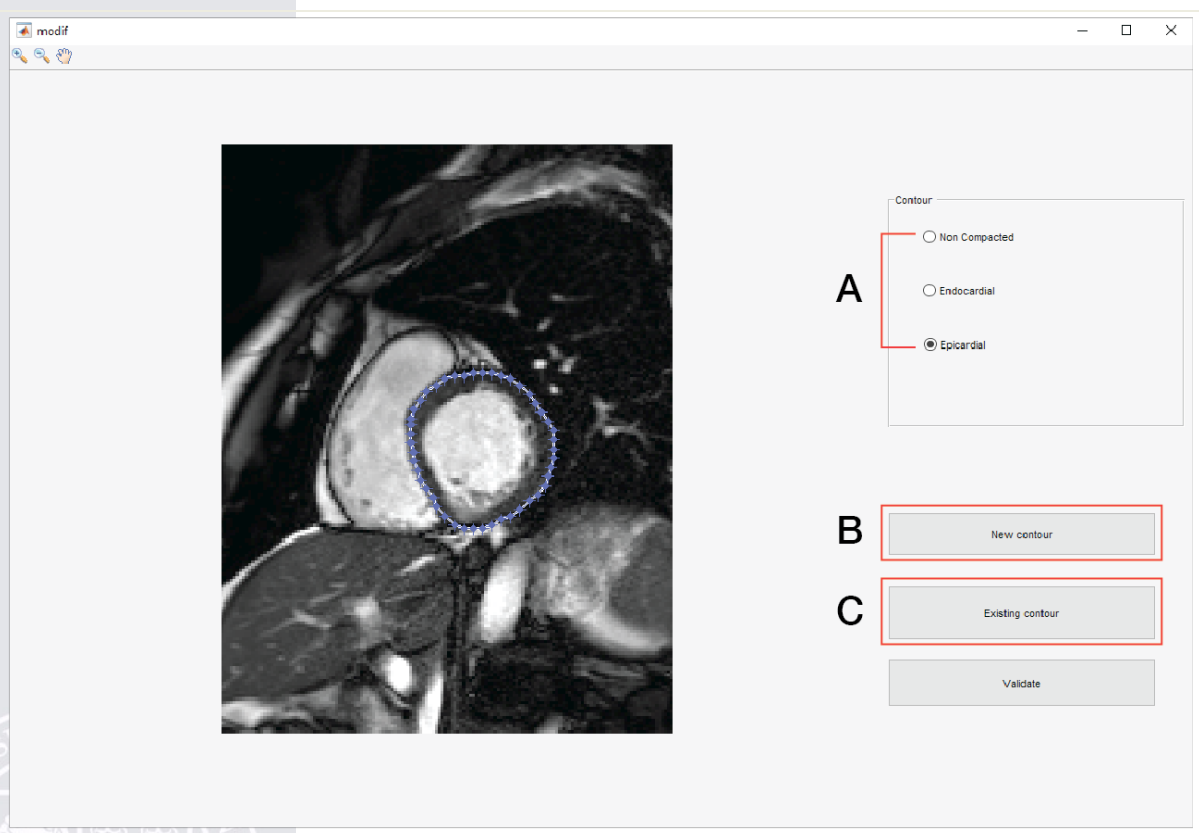


Figure 4: Manuel modification of the contours

Step 2: segmentation of the papillary muscles

a) Identification:

- The minimum size of a papillary muscle is a 2X2 pixels square.
- The papillary muscles appear in hyposignal.
- They are often circular in short axis images (consider the pathology of the treated subject).
- Use the overlying and underlying slices to decide.

b) Segmentation: (“Papillary muscles” button)

- Use the threshold slider on the right (Figure 5 A) to reveal the papillary muscles identified from step (a).



- Click on the “Papillary muscles” button (Figure 5 B) and select the revealed papillary muscles that appear as white pixels in the “Segmented” view.

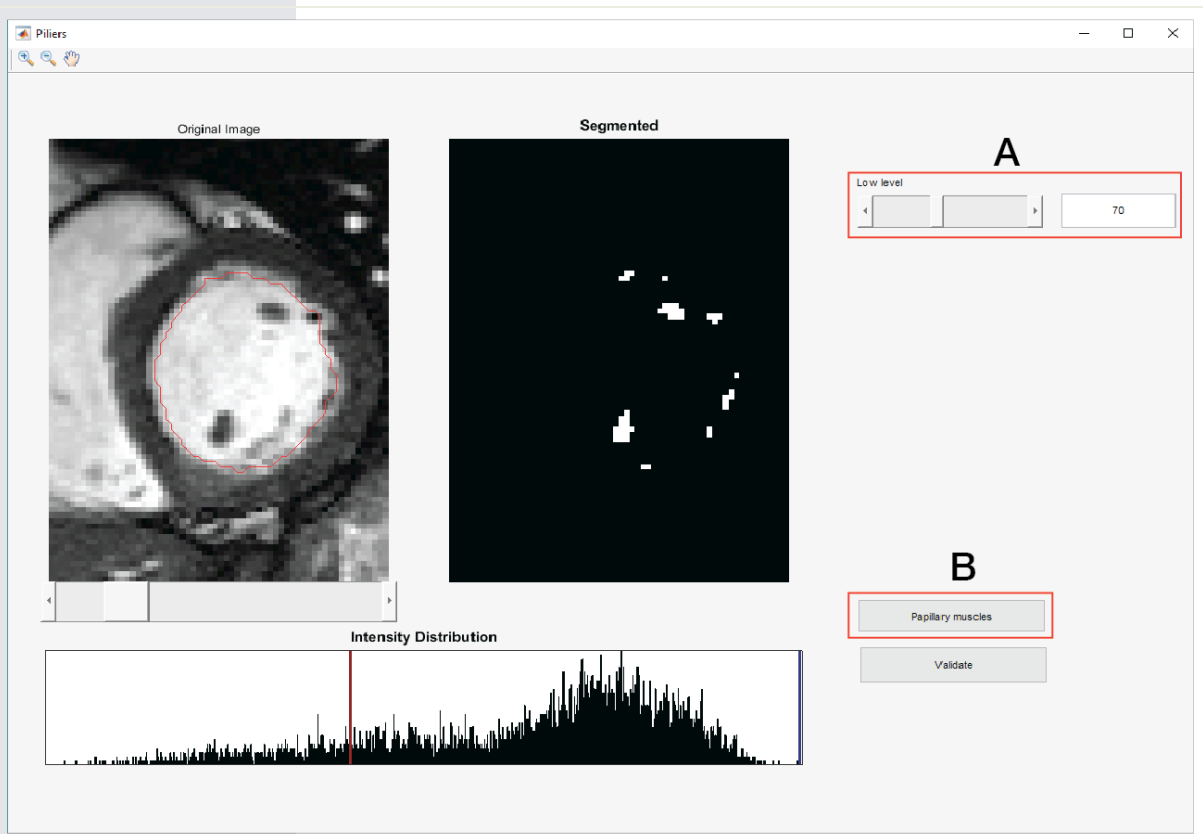


Figure 5: Identification and selection of the papillary muscles

- Use the “Eraser” tool to remove the pixels that are not considered as papillary muscles.

Description of the « Eraser » tool:

The tool has several options to help erase unwanted pixels:

- “Eraser” with a variable rubber size from 1 to 7X7 pixels (Figure 6 a).
- « Area » to remove all the pixels contained in a given area (Figure 6 b). To do so, draw a polygon around the pixels to be removed.
- “Erase slice” to remove all the pixels of the current slice (Figure 6 d).
- « Erase from slice » to erase all the pixels of the current slice and all the following slices (most apical) (Figure 6 e).
- Click on « Reinitialize slice » (Figure 6 c) to undo all the modifications.

PS: the “validate” button must be used only when all the slices had been processed, because it will close the papillary muscles segmentation window and validate the post-processing.



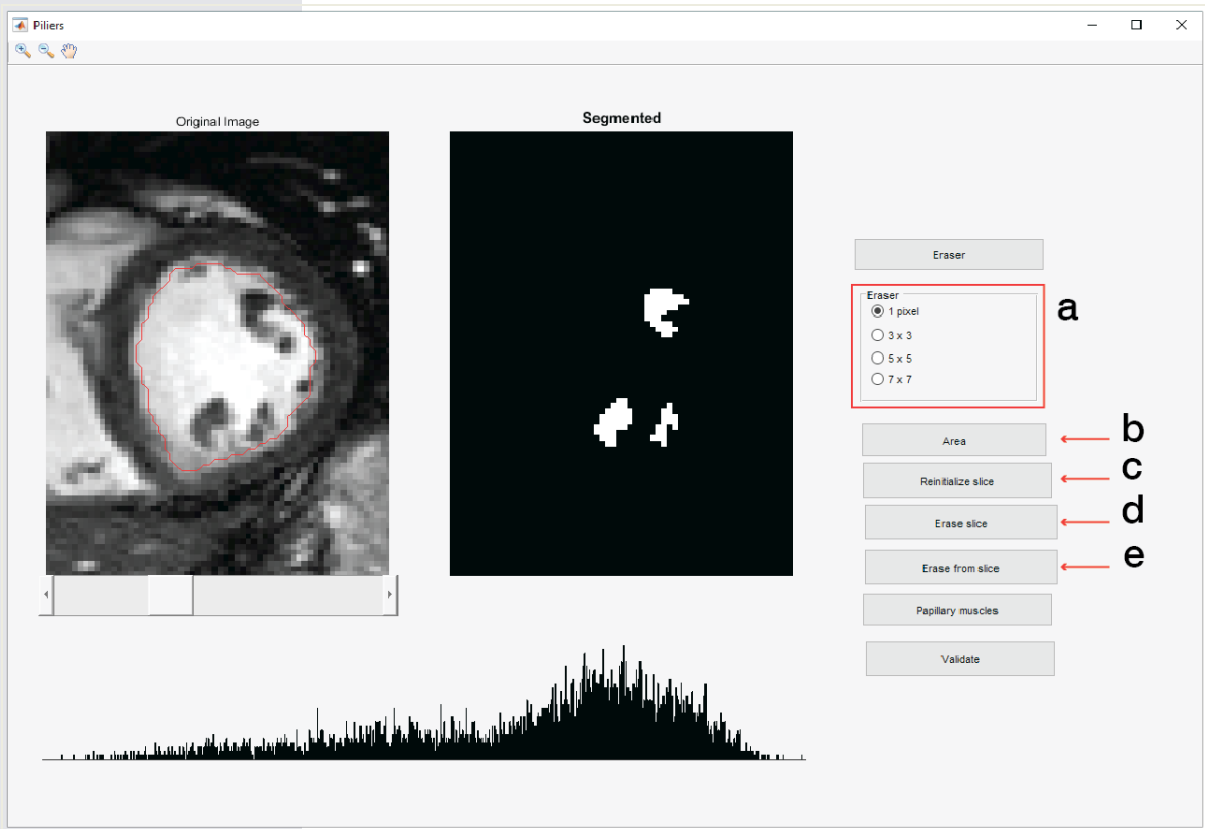


Figure 6: manual correction of the papillary muscles

Step 3: Segmentation of the trabeculations

a) Identification:

- One must be 100% sure to decide that a trabeculation is one, otherwise, exclude those pixels from the analysis.
- Must distinguish trabeculations and papillary muscles head. Make sure it's not a partial volume effect.

- Use the thresholding tool (see section « b ») to reveal or make disappear trabeculations (represented with gray pixels). Choose a threshold value according to the most trabeculated segment in each slice.
- Erode at least one time (Figure 7 B) to overcome the partial volume effect.

b) Description of the trabeculations segmentation tool:

- Set the value of the threshold according to the rules of section « a » (Figure 7 A).
- Erode, if necessary, by clicking on the “Erosion” button (Figure 7 B). This will reduce the contour size per 1 pixel. Use the “Cancel erosion” button to undo.
- Once satisfied with the threshold value as well as the erosion, click on “Validate threshold+erosion”



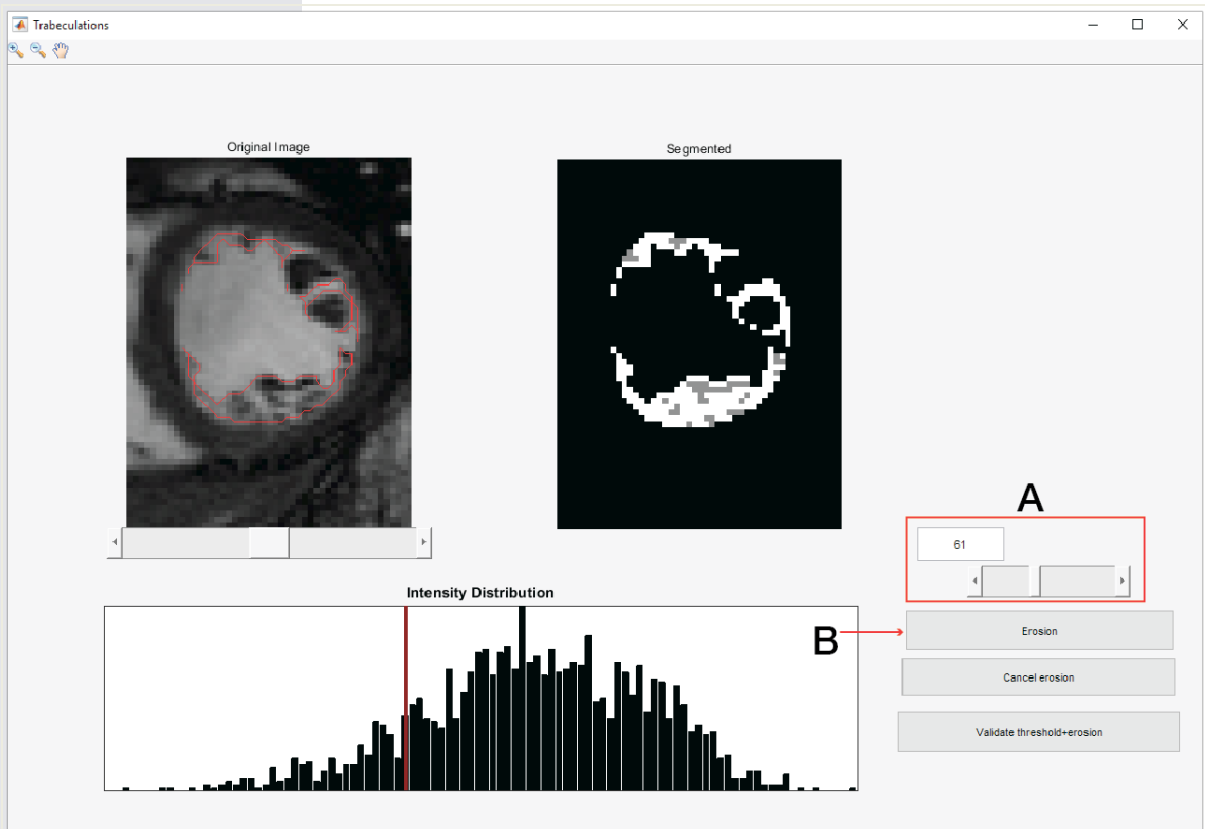


Figure 7: Identification of the trabeculations

- A new tool appears allowing to remove superfluous pixels. It's the same process as the papillary muscles erase tool (Figure 8). Choose a rubber size and erase the unwanted pixels (white pixels considered as blood).
- Validate to save the modifications and process the next slice.

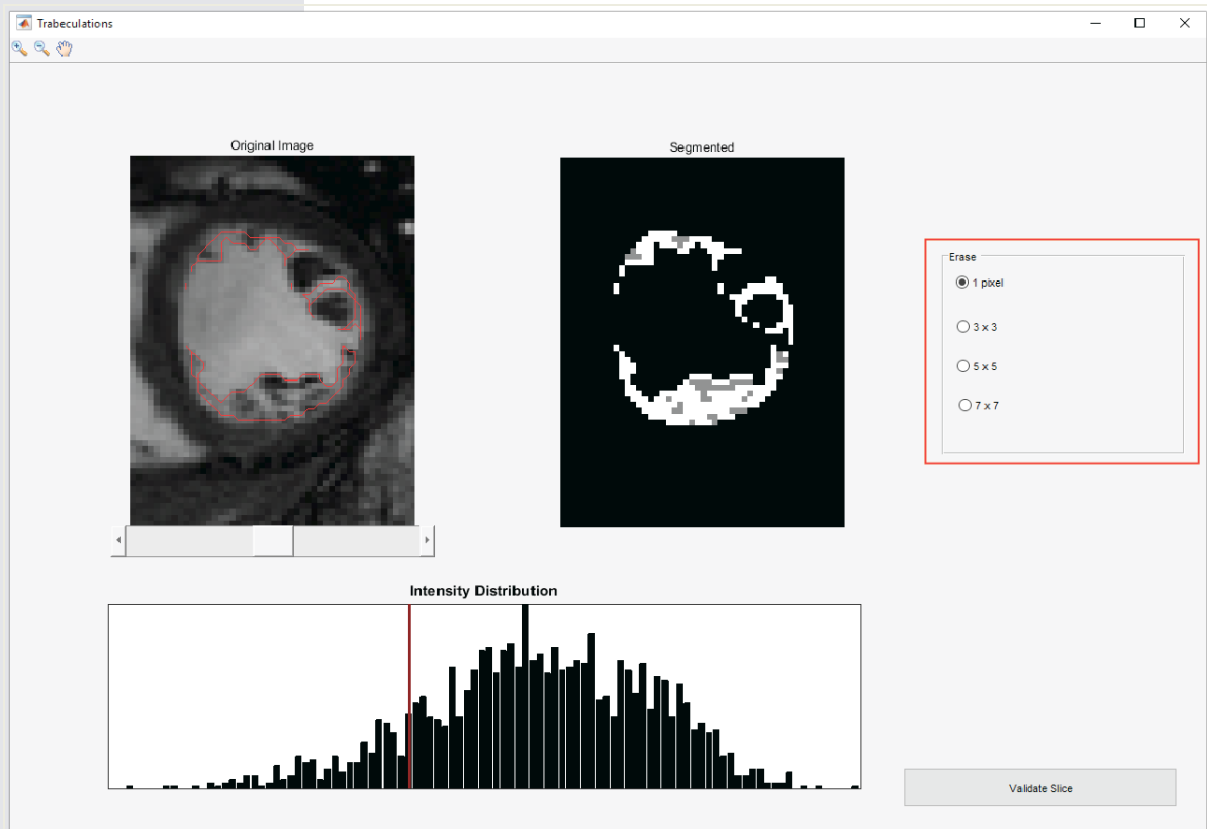


Figure 8: Blood suppression

Segmentation of the LV according the American Heart Association (AHA)

The « 16-segments segmentation » tool allows the segmentation and provide the analysis values of the 16 LV segments (AHA model: 17 segments, minus the apical segment, excluded from the analysis of short axis images). For an optimal segmentation, select a median slice and click on the insertion point of the anterior wall of the right ventricle on the interventricular septum.

Display and save results

Click on the « Volumes » button to display the compacted, noncompacted and end-diastolic volumes.

The last two buttons « Save results » and « Save contours » allow to save the analysis values in a text file and the contours in a “. mat” file, respectively.

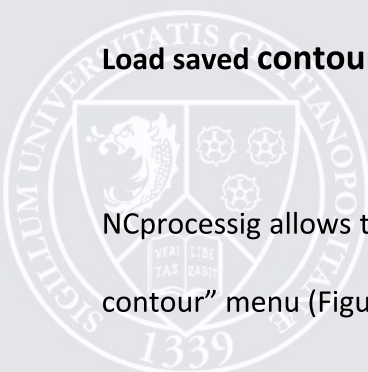
The text file stores the following data:

- Age, gender, weight, height and the body surface area (BSA).
- Compacted, noncompacted, end-diastolic and papillary muscles volumes.
- Compacted and noncompacted volumes of the 16 LV segments.

PS: Don't forget to multiply the compacted and noncompacted volumes per 1.05 (myocardium density) to get the masses in g (g/m^2 for the normalized values by the BSA).

Load saved contours:

NCprocessig allows to display the saved contours by opening the “.mat” file using the “Open contour” menu (Figure 1).



Annexe 2: article sur la validation technique



Semiautomatic Detection of Myocardial Contours in Order to Investigate Normal Values of the Left Ventricular Trabeculated Mass Using MRI

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Daniel Fagret, MD, PhD,⁶ Alexis Jacquier, MD, PhD,^{3,7} and Alain Lalande, PhD^{1,8}

Purpose: To propose, assess, and validate a semiautomatic method allowing rapid and reproducible measurement of trabeculated and compacted left ventricular (LV) masses from cardiac magnetic resonance imaging (MRI).

Materials and Methods: We developed a method to automatically detect noncompacted, endocardial, and epicardial contours. Papillary muscles were segmented using semiautomatic thresholding and were included in the compacted mass. Blood was removed from trabeculae using the same threshold tool. Trabeculated, compacted masses and ratio of noncompacted to compacted (NC:C) masses were computed. Preclinical validation was performed on four transgenic mice with hypertrabeculation of the LV (high-resolution cine imaging, 11.75T). Then analysis was performed on normal cine-MRI examinations (steady-state free precession [SSFP] sequences, 1.5T or 3T) obtained from 60 healthy participants (mean age 49 ± 16 years) with 10 men and 10 women for each of the following age groups: [20,39], [40,59], and [60,79]. Interobserver and interexamination segmentation reproducibility was assessed by using Bland-Altman analysis and by computing the correlation coefficient.

Results: In normal participants, noncompacted and compacted masses were 6.29 ± 2.03 g/m² and 62.17 ± 11.32 g/m², respectively. The NC:C mass ratio was $10.26 \pm 3.27\%$. Correlation between the two observers was from 0.85 for NC:C ratio to 0.99 for end-diastolic volume ($P < 10^{-5}$). The bias between the two observers was -1.06 ± 1.02 g/m² for trabeculated mass, -1.41 ± 2.78 g/m² for compacted mass, and $-1.51 \pm 1.77\%$ for NC:C ratio.

Conclusion: We propose a semiautomatic method based on region growing, active contours, and thresholding to calculate the NC:C mass ratio. This method is highly reproducible and might help in the diagnosis of LV noncompaction cardiomyopathy.

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Left ventricular noncompaction (LVNC) is a cardiomyopathy characterized by prominent trabeculations associated with deep recesses that communicate with the ventricular cavity.¹ LVNC has been linked to several gene mutations and an autosomal dominant inheritance mode is frequent.² The standard imaging tool for diagnosing LVNC is echocardiography. The diagnosis of LVNC has been made by applying the criteria established by Jenni et al,¹ but these

criteria seem to be too sensitive for the diagnosis of LVNC.³ Due to its high spatial resolution, magnetic resonance imaging (MRI) is a promising imaging technique for LVNC characterization.

MRI provides accurate anatomical and functional information to study cardiac function. This imaging technique is considered the reference standard for the evaluation of left ventricular (LV) volume and mass. LV segmentation is a

challenging task in MRI, especially for myocardial function characterization. Many algorithms have been proposed to determine endocardium and epicardium borders ranging from semiautomated to fully automated methods.⁴ However, most of the methods were not designed to take into account papillary muscles and trabeculae. Patel and Mor-Avi pondered if trabeculae and papillary muscles are an important part of cardiac anatomy or just distracting features to exclude while tracing endocardial boundaries.⁵ Chuang et al observed MRI-derived LV volumes in healthy adults using computer-aided analysis, but they included both trabeculae and papillary muscles in the LV cavity volume.⁶ This inclusion of papillary muscles and trabeculae in the blood volume can lead to a bias in the evaluation of ventricular volumes.⁷ Some studies have demonstrated that the inclusion of papillary muscles and trabeculae in either cavity or myocardial volumes produces notable differences in clinical and therapeutic indices and can have consequences for patient management.⁸ Papa-vassiliu et al showed that endocardial trabeculae markedly affect the quantification of LV volume.⁹

Petersen emphasized that currently there is no diagnostic tool for the accurate diagnosis of LVNC.¹⁰ Kawel et al studied the thickness of trabeculae in a large population-based cohort from the MESA study (Multi-Ethnic Study of Atherosclerosis).¹¹ They found that a ratio of noncompacted versus compacted higher than 2.3 measured at diastole on long-axis images in at least one myocardial region was common in their study participants, suggesting the need of a reevaluation of the current cardiac MR criteria for LVNC. Zemra et al studied the relationship of left ventricular trabeculae to ventricular function during a 10-year period in the MESA participants.¹² They found that LV trabeculae measured at end-diastole in an asymptomatic population appeared benign and was not associated with deterioration in LV volumes or function. Thus, a reliable method to delineate trabeculae is needed for LVNC diagnosis.

Jaspers et al proposed a semiautomatic threshold-based method to segment blood and muscle in the LV.¹³ Papillary muscles and trabeculae were excluded from the blood pool and included in the myocardial volume. This method needs a manually delineated epicardial contour as initialization and does not distinguish trabeculae from papillary muscles. Spreeuwers et al proposed a dedicated method to specifically segment the papillary muscles, based on a constrained region-growing algorithm, assuming that the myocardial boundaries of the LV are given, but they do not deal with trabeculae.¹⁴ Jacquier et al proposed a method to measure trabeculated LV mass using MRI, but they did not suppress blood in trabeculated regions.¹⁵ Chuang et al quantified trabeculae and papillary muscles using a fuzzy thresholding algorithm, but no difference was done between trabeculae and papillary muscles.¹⁶

The aim of this study is to propose a global framework for measuring noncompacted and compacted LV masses using MRI. The goals of the study were, on the one hand, to propose a semiautomatic method to quantify trabeculated mass and compacted mass, and on the other hand, to present normal noncompacted myocardial values acquired on a multicenter dual-vendor mode as well as to define inter- and intraobserver reproducibility of the presented method.

Materials and Methods

Preclinical Validation

To validate our method, we used four transgenic mice with mutations in the *Nkx 2.5* gene. The examination of these mice was approved by the local ethical committee on animal protection. These mice showed hypertrabeculation of the LV.¹⁷ MRI scans were performed 60 days after birth with a preclinical Bruker AVANCE 500 MHz/89 mm wide bore vertical imager (11.75T, Bruker Biospin, Ettlingen, Germany) and a 30-mm diameter tx/rf resonator. Anesthesia was maintained during MRI with 1–2% isoflurane in a constant flow of room air (270 mL/min) through a nose cone using a dedicated vaporizer (univentor anesthesia unit, univentor high precision instrument, Zejtun, Malta). Temperature was maintained at 37°C. Breath and cardiac rhythm were monitored and signals were used to trigger MRI acquisition using a monitoring and gating system (SA Instruments, Stony Brook, NY). High-resolution cine imaging (breath and ECG gating, repetition time 15 msec, echo time 1.68 msec, flip angle 30°, slice thickness 1 mm, pixel size 0.086 × 0.086 mm²) in small axis view, at the mid base-apex axis, was performed. Ten phases per heartbeat were acquired. The end diastole acquisition was used to segment myocardium with our software.

Mice were then sacrificed and the whole heart was removed and sectioned in the transversal axis using a cryostat. Then an immunofluorescence staining on mid-ventricular sections corresponding to the image acquired at the same level in the short axis by high-resolution MRI imaging was performed. Antibodies against endoglin to stain the endocardium in green, and against VEGFR2 to stain vessels in red, were used to better separate trabeculated mass in green and compacted mass in red.

The mean weight of the mice was 22.42 g. The mean heart weight was 206 mg. The mean diastolic LV volume was 143.81 μ L and the mean ejection fraction was 63.69%.

MR images were analyzed using custom made software developed in MatLab (MathWorks, Natick, MA), whereas histological images were manually analyzed using ImageJ (v. 1.49v, National Institutes of Health, Bethesda, MD). Analyses were performed by two observers (J.F., 3 years of experience; M.F., 1 year of experience). The noncompacted and compacted masses obtained on corresponding sections by these two methods were compared.

Subjects

The study was approved by the local ethical committee; all participants gave informed consent. From April 2013 to June 2014, 60 subjects were included with normal cardiovascular examination, no cardiac symptoms, no known risk factors or history of cardiac

TABLE 1. Baseline Characteristics of Normal Subjects Studied (Mean $\pm$ SD)

	Sex groups		Age groups		
	Males	Females	20–39 years	40–59 years	60–79 years
Height [cm]	175 $\pm$ 8	164 $\pm$ 6	170 $\pm$ 9	169 $\pm$ 8	169 $\pm$ 10
Weight [kg]	82 $\pm$ 18	65 $\pm$ 15	71 $\pm$ 18	75 $\pm$ 15	64 $\pm$ 14
BSA [m ²]	1.96 $\pm$ 0.22	1.7 $\pm$ 0.18	1.81 $\pm$ 0.24	1.85 $\pm$ 0.2	1.84 $\pm$ 0.27
EF [%]	63 $\pm$ 7	65 $\pm$ 7	65 $\pm$ 5	64 $\pm$ 7	64 $\pm$ 8

BSA, body surface area; EF, ejection fraction; SD, standard deviation.

disease. Their ejection fraction was above 55%, end-diastole volume less than 100 mL/m², LV mass less than 90 g/m², and LV wall thickness less than 12 mm. There was no late enhancement after injection of gadolinium-based contrast agent. Exclusion criteria were contraindications to MRI, arrhythmias, and pregnancy. Height and weight are reported in Table 1. Body surface area (BSA) was calculated using the Dubois and Dubois formula.¹⁸

The study population was homogeneous in terms of its ethnic origin (Caucasian). Subjects were selected in order to obtain 10 men and 10 women for each of the following age groups: [20,39], [40,59], and [60,79].

Image Acquisition

Imaging was performed on five clinical scanners from three different centers (Aera 1.5T, Trio Tim 3T, Skyra 3T, Siemens, Erlangen, Germany, and Achieva 1.5T, Intera 1.5T, Philips, Best, The Netherlands). MR images were acquired using a phased-array coil with conventional steady-state free precession (SSFP) sequences having the following parameters: repetition time between 3.07 and 3.57 msec, echo time between 1.17 and 1.79 msec, spatial resolution between 0.74*0.74 mm²/pixel and 1.95*1.95 mm²/pixel, and slice thickness between 5 and 10 mm. Six to 11 contiguous short-axis views covering the LV from the base to apex were acquired.

Image Analysis

The end-diastolic (ED) phase was defined as the first one after R-wave detection. The end-systolic (ES) phase was defined as the one where the cardiac cavity volume was visually the lowest. The most basal section was the section that, at ED and ES phases, still showed the LV myocardium extending over at least 50% of the myocardium circumference. The most basal section could differ between ED and ES.

To analyze mice and human MR images, we developed an in-house semiautomated software with a MatLab graphical user interface (MathWorks). This software enables detecting noncompacted, endocardial, and epicardial borders on each diastolic frame. This method only needed a seed for the initialization on the most basal slice. The user had to click in the left ventricular cavity on this slice. Using this seed, the noncompacted region, which is a relatively homogeneous area in terms of intensity, was obtained using a region-growing algorithm. From the seed, neighboring pixels were added to the region while their intensity comprised between $\pm 10\%$ of the mean intensity value of the area. This noncompacted

contour was then used for the initialization of an active contour to determine the endocardium border.¹⁹ This active contour was based on level-sets, and the implicit function was modeled as a continuous parametric function expressed on a B-spline basis. To compute the epicardial border, we mapped pixels from Cartesian to polar coordinates. Thus, the ring-shaped regions of interest were transformed to rectangular ones. An outer boundary was computed by dilating the endocardial border. Pixels on this boundary were then used as seeds for region-growing algorithms. The resulting contour was then smoothed and transformed to the original coordinate space to obtain the epicardial border (Fig. 1, top). If necessary, manual correction could be applied for each contour on each slice.

Papillary muscles were then segmented using a semiautomatic threshold tool based on the difference between bright blood and darker myocardial intensities. The user had to click on the papillary muscles on one slice and the segmentation was propagated on the other slices. When the papillary muscles could not be clearly distinguished from the trabeculae, they were considered trabeculae. Afterwards, blood was removed from trabeculae using the same thresholding tool. Trabecular compartment was defined as dark myocardium protruding from the LV wall into the bright LV

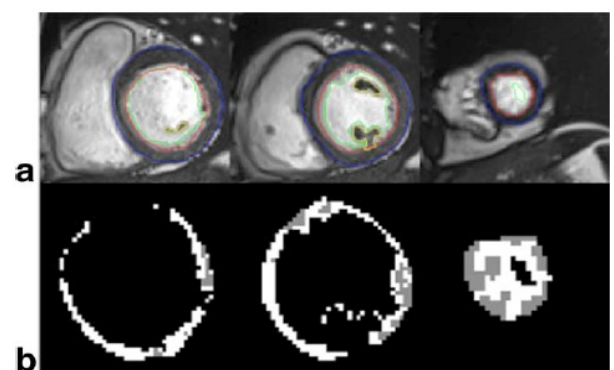


FIGURE 1: (A) Example of the left ventricular segmentation on several images from the base to the apex: epicardial border in blue, endocardial border in red, trabeculae border in green. Papillary muscles were segmented and included in the compacted mass (yellow line). **(B)** For the calculation of the non-compacted mass, trabeculae (gray) were segmented using a semiautomatic threshold tool to suppress blood (white).¹

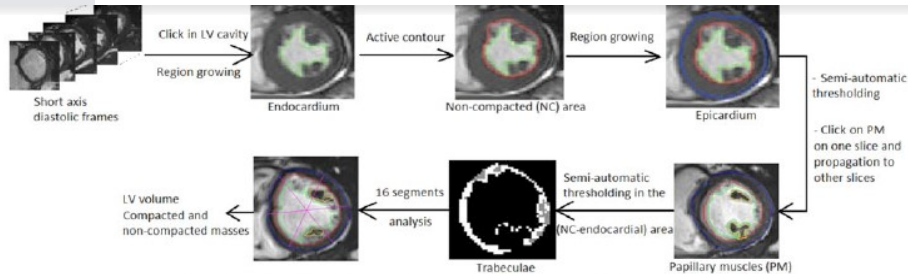


FIGURE 2: Workflow of proposed segmentation method.

cavity. For the two semiautomatic threshold tools, the default threshold was defined using the Otsu method.²⁰ This threshold could then be changed by the user. Finally, compacted mass, non-compacted mass, end-diastolic volume (EDV), and NC:C ratio were computed. For the calculation of the myocardial mass, a specific density of 1.05 g/mL was used. Papillary muscles were included in the compacted mass. For the calculation of the non-compacted mass, blood was removed to include only trabeculae.

To take into account the variations in body weight between the different subjects, LV volumes, compacted, and noncompacted

LV masses were indexed to the body surface area according to the formulae of Dubois and Dubois.¹⁸ The distribution of trabeculae was assessed by analysis of 16 segments according to the accepted nomenclature.²¹ Figure 2 shows the workflow of the proposed algorithm.

A first observer (A.L., 19 years of experience) performed the measurement for the 60 subjects. As the tool is not completely automatic, a second independent observer (J.F., 3 years of experience) repeated the different measurements for each dataset to evaluate interobserver measurement variability. To investigate intraobserver variability, observer 1 repeated the measurements in 20 subjects, at least 1 month after the first examination, to minimize recall bias. Each observer was blinded to the subject details (ie, patient sex and age) and to the other observer's findings.

Processing time was less than 15 minutes by subject for a complete examination, including user-interaction and computational time. Noncompacted, endocardial, and epicardial border detection took 3 minutes per subject. Semiautomatic papillary muscles detection took 3 minutes, whereas 8 minutes was required to segment trabeculations.

Statistical Analysis

All values are presented as mean $\pm$ SD. Averaged values were calculated for each sex and age groups. Interobserver measurement reproducibility, as well as the interexamination reproducibility, were assessed using Bland–Altman analysis²² and by computing Pearson's linear correlation coefficient. The significance of the correlation coefficient was tested using Student's *t*-test. To study the impact of the spatial resolution, we also compared the observer variability on the images with the lowest and highest pixel sizes.

Results were considered significant at $P < 0.05$.

Results

Preclinical Validation

Figure 3B shows an example of manual segmentation on histologic section compared with the corresponding MRI image segmented with our software (Fig. 3C).

The mean trabeculated mass on the studied slice was 1.72 mg for histology and 1.51 mg for MRI (agreement evaluation according to the Bland–Altman test: -0.2 ± 0.44 mg). The mean compacted mass was 11.93 mg for histology and 13.71 mg for MRI (agreement evaluation according to the Bland–Altman test: 1.7 ± 1.09 g). There was an

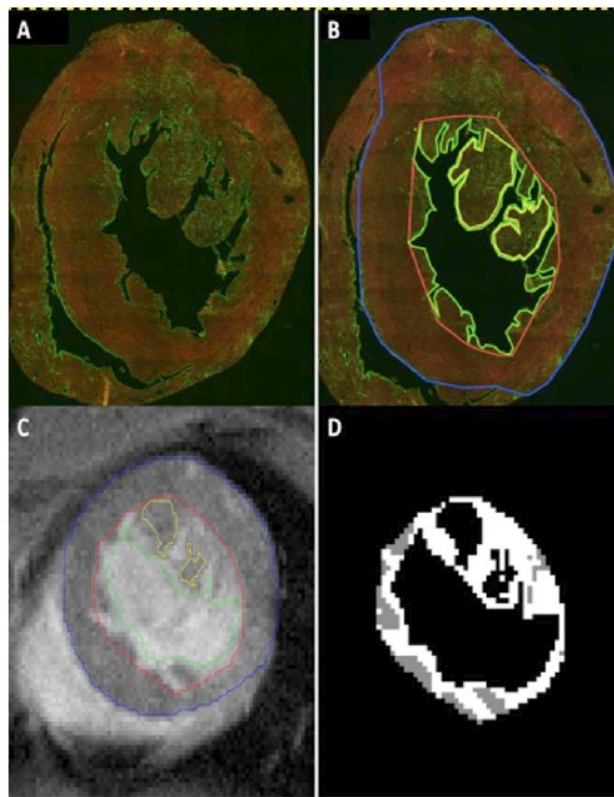


FIGURE 3: (A) Immunofluorescence of a histological section from an adult mouse heart showing endocardium in green and vessels in red. (B) Example of manual segmentation with ImageJ: epicardial border in blue, endocardial border in red, trabeculae border in green, and papillary muscles in yellow. (C) Example of a corresponding MRI slice and segmentation with our software using the same color code. (D) Example of blood removal for the calculation of NC mass.

TABLE 2. Left Ventricular Parameters by Age and Sex Groups

Measurement	20–39 years	40–59 years	60–79 years	Males	Females
NC [g]	11.72 ± 3.98	11.62 ± 3.88	11.20 ± 4.17	12.50 ± 4.19	10.53 ± 3.54
NC/BSA [g/m ²]	6.43 ± 1.95	6.35 ± 2.13	6.07 ± 2.04	6.38 ± 2.05	6.19 ± 2.02
C [g]	117.40 ± 30.33	113.98 ± 23.99	111.94 ± 28.95	130.47 ± 26.93	98.41 ± 17.46
C/BSA [g/m ²]	64.03 ± 10.49	61.63 ± 10.99	60.85 ± 12.42	66.49 ± 12.10	57.85 ± 8.59
EDV/BSA [mL/m ²]	75.04 ± 14.06	72.98 ± 11.52	66.33 ± 16.94	69.54 ± 15.46	73.35 ± 13.77
Ratio NC:C [%]	10.20 ± 3.18	10.44 ± 3.54	10.15 ± 3.16	9.71 ± 3.05	10.81 ± 3.42

Mean values ± SD. NC, noncompacted mass; C, compacted mass; EDV end-diastolic volume; BSA, body surface area; SD, standard deviation.

excellent correlation between MRI and histologic masses, $r = 0.98$, $P < 0.01$.

Clinical Validation

Table 1 summarizes the participant characteristics. All age groups were comparable in terms of weight, height, and BSA. There was no significant difference between men and women in terms of ejection fraction.

Figure 1 shows an example of the semiautomatic segmentation. Considering the whole subjects, the averaged compacted mass was 62.17 ± 11.32 g/m² and the averaged noncompacted mass was 6.29 ± 2.03 g/m². The averaged end-diastolic volume was 71.45 ± 14.71 mL/m². The averaged ratio of noncompacted to compacted masses was $10.26 \pm 3.27\%$.

The results according to age and sex are shown in Table 2. Compacted mass was 66.49 ± 12.10 g/m² for men and 57.85 ± 8.59 g/m² for women. The NC:C ratio was $9.71 \pm 3.05\%$ for men and $10.81 \pm 3.42\%$ for women.

Pixel size varied from 0.74×0.74 mm² to 1.95×1.95 mm². However only three cases had pixel size less than 1.2×1.2 mm², and three cases had pixel size higher than

1.8×1.8 mm². The mean noncompacted indexed mass was 5.79 g/m² for the lowest pixel size and 5.28 g/m² for the highest pixel size. The mean compacted indexed mass was 58.29 g/m² for the lowest pixel size and 57.63 g/m² for the highest pixel size. The mean value of the differences for noncompacted mass was -0.21 g/m² for the lowest pixel size and -0.65 g/m² for the highest pixel size. The mean value of the differences for compacted mass was -4.37 g/m² for the lowest pixel size and -4.60 g/m² for the highest pixel size.

Trabeculae were more prominent on the apical segment than on medial and basal segments (Fig. 4). For basal segments and segments 8–10, the NC:C ratio was lower than 10%. For segments 13 and 16 the ratio was higher than 20%. The less trabeculated segment was segment 3, with a ratio of $0.44 \pm 1.48\%$ corresponding to a septal localization. The most trabeculated segment was segment 13, with a ratio of $24.27 \pm 11.13\%$. The corresponding illustrations are displayed in Figs. 5 and 6 for age and sex groups. For each segment, no significant differences were found between age groups or between women and men.

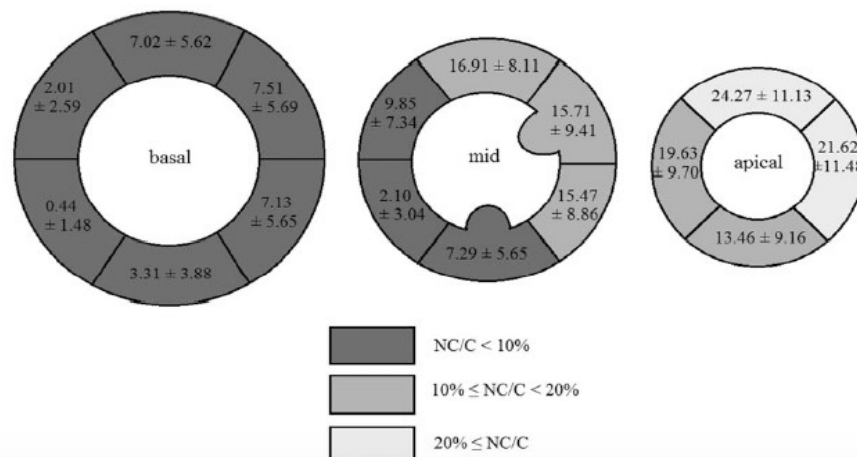


FIGURE 4: Segmental representation of the mean (±SD) ratio of noncompacted to compacted masses for all subjects.

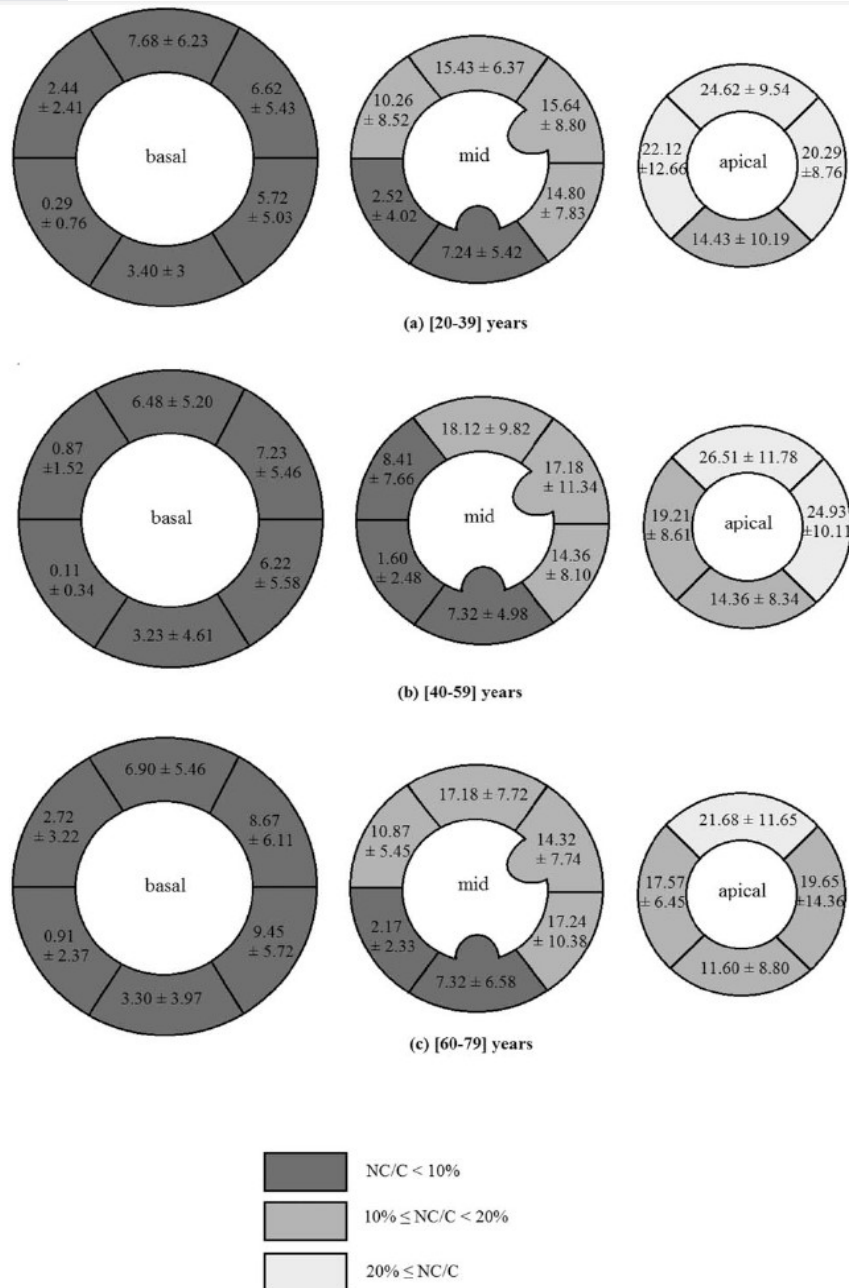


FIGURE 5: Segmental representation of the mean ($\pm$ SD) ratio of noncompacted to compacted masses for age groups.

Analysis of the agreement between measurements obtained by the two independent observers was performed (Table 3). The correlation between the two observers was high for all parameters, from 0.85 for the NC:C ratio to 0.99 for the end-diastolic volume ($P < 10^{-5}$). The bias between the two observers was -1.06 ± 1.02 g/m² for the trabeculated mass, -1.41 ± 2.78 g/m² for the compacted mass, and $-1.51 \pm 1.77\%$ for the NC:C ratio. Analysis of the reproducibility between the two measurements obtained

by observer 1 was also performed (Table 4). The correlation between the two measurements of the same observer 1 was between 0.80 and 0.99 ($P < 10^{-4}$). For the ratio of non-compacted to compacted mass, the correlation for the reproducibility was 0.82, whereas the correlation between the two observers was 0.77 for the same 20 subjects. The bias between the two analyses of observer 1 was -0.9 ± 1.02 g/m² for the trabeculated mass, -1.02 ± 2.98 g/m² for the compacted mass, and $-1.53 \pm 1.51\%$ for the NC:C ratio.

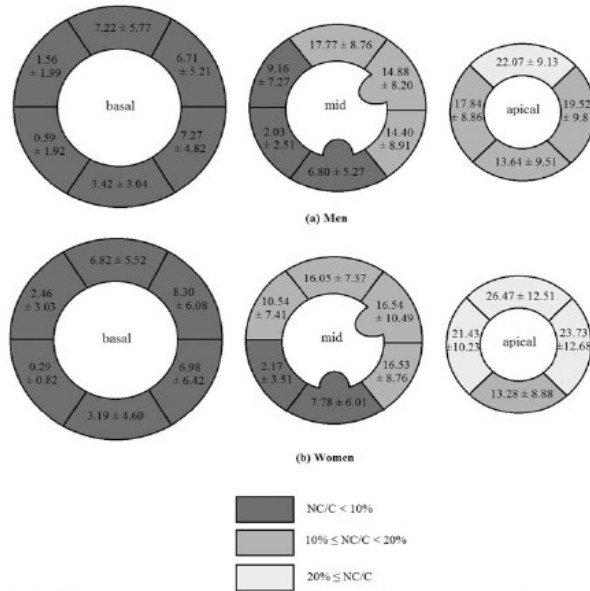


FIGURE 6: Segmental representation of the mean ($\pm$ SD) ratio of noncompacted to compacted masses for sex groups.

Discussion

We present a new method allowing estimation of the noncompacted mass of the LV. The method that we propose combines region-growing, level-sets, and thresholding with minimal user interaction. This method requires only one click in the left ventricular cavity for the initialization of the region-growing algorithm to detect the noncompacted border. Interactive thresholding was used to segment papillary muscles and to extract blood from noncompacted area. This method was accurate, reproducible, and allowed evaluating the compacted and noncompacted masses. The choice of various image resolutions was intended in order to evaluate our method on a maximum of plausible set-ups. Spatial resolution and pixel size seem not to affect the segmentation results.

This study provided a precise range and distribution of noncompacted mass in healthy subjects. We chose to

exclude the papillary muscles from trabeculae because of their active role during systole and we decided to include them in the compacted mass. Papillary muscles and trabeculae could represent on average 10% of cardiac mass in healthy volunteers, as shown by Papavassiliu et al.⁹ We wanted to make the distinction between papillary muscles and trabeculae in our results to allow a more precise study of trabeculae. Kohli et al and Kawel et al studied respectively echocardiographic and MRI criteria for noncompaction.^{3,11} We opted for a 3D approach in order to take into account the pitfalls of a 2D analysis shown by these two studies.

From a multiethnic study of atherosclerosis involving 800 healthy participants, the results from MRI showed that normal LV differed in mass and volume between sex and among certain ethnic groups and that LV mass was independent of age.²³ Our results were consistent with a compacted mass higher in men and no difference between age groups. We found no difference for the noncompacted mass and NC:C ratio between sex and age groups. These results can also be matched to those of Dawson et al, who showed no difference for trabeculated layer in 120 volunteers using the LV wall thickness.²⁴

Jacquier et al found a body-indexed trabeculated mass of 9 g/m² in healthy patients, but they did not suppress blood from the trabeculae.¹⁵ Thus, these results were in concordance with our value of 6.3 g/m² for the noncompacted indexed mass. The absolute differences were small because there were very few trabeculae in healthy subjects and only a small amount of blood to suppress. Our results are consistent with those of Grothoff et al, who found a body-indexed trabeculated mass of 5.3 g/m² by contouring manually trabeculae on diastolic short-axis view.²⁵ Moreover, the most trabeculated segments were the most apical and lateral ones, as previously published.^{11,15,26} Choudary et al, with a semiautomated method based on signal intensity, recently found a noncompacted mass of 30.9 g and an NC:C ratio of 26% in healthy volunteers at end-diastole. It

TABLE 3. Interobserver Reproducibility of Measurements in 60 Subjects

Measurement	Observer 1 Mean $\pm$ SD	Observer 2 Mean $\pm$ SD	R	P value	Bland-Altman Mean $\pm$ SD
NC [g]	12.46 $\pm$ 3.88	10.57 $\pm$ 3.89	0.89	<10 ⁻⁵	-1.89 $\pm$ 1.84
NC/BSA [g/m ²]	6.82 $\pm$ 2.01	5.76 $\pm$ 1.92	0.87	<10 ⁻⁵	-1.06 $\pm$ 1.02
C [g]	115.81 $\pm$ 28.38	113.07 $\pm$ 27.27	0.98	<10 ⁻⁵	-2.74 $\pm$ 5.22
C/BSA [g/m ²]	62.88 $\pm$ 11.39	61.47 $\pm$ 11.29	0.97	<10 ⁻⁵	-1.41 $\pm$ 2.78
EDV/BSA [mL/m ²]	71.67 $\pm$ 14.89	71.23 $\pm$ 14.64	0.99	<10 ⁻⁵	-0.44 $\pm$ 1.38
Ratio NC:C [%]	11.02 $\pm$ 3.27	9.51 $\pm$ 3.12	0.85	<10 ⁻⁵	-1.51 $\pm$ 1.77

NC, noncompacted mass; C, compacted mass; EDV end-diastolic volume; BSA, body surface area; SD, standard deviation.

TABLE 4. Intraobserver Reproducibility of Measurements in 20 Subjects

Measurement	Observer 1 Segmentation 1 Mean $\pm$ SD	Observer 1 Segmentation 2 Mean $\pm$ SD	<i>R</i>	<i>P</i> value	Bland-Altman Mean $\pm$ SD
NC [g]	12.77 $\pm$ 3.11	11.08 $\pm$ 2.58	0.80	$<10^{-4}$	-1.68 $\pm$ 1.86
NC/BSA [g/m ²]	7.04 $\pm$ 1.73	6.10 $\pm$ 1.40	0.81	$<10^{-4}$	-0.9 $\pm$ 1.02
C [g]	112.28 $\pm$ 31.34	114.14 $\pm$ 31.81	0.99	$<10^{-5}$	1.86 $\pm$ 5.25
C/BSA [g/m ²]	61.34 $\pm$ 14.91	62.35 $\pm$ 15.21	0.98	$<10^{-5}$	1.02 $\pm$ 2.98
EDV/BSA [mL/m ²]	70.41 $\pm$ 11.60	69.90 $\pm$ 12.49	0.99	$<10^{-5}$	-0.52 $\pm$ 1.91
Ratio NC:C [%]	11.77 $\pm$ 2.61	10.23 $\pm$ 2.30	0.82	$<10^{-3}$	-1.53 $\pm$ 1.51

NC, noncompacted mass; C, compacted mass; EDV end-diastolic volume; BSA, body surface area; SD, standard deviation.

is much higher than our results and the results of other studies, but they might have included a lot of partial volume and blood in the noncompacted mass.²⁷ Moreover, their method needs a manual delineation of endocardial and epicardial borders.

Papavassiliu et al demonstrated that trabeculae markedly affected quantification of LV volume, and inclusion of trabeculae in the cavity provided a better reproducibility.⁹ This could explain the highly interobserver reproducibility of epicardial and endocardial contours and the slightly less good reproducibility of trabeculae mass estimation. Indeed, due to their small size, partial volume effects, and flow artifacts, trabeculae were sometimes difficult to differentiate from blood, and it is challenging in these cases to determine the noncompacted contour and to suppress blood.

Recently, Captur et al proposed two studies on a novel way to characterize trabeculae.^{28,29} Instead of calculating a mass or studying the repartition of trabeculae, they were interested in their pattern and calculated a fractal dimension. We could not compare our study with those two because their method was quite different from ours: 1) they did not use the 3D acquisition of cardiac MR but only calculated the 2D measurements, 2) they did not use the whole heart but only the mid and apical parts, 3) they did not calculate masses, and their software did not perform the segmentation of noncompacted and compacted mass, and 4) they present results on patients with different pathologies, whereas our method was only applied on normal participants.

There were some limitations to our study. First, the sample size of examined subjects was relatively limited, as compared to the study of Hautvast et al.³⁰ Nevertheless, the sample represented a population with a wide and uniform range of ages. Second, the trabeculated and compacted masses were measured in only one ethnic group. Thus, references values should be computed in other ethnic groups such as a black African population.^{3,11}

In conclusion, we propose a method allowing evaluating compacted and trabeculated masses in a fast and highly reproducible manner. This method was validated on transgenic mice with hypertrabeculated hearts due to a mutation in the *Nkx 2.5* gene and then applied to a wide range of volunteers. This semiautomatic method could be used to help diagnosis of left ventricular noncompaction patients and in other pathologies, in particular for patients with hypertrophic cardiomyopathy or dilated cardiomyopathy. We intend to provide free software allowing evaluation of LVNC by computing different clinical indexes such as NC:C mass ratio.

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Annexe 3: article sur la validation histologique



Semi-automatic detection of myocardial trabeculation using cardiovascular magnetic resonance: correlation with histology and reproducibility in a mouse model of non-compaction

AQ1

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Background

The definition of left ventricular (LV) non-compaction is controversial, and discriminating between normal and excessive LV trabeculation remains challenging. Our goal was to quantify LV trabeculation on cardiovascular magnetic resonance (CMR) images in a genetic mouse model of non-compaction using a dedicated semi-automatic software package and to compare our results to the histology used as a gold standard.

Methods

Adult mice with ventricular non-compaction were generated by conditional trabecular deletion of *Nkx2-5*. Thirteen mice (5 controls, 8 *Nkx2-5* mutants) were included in the study. Cine CMR series were acquired in the mid LV short axis plane (resolution $0.086 \times 0.086 \times 1 \text{ mm}^3$) (11.75 T). In a sub set of 6 mice, 5 to 7 cine CMR were acquired in LV short axis to cover the whole LV with a lower resolution ($0.172 \times 0.172 \times 1 \text{ mm}^3$). We used semi-automatic software to quantify the compacted mass (M_c), the trabeculated mass (M_t) and the percentage of trabeculation (M_t/M_c) on all cine acquisitions. After CMR all hearts were sliced along the short axis and stained with eosin, and histological LV contouring was performed manually, blinded from the CMR results, and M_t , M_c and M_t/M_c were quantified. Intra and interobserver reproducibility was evaluated by computing the intra class correlation coefficient (ICC).

Results

Whole heart acquisition showed no statistical significant difference between trabeculation measured at the basal, midventricular and apical parts of the LV. On the mid-LV cine CMR slice, the median M_t was 0.92 mg (range 0.07–2.56 mg), M_c was 12.24 mg (9.58–17.51 mg), M_t/M_c was 6.74% (0.66–17.33%). There was a strong correlation between CMR and the histology for M_t , M_c and M_t/M_c with respectively: $r^2 = 0.94$ ($p < 0.001$), $r^2 = 0.91$ ($p < 0.001$), $r^2 = 0.83$ ($p < 0.001$). Intra- and interobserver reproducibility was 0.97 and 0.8 for M_t ; 0.98 and 0.97 for M_c ; 0.96 and 0.72 for M_t/M_c , respectively and significantly more trabeculation was observed in the M_c Mutant mice than the controls.

Conclusion

The proposed semi-automatic quantification software is accurate in comparison to the histology and reproducible in evaluating M_c , M_t and M_t/M_c on cine CMR.

Keywords

Left ventricular non-compaction
Semi-automatic segmentation
CMR
Genetic mouse model
CMR

Abbreviations

CI	Confidence interval
CMR	Cardiovascular magnetic resonance
Cx40	Connexin 40
D	Day
ICC	Intra-class correlation coefficient
LV	Left ventricle/left ventricular
LVEF	Left ventricular ejection fraction
LVNC	Left ventricular non-compaction
M_c	Compacted mass
M_t/M_c	Percent of trabeculation computed by $M_t/M_c \times 100$
M_t	Trabeculated mass
P1	Postnatal day 1
PCR	Polymerase chain reaction
RV	Right ventricle/right ventricular

Background

Left ventricular (LV) non-compaction (LVNC) is suspected on echocardiography or cardiovascular magnetic resonance (CMR) when a characteristic double-layered aspect of the myocardium with a thick, non-compacted endocardial layer, prominent trabeculations and deep recesses are observed [1, 2, 3]. Untreated, LVNC can lead to heart failure, cardioembolic events, and tachyarrhythmia; it may occur as an isolated cardiomyopathy or in association with other congenital heart diseases [4]. Quantification of the total amount of LV trabeculation on CMR has

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been proposed as a method to diagnose LVNC [3, 5], but manual contouring of all LV trabeculation and compacted LV is time consuming. Recently, Bricq et al. developed a semi-automatic software package to calculate the non-compacted mass (M_c not M_c but M_t , non compacted mass is trabeculated mass) that suppresses blood from the trabeculae and evaluate the total amount of LV trabeculation as well as LV mass [6]. The feasibility of this trabeculation quantification algorithm was illustrated in a small group of mice along with their histology data, but no assessment of reproducibility or validation in an animal group of sufficient size was provided.

In this study, the quantification software was compared with histology and tested for accuracy and reproducibility in a mouse model of non-compaction. Nkx2-5 is a homeodomain transcription factor, which drives the early stages of cardiac morphogenesis. Global Nkx2-5 knock-out in mice causes early embryonic lethality with delayed cardiac development [7, 8], though heterozygous Nkx2-5 knock-out mice present a spectrum of cardiac anomalies including signs of ventricular non-compaction and cardiac conduction system defects [8, 9, 10, 11]. A previous animal study has shown that inducible ventricular knock-out of Nkx2-5 generates adult mice with an LVNC phenotype [12]. Mutations of Nkx2-5 have been identified in numerous patients with congenital heart diseases, and some were associated with LVNC [13]. Our goal was to quantify LV trabeculation on CMR in a genetic mouse model of non-compaction using a dedicated semi-automatic software package and to compare our results to the histology used as a gold standard.

Methods

Conditional trabecular Nkx2–5 knock-out mouse model (Fig. 1)

The animal procedures were approved by the University's ethics committee for animal experimentation (n°40–10,102,012). Animal care was given in compliance with the national and institutional guidelines and this study was performed under the authorization of the local ethical committee. Mice were part of the project “mouse cardiovascular development system” (n° 01055.02). During embryonic development, Connexin-40 (Cx40) is expressed in the trabeculae, therefore we crossed Nkx2–5 floxed mice [11] with Cx40^{cre/+} mice to allow Cre-inducible deletion of the floxed gene specifically in the trabeculae [14]. Cre recombinase mediated-deletion of the Nkx2–5-Flox gene was induced with tamoxifen (20 mg/ml, Sigma-Aldrich, St. Louis, Missouri, USA) injected intraperitoneally [15] into pregnant mice (200 µL) for 2 consecutive days or once subcutaneously at 1 day postnatally (P1) (20 µL). The first day of gestation was defined as the morning on which the vaginal plug was found. Thirteen mice were

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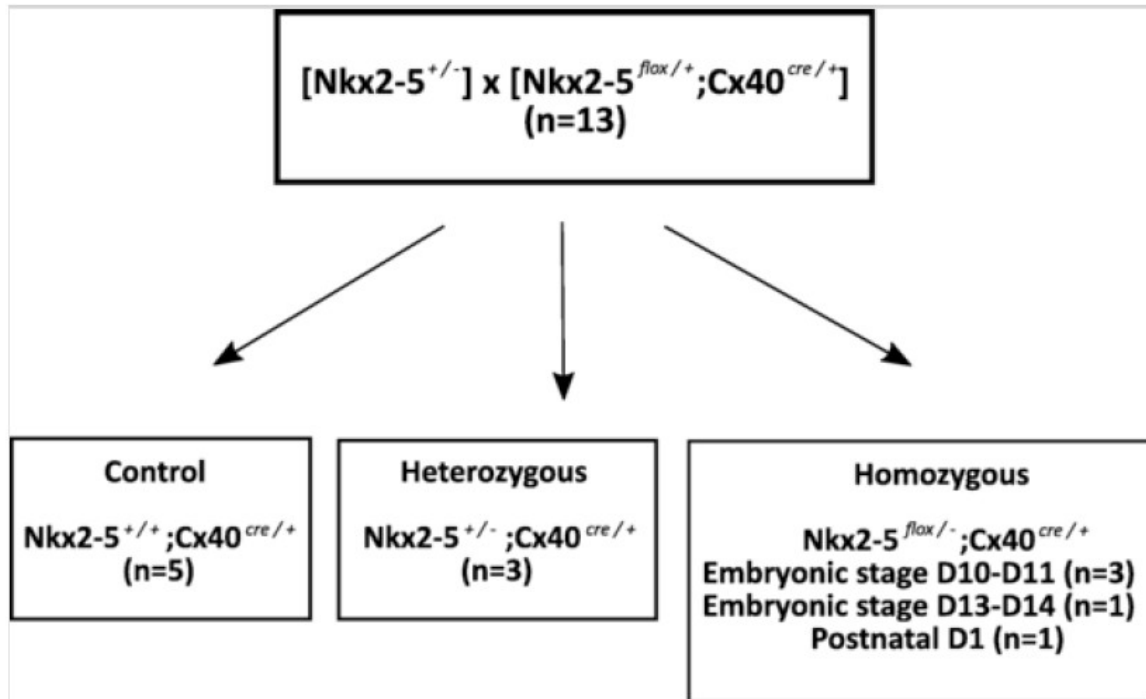
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analyzed in 2 groups (flow chart): 5 controls Nkx2–5^{+/+};Cx40^{cre/+}, and 8 mutant mice: 3 heterozygous Nkx2–5^{+/-};Cx40^{cre/+} [9], and 5 homozygous Nkx2–5^{flox/-};Cx40^{cre/+} at different stages (4 injected at embryonic stages, and 1 at P1). All mice were sacrificed after CMR and whole hearts were dissected and sectioned in the transversal axis using a cryostat. The eosin stained slices were incubated for 5 min in a 5% aqueous solution (Sigma-Aldrich).

Fig. 1

Flow chart



CMR

CMR scans were performed on adult mice when they weighed 20–30 g, i.e. after 2 to 4 months depending on the strains, using a preclinical 11.75 T system (AVANCE 500 MHz/89 mm wide bore vertical imager (11.75 Bruker Biospin GmbH, Ettlingen, Germany) and a 30 mm diameter transmit-receive radiofrequency resonator. Anesthesia was maintained during CMR with 1–2% isoflurane in a constant flow of ambient air (270 ml/min) through a nose cone using a dedicated vaporizer (Univentor anaesthesia unit, Univentor high precision instruments, Zejtun, Malta). Body temperature was maintained at 37 °C. Respiration rate and heart rate were monitored and the signals were used to trigger the CMR acquisitions using a monitoring and gating system (SA Instruments, Inc. Stony Brook, New York, USA). High resolution spoiled gradient echo cine CMR (repetition time 15 ms, echo time 1.68 ms, flip angle

30°, slice thickness 1 mm, in-plane resolution $0.086 \times 0.086 \text{ mm}^2$) was performed in short axis view, at mid base-apex level.

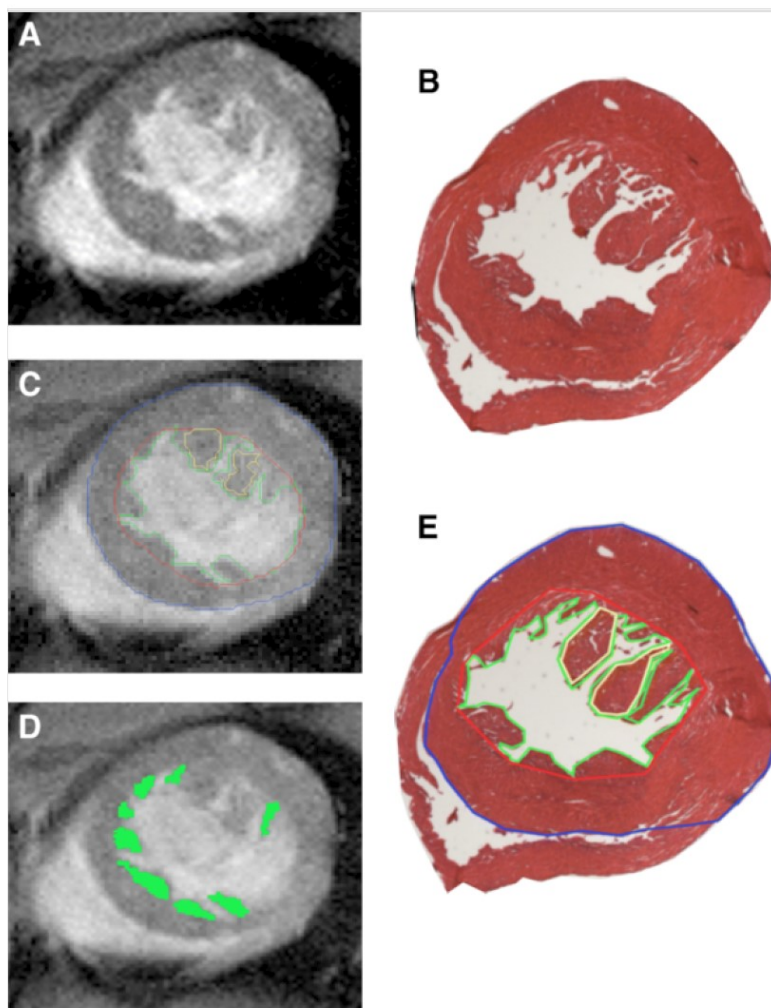
In a subset of 6 mice (1 control, 5 mutant), whole heart spoiled gradient echo cine CMR with lower spatial resolution was added to the high resolution acquisition (repetition time 5 ms, echo time 1.51 ms, flip angle 20°, slice thickness 1 mm, in-plane resolution $0.172 \times 0.172 \text{ mm}^2$) in short axis view. Five to seven slices were acquired from base to apex to cover the whole left ventricle. The end-diastolic frames were segmented with the software to assess the extent of the trabeculation across LV.

Image analysis

For the evaluation of LV trabeculation, the end diastolic frame from the cine imaging was used to segment the myocardium with the dedicated software previously described [6]. The method requires a seed indicated by the user to initialize the selected slice. The non-compacted region was obtained using this seed and a region-growing algorithm. This non-compacted contour was then used to initialize an active contour to determine the endocardial and epicardial borders. When necessary, each contour was manually corrected. The papillary muscles were then segmented using a semi-automatic threshold tool based on the difference between bright blood and darker myocardial intensities. Afterwards, blood was removed from the trabeculae using the same thresholding tool. The trabeculae were defined at the end-diastolic phase as dark myocardium protruding from the LV wall into the bright LV cavity. Papillary muscles were included in the myocardial mass. If the papillary muscles could not be clearly distinguished from the trabeculation on the short-axis images, they were treated as trabeculation (Fig. 2) [6].

Fig. 2

Example of left ventricular (LV) high resolution CMR segmentation and corresponding eosin stained histological images. **a-b**: Example of a midventricular short axis, cine image from a mutant mouse heart (**a**) and slice at the same level from eosin stained histological slide (**b**). **c-d**: Semi-automatic LV segmentation of the cine image: the epicardial border appears in blue, the endocardial border in red, the trabecular border in green (**c**) and the trabecular surface in green (**d**). The papillary muscles were segmented and included in the compacted area (yellow line) (**c**). **e**: Manual LV segmentation of the histological slice using the same segmentation procedure: epicardial border in blue, endocardial border in red, trabecular border in green. The papillary muscles were segmented and included in the compacted area (yellow line)



The trabeculated area (M_t) was defined as the area including all LV trabeculations excluding the papillary muscles and LV blood between the trabeculae. Compacted area (M_c) was defined by the myocardial area including the papillary muscles and excluding LV trabeculation. The knowledge of the slice thickness of 1 mm allows the calculation of the trabeculated and compacted mass in each slice. Percentage of LV trabeculation (M_t/M_c) was expressed as a percentage of the trabeculated area computed by $M_t/M_c \times 100$.

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To investigate intraobserver variability, observer 1 (J.F., 5 years of experience) repeated the measurements on 10 cine images at least 1 month after the first examination to minimize recall bias. A second independent observer (Z.B., 2 years of experience) repeated the different measurements on the same acquisitions to assess interobserver variability.

All mice were sacrificed after CMR and whole hearts were dissected and sectioned in the transversal axis using a cryostat. Cryosections were 20 μm thick. From these cryosections, we visually selected the one that best corresponded to the CMR slice level. Papillary muscle shape and size and trabeculation distribution were selected as land marks to perform a visual co-registration between CMR and the histology. In the subset of 6 mice, 5 to 7 histological sections were selected per animal to be consistent with the 5 to 7 CMR stacks acquired from base to apex using the same co-registration method.

Each histological slice was analyzed by observer 1 (J.F.) who was blinded to the CMR results. The segmentation was done manually with Image J (version 1.49 v, National Institute of Health, Bethesda, Maryland, USA) using the same segmentation procedure (Fig. 2).

Statistical analysis

All mouse group characteristics are reported as median values and range. The values were compared between the groups using Kruskal-Wallis and Mann Whitney U tests. The results were considered as significant when $p < .05$. Linear regression and Pearson correlation coefficients between the histology and CMR were assessed. The histological and CMR results for M_c , M_t and M_t/M_c were compared using Bland-Altman analysis and taking the histological data as a reference. Interobserver and intraobserver reproducibilities were assessed by computing the intraclass correlation coefficient (ICC) with a confidence interval (CI) of 95% using Bland-Altman analysis.

Results

Mice characteristics

There were no significant differences between the control and mutant groups in terms of age, body weight or heart weight (Table 1). CMR was feasible with a good quality of image in all mice. A total of 13 cine images were evaluated. CMR was performed on adult mice on median day 63 (range: 47–120 days). Median body and heart weight at the time of CMR were 26.5 g (range 16.5–34.0 g) and 185 mg (range 124–233 mg), respectively. Histological staining was successful in all cases.

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Table 1

Mice characteristics at time of CMR: median values (min-max)

	Control (n = 5)	Mutant (n = 8)	P value
Age at time of CMR (days)	118 (60–120)	60 (47–112)	0.17
Total weight (g)	30.6 (24–33)	24 (16.5–34)	0.30
Heart weight (mg)	132 (124–181)	196 (153–233)	0.07

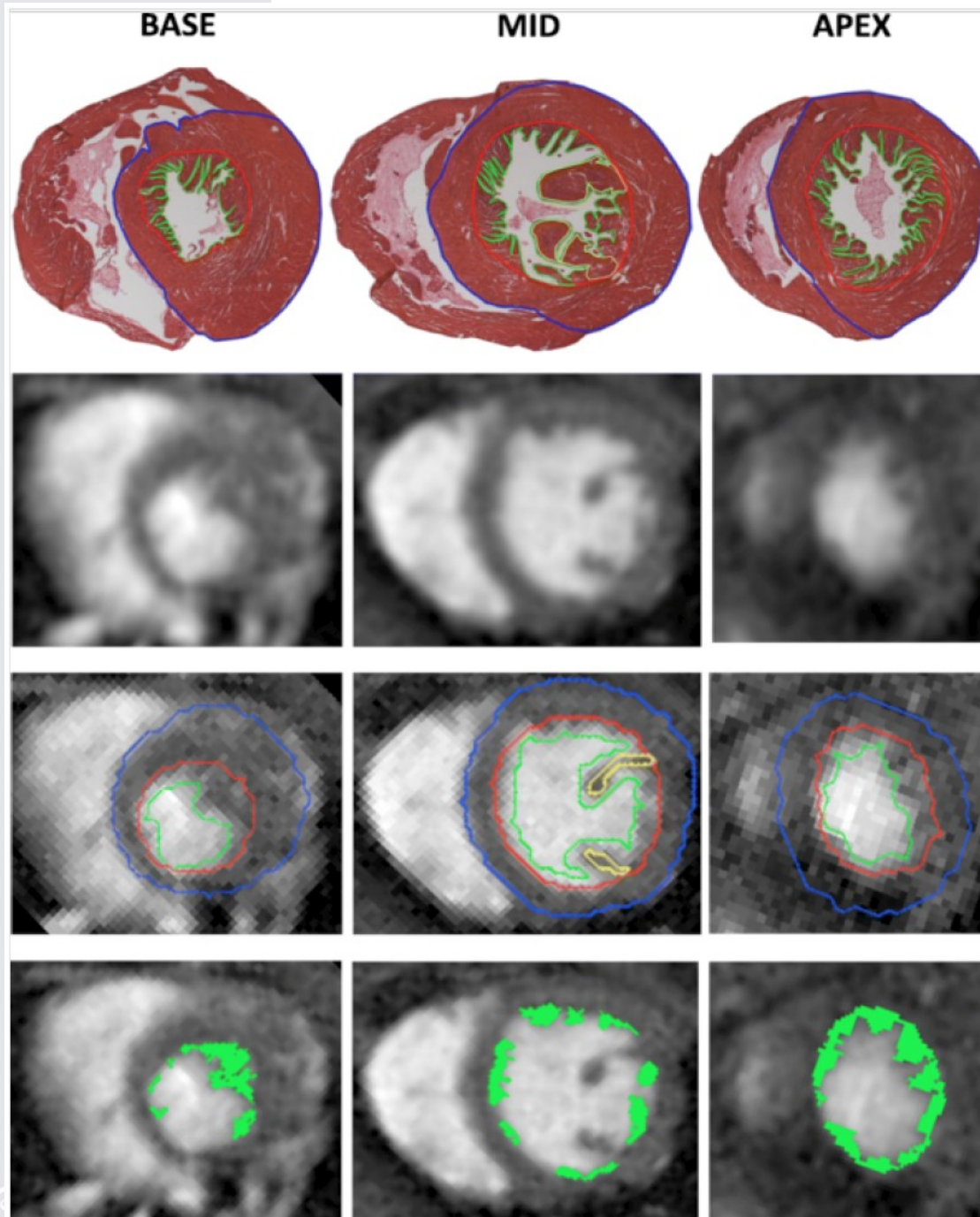
Study of trabeculation across the whole LV

Processing took 15 ± 0.5 min for one whole heart examination, including computation time and user interaction. Automatic epicardial, endocardial and trabeculation border detection took less than 1 min per slice. Semi automatic papillary muscles detection took less than 30 s, whereas around 1.5 min were required to segment blood within the trabeculation net per slice (Fig. 3).

Fig. 3

Example of left ventricular whole heart CMR segmentation and corresponding eosin stained histological images. Example of a basal (left), midventricular (middle) and apical (right), histological slices with the corresponding short axis cine image from a mutant mouse heart. The epicardial borders appear in blue, the endocardial borders in red, the papillary muscles in yellow, the trabecular borders in green and the trabecular surface after manual deletion of blood in green (bottom line)





The results of whole heart segmentation for M_t , M_c and M_t/M_c are summarized in Table 2. There was no statistical difference between basal, midventricular and apical parts for M_t ($p \neq 0.61$), M_c ($p \neq 0.48$) and M_t/M_c ($p \neq 0.35$).

Table 2

Trabeculae segmentation: whole heart CMR vs histology

	CMR	Histology	P value
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	CMR	Histology	P value
Whole heart M_t (mg) median values (min–max)	6.31 (4.76–10.22)	4.68 (2.3–8.4)	0.16
Apical M_t (mg)	2.68 (1.56–4)	1.68 (0.6–2.93)	0.86
Midventricular M_t (mg)	2.59 (1.57–5)	1.75 (0.65–2.47)	0.94
Basal M_t (mg)	2.28 (1.21–5)	0.97 (0.36–2.06)	0.41
Whole heart M_c (mg)	57.52 (36.72–90.8)	52.13 (34.63–86.59)	0.7
Apical M_c (mg)	17.07 (8.87–27)	17.13 (12.26–25.97)	0.89
Midventricular M_c (mg)	24.1 (15.73–30)	21.7 (16.09–32.34)	0.56
Basal M_c (mg)	19.42 (12.1–29.5)	17.95 (7.12–28.28)	0.73
Whole heart M_t/M_c (%)	12.95 (6.36–17.57)	9.19 (3.12–16)	0.75
Apical M_t/M_c (%)	15.75 (7.61–33.03)	10.85 (7.15–23.9)	0.9
Midventricular M_t/M_c (%)	10.59 (6.9–16.67)	8.51 (2.51–12.02)	0.73
Basal M_t/M_c (%)	9.12 (7.99–14.67)	5.4 (3.66–7.67)	0.44

The results of the Bland-Altman test performed with the histological data as reference showed good agreement with a subtle overestimation of myocardial mass compared to the histology: $+1.89 \pm 1.08$ mg for M_c , $+4.26 \pm 2$ mg for M_t and $+2.35 \pm 1.83\%$ for M_t/M_c . There was a strong correlation between the histology and the whole heart cine imaging for M_c : $r^2 = 0.98$ ($p \leq 0.001$); and good for M_t : $r^2 = 0.74$ ($p \leq 0.017$); and M_t/M_c : $r^2 = 0.81$ ($p \leq 0.009$).

Trabecular segmentation and histological correlation on high resolution CMR

Segmentation was feasible in all mice (Table 3). The median value of M_t in all mice by CMR showed a high agreement with histology (0.92 mg CMR vs 0.8 mg histology, $r^2 = 0.94$, $p < 0.001$). The median value of M_c in all mice by CMR showed a high agreement with histology (12.24 mg CMR vs 12.16 mg histology, $r^2 = 0.91$, $p < 0.001$). The median value of M_t/M_c in all mice by CMR showed a good agreement with histology (6.74% CMR vs 6.17% histology, $r^2 = 0.83$, $p < 0.001$, Table 3, Fig. 4). The results of the Bland-Altman test using the histological data as a reference showed high agreement (Fig. 4).

Table 3

Trabeculae segmentation: high resolution CMR vs histology

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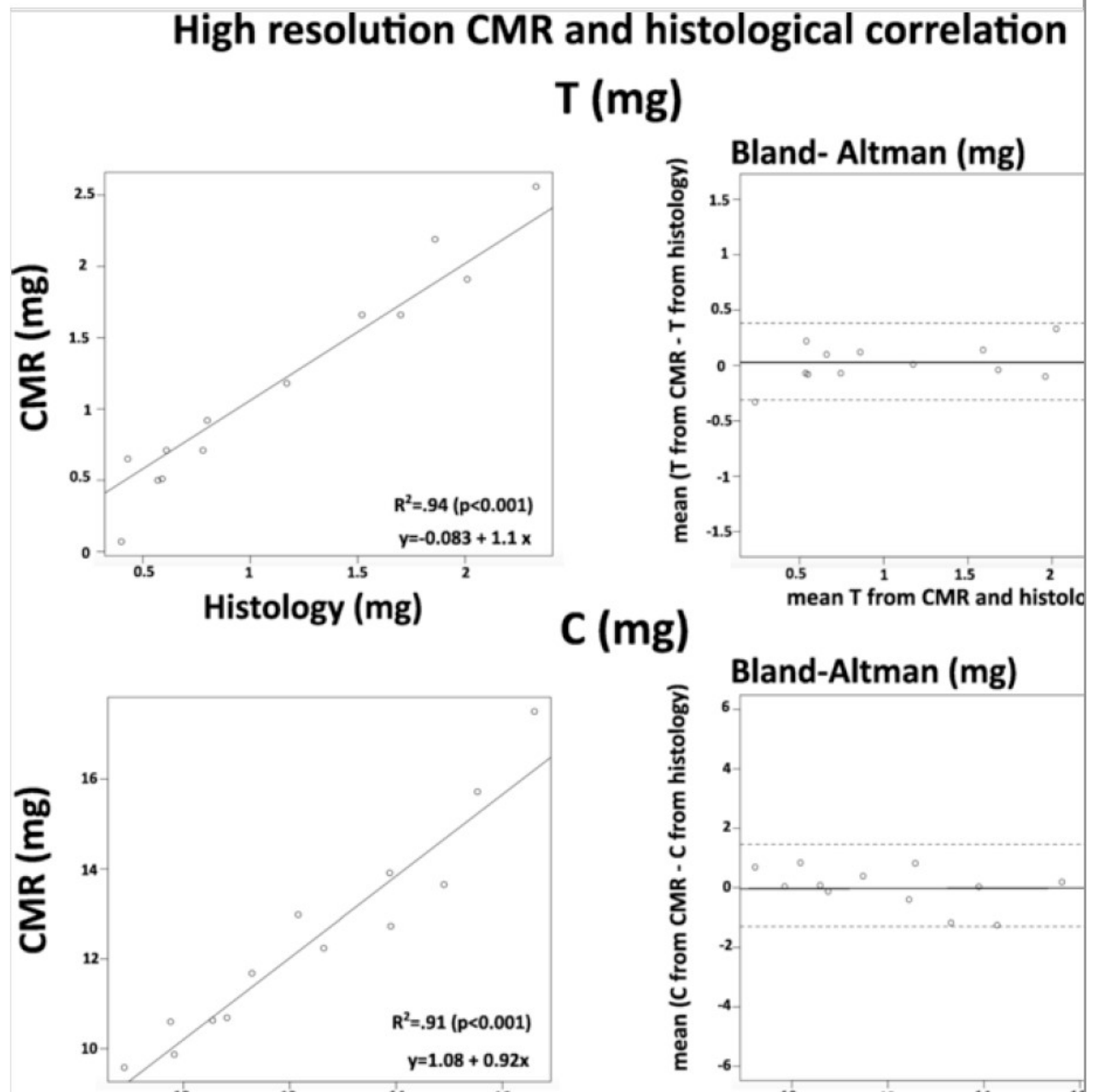
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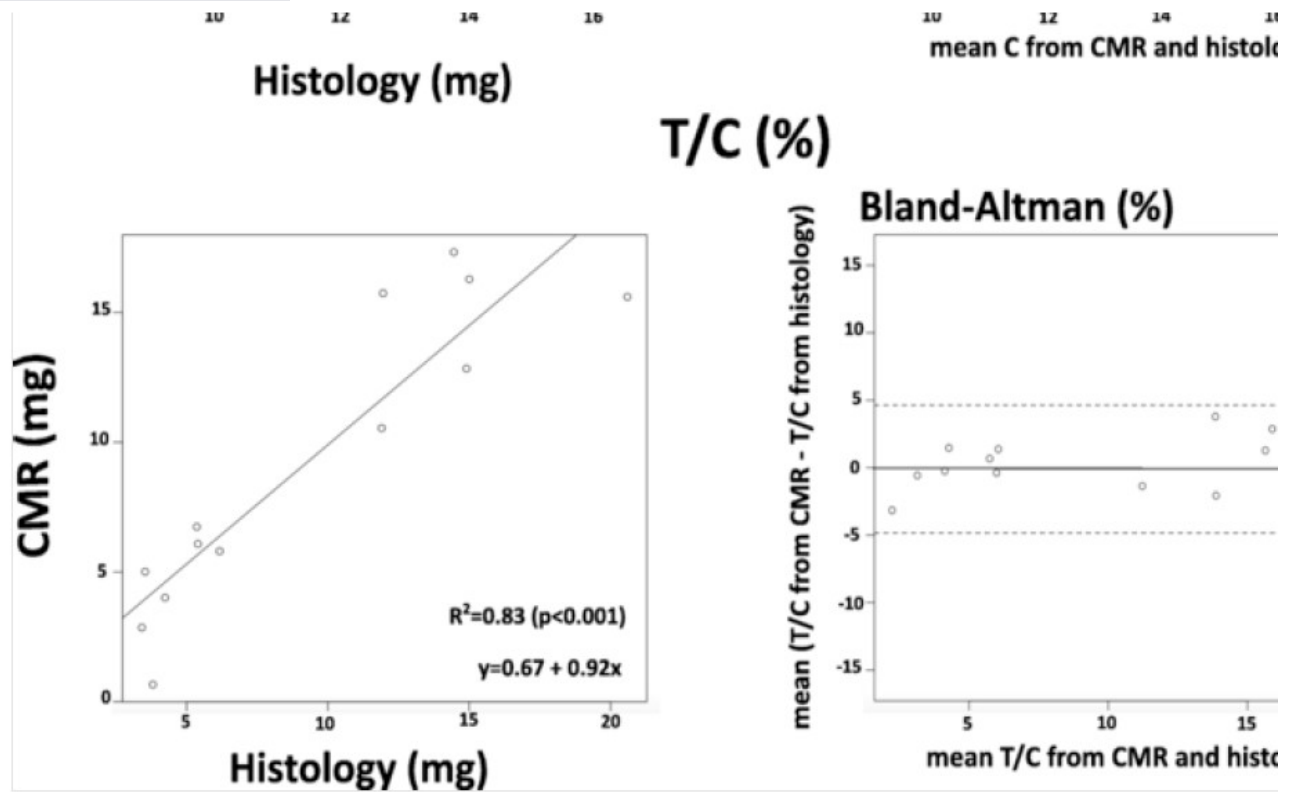
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	CMR	Histology	P value
M_t (mg) median values (min–max)	0.92 (0.07–2.56)	0.8 (0.4–2.23)	0.88
M_c (mg)	12.24 (9.58–17.51)	12.16 (8.89–16.6)	0.74
M_t/M_c (%)	6.74 (0.66–17.33)	6.17 (3.42–14.45)	0.83

Fig. 4

Correlation between high resolution CMR and histology. Linear regression (left) showing the relationship between histology and CMR quantifications of M_t (T) (top), M_c (C) (middle) and M_t/M_c (T/C) (bottom). Bland-Altman plots (right) showing the agreement between histology and CMR quantifications of T (top), C (middle) and T/C (bottom)





Intra- and interobserver reproducibility

Intraobserver reproducibility assessed by ICC was high for M_t , M_c and M_t/M_c with 0.97 (0.6–0.99), 0.98 (0.94–0.99) and 0.93 (0.84–0.97), respectively (Fig. 5). Interobserver reproducibility was high for M_c : 0.97 (0.88–0.99) and moderate for M_t and M_t/M_c : 0.80 (0.55–0.85), 0.72 (0.49–0.85), respectively. The results of the Bland-Altman analysis comparing the 2 observers showed very good agreement with a mean difference close to 0: 0.01 ± 0.54 mg (coefficient of variation: $\pm 33.23\%$) for M_t , 0.63 ± 0.45 mg ($\pm 7.63\%$) for M_c and $-0.56 \pm 4.14\%$ ($\pm 37.69\%$) for M_t/M_c .

Fig. 5

Intra- and Interobserver agreement. Bland and Altman plots showing the agreement for intraobserver measurements (left), and interobserver measurements (right) for CMR quantification of M_c (top), M_t (middle), M_t/M_c (bottom). Observer 1 first measurements were chosen as reference

Intraobserver agreement

Bland-Altman T (mg)



Interobserver agreement

Bland-Altman T(mg)

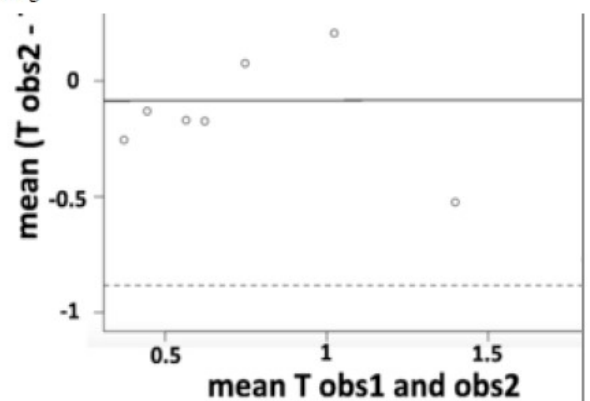
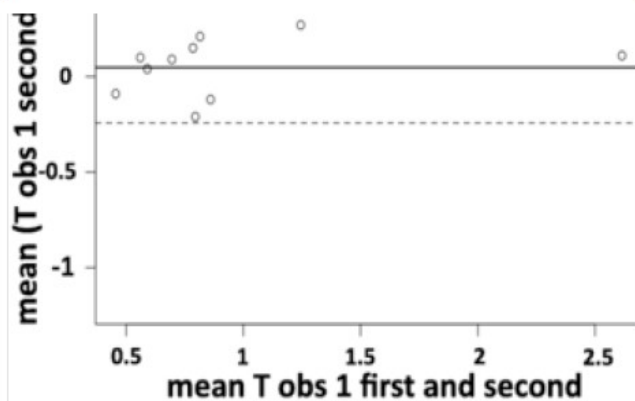


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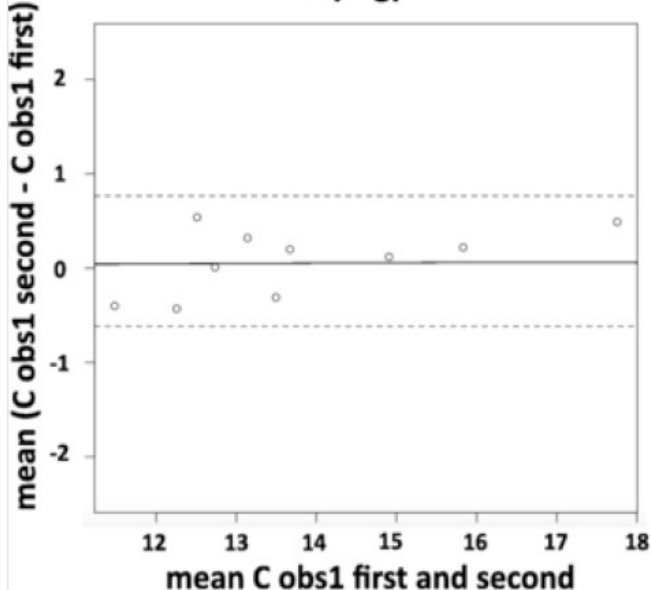
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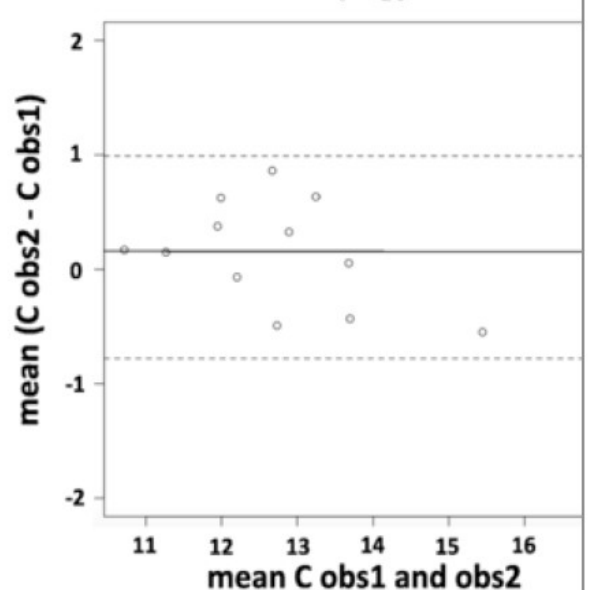
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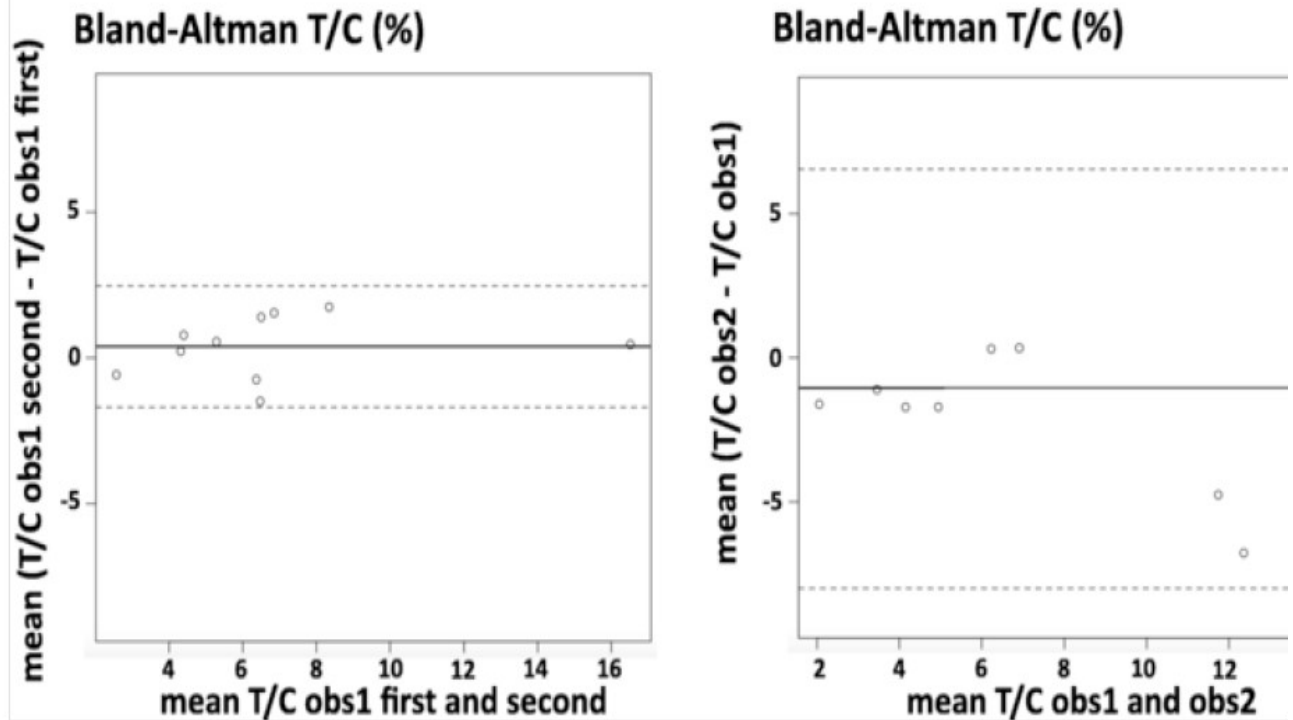


Bland-Altman C (mg)



Bland-Altman C (mg)





Trabecular segmentation and mutant mice on high resolution CMR

The M_c part of the myocardium from the CMR mid-LV cine images showed no significant difference when the mutant mice: median 12.17 mg (range 9.58–

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17.51 mg) were compared to the control mice 12.24 mg (range 10.63–12.98 mg) ($p \neq 0.94$) (Table 4). The M_t and M_t/M_c calculated using CMR were significantly higher in the mutants in comparison to the control mice with 1.66 mg (range 0.5–2.56 mg) vs 0.65 mg (range 0.07–0.71 mg) ($p \neq 0.02$) for M_t ; and 14.22% (range 2.86–17.33 mg) vs 5% (range 0.66–6.08%) ($p \neq 0.02$) for M_t/M_c (Fig. 6).

Table 4

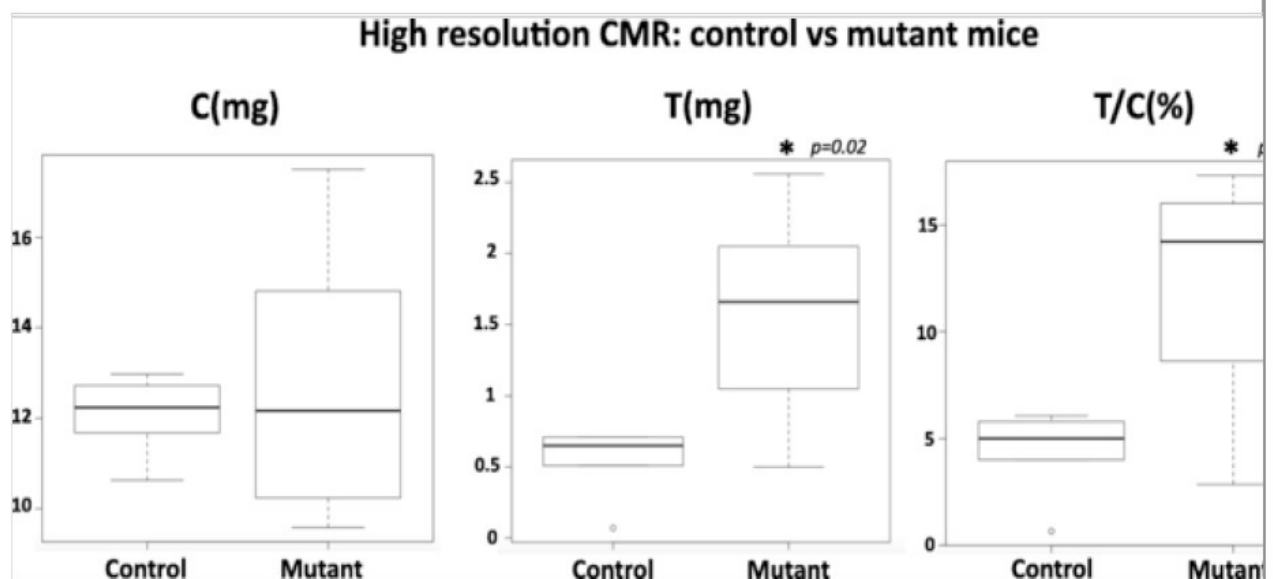
Trabecular segmentation on high resolution CMR: control vs mutant mice

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	Control (n = 5)	Mutant (n = 8)	P value
M_t from histology (mg) median values (min–max)	0.59 (0.04–0.78)	1.61 (0.57–2.33)	0.01 0.01
M_t from CMR (mg)	0.65 (0.07–0.71)	1.66 (0.5–2.56)	0.02 0.02
M_c from histology (mg)	12.16 (10.55–13.91)	12.35 (8.89–15.53)	1.00
M_c from CMR (mg)	12.24 (10.63–12.98)	12.17 (9.58–17.51)	0.94
M_t/M_c from histology (%)	4.24 (3.54–6.17)	13.72 (3.43–19.12)	0.06
M_t/M_c from CMR (%)	5.01 (0.66–6.08)	14.21 (2.86–17.33)	0.02 0.02

Fig. 6

comparison between control and mutant mice on high resolution CMR. Box-and-whisker plots comparing M_c (left), M_t (middle) and M_t/M_c (right) quantification on CMR, between control and mutant mice



Discussion

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In this murine CMR study, we assessed the performance of a semi-automatic software program dedicated to the quantification of compacted, trabeculated mass and found a high correlation between the histology and CMR. The software also showed a low intra- and intrerobserver variability.

A few earlier studies assessed trabeculation in mice using different methods. Kosaka et al. assessed trabeculation by measuring the M_t area [16], however, their histological validation was performed on sliced hearts in 4-chamber views. Yang et al. and Chen et al. also studied trabeculation in embryonic mice hearts [17, 19] mistake, not 19 but 18 (19 refers to Kawel and his article on the mesa study) ... by measuring M_t thickness on histological pseudo 4-chamber slices. These data are difficult to compare with our results.

In our study, the LV of mice seemed to be less trabeculated compared to human LV with a more homogeneous distribution of trabeculation over the entire LV. In humans there is a basal to apical gradient with increase of trabeculation from base to apex [6, 19]. In mice, the apical and midventricular part of the LV seemed to be hypertrabeculated. We chose a midventricular section for high resolution cine imaging to facilitate co registration with the histology using the papillary muscles as landmarks. A comparison between CMR and the histology was feasible in all mice and the correlation was high, however, the correlation with the histology was slightly lower than for the compacted part due to the small size and amount of trabeculation, partial volume effects and flow artifacts.

The software we used for this purpose quantifies trabeculation for the whole LV and requires just one click in the LV cavity to initialize the region-growing algorithm to detect the non-compacted borders. We used interactive thresholding to segment the papillary muscles and to extract blood from the non-compacted area. The software provided an accurate, reproducible, non-invasive semi-automatic calculation of LV compacted and trabeculated mass in adult mice on the cine imaging. We were able to show a significant increase of LV trabeculation in mice with conditional trabecular deletion of the *Nkx2.5* gene compared to control mice. The semi-automatic software was capable of differentiating between control and mutant mice. Assessing the correlation between the amounts of trabeculae and clinical phenotypes may contribute to a better understanding of hypertrabeculation in humans.

The reproducibility for M_c was high as shown earlier by Bricq et al. in their evaluation of normal trabeculation in humans (60 volunteers) [6]. Interobserver reproducibility was moderate for M_t and M_t/M_c mainly due to additive systematic measurement errors between M_t and M_c . This could be explained by the difference of segmentation due to partial volume around trabecules between

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the two observers. Intraobserver reproducibility remained very high for M_t , M_c , and M_t/M_c ; this emphasizes the necessity for a strict method of segmentation, notably for trabeculation. Measurement variation for M_t and M_t/M_c could be clinically significant for small amounts of trabeculae.

The quantification of trabeculated mass has previously been used in several human studies assessing hypertrabeculation [3, 5, 6]. The global trabeculation ratio of 6.74% was low compared to human reference even for mutant mice with a T/C of 14.22%. In their study on 60 healthy subjects, Briq et al. found a M_t/M_c ratio of 10.26% with more trabeculation in the apex and lateral parts [6]. In human studies, the diagnosis of LVNC is still a matter of debate and there is no standard of reference for LV trabeculation quantification. Petersen et al. measured the M_t/M_c thickness on human subjects at the point where trabeculation was the most pronounced [2]. A threshold M_t/M_c thickness of 2.3 has been proposed to diagnose non-compaction [2], however Kawel et al. have shown that 43% healthy subjects from the Multiethnic Study of Atherosclerosis (MESA) Study have a $M_t/M_c > 2.3$ [19]. In this study, the evaluation was based on area segmentation and mass calculation. We arbitrarily selected a mid-LV slice to compare with the histological data. Captur et al. characterized the trabeculae differently, using a parameter correlated with LV endocardial complexity to calculate a fractal index that they applied to embryonic mice hearts by using high-resolution microscopy [20, 21, 22]. Hypertrabeculation is also a phenotype that could be encountered in several other cardiomyopathies such as hypertrophic cardiomyopathy. Furthermore some first-degree relatives of patients with LVNC may express a hypertrabeculated phenotype. An accurate tool to quantify LV trabeculation could prove to be useful to define the phenotype of the patient more precisely.

The principal limitation of the study is the fact that our software is not fully automatic and this next step would require further resources such as artificial intelligence tools to allow completely automated segmentation of LV trabeculation. The co-registration between CMR and histology was carried out visually. Despite the care and accuracy we put into that step a misregistration bias may have been included.

Furthermore, we quantify trabeculation as a mass. This is an approximation of the truth due to the partial volume effect. M_t seen on CMR may not always extend through the entire thickness of the slice. It is more likely that trabeculation represents a partial volume through the slice thickness. What we express as M_t is thus some uncertain value between blood and myocardium. To be consistent with the literature we have used the term trabeculated mass.

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The method requires assessment in clinical practice to define cut off values for normal LV trabeculation and excess trabeculation.

Conclusions

The proposed post-processing software allowed accurate and reproducible quantification of the amount of LV trabeculation in mice from CMR. In comparison to the histology, the semi-automatic software was accurate in its evaluation of M_c , M_t and M_t/M_c . Trabeculation seems to increase dramatically when the deletion of the *Nkx2.5* gene is induced at different embryonic development stages.

Clinical competences

This study assesses the accuracy and reproducibility of a new software program to evaluate left ventricular T. The semi-automatic software shows a high degree of accuracy and reproducibility compared to the histology as a reference. This software program could be used in humans to quantify the total amount of trabeculation.

Translational outlook

We showed that it was possible to assess trabeculation in a non-invasive manner and that we could differentiate mutant from control mice. LV non-compaction remains controversial and has no gold standard diagnosis. This semi-automatic software could help to discriminate normal from hypertrabeculated patients due to its accuracy in calculating the amount of LV trabeculation.

Availability of data and materials All datas and materials are available. Please contact for data requests.

Authors' contributions

JF: conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the manuscript. LM, MF, ZB: acquisition of data, analysis and interpretation of data. SB, AL, MB, PAB, FK, LM, AJ: conception

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and design of the study, revision of manuscript. SB, AL: Management of the post-processing tools. DF, GH: revision of manuscript. All authors read and approved the final manuscript.

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Ethics approval The animal procedures were approved by the University's ethics committee for animal experimentation (n°40–10,102,012). Animal care was given in compliance with the national and institutional guidelines and this study was performed under the authorization of the local ethical committee. Mice were part of the project “mouse cardiovascular development system” (n° 01055.02).

Consent for publication Not applicable

Competing interests The authors declare that they have no competing interests.

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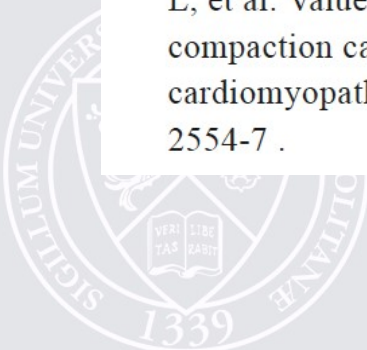
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Annexe 4: article sur la distribution normale des trabécules



Distribution of left ventricular trabeculation across age and gender in a cohort of 140 healthy Caucasian subjects

Abstract

Rationale and objectives

This study sought to quantify the distribution of trabeculated (T) and compact (C) left ventricular (LV) myocardium masses in a healthy Caucasian population against age, gender and LV parameters, and to provide normal values for T, C and T/C.

Materials and methods

One hundred and forty healthy subjects were prospectively recruited and underwent cardiac MRI at 1.5T with a stack of short-axis cine sequences covering the entire LV. End-diastolic volume (EDV), C and T masses were quantified using a semiautomatic method. Ejection fraction (EF) and T/C ratio were computed.

Results

We included 70f/70m, mean age: 44 years $\pm$ 14, range: [20-69]. The mean EF was 63.7 $\pm$ 6.3%[50.7-82.0], the mean EDV was 75.9 $\pm$ 16.2ml/m²[36.4-112.2], the mean C mass was 53.9 $\pm$ 11.2g/m²[26.5-93.4] and the mean T mass was 4.9 $\pm$ 2.4g/m²[1.1-11.4]. The T/C ratio was 9.2 $\pm$ 4.5%[2.0-29.4]. Multivariate ANOVA test showed that the compact mass was influenced by EDV ($P<0.0001$), EF ($P=0.001$) and gender ($P<0.0001$), and the trabeculated mass depended on EDV ($P<0.0001$), gender ($P=0.002$) and age ($P<0.0001$), while the T/C ratio was only impacted by age ($P=0.0003$). Spearman test showed a correlation between EDV and C ($r=0.60$, $P<0.0001$), T ($r=0.46$, $P<0.0001$) and T/C ratio ($r=0.26$, $P=0.0023$).

Only T and T/C ratio were correlated to EF ($r=-0.18$, $P=0.0373$; $r=-0.18$, $P=0.0321$ respectively).

Conclusion

While the compact and trabeculated myocardium masses appear to relate separately to the cardiac function, age and gender, their ratio T/C appears to only decrease with age. Furthermore, we propose here normal values for T, C and T/C in a cohort of healthy Caucasians subjects.

Running title: Distribution of LV trabeculations in healthy Caucasian subjects.

Key words

Myocardium; Left Ventricular Remodeling; Isolated Noncompaction of the Ventricular Myocardium; Left Ventricular Function; Healthy Volunteers.

Abbreviations and acronyms

BSA: body surface area

bSSFP: balanced steady-state free precession

C: compact myocardium mass

CI: confidence interval

DCM: dilated cardiomyopathy

ECG: electrocardiogram

ED: end-diastolic

EDV: end-diastolic volume

EF: ejection fraction

ES: end-systolic

FOV: field of view

HCM: hypertrophic cardiomyopathy

ICC: intraclass correlation coefficient

LV: left ventricle

LVNC: left ventricular non-compaction

MRI : magnetic resonance imaging

SD: standard deviation

T: trabeculated myocardium mass



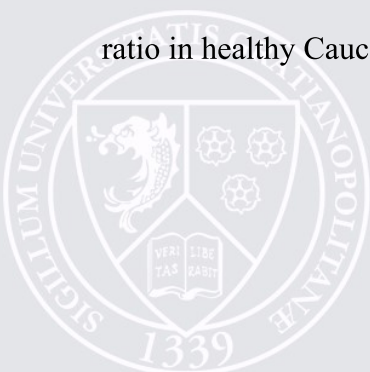
Introduction

Cardiac MRI has emerged as a reference modality for heart diseases investigation. It's a valuable non-invasive tool allowing an accurate morphological and functional characterization of the heart in several clinical conditions (39,40).

Excess of trabeculation is a trait shared by several cardiomyopathies such as left ventricular non compaction (LVNC) (41) or hypertrophic cardiomyopathy (HCM) (42). Excess of trabeculation was also described in pregnant women (43) or highly trained athlete (44). Trabeculations are normal constituent of the left ventricle (LV) endocardial layer and discriminating between normal and excess of trabeculation is still a matter of debate.

Therefore, there is a need for reliable quantification of trabeculations in the LV myocardium and its variations in a healthy population. Indeed, to date, there is no criteria or method considered as gold-standard for differentiating between normal and pathological trabeculation of the myocardium. Recent semi-automatic method to quantify compact (C) and trabeculated (T) LV myocardium masses from cardiac magnetic resonance imaging (MRI) has been established and validated (45). However, this method has yet to be evaluated in terms of the range of "normal" healthy values of LV masses in a large cohort.

The aim of this paper was to quantify the distribution of T and C myocardium masses across age and gender in a healthy population and to provide normal values for T, C and T/C ratio in healthy Caucasian subjects.



Materials and methods

Recruitment

This study was approved by the local Institutional Review Board in accordance with the guidelines outlined in the Declaration of Helsinki. One hundred and forty healthy volunteers were enrolled for a cardiac MRI examination after providing informed consent. Among them, forty-one were already included in an initial study by Bricq et al. (45). An additional ninety-nine healthy subjects (clinical trial NCT01712581, clinicaltrials.gov) were prospectively recruited from October 2012 to June 2014 and included in this cohort. A pregnancy test was performed for all female participants of childbearing age. Inclusion criteria were: age over 18 and below 70 years old, ability to understand and sign the consent form, ability to maintain supine position for 20 minutes, no cardiac symptoms, normal cardiovascular examination results and no known risk factors or history of cardiac disease. Exclusion criteria were: claustrophobia, transfused subjects, haematological disease, iron deficiency, high blood pressure, contraindication for MRI, pregnancy, diabetes and arrhythmias.

Image acquisition

Magnetic resonance (MR) examinations were performed on a 1.5 T Avanto[®] scanner (Siemens Healthineers, Erlangen, Germany) using a multichannel body array coil combined with a spine array coil with the subjects in supine position. A stack of balanced steady-state free precession (bSSFP) cine sequences were acquired in short axis view in order to cover the whole LV from base to apex with a total of 7 to 14 slices. Acquisitions were

electrocardiogram (ECG) synchronized and performed under inspiratory breath hold using the following typical parameters set: flip angle: 77° , pixel bandwidth: 930Hz, spatial resolution between $1.37 \times 1.37 \text{ mm}^2$ and $1.48 \times 1.48 \text{ mm}^2$, slice thickness: 6mm, gap between slices: 9mm, repetition time: 3.62ms, echo time: 1.51ms, parallel imaging 2x (GRAPPA (46)). The resolution and the flip angle were adapted to subject morphology for each individual.

Image analysis

Argus[®] Ventricular Function software (Siemens Healthineers, Erlangen, Germany) was used to quantify end diastolic volume (EDV), end systolic volume (ESV), end diastolic LV mass and to compute the ejection fraction (EF) for all subjects. The end-diastolic (ED) image was defined as the frame with the largest blood pool volume in the LV cavity. The end-systolic (ES) image was determined as the frame with the smallest blood pool volume in the LV cavity. The most basal slice of the LV was defined as the slice showing at least 50 % of the LV myocardium circumference at ED. The most basal section could differ between ED and ES.

LV trabeculations, endocardial and epicardial borders were defined, in the whole stack of the diastolic phase, using the semi-automatic method proposed by Bricq et al. (45) (Fig1). Compact (C) and trabeculated (T) myocardium masses were defined as the difference between the epicardium and the endocardium and between the trabeculation contour and the endocardium respectively, as described in (45). Papillary muscles were included in the compact LV myocardium mass. C and T masses were indexed to the body surface area (BSA) to take into account variation of height and weight between subjects. The T/C ratio was then computed and expressed as a percentage. All left ventricular parameters, C and T

measurements were done by an operator (Op-1, three-year experience, Image Processing Engineer). A second operator (Op-2, two-year experience, Doctor) repeated the measurements on 10 randomly assigned subjects to evaluate inter-observer variability. To assess intra-observer variability, measurements were repeated by the first operator on 20 randomly selected subjects, at least 3 weeks later. All operators were blinded to the subject characteristics (age and gender) and to the other operator measurements. A representation of T/C ratio mean values across age and gender was produced for the 16 LV segments.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 7.0a (GraphPad software Inc., San Diego, CA). The intraclass correlation coefficient (ICC) and Bland-Altman method comparison were used to evaluate the reproducibility between two segmentations by Op-1 and between segmentations by Op-1 and Op-2. Values were reported as mean $\pm$ standard deviation (SD). The mean values were calculated according to gender (70 males and 70 females) and age (5 groups of 28 subjects, 14 men and 14 women). BSA was calculated using the formula of Dubois and Dubois (47). D'Agostino & Pearson normality test was performed for the 5 age groups and each gender. For each variable (EF, EDV, ESV, T, C and T/C ratio), a non-parametric analysis (Mann-Whitney test for genders, Kruskal-Wallis test and Dunn multiple comparisons post-hoc test for age groups) was used to compare groups who did not pass the normality test. Groups who passed the normality test were analysed using a parametric test (Welch's t test for genders, ordinary one-way ANOVA with Tukey's multiple comparisons test for age groups). A Multivariate ANOVA (MANOVA) was performed to test the influence of gender and age on quantitative cardiac MRI measurements. A Spearman test

was also performed in order to measure the correlation between LV masses and the other LV parameters (EF and EDV). Significance was set to $P<0.05$.

Results

Participant Characteristics

Volunteers (70 men and 70 women) were organised so as to include 14 men and 14 women in each of the 5 following groups: first group, age between 20-29 years old; second group, age between 30-39 years old; third group, age between 40-49 years old; fourth group, age between 50-59 years old and the fifth group, age between 60-69 years old. Subject characteristics were summarized in Table 1. All quantitative variables were expressed as mean $\pm$ SD and ranges. There was no significant difference ($P>0.05$) between any age group in terms of height, weight and BSA. Men had significantly greater BSA than women ($P<0.0001$).

Left ventricular parameters

Regarding the ejection fraction (EF), all age groups were similar, but there was a significant difference ($P=0.0178$) between men (62.4 ± 6.1 %) and women (64.9 ± 6.2 %) as shown in Table 2. EF increased significantly with age ($P=0.0184$) (Fig. 2a). The mean value of end-diastolic volume (EDV) was significantly greater in men (78.8 ± 18.0 ml/m²) than in women (72.8 ± 13.6 ml/m², $P=0.0280$). When comparing EDV across age groups, it decreased significantly with age from 87.13 ± 16.74 ml/m² in the youngest to 67.37 ± 17.09 ml/m² in the oldest ($P<0.0001$). ESV also decreased significantly with age ($P<0.0001$) (Fig. 2b) with significant difference between men and women ($P=0.0052$) and the age group 20-29 and 40-49 ($P=0.0005$), 50-59 ($P=0.0017$) and 60-69 ($P<0.0001$).

Left ventricular masses across gender and age groups

There was no significant difference between age groups in terms of compact myocardium mass. The mean value of C was significantly greater in men ($60.0 \pm 9.1 \text{ g/m}^2$) than in women ($47.7 \pm 9.8 \text{ g/m}^2$, $P < 0.0001$) (Table 2). While on the other hand, C remained stable across age (Fig. 2c).

Trabeculated myocardium mass decreased significantly with age from $6.85 \pm 2.56 \text{ g/m}^2$ in the first age group 20-29 to $3.97 \pm 1.99 \text{ g/m}^2$ in the oldest (fifth age group: 60-69) ($P < 0.0001$) (Fig. 2c) with significant difference between the first group (age between 20-29) and all the other age groups (30-39, $P = 0.0072$; 40-49, $P = 0.0025$; 50-59, $P = 0.0001$; and 60-69, $P < 0.0001$). The difference was also significant between genders with greater values for men: $5.4 \pm 2.3 \text{ g/m}^2$, compared to women: $4.0 \pm 2.3 \text{ g/m}^2$ ($P = 0.0052$) (Table 2).

T/C ratios were not significantly different between genders ($9.1 \pm 3.7 \%$ for men and $9.2 \pm 5.2 \%$ for women). The T/C ratio was decreasing with age from $12.33 \pm 4.57 \%$ in the youngest to $7.55 \pm 3.49 \%$ in the oldest ($P = 0.0003$) (Fig. 2d). There was also a significant difference between the first age group 20-29 and the age groups 40-49 ($P = 0.0390$), 50-59 ($P = 0.0009$) and 60-69 ($P = 0.0006$).

Values of the T/C ratio for men and women in each age group are listed in Table 3, as mean $\pm$ SD and 95% confidence interval (CI).

Observer variability

Mean and SD of T, C and T/C ratio for the intra-observer variability, are provided in Table 4. The ICC (Table 4, ICC) was high (> 0.90) for all parameters (T, C and T/C ratio). The Bland-Altman bias between the segmentations was $-0.16 \pm 0.39 \text{ g}$ for T mass, $0.70 \pm 2.00 \text{ g}$ for C mass and $-0.21 \pm 0.47 \%$ for the T/C ratio.

Table 4 displays also the variability between the two experts Op-1 and Op-2 where the ICC was computed. We had a high ICC (≥ 0.80) for C, T and T/C ratio. Bland-Altman bias was found to be -0.64 ± 0.39 g for the T mass, -3.61 ± 6.04 g for the compact myocardium mass and -0.58 ± 1.81 % for the T/C ratio. The mean values $\pm$ SD for the three parameters (T, C, and T/C ratio) are also given in Table 4.

Relationship between LV masses and age, gender and LV parameters

In terms of trabeculated myocardium mass, there was a significant correlation with age, gender and EDV, but not with EF. The only factor independently linked with the T/C ratio variation was age (Table 5).

The Spearman test showed a positive correlation between EDV and C, T and T/C ratio ($r=0.60$, $P<0.0001$; $r=0.46$, $P<0.0001$; $r=0.26$, $P=0.0023$ respectively), but only T and T/C ratio were negatively correlated to EF ($r=-0.18$, $P=0.0373$; $r=-0.18$, $P=0.0321$ respectively).

Values of the T/C ratio across the 16 LV segments

The distribution of the highest T/C ratios (over 20 %) was found in apical anterior and apical lateral segments. T/C ratios in the basal third did not reach 10 % for any subject regardless of gender and age as shown in Fig. 3.



Discussion

The main findings of this study were: 1) Local distribution of trabeculations showed that T/C ratios vary substantially across the LV, with higher ratios in apical anterior and apical lateral segments. 2) The T/C ratio was inversely correlated with age and independent to LV volumetric parameters in that cohort.

Trabeculations are normal constituent of LV and discrimination between normal trabeculation and hypertrabeculation remains a matter of debate (48). The presence of hypertrabeculation has been linked with a response to mechanical load which may disappear as the mechanical load dissipates (43,44). Therefore, the evocation of hypertrabeculation might have important consequence in highly trained athletes. Furthermore, hypertrabeculation was diagnosed in asymptomatic subjects with normal or nearly normal ejection fraction (49). Criteria based on segmental analysis using ultrasound (US), used to diagnose hypertrabeculation, are poorly correlated and are prone to confounding factors (50). Kohli et al. showed that segmental measurements of T and C thickness using US are too sensitive to partial volume effect and slice selection. Authors concluded that those criteria result in over diagnosis of LVNC. Using MRI, Kawel et al. showed in 323 healthy subjects without cardiac disease or hypertension that more than 40% of subjects presented a trabeculated over compact myocardium thickness ratio higher than 2.3 [16]. However, the thickness ratio was assessed in only one segment in that study. The present study confirmed that LV trabeculations are not homogeneously distributed over LV cavity and a segmental measurement of trabeculation might reflect this variation. André et al. (51) showed similar result using MRI in 117 healthy

subject using only the long axis view. Quantification of the total amount of LV trabeculation appears preferable to assess normal LV trabeculation and already showed ability to discriminate between normal and diseased patients (52). Our results were in line with the current literature in which the T/C ratio varies from 9.7 to 12.0 % (52–54). Papavassiliu et al. found that trabeculae account for 10 % of the left ventricular mass (53), but only 20 healthy volunteers were included using a fast-gradient echo sequence, whereas bSSFP sequences with higher resolution would be beneficial (55). Using a manual segmentation and bSSFP sequences, Jacquier et al. found a T/C ratio of 12 ± 5 % for healthy subjects (52). Grothoff et al. assessed the T/C ratio by manual contouring in 24 healthy volunteers and found a mean value of 9.9 ± 4.4 % (54).

Obtaining normal values and ranges for trabeculated mass and T/C ratio is crucial as trabeculated myocardium is a determinant phenotypic expression in several cardiac diseases. In an early study, Kozor et al. showed that, in Fabry disease and in HCM, both relative papillary muscle mass and T mass values were increased (38). In contrast, these authors also showed that neither in hypertrophy linked to afterload increase (aortic stenosis and hypertension) nor in amyloidosis were there increased trabeculations. Furthermore, Choudhary suggested that a higher T/C ratio was associated with a higher prevalence of electrical disturbances including sustained ventricular tachycardia in patients suffering from LVNC (56). Therefore a precise quantification of LV trabeculation might be clinically relevant to discriminate between normal and excess of trabeculation in specific cardiomyopathies, despite negative findings in the follow-up of healthy subjects (57).

An additional finding of our study is that T mass and T/C ratio decreased with age, with no correlation with either LV volumes, nor EF. The compact myocardium mass remained

stable across age. This might be due to the physiological process of aging. This result was in line with findings from André et al. who also showed a decrease of LV trabeculated mass with age (51). Dawson et al. suggested a decrease in the T/C thickness ratio with age, using the T/C thickness ratio measured in the end systolic phase with MRI (58), and this trend was confirmed in our study. Lastly, no gender differences have been reported in the literature regarding T/C ratio (45,58), which is in accordance with our results. The T/C ratio has been shown to be different between ethnic groups and seems to be higher in Chinese and African-American populations (59), therefore our values may be only applicable in Caucasian populations.

The non-compaction of the myocardium has been studied in a large cohort of patients in the MESA study, but was limited to the measurement of the thickness of the compact and trabeculated LV wall (57) or using the global fractal approach (60). The proposed segmentation tool integrates other crucial functional parameters like EDV and LV wall mass which could help in patients stratification. Indeed, some of these parameters are prone to affect the morphology and amount of LV trabeculations (51,56,59).

Our method has yet to be applied in a larger population, prospectively, using a multicentric design. In our study, only one ethnic group was included.

We have proposed normal values for T, C and T/C mass ratio in a cohort of healthy Caucasian subjects. These normal values of the T/C ratio are 9.1 ± 3.7 % for men and 9.2 ± 5.2 % for women; within each gender, T/C decreases with greater age. While the compact and trabeculated myocardium masses appear to relate to both cardiac function and gender, there was no correlation between these parameters and the T/C ratio.

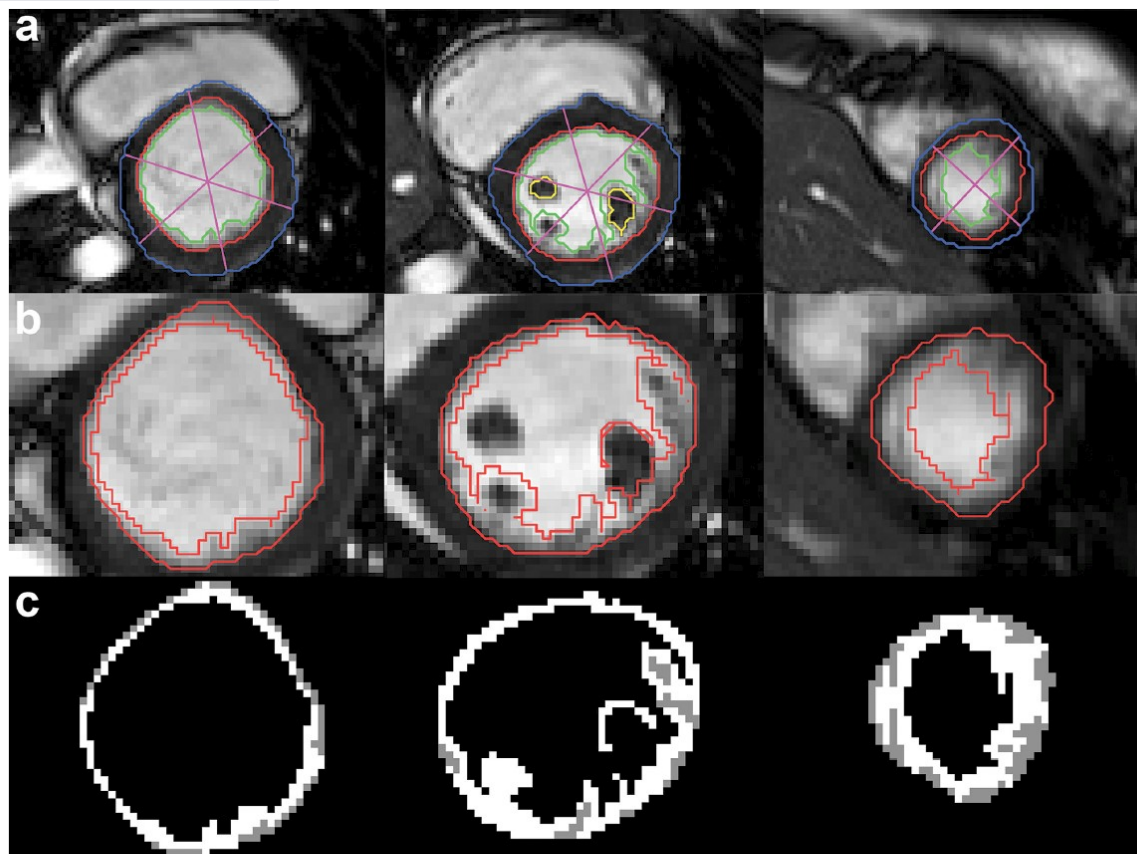


Figure 1. Example of the 16-segments left ventricular segmentation over base (left), median (middle) and apex (right). a: epicardial border in blue (manually drawn), endocardial border in red (manually drawn), trabeculae border in green (automatically segmented), papillary muscles in yellow (included in the compact myocardium mass, semi-automatically segmented) and the 16-segments delineations in magenta; b: to help the operator refine the segmentation of the trabeculae, the original image is displayed with the endocardial and the trabeculae borders (red line); c: a semi-automatic thresholding tool helps suppress blood (white pixels) and isolate trabeculae (gray pixels). (b) and (c) are zoomed for better visibility.

Table 1 Baseline characteristics of 140 healthy volunteers.

	All	Gender groups		P-value	Age groups (years)					P-value
		Male (n = 70)	Female (n = 70)		20–29 (n = 28)	30–39 (n = 28)	40–49 (n = 28)	50–59 (n = 28)	60–69 (n = 28)	
Height, (cm)	170 ± 10 (149–192)	177 ± 7 (163–192)	163 ± 7 (149–176)	<0.0001 ^a	172 ± 9 (155–187)	170 ± 8 (154–183)	169 ± 12 (150–192)	169 ± 10 (155–189)	169 ± 10 (149–176)	NS ^b
Weight, (kg)	70 ± 15 (45–138)	80 ± 14 (50–138)	61 ± 10 (45–94)	<0.0001 ^a	67 ± 12 (51–110)	69 ± 12 (48–94)	71 ± 16 (47–105)	71 ± 16 (45–103)	74 ± 19 (49–138)	NS ^b
BSA, (m ²)	1.80 ± 0.23 (1.40–2.58)	1.96 ± 0.18 (1.57–2.58)	1.65 ± 0.15 (1.40–2.04)	<0.0001 ^a	1.79 ± 0.19 (1.51–2.29)	1.79 ± 0.19 (1.46–2.16)	1.81 ± 0.26 (1.40–2.35)	1.80 ± 0.24 (1.40–2.25)	1.83 ± 0.26 (1.45–2.58)	NS ^b

Note. Data are expressed as mean ± SD. Numbers in brackets are ranges. DCM: dilated cardiomyopathy; BSA: body surface area; SD: standard deviation; NS: non-significant P-value.

^a Mann-Whitney test.

^b One-way ANOVA.

Table 2 Left ventricular parameters and masses in 140 healthy volunteers.

	Male	Female	P-value	All
EF, (%)	62.4 ± 6.1 (60.9–63.9)	64.9 ± 6.2 (63.4–66.4)	0.0178 ^a	63.6 ± 6.3 (62.6–64.7)
EDV, (mL/m ²)	78.8 ± 18.0(74.5–83.1)	72.8 ± 13.6(69.6–76.1)	0.0280 ^b	75.8 ± 16.2(73.1–78.5)
ESV, (mL/m ²)	29.8 ± 8.8 (27.7–31.9)	25.9 ± 7.4 (24.1–27.6)	0.0051 ^b	27.8 ± 8.3 (26.4–29.2)
T/BSA, (g/m ²)	5.4 ± 2.3 (4.9–6.0)	4.0 ± 2.3 (3.8–4.9)	0.0052 ^b	4.9 ± 2.4 (4.5–5.3)
C/BSA, (g/m ²)	60 ± 9.1 (57.8–62.2)	47.7 ± 9.8 (45.4–50.0)	<0.0001 ^a	53.9 ± 11.2(52.0–55.7)
T/C ratio, (%)	9.1 ± 3.7 (8.2–10)	9.2 ± 5.2 (8.0–10.5)	NS ^a	9.2 ± 4.5 (8.4–9.9)

Note. Data are expressed as mean ± SD. Numbers in parentheses are 95% confidence intervals. EF: ejection fraction; EDV: end-diastolic volume; ESV: end-systolic volume; BSA: body surface area; T: trabeculated myocardium mass; C: compacted myocardium mass; SD: standard deviation; NS: non-significant P-value.

^a Mann-Whitney test.

^b t test with Welch's correction.

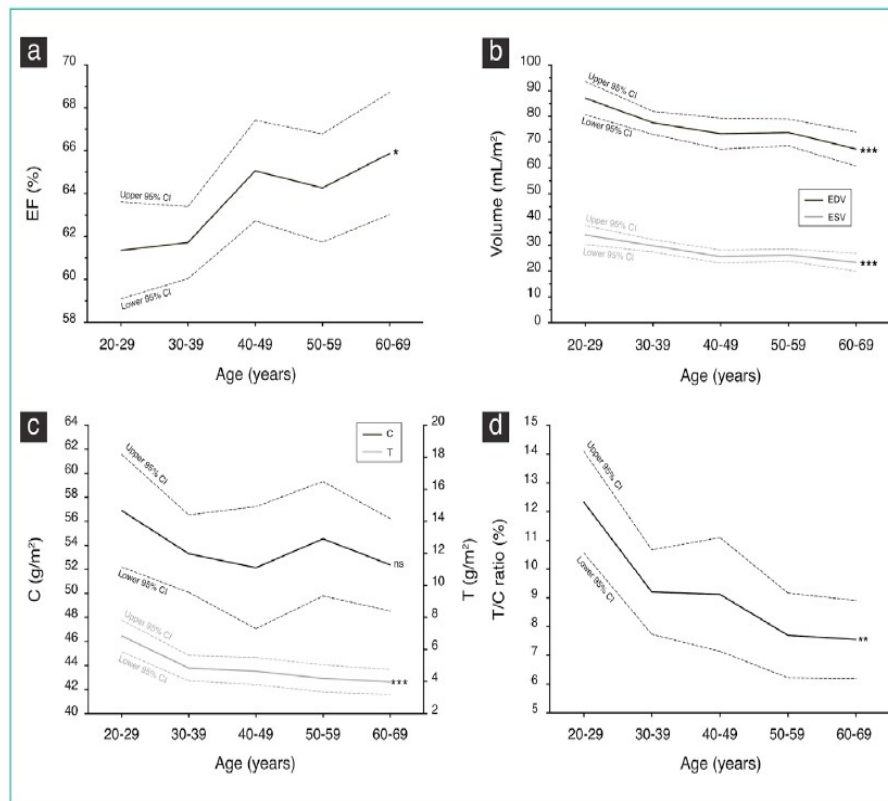


Figure 2. Diagrams show the influence of age on left ventricular parameters and masses. a: distribution of ejection fraction (EF), and; b: end-diastolic volume (EDV) and end-systolic volume (ESV) measurements by age group; c: distribution of left ventricular compact (C) and trabeculated myocardium masses (T), and (d) T/C ratio by age groups. The values are represented as connected means ± SD for age groups 20–29; 30–39; 40–49; 50–59 and 60–69 years. (ns: non-significant P-value; *, $P = 0.0184$; **, $P = 0.0003$; ***, $P < 0.0001$).

Table 3 Values of T/C ratio for men and women by age groups.

	Age groups (years)					<i>P</i> -value ^b
	20–29 (<i>n</i> = 28)	30–39 (<i>n</i> = 28)	40–49 (<i>n</i> = 28)	50–59 (<i>n</i> = 28)	60–69 (<i>n</i> = 28)	
Male	12.4 ± 3.4 (10.5–14.4)	9.3 ± 3.9 (7.1–11.6)	8.3 ± 3.4 (6.3–10.3)	7.7 ± 3.2 (5.9–9.6)	7.7 ± 2.7 (6.2–9.3)	0.0064
Female	12.2 ± 5.6 (9.0–15.5)	9.1 ± 3.8 (6.9–11.3)	9.9 ± 6.4 (6.2–13.6)	7.6 ± 4.4 (5.1–10.2)	7.3 ± 4.2 (4.9–9.8)	NS
<i>P</i> -value ^a	NS	NS	NS	NS	NS	

Note. Data are expressed as mean ± SD. Numbers in parentheses are 95% confidence intervals. T: trabeculated myocardium mass; C: compacted myocardium mass; SD: standard deviation; NS: non-significant *P*-values.

^a Mann-Whitney test.

^b Kruskal-Wallis test.

Table 4 Intra-observer and inter-observer variability.

Measurement	Intra-observer				Inter-observer			
	Op-1 Segmentation 1 (mean ± SD)	Op-1 Segmentation 2 (mean ± SD)	ICC	Bland- Altman bias ^a (mean ± SD)	Op-1 Segmentation (mean ± SD)	Op-2 Segmentation (mean ± SD)	ICC	Bland- Altman bias ^a (mean ± SD)
T, (g)	6.43 ± 4.28	6.59 ± 4.49	0.99	−0.16 ± 0.39	8.13 ± 4.50	8.77 ± 4.01	0.94	−0.64 ± 1.35
C, (g)	82.26 ± 26.02	81.56 ± 25.81	0.98	0.70 ± 2.00	98.06 ± 33.91	101.68 ± 34.00	0.98	−3.61 ± 6.01
T/C ratio, (%)	7.65 ± 4.15	7.87 ± 4.32	0.99	−0.21 ± 0.47	7.49 ± 3.23	8.50 ± 2.44	0.80	−0.58 ± 1.81

Note. Data are expressed as mean ± SD. C: compact myocardium mass; T: trabeculated myocardium mass; SD: standard deviation; ICC: intraclass correlation coefficient.

^a Difference (Op-1 Segmentation 1–Op-1 Segmentation 2) vs. average.

Table 5 Impact of age, gender, ejection fraction and end-diastolic volume on left ventricular masses.

Multivariate ANOVA					
	Whole model <i>P</i> -value	Age <i>P</i> -value	Gender <i>P</i> -value	EF <i>P</i> -value	EDV <i>P</i> -value
C	<0.0001	0.67	<0.0001	0.001	0.0001
T	<0.0001	0.007	0.02	0.91	0.0001
T/C ratio	0.0009	0.008	0.53	0.26	0.27

C: compact myocardium mass; T: trabeculated myocardium mass; EF: ejection fraction; EDV: end-diastolic volume.

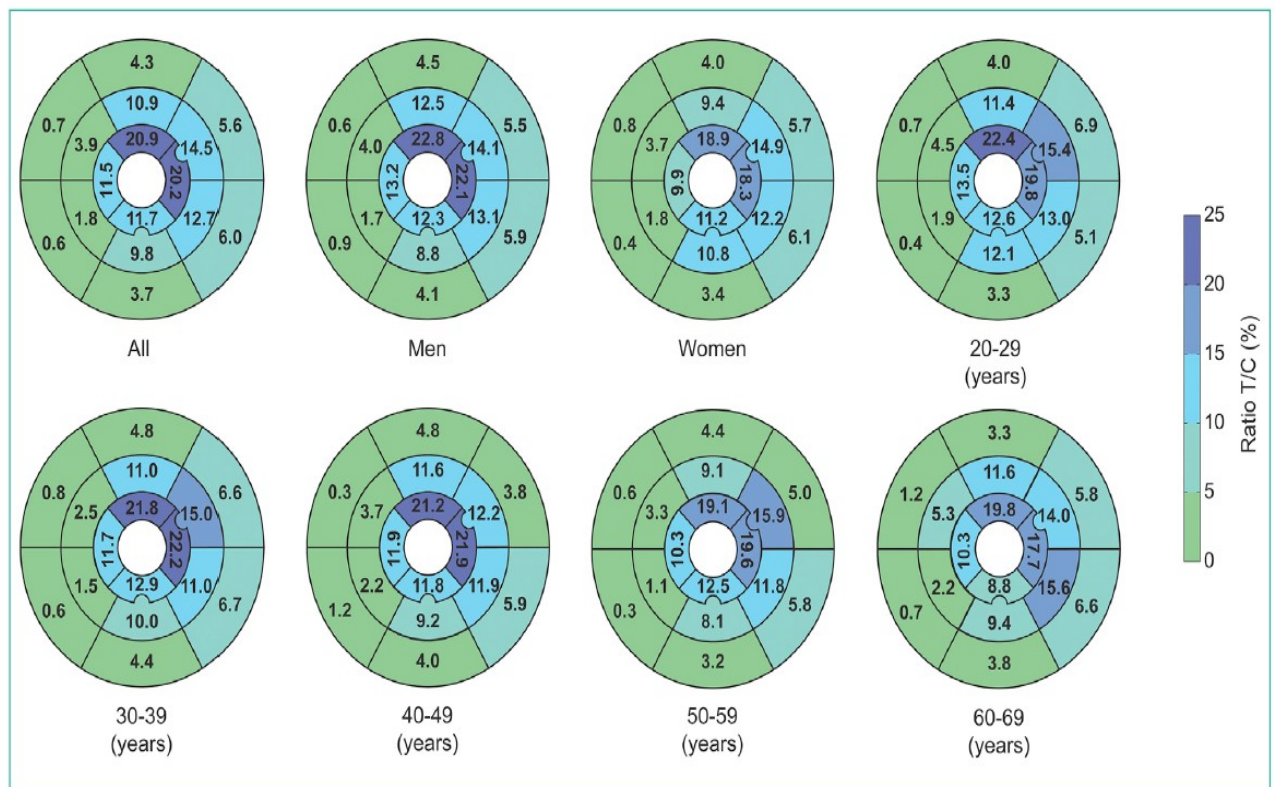


Figure 3. Figures show mean measurement values of the ratio T/C for the 16 LV segments. C, compact myocardium mass; T, trabeculated myocardium mass.

Table and figure legends

Table 1. Baseline characteristics of the healthy volunteers (mean $\pm$ SD & ranges)

Table 2. Left ventricular parameters and masses (mean $\pm$ SD & 95% CI).

Table 3. Values of T/C ratio for men and women by age groups (mean $\pm$ SD & 95% CI).

Table 4. Intra-observer & inter-observer variability.

Table 5. Impact of age, gender, ejection fraction and end-diastolic volume on left ventricular masses.

Figure 1. Example of the 16-segments left ventricular segmentation over base (left), median (middle) and apex (right). (a) Epicardial border in blue (manually drawn), endocardial border in red (manually drawn), trabeculae border in green (automatically segmented), papillary muscles in yellow (included in the compact myocardium mass, semi-automatically segmented) and the 16-segments delineations in magenta. (b) To help the operator refine the segmentation of the trabeculae, the original image is displayed with the endocardial and the trabeculae borders (red line). (c) A semiautomatic thresholding tool helps suppress blood (white pixels) and isolate trabeculae (gray pixels). (b) and (c) are zoomed for better visibility.

Figure 2. Influence of age on left ventricular parameters and masses. (a) Distribution of ejection fraction (EF), and (b) end-diastolic volume (EDV) and end-systolic volume (ESV) measurements by age groups. (c) Distribution of left ventricular compact (C) and trabeculated

myocardium masses (T), and (d) T/C ratio by age groups. The values are represented as connected means $\pm$ SD for age groups 20-29, 30-39, 40-49, 50-59 and 60-69 years old. ns, non-significant p value; *, $P=0.0184$; **, $P=0.0003$; ***, $P<0.0001$.

Figure 3. Mean measurement values of the ratio T/C for the 16 LV segments. C, compact myocardium mass ; T, trabeculated myocardium mass.

Conflict of interest:

The authors of this manuscript declare no relationships with any companies, whose products or services may be related to the subject matter of the article.

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Statistics and biometry:

One of the authors has significant statistical expertise.

Informed consent:

Written informed consent was obtained from all subjects in this study.

Ethical approval:

Institutional Review Board approval was obtained.

Study subjects or cohorts overlap:

Some study subjects or cohorts have been previously reported in Bricq et al. article (Semiautomatic detection of myocardial contours in order to investigate normal values of the left ventricular trabeculated mass using MRI, JMRI 2015)

Trial registration: clinicaltrials.gov NCT01712581. Registered 17 October 2012.



Annexe 5: Thèse de Mathieu Finas sur la trabeculation chez le patient avec une cradiomyopathie hypertrophique.



Quantification of left ventricle trabeculae in Hypertrophied

Cardiomyopathy (HCM), in comparison with healthy subjects

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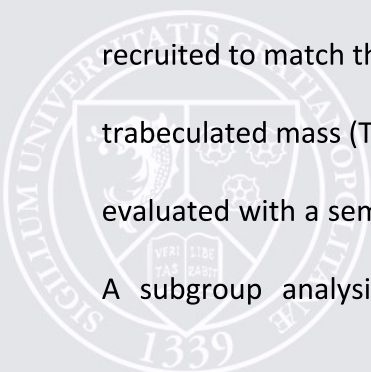
Abstract

Introduction:

Hypertrabeculation is the main symptom of left ventricular non-compaction (LVNC) however the current literature advocate that LV hypertrabeculation might be a phenotype trait overlapping in other cardiomyopathies, such as hypertrophic cardiomyopathy (HCM). We used a semi-automated method to quantify global and segmental trabeculated mass (T) across the LV in a cohort of HCM patients, in comparison with healthy subjects.

Method:

HCM patients were retrospectively recruited from January 2015 to December 2015 in our institution (CHRU La Timone, Marseille). Healthy volunteers were prospectively recruited to match the patients regarding baseline characteristics. For each subject trabeculated mass (T), compacted mass (C) and papillary muscles (PM) masses were evaluated with a semi-automated software. The T/C and PMT/C ratios were then computed. A subgroup analysis was performed comparing Patients with severe LV hypertrophy



(«LVH+», C mass>85g/m²), Patients with moderate hypertrophy («LVH-», C mass<85g/m²) and Controls. We further studied the segmental distribution of T/C across the LV.

Results:

75 subjects were recruited in each group (37 «LVH+» and 38 «LVH-» patients in each subgroup). T/C and PMT/C ratios differed significantly between Patients and Controls (13.4+/-3.1% versus 9.9+/-2.6%, p<0.001 and 17.8+/-3.2%, versus 13.9+/-2.9% p<0.001).

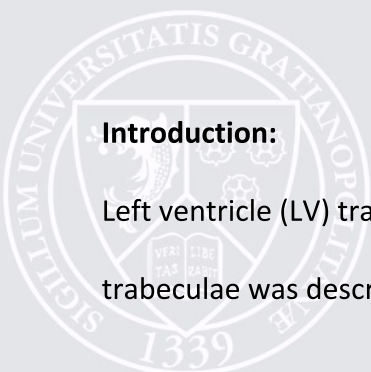
These ratios didn't differ between «LVH+» and «LVH-» Patients, however a significant difference was noticed between both subgroups of Patients and Controls («LVH-»: 13.9+/-3.4% and 18.3+/-3.5%; «LVH+»: 12.9+/-2.8% and 17.3+/-2.9%; Controls: 9.9+/-2.6% and 13.9+/-2.9, p<0.001). Among patients, the most trabeculated segments were 12, 13 and 16 and the most compacted segments were 1, 3 and 9. A positive correlation between T and C in 14 segments. No correlation was found between T/C ratio and LV function parameters.

Conclusion:

T/C and PMT/C ratios are different in Patients comparing to Controls, suggesting that hypertrabeculation might be a phenotypic trait of HCM, evenmore in patients with moderate LV hypertrophy. Global and segmental distribution of T and C are correlated in HCM patients.

Introduction:

Left ventricle (LV) trabeculae are a normal constituent of the endocardial layer. Excess of LV trabeculae was described in several cardiomyopathies like left ventricular non compaction



(LVNC), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM) and HCM [25].

Other conditions like pregnancy or high intensity training can also involve trabeculated phenotypes. However, there is no standardized method to quantify LV trabeculae. Several MRI methods for trabeculated mass quantification have been proposed in the last decade: semi-quantitative 2D analysis (T and C thickness ratio) [4], [6], 3D quantitative analysis (volume and mass quantification) [7]–[9], and indirect quantification, by fractal dimension of the LV endocardial border (FD) [10]. Recently, a new trabeculae quantification tool, using semi-automated segmentation, has been introduced. However, the method was only validated in healthy subjects [24]. This method provides a finer assessment of T mass and subsequent T/C ratio quantification.

LV Hypertrabeculation has been described as a potential phenotypic marker in hypertrophic cardiomyopathy (HCM). In HCM patients, literature suggests a higher mass of both trabeculae and papillary muscles [18] and a higher rate of myocardial crypts [21]. This last phenotypic specificity could help to identify patients carrying a sarcomeric mutation for HCM, without expressing the usual phenotype of this disease [26], as well as the increase of endocardial complexity, assessed by FD, in subjects carrying sarcomeric mutation without myocardial hypertrophy (Captur, Circ Cardiovasc Genet., 2014). Thus, we aimed to study T and C masses and T/C ratio on a cohort of HCM patients in comparison with healthy subjects with a semi-automatic post treatment solution. Furthermore, we aimed at benefiting from the refined analysis to study the segmental distribution of T mass and T/C ratio along the LV cavity.

Material and methods:

Population:

This retrospective study gathered data from 75 Patients suffering from HCM, who received a follow-up at our institution (La Timone, Centre Hospitalier Régional Universitaire, Marseille, France) and underwent a cardiac MRI between January 2015 and December 2015. The study was approved by the local ethical committee in accordance with the guidelines outlined in the Declaration of Helsinki and signed informed consent was obtained for each patient. Inclusion criteria were: patient with hypertrophic cardiomyopathy defined by an end-diastole left ventricle wall thickness > 15mm on at least 1 segment and age >18 yo. Exclusion criteria were: patient suffering from another cardiac disease likely to cause myocardial hypertrophic phenotype (Hypertension, amyloidosis, aortic stenosis, Fabry disease) or pregnancy. A pregnancy test was performed for all female participants of childbearing age. Seventy-five volunteers from the Heatmap cohort, prospectively recruited from September 2012 to June 2014 were included in this cohort as Controls, and paired with patients by age and gender. For volunteers, inclusion criteria were: age over 18 and below 70 year-old, ability to understand and sign the consent form, ability to maintain supine position for 20 minutes, no cardiac symptoms, normal cardiovascular examination results and no known risk factors or history of cardiac disease. Exclusion criteria were: claustrophobia, transfused subjects, haematological disease, martial balance disruption, high blood pressure, contraindication for MRI, pregnancy, diabetes and arrhythmias.

For subgroup analysis, Patients' group was split into two subgroups of 37 Patients with a global hypertrophy ($C_{mass} > 85 \text{ g/m}^2$) and 38 patients with only segmental hypertrophy (normal $C_{mass} < 85 \text{ g/m}^2$). The threshold of 85 g/m^2 was set based on the normal LV wall mass across the literature [27].

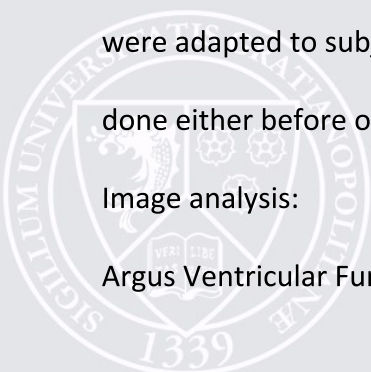
Image acquisition:

Images acquisitions were performed both on a 3T Skyra scanner (Siemens Healthineers, Erlangen, Germany) and on a 1.5 T Avanto scanner (Siemens Healthineers, Erlangen, Germany) using multichannel body array coils combined with a spine array coil with the subjects in supine position. A stack of balanced steady-state free precession (bSSFP) cine sequences were acquired in short axis view, covering the whole LV from base to apex with 7mm slices. Acquisitions were ECG gated and performed during inspiratory breath hold.

Following parameters were used for 1.5T scanner: flip angle: 77° , pixel bandwidth: 930Hz, spatial resolution between $1.37 \times 1.37 \text{ mm}^2$ and $1.48 \times 1.48 \text{ mm}^2$, slice thickness: 6mm, gap between slices: 9mm, repetition time: 3.62ms, echo time: 1.51ms, parallel imaging (GRAPPA). Following parameters were used for 3T scanner: flip angle: 46° , matrix: $120 \times 150 \times 208$, pixel bandwidth: 1095Hz, gap between slices: 7mm, TE/TR: 1.2/2.4 ms, 11 segments per cardiac phase, pixel resolution: $2 \times 1.6 \text{ mm}^2$. The resolution and the flip angle were adapted to subject morphology for everyone. In patients, cine BSSFP sequences were done either before or after Gadolinium chelate injection.

Image analysis:

Argus Ventricular Function software (Siemens Healthineers, Erlangen, Germany) was used to



quantify end diastolic volume (EDV), end systolic volume (ESV), end diastolic LV mass and to compute the ejection fraction (EF) for all subjects. The end-diastolic images were defined as the frame with the largest blood pool volume in the LV cavity. The end-systolic (ES) images were determined as the frame with the smallest blood pool volume in the LV cavity.

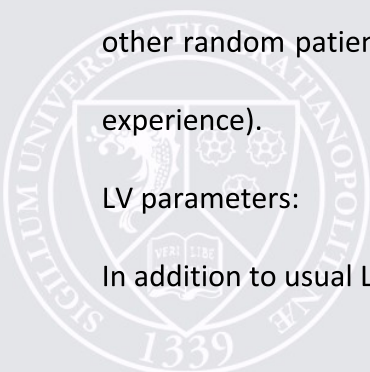
The most basal slice of the LV was set as the one that still showed at least 50% of the LV myocardium circumference at ED. The most basal section could differ between ED and ES.

LV trabeculations, endocardial and epicardial borders were defined using the semi-automatic method proposed by Bricq et al. [24]. Compacted (C) and trabeculated (T) masses were defined as the difference between the epicardium and the trabeculations contour and between the trabeculation contour and the endocardium respectively, as described in [ibid].

Figure 1 is an example of LV processing with our software. C and T masses were indexed to the body surface area (BSA). The T/C ratio was then computed for 16 of the AHA cardiac segments [28] (apical segment excluded from the analysis) and the whole LV cavity. All left ventricular parameters, C and T measurements were done by an operator specialized in cardiovascular MRI (M.F., two-year experience). Image analysis was blinded to the operator. Intraobserver reproducibility was assessed by iterative processing on 10 random patients, 3 months later than the first processing. Interobserver reproducibility was assessed on 10 other random patients whom images were processed by another operator (Z.B., three-year experience).

LV parameters:

In addition to usual LV function parameters, we studied the following parameters for each



subject:

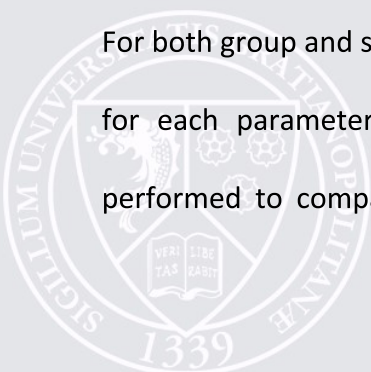
- T and C: trabeculated and compacted masses,
- PM: Papillary muscles mass, PMT: sum of PM and T,
- T/C, PM/C and PMT/C ratios: T, PM and PMT divided by C and expressed as a percentage, giving the relative amount of each of these parameters.

Segmental LV wall thickness at end diastolic phase was also measured following standard recommendation [20] in Patients in order to compare semi-quantitative thickness analysis and quantitative volume assessment regarding the distribution of T and C masses across the LV.

Statistical analysis:

Statistical analysis was performed using GraphPad Prism version 7.0b (GraphPad software Inc., San Diego, CA). Values are reported as mean±standard deviation (SD). The mean values were calculated on Patients and Controls groups. BSA was calculated using Dubois and Dubois formula. Volunteers were selected to match as much as possible to Patients in term of age and gender. Mann-Whitney tests were performed on age, weight, height, EF and EDV. An unpaired t test was performed on BSA to assess these baseline characteristics for both groups.

For both group and subgroups analysis, a d'Agostino-Pearson normality test was performed for each parameter. Subsequently, an Unpaired t test or a Mann-Whitney test were performed to compare T, C, T/C, PM, PM/C, PMT and PMT/C. Finally, ROC curves were



performed to figure out potential diagnosis performances of T/C and PMT/C ratios for group and subgroup analysis.

Results:

Baseline clinical characteristics:

Seventy-five Patients and seventy-five Volunteers were included. Baseline characteristics are listed in Table 1. There was no significant difference between the groups regarding age, gender, height, weight and body surface area (BSA). The ejection fraction (EF) and the enddiastolic volume (EDV) were slightly higher in the Patients group.

Global analysis: T and C masses and T/C ratio

All mass results are given indexed to the BSA and are summarized in Table 2. T and C masses were significantly higher in the Patients group compared to the Volunteers. The T/C ratio was higher for the HCM patients: $13.4 \pm 3.1\%$ versus $9.9 \pm 2.6\%$ for Volunteers ($p < 0.001$).

Papillary muscles mass (PM) was higher in the Patients group compared to Volunteers, but there was no statistical difference between the groups regarding PM reported to C (PM/C). If T mass and PM were summed, there was still an increase in PMT in the Patients group, even reported to C compared to the Volunteers. All these results are showed in Figure 2. In

addition, there was a strong correlation between T and C in the whole cohort of HCM patients (Spearman's coefficient = 0.81, $p < 0.001$).

Twenty-five females and fifty males took part of our Patients cohort. Regarding gender, there was no significant difference for T (12.1 ± 5.8 g/m² for female, versus 11.85 ± 3.5 g/m²

for male, $p=0.63$). However, female had a slightly lower C (84.2 ± 39 g/m² versus 94.3 ± 27.8 g/m² for male, $p=0.025$), a slightly higher T/C ratio ($14.5\pm 3.5\%$ versus $12.8\pm 2.8\%$, $p=0.025$), and a lower EF (54 ± 14.5 versus 66.7 ± 9.1 , $p<0.001$). A weak negative correlation was noted between T/C ratio and age in the Volunteers group. A weak correlation was observed between age and EF in the Patients group, while a weak negative correlation between age and LVEDV and a weak positive correlation between BSA and LV-EDV were noted among the Volunteers. Considering the whole cohort, there was a positive weak correlation between EF and age.

ROC curves, pictured in Figure 5, showed an area under curve (AUC) of 0.81 (IC[0.74;0.87], $p<0.001$) for T/C and 0.82 (IC[0.75;0.88], $p<0.001$) for PMT/C, suggesting that these ratio are discriminant between groups. These curves showed that a $T/C>15.2\%$ had a specificity (Sp) of 100% for HCM, and thus a Predictive Positive Value (PPV) of 100%. 21.3% of patients had a $T/C>15.2\%$.

Subgroup analysis: «LVH-» patients versus «LVH+» patients and Controls

Among Patients, there was thirty-seven patients with severe LV Hypertrophy («LVH+») and thirty-eight patients without total LV mass increase («LVH-»). See Table 2 and Figure 4 for the sub-group analysis results.

«LVH+» versus «LVH-»:

This analysis showed that «LVH+» patients had higher T mass, C mass, PM mass and PMT compared to «LVH-» patients. Nevertheless, neither T/C nor PM/C ratios were different between these subgroups. Neither was the PMT/C ratio.

«LVH-» versus Healthy:

«LVH-» patients had higher T/C and PMT/C ratios compared to healthy controls. ROC curves showed AUC of 0.83 (IC[0.74;0.91], $p<0.001$) for T/C and 0.84 (IC[0.76;0.91], $p<0.001$) for PMT/C. Among «LVH-» subgroup, 11 (29%) patients had a $T/C>15.2\%$, with a 100% Sp.

Segmental analysis: T/C ratio across the LV

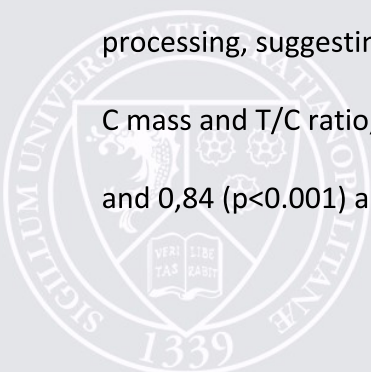
Segmental results for T and C masses, T/C ratio, and segmental correlation between T and C across the LV are pictured in Figure 3. The most hypertrophied segments were the segments 1 ($13.7\pm5.1\text{g}$), 3 ($12.1\pm4.9\text{g}$) and 9 ($12.5\pm5.6\text{g}$): anterior and postero-septal basal and midcavity postero-basal). The most trabeculated segments were segments 12 ($2.0\pm/-1.2\text{g}$), 13 ($2.9\pm1.1\text{g}$) and 16 ($2.5\pm1.1\text{g}$) (postero-lateral midcavity, and anterior and lateral apical).

There was a basal to apical gradient in the T mass distribution. Distribution of T/C ratio across the LV showed a higher ratio in segments 7 ($23.2\pm11.8\%$), 13 ($30.1\pm9.7\%$) and 16 ($27.8\pm9.0\%$) (mid-anterior and anterior and lateral apical segments).

There was a positive correlation between segmental T and C masses in 14 segments among 16. This correlation was strong in segments

Reproducibility:

Intra and interobserver reproducibility showed good concordance between the different processing, suggesting that our method is robust also in pathological conditions. For T mass, C mass and T/C ratio, intra and interobserver reproducibility's were respectively 0,97, 0,99 and 0,84 ($p<0.001$) and 0.92, 0,99 and 0.86 ($p<0.001$), showing good reproducibility despite



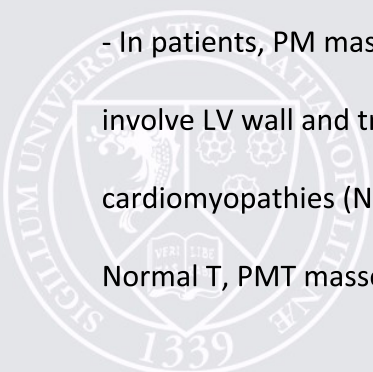
measure error enhancement for T/C ratio, due to the multiplication of the both T and C masses measurement errors.

Discussion:

The main findings of our study are:

- T mass is significantly increased in HCM patients compared to healthy volunteers and T/C and PMT/C ratios are also significantly increased, suggesting that hypertrabeculation could be a phenotypic marker of HCM.
- This finding is heightened when considering patients without global LV mass increase («LVH-», LV mass < 85g/m²). Indeed, we found larger differences in T/C and PM+T/C comparing «LVH-» patients with healthy subjects suggesting that trabeculations could provide a diagnostic help in patients with none or moderate hypertrophy in addition to well-known morphologic criterions of HCM. This could be of interest particularly in patients eligible for proband screening.
- T and C masses were positively correlated in most segments, suggesting that hypertrabeculation is prevailing in diseased segments. This data could be confirmed by studying segmental T analyses coupled with other parameters including late gadolinium enhancement, myocardial strain, EVC or T1 mapping.
- In patients, PM masse is increased, but not PM/C ratio suggesting that hypertrophy only involve LV wall and trabeculation. PM are nevertheless involved in other non-ischemic cardiomyopathies (NICM), like Fabry disease for instance [16].

Normal T, PMT masses and T/C, PM+T/C ratios:



Across the literature, results in quantifying T and C masses and their subsequent T/C ratio, are very heterogenous and depending on the method of segmentation. Main results of previous works are listed in Tables 3 and 4. Big gaps between values are seen throughout them. In one hand, our values are lower than the values provided by Chuang et al. [22] and André et al. [23] for instance. However, with careful attention for their contouring and segmentation methods, their analyses considered both blood inside trabeculae and partial volume effects, whereas our thresholding method is made for managing partial volume effects. For Andre et al. [23] the values of the T/C ratio are very high (above 100% for female), hence their segmentation method is questionable. Our method allows a quick intertrabecular blood removal and decreases the incidence of partial volume artifacts in trabeculation masse assessment. On another hand, our values are higher than these provide by Janik et al.[17] who found that PM and T masses account for only 6.2% of the LV wall mass, what seems really few. Finally, our values are concordant with Papavassiliu et al. [29], with a manual segmentation approach. These data were confirmed by Golfín et al. [30], Grothoff et al. [8] and Kozor et al. [18] suggesting that our method is relevant.

PM+T masses and T/C, PMT/C ratios in HCM patients:

For HCM patients, literature data is also really heterogenous. Firstly, for the PMT/C ratio,

Janik et al.[17] found values depending on the LV remodeling (concentric versus eccentric

hypertrophy) and increased in LVH comparing to healthy subjects. Golfín et al. [30] and

Grothoff et al.[8] found respectively a PMT/C and a T/C ratios decreased for the LVH

patients comparing to controls. Jacquier et al. [7] found a T/C ratio similar for HCM patients

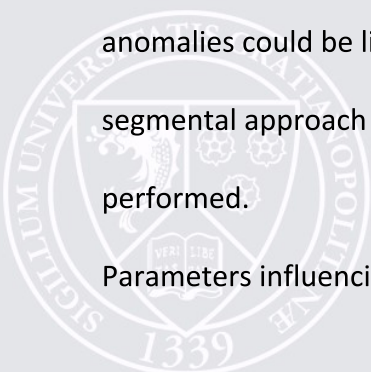
and healthy controls. However, these studies include partial volume effect and analyses were made on small patient's samples. Elsewhere, Captur et al [31] showed that FD increase were independently correlated to LV mass and LV wall thickness in a large cohort of the MESA study, suggesting that patients with LV hypertrophy might have more LV trabeculations. But the FD assessment should be conducted on HCM patients. In the present study we showed that the quantity of LV trabeculation is increased in hypertrophic cardiomyopathy independently to the magnitude of C mass increase. For «LVH-» patients ($C\text{ mass} < 85\text{g/m}^2$), the T/C and PMT/C ratios could provide a diagnostic help, as well as the endocardial border FD assessment (Captur, Circ Cardiovasc Genet., 2014). This technic has thus to be tested on a larger normal LV mass population of HCM patients as well as patient with genotype and no phenotype of HCM to assess the value of this phenotype for proband screening.

Regional analysis:

Local distribution of C mass and LV wall thickness increase is concordant with the literature.

Indeed, the most compacted segments were anterior, antero-septal and postero-septal basal [21]. We found a positive correlation between global and segmental T and C masses, suggesting that NC increase occur in hypertrophied segments (see Figure 3). Wall motion anomalies could be linked to hypertrabeculation as suggested by Chuang et al. [22] and a segmental approach of T/C mass ratio combined to wall motion evaluation has further to be performed.

Parameters influencing T/C and PMT/C ratio:



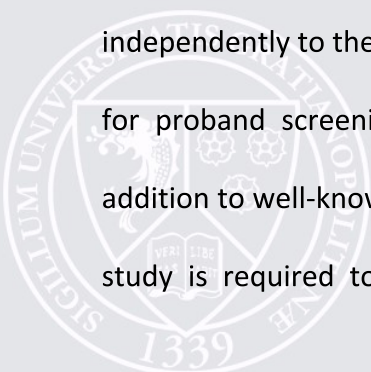
We didn't find any association between neither T/C ratio, nor [PM+T]/C ratio and EF, age, gender, LV EDV nor BSA. Other works suggest that T/C ratio, evaluated directly by mass assessment or even indirectly by Petersen's method [6], and EF should be inversely correlated [9], [23], [32].

Limitations and perspectives:

T/C ratio analyses on large cohort [32], as well as fractal dimension endocardial border assessment [10], [31] showed ethnical discrepancies since Asian and Afro-American people are "more trabeculated". Our study is conducted in a population of Caucasian subjects and results should be applied with care to other populations. [26]. We only included patients with good LV function parameters. Hence, T/C and PMT/C should be also considered in patients with cardiac compensation mechanisms overrun. Finally, our processing, despite being semiautomated, stays time consuming with an average of 10 minutes spent per patient. Finally, our retrospective designed study is subject to bias and our findings should be confirmed with a prospective approach.

Conclusion:

Results of this study suggest that hypertrabeculation is a phenotypic trait in HCM patient independently to the magnitude of the LV compacted mass increase. T/C and PMT/C ratios for proband screening should thus be relevant and could provide a diagnostic help in addition to well-known imaging diagnosis criterions of this prevalent cardiac disease. Further study is required to assess the value of trabeculation quantification in a population of



patients with genotype and no classical phenotype of HCM to assess the value of the described method for proband screening.

TABLES AND FIGURES

Figure 1. Example of processing of the LV cavity

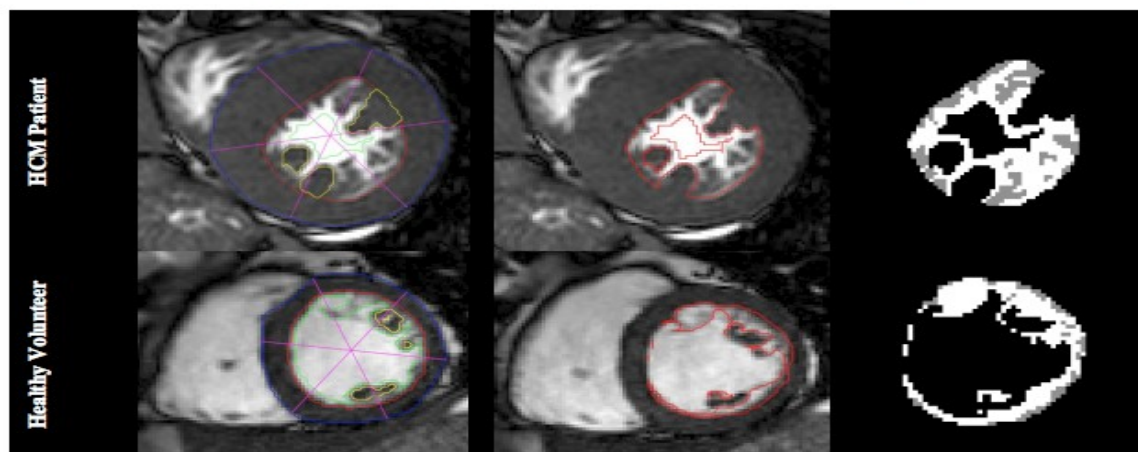


Figure 1A. Left column: Contouring of epicardium (blue line), endocardium (red line), trabeculated area (green line) and papillary muscles (yellow line); Middle column: extraction of trabeculated area before semi-automated thresholding; Right column: trabeculation thresholding, removing the blood and partial volume effects.

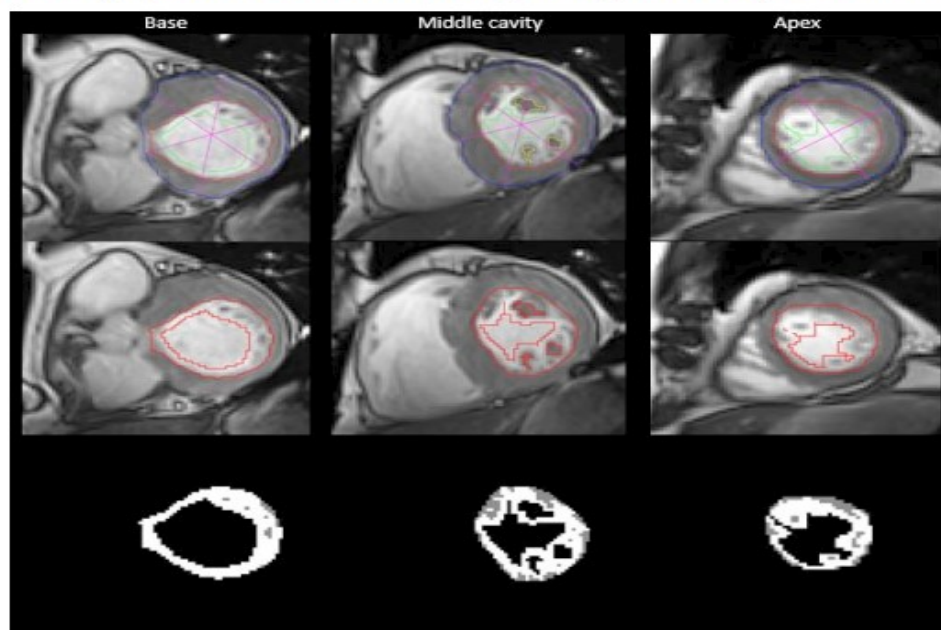


Figure 1B. Example of contouring and trabeculation thresholding at three levels in the LV cavity.

Figure 2. Results of the group analysis

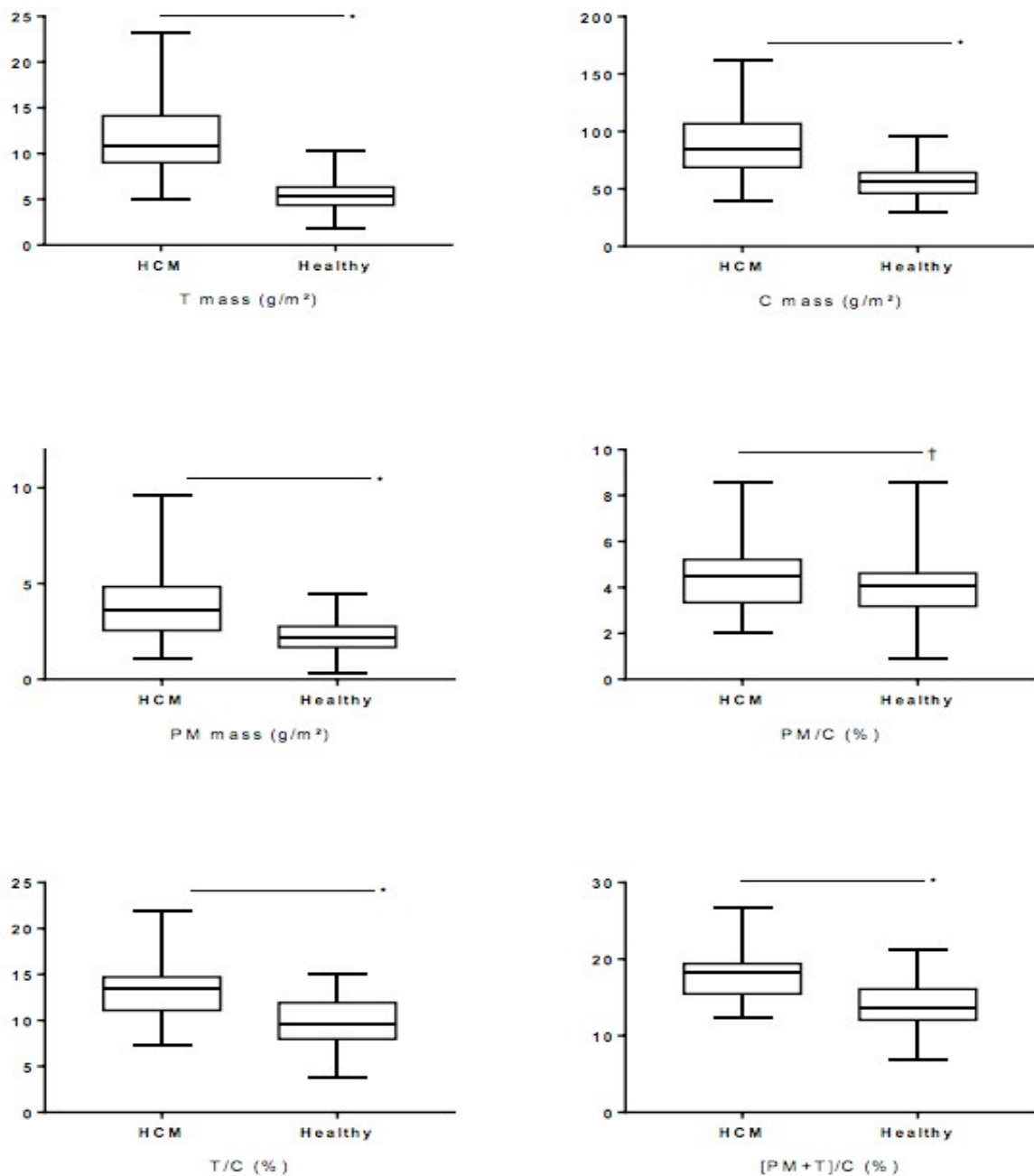
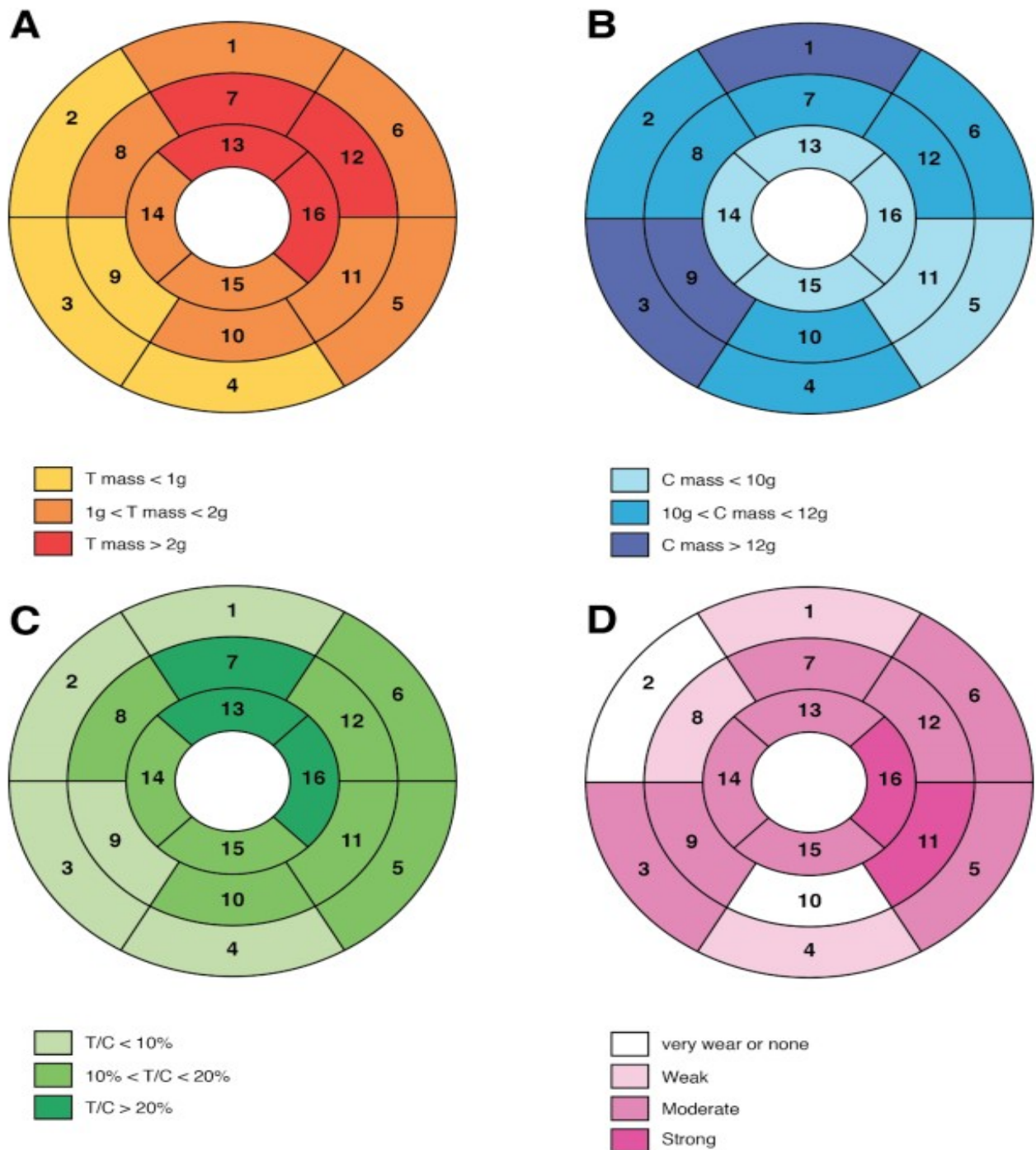


Figure 2. Showing parameters of interest of the group analysis; *: p<0.001, †: p=ns; HCM: hypertrophied cardiomyopathy group; Healthy: Healthy volunteers group

Figure 3. Segmental distribution of T, C, T/C ratio, and correlation between T and C



Local distribution of 4 parameters across the LV in HCM patients, through 16 segments of the AHA 17 segments model. A: T mass; B: C mass; C: T/C ratio; D: correlation between NC and C in each segment. Apical segment (17) was not taken into consideration since its analyze is complex in short axe stacks.

Figure 4. Results of the sub-group analysis

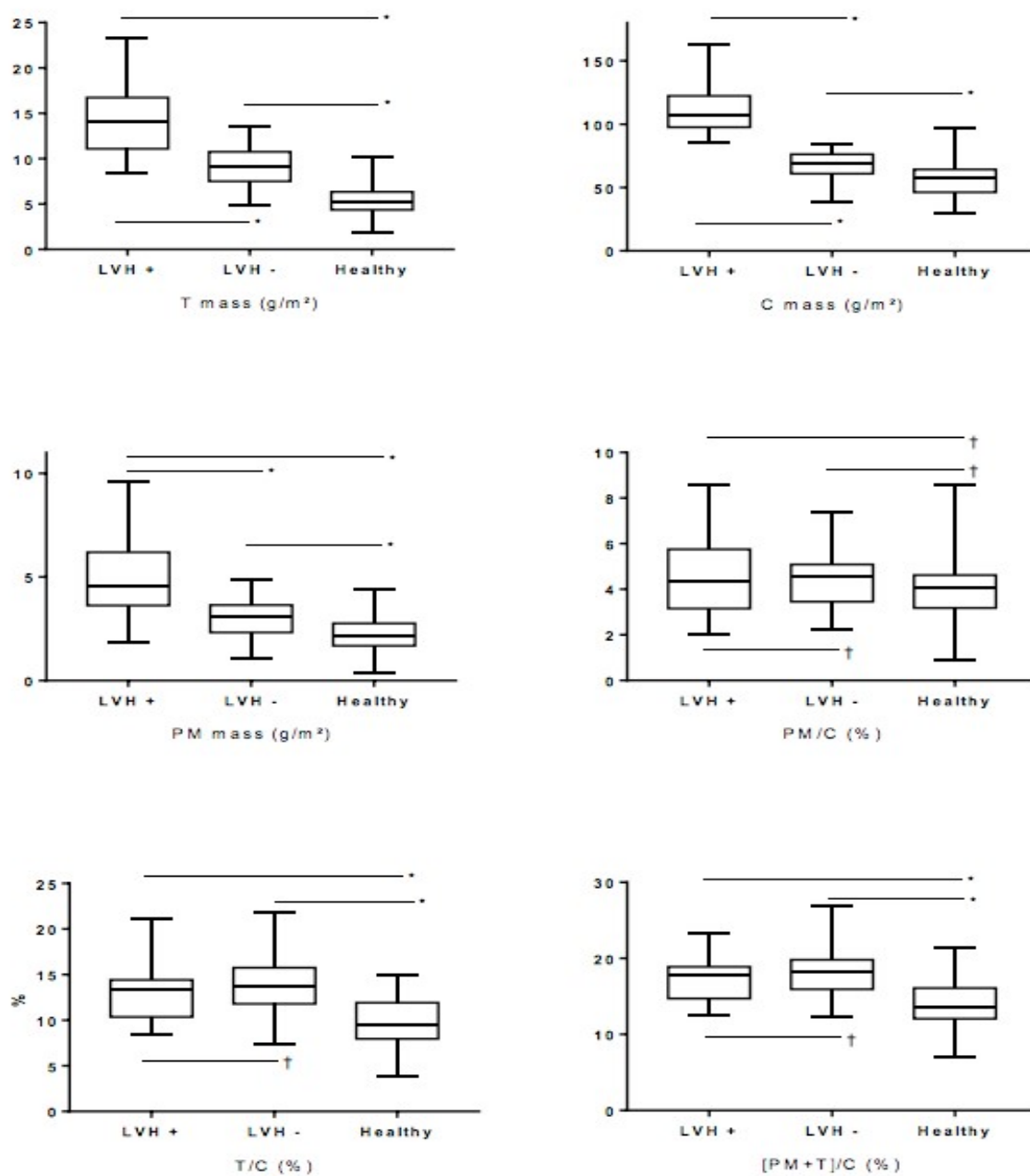


Figure 4. Showing parameters of interest of the subgroup analysis; *: $p < 0.001$; †: $p = ns$; LVH+: patients with $C > 85g/m^2$; LVH-: patients with $C < 85g/m^2$; Healthy: healthy volunteers.

Figure 5. ROC curves for T/C and [PM+T/C] ratios

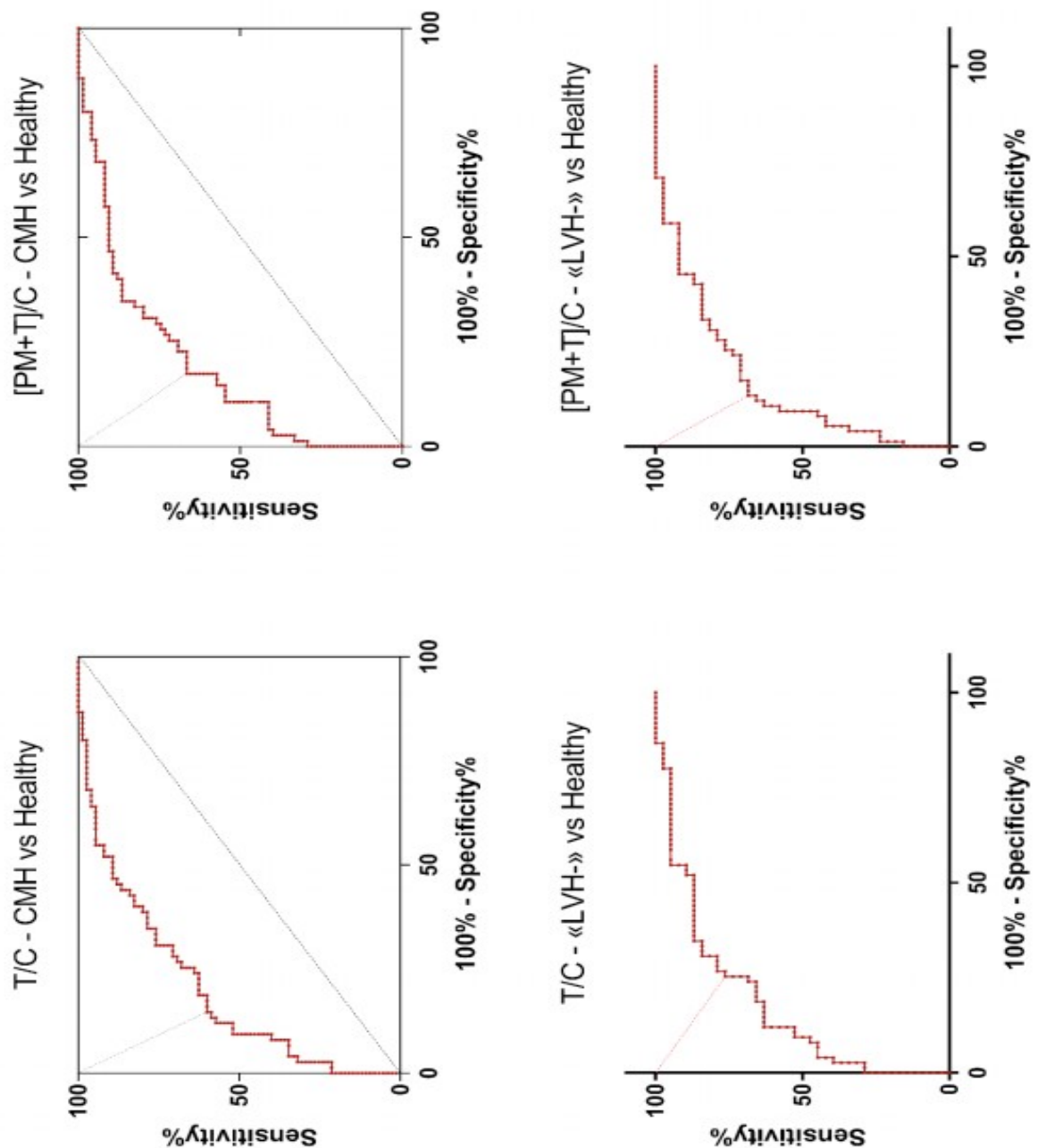


Figure 5. ROC curves for Group and Subgroup analysis, showing diagnostic performances of T/C and [PM+T]/C ratios for group analysis (HCM patients vs Healthy volunteers) and two of the three subgroups («LVH-» patients [patients without global hypertrophy, ie $C < 85 \text{ g/m}^2$] vs Healthy volunteers).

Table 1. Baseline characteristics

Data/Group	HCM	Healthy Volunteers	p-value
Age	51.6+/-14.5	50.6+/-13.3	ns
Gender (f/m)	25/50	25/50	ns
Height (cm)	171.7+/-7.9	172.3+/-10.1	ns
Weight (Kg)	78.4+/-15.0	74.8+/-11.8	ns
BSA (m ²)	1.91+/-0.19	1.88+/-0.20	ns
EF (%)	66.1+/-9.6	62.9+/-5.6	p=0.002
LVEDV (mL/m ²)	82.5+/-20.2	74.9+/-15.9	p=0.028

Results given as mean+/-SD, HCM: hypertrophic cardiomyopathy, BSA: body surface area, EF: ejection fraction, LVEDV: Left ventricle end-diastolic volume

Table 2. Main results of group and sub-group analysis

Parameters	HCM patients	Healthy volunteers	LVH+	LVH-
T mass indexed (g/m ²)	11.7 +/-3.9	5.5+/-1.7	14.2+/-3.6	9.2 +/-2.3
C mass, indexed (g/m ²)	88.8 +/-26.7	56.1 +/-11.9	110.5+/-19.3	67.6 +/-11.3
T/C ratio (%)	13.4 +/-3.1	9.9 +/-2.6	12.9+/-2.8	13.9 +/-3.4
PM mass, indexed (g/m ²)	3.9 +/-1.8	2.2+/-0.8	4.9+/-1.9	3.0 +/-1.0
PM/C ratio	4.4 +/-1.5	4 +/-1.3	4.4+/-1.6	4.5 +/-1.3
T+PM mass (g)	15.6 +/-4.9	7.7 +/-2.1	19.1+/-4.2	12.3 +/-2.5
[T+PM]/C ratio (%)	17.8 +/-3.2	13.9 +/-2.9	17.3+/-2.9	18.3 +/-3.5
Mean FE (%)	66.1+/-9.6	62.8+/-5.6	64.6+/-10.2	67.6+/-8.8

Table 3. Results of the literature
Table 3A. Healthy subjects

Author	T mass (g/m ²)	T/C (%)	T+PM mass (g/m ²)	[T+PM]/C (%)
Papavassiliu [29]	8.1*	10		
Jacquier [7]	9+/-4 g/m ²	12+/-5 %		
Grothoff [8]	5.3+/-2.4 g/m ²	9.9+/-4.4		
Bricq [24]	Male: 6.4+/-2.1 g/m ² Female: 6.2+/-2.0 g/m ²	Male: 9.7+/-3.1 Female: 10.8+/-3.4		
Janik [17]			3.1+/-0.7	6.2
Golfin [30]			23.4+/-9.8	17.2+/-4.9
Chuang [22]			20**	30
Kozor [16]			8	13+/-2
Andre [23]			Male: 43.1+/-8.7 Female: 38.1+/-5.9	Male: 88.0+/-17.7 Female: 105.5+/-16.3

*Result given in term of volume in the original article. Indexed mass obtained by formula: Volume (mL) times Myocardial density (1.05g/m³) times mean Body Surface Area in a Caucasian population (BSA, 1.73m²); ** Results given in term mass in the original article. Indexed mass obtained by multiplying mass by mean BSA in a Caucasian population (1.73m²).

Table 3B. HCM patients

Author	T mass (g/m ²)	T/C (%)	T+PM mass (g/m ²)	[T+PM]/C (%)
Jacquier [7]	14+/-5	12+/-4		
Grothoff [8]	7.0+/-3.1	8.4+/-4.2		
Janik [17]		Con. Hypertr. Ecc. Hypertr.	12 20.9	10.8 18.6
Han [12]			17	17
Golfin [30]			24.3+/-15.1	13.2+/-8.8
Park [13]			20.2+/-5.8	20