123I-mIBG assessed cardiac sympathetic activity: standardizing towards clinical implementation
Verschure, D.O.

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123I-mlBG assessed cardiac sympathetic activity: standardizing towards clinical implementation

Derk Otto Verschure

Uitnodiging
Voor het bijwonen van de openbare verdediging van het proefschrift

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door Derk Otto Verschure

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standardizing towards clinical implementation

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standardizing towards clinical implementation

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$^{123}$I-mIBG assessed cardiac sympathetic activity: standardizing towards clinical implementation

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Faculteit:  Geneeskunde

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Ter herinnering aan mama
Voor Saar, Olivier en Stijn
LIST OF ABBREVIATIONS

123I-mIBG  123I-meta-iodobenzylguanidine
22q11.2 DS  22q11.2 deletion syndrome
ACE-I  angiotensin converting enzyme inhibitor
AMPT  alpha-methyl-para-tyrosine
AR  adrenergic receptor
ARB  angiotensin II receptor blocker
CCB  calcium channel blocker
CHD  congenital heart disease
CHF  chronic heart failure
COMT  catechol-O-methyl-transferase
DA  dopamine
CRP  C-reactive protein
e-CC  estimated creatinine clearance
e-GFR  estimated glomerular filtration rate
HF  heart failure
HR  hazard ratio
H/M ratio  heart-to-mediastinum ratio
LVEF  left ventricle ejection fraction
MDRD  modification of diet in renal disease
NE  norepinephrine
NET  norepinephrine transporter
NT-proBNP  N-terminal pro B-type Natriuretic Peptide
p.i.  post injection
ROI  region of interest
SPECT  single photon emission computed tomography
TCM  tako-tsubo cardiomyopathy
WO  washout
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General introduction and outline thesis
CHRONIC HEART FAILURE

Heart failure (HF) is a life-threatening disease affecting approximately 26 million people worldwide. The incidence of HF in the Netherlands ranges between 28,000 and 44,000 cases per year and increases with age; the majority of HF patients are older than 75 years. Currently, there are between 100,000 and 150,000 patients with HF in the Netherlands. It is the only cardiovascular disease with both growing incidence and prevalence. Reasons for this trend are related to increased life expectancy, improvement of survival after myocardial infarction and better treatment options for HF (Figure 1). It is expected that the total number of HF patients in the Netherlands will increase to 275,000 in 2040. As a consequence, the costs related to HF care will increase: in 2007 these costs were 455 million euro which rose to 940 million in 2011. For 2025, these costs are estimated at 10 billion euros.

Despite the successful introduction of treatment with a combination of beta-blockers and angiotensin-converting-enzyme inhibitors or angiotensin receptor blockers together with loop diuretics, the prognosis of chronic HF (CHF) remains unfavourable. The most recent European data (ESC-HF pilot study) demonstrate that 12-month all-cause mortality rates for hospitalised and stable/ambulatory HF patients were 17% and 7%, respectively. The majority of these deaths are caused by progression of HF, lethal arrhythmia and sudden cardiac death. The use of implantable devices such as implantable cardioverter defibrillators (ICD) and cardiac resynchronisation therapy (CRT) has improved the overall survival of CHF patients. Current European guidelines recommend ICD for primary prevention of fatal arrhythmias in CHF subjects with an ejection fraction <35% and symptomatic HF NYHA class ≥ 2 under optimal pharmacological therapy. In addition, CRT is recommended in CHF patients who remain symptomatic in NYHA class ≥ 2 under optimal pharmacological therapy, with a left ventricular ejection fraction (LVEF) < 35% and wide QRS complex (≥ 130 ms).

ICDs applied for primary or secondary (i.e. already proven ventricular arrhythmias) prevention reduce the relative risk of death by 20%. However, the MADIT II (Second Multicenter Automated Defibrillator Implantation Trial) has shown that the absolute reduction of fatal events was only 5.6% (mortality was 19.8% in the control group and 14.2% in the ICD group during a mean follow-up of 20 months). In addition, the SCD-HeFT (Sudden Cardiac Death in Heart Failure Trial) study showed that the annual number of ICD shocks was 7.1% of which 5.1% were appropriate in the first year rising to 21% in the 5th year post-implantation. However, three years after ICD implantation for primary prevention, a remarkably high percentage of 65% had never received appropriate ICD therapy. Moreover, there is also a risk of malfunction and operative complications, e.g. inappropriate shocks, infection.
Last but not least is the relative high cost of these devices. Therefore, it is essential, not only from a clinical but also from a socioeconomic point of view, to optimise the current selection criteria for CRT and ICD for primary prevention aimed at better identification of patients who will benefit from implantation.

Currently one of the selection criteria for CRT and ICD implantation for primary prevention is an LVEF < 35%. However LVEF assessed by cardiovascular magnetic resonance imaging (CMR) is significantly lower compared with echocardiography. Therefore CMR would significantly increase the number of CHF patients eligible for CRT or ICD implantation. This illustrates that the method to assess LVEF has substantial impact on the selection of ‘appropriate’ patients for CRT and ICD implantation. The lack of uniformity among imaging modalities to assess LVEF raises the question if other parameters may be useful to better identify those patients who will benefit from CRT or ICD implantation. One of those alternative parameters might be cardiac sympathetic hyperactivity, which is related to poor prognosis and fatal arrhythmias in CHF.

**Figure 1.** Number of deaths as a result of acute myocardial infarction and heart failure in the Netherlands from 1980 to 2010. The decrease in the number of deaths after myocardial infarction declines more rapidly than the increase in number of deaths due to heart failure. Source: Centraal Bureau voor de Statistiek (CBS), the Netherlands
Chapter 1

Figure 2. Schematic representation of the sympathetic synapse. Norepinephrine is synthesised within neurons by an enzymatic cascade. Dihydroxyphenylalanine (DOPA) is generated from tyrosine and subsequently converted to dopamine by DOPA decarboxylase. Dopamine is transported into storage vesicles by the energy-requiring vesicular monoamine transporter (VMAT). Norepinephrine is synthesised by dopamine β-hydroxylase within these vesicles. Neuronal stimulation leads to norepinephrine release through fusion of vesicles with the neuronal membrane (exocytosis). Apart from neuronal stimulation, release is also regulated by a number of presynaptic receptor systems, including α2-adrenergic receptors, which provide negative feedback for exocytosis. Most norepinephrine undergoes reuptake into nerve terminals by the presynaptic norepinephrine transporter (NET) and is re-stored in vesicles (following uptake by vesicular amine transporter 2 (VMAT2)) or is metabolised in cytosol dihydroxyphenylglycol (DHPG) by monoamine oxidase (MAO).
CARDIAC SYMPATHETIC ACTIVITY

Norepinephrine is the neurotransmitter of the cardiac sympathetic system and is stored in vesicles in the presynaptic nerve terminals (Figure 2). On the basis of tissue norepinephrine content, the heart is characterised by dense sympathetic innervation with a gradient from atria to base of the heart and from base to apex of the ventricles. Via exocytosis, norepinephrine is released into the synaptic cleft. Only a small amount of the released norepinephrine in the synaptic cleft is available to stimulate the postsynaptic β-adrenergic receptors (β-AR) on the myocytes. Most of the norepinephrine undergoes reuptake into the nerve terminals via the uptake-1 mechanism, member of the solute carrier family of transporter SLC6A2. This transport system, i.e. norepinephrine transporter (NET), is sodium and chloride dependent and responsible for approximately 70–90% of the norepinephrine re-uptake from the myocardial synaptic cleft.

The cardiac sympathetic system is one of the neurohormonal compensation mechanisms that plays an important role in the pathogenesis of CHF with impaired LVEF. Patients with CHF have increased cardiac sympathetic activity with increased exocytosis of norepinephrine from the presynaptic vesicles. In addition, the norepinephrine re-uptake via uptake-1 (NET) in the sympathetic terminal nerve axons is decreased resulting in elevated synaptic levels of norepinephrine. Eventually this results in increased plasma and urinary levels of norepinephrine concomitant with the severity of left ventricular dysfunction. Initially, β-AR stimulation by increased norepinephrine levels helps to compensate for impaired myocardial function, but long-term norepinephrine excess has detrimental effects on myocardial structure and gives rise to a downregulation and decrease in the sensitivity of post-synaptic β-AR. This downregulation leads to left ventricular remodelling and is associated with increased mortality and morbidity. Increased norepinephrine plasma levels are associated with poor prognosis in CHF. However, these levels do not specifically reflect the sympathetic activity at a cardiac level. In addition, these measurements are time consuming and there is a high variability in measurements. However, cardiac sympathetic activity can be non-invasively visualised by nuclear techniques. To date, most commonly used tracers are norepinephrine analogues (123I-mIBG) for single photon emission tomography (SPECT) and ¹¹C-hydroxyephedrine for positron emission tomography (PET). Both radiotracers are resistant to metabolic enzymes and show high affinity for presynaptic norepinephrine uptake-1 (NET) allowing the visualisation of presynaptic sympathetic nerve function. Other presynaptic PET tracers include ¹³C-epinephrine, ¹³C-phenylephrine, and ¹⁸F-LMI1195. ¹¹C-CGP12177 is the most commonly used tracer for postsynaptic β-ARs. However, unlike 123I-mIBG, which can be centrally manufactured and then distributed, most PET agents are labelled with short half-life isotopes and are therefore only available in institutions with an on-site cyclotron. Although the early development of an ¹⁸F-labelled compound for sympathetic PET imaging is continuing, for the foreseeable future ¹²³I-mIBG scintigraphy will remain the only widely available method.
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Figure 3. Example of placing a region-of-interest (ROI) over the heart (H) and fixed rectangular mediastinal ROI placed on the upper part of the mediastinum (M) for calculating H/M ratio.

Figure 4. Example of late $^{123}$I-mIBG SPECT imaging. On the left the conventional short, vertical and horizontal axis, in the middle the corresponding 17-segment model polar map and on the right a 3D reconstruction. There is impaired regional $^{123}$I-mIBG uptake in the inferior wall from the myocardial base until the apex with extension to both inferoseptal and inferolateral regions.
available nuclear imaging method for assessing global and regional myocardial sympathetic innervation. In addition, myocardial $^{123}$I-$m$IBG scintigraphy is easily implemented in any department of nuclear medicine and thereby readily available for CHF patients.

$^{123}$I-$m$IBG SCINTIGRAPHY

$^{123}$I-$m$IBG is a norepinephrine analogue that shares the same presynaptic uptake, storage and release mechanism as norepinephrine. Because $^{123}$I-$m$IBG is not metabolised, its accumulation over several hours is a measure of neuronal sympathetic integrity of the myocardium. Since the introduction of cardiac $^{123}$I-$m$IBG scintigraphy, parameters of $^{123}$I-$m$IBG myocardial uptake and washout have been shown to be of clinical value in many cardiac diseases, especially for the assessment of prognosis.24-27

$^{123}$I-$m$IBG scintigraphy planar acquisition and analysis

To block uptake of free $^{123}$I by the thyroid gland, subjects are pretreated with 250 mg of oral potassium iodide 30 min before intravenous injection of 185 MBq $^{123}$I-$m$IBG. Fifteen minutes (early acquisition) and 4 hours (late acquisition) after administration of $^{123}$I-$m$IBG, 10-min planar images are acquired with the subjects in a supine position using a gamma camera equipped with a low energy high resolution or medium collimator. Based on the obtained planar (2D) images, three major outcomes of cardiac $^{123}$I-$m$IBG uptake can be determined: the early and late heart/mediastinal (H/M) ratio and cardiac washout rate (WO). The H/M ratio is calculated from planar $^{123}$I-$m$IBG images using a regions-of-interest (ROI) over the heart (Figure 3). Standardised background correction is derived from a fixed rectangular mediastinal ROI placed on the upper part of the mediastinum.28 The location of the mediastinal ROI is determined in relation to the lung apex, the lower boundary of the upper mediastinum, and the midline between the lungs. The H/M ratio is calculated by dividing the mean count density in the cardiac ROI by the mean count density in the mediastinal ROI.28 The $^{123}$I-$m$IBG WO can be calculated using early and late H/M ratio (A). There are variations to the WO calculation using the myocardial count densities only, requiring a time-decay correction (factor of 1.21), without (B) or with background correction (C):

\[
\text{(A) WO} = \left\{ \frac{\text{(early H/M ratio – late H/M ratio)}}{\text{early H/M ratio}} \right\} \times 100
\]

\[
\text{(B) WO} = \left\{ \frac{\text{(early H) – (late H \times 1.21)}}{\text{early H}} \right\} \times 100
\]

\[
\text{(C) WO} = \left\{ \frac{\text{(early H – early M) – (late H – late M)\times 1.21}}{\text{early H – early M}} \right\} \times 100
\]
Chapter 1

The early H/M ratio predominantly reflects the integrity of sympathetic nerve terminals (i.e. number of functioning nerve terminals and intact uptake-1 mechanism). The late H/M ratio particularly offers information about neuronal function resulting from uptake, storage and release. The $^{123}$I-mIBG WO reflects predominantly neuronal integrity of sympathetic tone/adrenergic drive.  

$^{123}$I-mIBG scintigraphy SPECT acquisition and analysis

Further, compared with the H/M ratio derived from two-dimensional planar images, the results of three-dimensional imaging using SPECT provide a more complete understanding of global dysinnervation.  

Preclinical and animal studies suggested that myocardial regions with damaged or dysfunctional neurons but preserved perfusion can be a source of arrhythmias. Therefore, volumetric data such as SPECT may be of added value. The specific SPECT acquisition parameters have been described elsewhere but are largely comparable with those used for myocardial perfusion SPECT imaging. Images can be processed and prepared for display and interpretation using the available commercial software packages (e.g. Emory Cardiac Toolbox and Cedar-Sinai Quantitative Perfusion SPECT). While there is no officially established method for scoring $^{123}$I-mIBG SPECT images, analysis can be performed similar to the conventional 17-segment/5-point model used for SPECT myocardial perfusion imaging (MPI) (Figure 4). Therefore the $^{123}$I-mIBG SPECT images can easily be compared with MPI SPECT images in order to investigate the difference between regional innervation and possible myocardial perfusion abnormalities.

$^{123}$I-mIBG scintigraphy and prognosis in CHF

Cardiac sympathetic hyperactivity is reflected by a decreased $^{123}$I-mIBG late H/M ratio and increased WO. Both are associated with increased fatal arrhythmia and cardiac mortality. Initially $^{123}$I-mIBG scintigraphy assessed cardiac sympathetic activity in CHF has extensively been studied in small, single centre studies. However, the ADMIRE-HF study (ADreView Myocardial Imaging for Risk Evaluation in Heart Failure), a large multicentre, prospective study, reported that decreased late H/M ratio was associated with the composite endpoint of HF progression, ventricular tachyarrhythmia and death.

AIM OF THIS THESIS

In this thesis several aspects of cardiac $^{123}$I-mIBG imaging and the prognostic value in CHF are discussed. Although a large number of studies on $^{123}$I-mIBG assessed cardiac sympathetic activity has published, methodological and analytical limitations have hampered wide scale clinical implementations of cardiac $^{123}$I-mIBG scintigraphy. Essential for large scale implementation of cardiac $^{123}$I-mIBG imaging is adequate
reproducibility, standardization and validation. The lack of standardisation of acquisition and post-acquisitions analysis have hampered comparison between different institutions. Moreover, most of these data are acquired from single centre experience and do not necessarily allow extrapolation of the obtained results to other institutions. **Part I** of this thesis focusses on the standardization and validation of planar cardiac $^{123}$I-mIBG scintigraphy and describes several factors that could influence the $^{123}$I-mIBG derived parameters. In **part II** the prognostic value of cardiac $^{123}$I-mIBG imaging in patients with CHF is studied. Finally in **part III** the use of cardiac $^{123}$I-mIBG scintigraphy in populations other than CHF is discussed.

**OUTLINE OF THIS THESIS**

In **part I** of this thesis several aspects are studied related to standardization of image acquisition. High-energy photon emission of $^{123}$I leads to penetration of collimator septa and subsequently affects the accuracy of the H/M ratio. It is therefore apparent that differences in collimator, essential in nuclear medicine techniques, influence $^{123}$I-mIBG myocardial derived parameters. To correct for these differences in collimators a European cross-calibration study was performed. This cross-calibration enables a better comparison between institutions which is important for identifying appropriate thresholds for differentiating high and low risk heart failure patients. Standardizing the post-acquisition processing of planar cardiac $^{123}$I-mIBG scintigraphy is also essential. Therefore the impact of differences in region of interest (ROI) placement (e.g. a fixed mediastinal ROI) on the accuracy were studied. In addition different patient factors such as polymorphism of the SLC6A2 gene encoding for the NE re-uptake, renal function and the relationship between changes in heart (H) and mediastinal (M) counts and the change in vascular $^{123}$I-mIBG activity were studied.

**Part II** evaluates the prognostic value of cardiac $^{123}$I-mIBG scintigraphy in CHF. First, a meta-analysis using individual patient data from 6 different published studies looked at the prognostic value of cardiac $^{123}$I-mIBG scintigraphy. Furthermore the relationship between cardiac sympathetic activity and inflammation in stable CHF and their prognostic value was evaluated. Finally, an European multicentre study was performed to study cardiac $^{123}$I-mIBG scintigraphy in stable CHF patients eligible for ICD implantation for primary prevention with the goal to optimize the current selection criteria for this specific indication.

**Part III** describes $^{123}$I-mIBG scintigraphy assessed cardiac sympathetic activity in patients with 22q11.2 deletion syndrome which affects the degradation of NE, the neurotransmitter of the cardiac sympathetic system. Furthermore we discussed the possible pathophysiology and the potential role of cardiac $^{123}$I-mIBG scintigraphy in Tako-tsubo cardiomyopathy.
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2010;55:2769-77
General introduction and outline thesis


PART I

Standardization and validation of cardiac $^{123}$I-mIBG scintigraphy
Chapter 2

$^{123}$I-$m$IBG heart-to-mediastinum ratio is influenced by high-energy photon penetration of collimator septa from liver and lung activity.

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Chapter 2

ABSTRACT

Aim
The 123I-meta-iodobenzylguanidine (123I-mIBG)-derived late heart-to-mediastinum (H/M) ratio is a well-established prognostic parameter in patients with chronic heart failure (CHF). However, 123I presents imaging problems owing to high-energy photon emission leading to penetration of collimator septa and subsequent reduction in image quality. Most likely this affects the H/M ratio and may subsequently lead to incorrect patient risk classification. In this prospective study we assessed the intrapatient variation in late H/M ratio between low-energy high-resolution (LEHR) and medium-energy (ME) collimators in patients with CHF.

Materials and methods
Fifty-three patients with CHF (87% male, age 63 ± 8.3 years, LVEF 29 ± 7.8) referred for cardiac 123I-mIBG scintigraphy were enrolled in the study. In each patient, after the administration of 185 MBq 123I-mIBG, early (15 min after injection) and late (4 h after injection) planar anterior thoracic images were acquired with both LEHR and ME collimators. Early and late H/M ratios were calculated on the basis of the mean count densities from the manually drawn regions of interest (ROIs) over the left ventricle and a predefined fixed ROI placed in the upper mediastinum. Additional ROIs were drawn over the liver and lungs. Liver/lung to myocardium and liver/lung to mediastinal ratios were calculated to estimate the effect of collimator septa penetration from liver and lung activity on the myocardial and mediastinal ROIs.

Results
The mean LEHR collimator-derived parameters were lower compared with those from the ME collimator (late H/M ratio 1.41 ± 0.18 vs. 1.80 ± 0.41, p < 0.001). Moreover, Bland–Altman analysis showed that with increasing late H/M ratios the difference between the ratios from the two collimator types increased (R² = 0.73, p = 0.001). Multivariate regression analysis showed that almost 90% of the variation in the difference between ME and LEHR late H/M ratios could be explained by scatter from the liver in both the mediastinal and myocardial ROIs (R² = 0.90, p = 0.001). Independent predictors for the difference in the late H/M ratio between ME and LEHR collimator were the liver-to-heart ratio and the liver-to-mediastinum ratio assessed by ME collimator (standardized coefficient of −1.69 and 1.16, respectively) and LEHR collimator (standardized coefficient of 1.24 and −0.90, respectively) (p < 0.001 for all).

Conclusion
Intra-patient comparison in H/M ratio between the ME and LEHR collimators in patients with CHF showed that with increasing H/M ratio the difference between the ratios increased in favour of the ME collimator. These differences could be explained by septal penetration of high-energy photons from both the liver and the lung in the mediastinum and myocardium, being lowest when using the ME collimator. These results strengthen the importance of the recommendation to use ME collimators in semiquantitative 123I-mIBG studies.
INTRODUCTION

Myocardial $^{123}$I-meta-iodobenzylguanidine ($^{123}$I-mIBG) scintigraphy has been used extensively to assess cardiac sympathetic activity in patients with chronic heart failure (CHF). Numerous single-centre studies have demonstrated that a low late heart-to-mediastinum (H/M) ratio in CHF patients is an independent predictor for ventricular arrhythmia, appropriated ICD therapy, sudden cardiac death and mortality. The prognostic value of the late H/M ratio has been confirmed in the large prospective multicentre ADMIRE-HF study. Although reproducibility and inter- and intraobserver variability have been proven to be adequate, the methods used to obtain the H/M ratio show substantial variation in both acquisition and image analysis. These interinstitutional differences have hampered multicentre comparison of H/M ratio and have made the extrapolation of single-centre results difficult. The relatively recent proposal by Flotats et al. to standardize cardiac sympathetic imaging with $^{123}$I-mIBG scintigraphy will most likely reduce the interinstitutional variation.

Of special concern with $^{123}$I scintigraphy is the fact that, in addition to the main photopeak of 159 keV (83%), $^{123}$I emits high-energy photons of 529 keV (1.4%). These high-energy photons may lead to septal penetration of the collimator and cause scatter that is detected in the 159 keV energy window. In patients with CHF the myocardial uptake of $^{123}$I-mIBG can be low. This means that, especially when there is relatively high $^{123}$I-mIBG uptake in other organs in the direct vicinity of the myocardium (i.e. liver and lungs), septal penetration will degrade image quality and the quantitative accuracy of the H/M ratio (Figure 1).

Medium-energy (ME) collimators have thicker septa compared with low-energy (LE) collimators; ME collimators are therefore better equipped to stop high-energy photons. The effect of collimator selection has been evaluated in a number of phantom studies, and significantly higher H/M ratios have been demonstrated when using the ME collimator compared with the LE collimator. Recently, Inoue et al. demonstrated the same difference in H/M ratio between low-energy high-resolution (LEHR) and low-to-medium-energy collimators in 40 patients with neurodegenerative disorders. However, data on the impact of collimator type on H/M ratio in patients with CHF are limited. Fletcher et al. demonstrated the difference between ME and low-energy high-sensitivity collimator types in 100 CHF patients. However, the planar $^{123}$I-mIBG images were not assessed as proposed by Flotats et al. Therefore, data on direct intra-individual comparison between collimators in CHF patients assessed on planar $^{123}$I-mIBG imaging 15 min and 4 h after injection of $^{123}$I-mIBG are still lacking.

The primary objective of this prospective study was to assess the intra-individual variation of collimator choice on planar $^{123}$I-mIBG early and late H/M ratios in CHF patients. As a secondary objective, an estimation of septal penetration by high-energy photons emerging from other organs than the myocardium (i.e. liver and lungs) was tested as a possible explanation for the inter-individual differences.
Chapter 2

MATERIALS AND METHODS

Study population
The study population consisted of CHF patients with New York Heart Association functional class II–III/IV and an impaired left ventricle ejection fraction (LVEF) of less than or equal to 35% who were referred for 123I-mIBG myocardial scintigraphy to the department of Nuclear Medicine at the Diakonessenhuis, Utrecht, the Netherlands, and the Wilhelminenspital, Vienna, Austria. Both centres are larger teaching hospitals with a large regional adherence area. All patients were optimally treated according to the European guidelines for heart failure.18

Data acquisition
All patients were pretreated with 250 mg of oral sodium perchlorate 30 min before intravenous injection of 185 MBq 123I-mIBG (AdreView™, GE Healthcare, Eindhoven,

Figure 1. Examples of early and late planar 123I-mIBG images from the same patient assessed with both an LEHR and an ME collimator. (a, b) The early and late planar 123I-mIBG images derived with an LEHR collimator are shown. (c, d) The early and late planar 123I-mIBG images from the same patient derived with an ME collimator are shown. Compared with the LEHR collimator-derived images the ME collimator-derived image shows less background noise and better contrast between the organs. The images show decreasing lung 123I-mIBG uptake over time while the liver uptake remains stable. 123I-mIBG, 123I-meta-iodobenzylguanidine; LEHR, low-energy high-resolution; ME, medium-energy.
The Netherlands) to block uptake of free $^{123}$I by the thyroid gland. $^{123}$I-mIBG planar images were acquired with the patient in the supine position. A duration of 15 min (early images) and 4 h (late images) after intravenous injection of $^{123}$I-mIBG, 10-min planar images were acquired from an anterior thoracic view using a zoom factor of 1 and a matrix of $256 \times 256$. All images were acquired with a 15% energy window centred at the 159 keV photopeak of $^{123}$I. Images were acquired using a dual-headed gamma camera (Philips Skylight; Philips, Milpitas, California, USA and Siemens Symbia T6; Siemens, Erlangen, Germany). Per-time-point images were acquired using an LEHR collimator, directly followed by image acquisition using an ME collimator. In one centre (Diakonessenhuis, Utrecht) a dual-headed gamma camera was used. By mounting an LEHR collimator on one head of the gamma camera and the ME collimator on the second gamma camera head it was possible to quickly switch between two different acquisitions. After the LEHR acquisition the heads of the gamma camera rotated so that the ME collimator was in the same anterior position as the earlier LEHR acquisition. In the second centre (Wilhelminenspital, Vienna) the dual-headed gamma camera could only be mounted by one type of collimator. Therefore, the collimators had to be changed before the next acquisition could be made. In both situations, care was taken that the positioning of the patient was left unchanged between the LEHR and ME collimated acquisitions.

**Planar $^{123}$I-mIBG images analysis**

All planar $^{123}$I-mIBG images were analyzed by one experienced observer from the Academic Medical Center in Amsterdam using post-processing software (Hermes Hybrid Viewer v1.4; Hermes Medical solutions, Stockholm, Sweden). The observer was blinded to patient data. All regions of interest (ROIs) except the cardiac ROI were predefined. The cardiac ROI was manually drawn over the myocardium including the left ventricular cavity. The mediastinal ROI with a rectangular shape ($10 \times 5$ pixels) was placed on the upper part of the mediastinum. The location of the mediastinal ROI was determined in relation to the lung apex, the lower boundary of the upper mediastinum and the midline between the lungs. The H/M ratio was calculated by dividing the mean count density in the cardiac ROI by the mean count density in the mediastinal ROI. The $^{123}$I-mIBG washout (WO) was calculated using the early and late H/M ratio with the following formula:

\[
WO = \left( \frac{\text{early H/M ratio} - \text{late H/M ratio}}{\text{early H/M ratio}} \right) \times 100
\]

Additional ROIs were placed over the liver and both lungs (Figure 2). The liver ROI with a rectangular shape ($13 \times 8$ pixels) was placed on the right liver lobe. Left and right lung ROIs with a rectangular shape ($12 \times 8$ pixels) were placed on the mid part of each lung. The mean count density in the liver and in both lung ROIs was used to calculate the liver/heart (Li/H) ratio, the liver/mediastinum (Li/M) ratio, the lung/heart (Lu/H) ratio and the lung/mediastinum (Lu/M) ratio.
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Statistical analysis
All continuous variables are expressed as average ± SD. The difference in ratio of each collimator type was evaluated with a paired t-test. Bland–Altman analysis was used to compare the differences between the LEHR and ME collimators for both mean early and mean late H/M ratios. Multivariate logistic forward regression analysis was performed to investigate several early and late parameters (i.e. Li/H, Li/M, Lu/H and Lu/M derived from both collimator types) as possible independent predictors for the difference between LEHR and ME collimators for the early and late H/M ratios. All statistical analyses were performed using the software package SPSS, version 20.0 (SPSS Inc., Chicago, Illinois, USA).

Figure 2. Example of post-processing planar 123I-mIBG images. The positioning of the predefined mediastinum ROI (M) is determined in relation to the lung apex, the lower boundary of the upper mediastinum and the midline between the lungs. The manually drawn cardiac ROI (H) is placed over the myocardium, including the left ventricular cavity. The predefined liver ROI (Li) is placed over the right liver lobe. The predefined left and right lung ROIs (L) are placed over the mid part of both lungs. ROI, region of interest.
RESULTS

Study population
A total of 53 patients with CHF were included in the study and they underwent early and late ¹²³I-mIBG scintigraphy (35 patients in Utrecht and 18 patients in Vienna). The majority of patients were male (87%) with a mean age of 63 ± 8.3 years (Table 1). The mean New York Heart Association functional class was 2.3 ± 0.4 and the mean LVEF was 29 ± 8.0%. The majority of patients had an ischaemic origin of CHF (n = 38, 72%). Medication use consisted of β-blockers (89%), angiotensin-converting enzyme inhibitors (ACE-I) or angiotensin II receptor blocker (ARB) (98%), diuretics (76%) and lipid-lowering agents (72%). Planar ¹²³I-mIBG images analysis Figure 1 illustrates increase septal penetration of high-energy photons when using an LEHR collimator with increased background noise and consequently decreased image quality compared with the ME collimator. The LEHR collimator-derived early and late H/M ratios were significantly lower compared with the ME collimator derived ratios (early H/M ratio 1.51 ± 0.23 vs. 2.08 ± 0.42, p < 0.001, and late H/M ratio 1.41 ± 0.18 vs. 1.80 ± 0.41, p < 0.001, for LEHR and ME, respectively). Interestingly, Bland–Altman analysis showed a linear increase in the difference between LEHR and ME collimators with increasing mean early H/M ratio (R² = 0.391, p < 0.001). For the late H/M ratio a similar pattern between LEHR and ME collimators was seen (R² = 0.733, p < 0.001) (Figure 3).

The additionally calculated ratios using ROIs of the liver and lung compared with those of the mediastinum and the heart are shown in Table 2. In line with the H/M ratio, the additional ratios derived from the late planar images using the ME collimator are significantly higher than those obtained with the LEHR collimator. However, the early Lu/H and Lu/M ratios showed no significant difference between the ME and LEHR collimators. In addition to the early and late H/M ratios, the ¹²³I-mIBG WO derived with the ME collimator was significantly higher compared with that obtained with the LEHR collimator (13.5 ± 10.6 vs. 5.4 ± 17.2, p = 0.001, respectively, for ME and LEHR).

Multivariate regression analysis
Multivariate regression analyses showed that Li/H and Li/M ratios from both collimator types and the Lu/H and Lu/M ratios from the ME collimator were independent predictors of the difference in early H/M ratio between the LEHR and ME collimators. The combined model containing variables of early Li/H, early Li/M from both LEHR and ME collimators and early Lu/M from the ME collimator explained ~90% of the variation in the early H/M ratio difference between the two collimator types (adjusted R² = 0.888, p = 0.001). The difference in late H/M ratio between the two collimator types could be independently explained by Li/H and Li/M from both collimators. As for the difference in early H/M ratio, the combined model explained ~ 90% of the variation in the late H/M ratio difference between the two collimator types (adjusted R² = 0.897, p < 0.001). As the ¹²³I-mIBG WO is

\[ 123I-mIBG \text{ scintigraphy and influence of collimators} \]
Table 1. Patient characteristics of the study population.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>(n = 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male), [n (%)]</td>
<td>46 (87)</td>
</tr>
<tr>
<td>Age mean ± SD (years)</td>
<td>63 ± 8.3</td>
</tr>
<tr>
<td><strong>Heart failure characteristics [n (%)]</strong></td>
<td></td>
</tr>
<tr>
<td>Ischaemic cardiomyopathy</td>
<td>38 (72)</td>
</tr>
<tr>
<td>Non-ischaemic cardiomyopathy</td>
<td>15 (28)</td>
</tr>
<tr>
<td>NYHA functional class (mean ± SD)</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>LVEF (mean ± SD)(%)</td>
<td>29 ± 8.0</td>
</tr>
<tr>
<td><strong>Clinical cardiovascular risk factors [n (%)]</strong></td>
<td></td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>17 (32)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>34 (64)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>34 (64)</td>
</tr>
<tr>
<td><strong>Medication [n (%)]</strong></td>
<td></td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>47 (89)</td>
</tr>
<tr>
<td>ACE-I or ARB</td>
<td>52 (98)</td>
</tr>
<tr>
<td>Loop diuretics</td>
<td>40 (76)</td>
</tr>
<tr>
<td>Lipid-lowering agent</td>
<td>38 (72)</td>
</tr>
</tbody>
</table>

ACE-I: angiotensin-converting enzyme inhibitors; ARB: angiotensin II receptor blocker; LVEF: left ventricular ejection fraction; NYHA: New York Heart Association.

Table 2. Mean ratios derived from early (15 min after injection) and late (4 h after injection) planar 123I-mIBG acquisition using LEHR and ME collimator.

<table>
<thead>
<tr>
<th></th>
<th>LEHR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Early acquisition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver/Heart</td>
<td>1.97 ± 0.51</td>
<td>2.74 ± 1.01</td>
</tr>
<tr>
<td>Liver/Mediastinum</td>
<td>2.95 ± 0.71</td>
<td>5.43 ± 1.46</td>
</tr>
<tr>
<td>Lung/Heart</td>
<td>1.34 ± 0.82</td>
<td>1.51 ± 0.53</td>
</tr>
<tr>
<td>Lung/Mediastinum</td>
<td>1.99 ± 1.27</td>
<td>3.02 ± 0.87</td>
</tr>
<tr>
<td><strong>Late acquisition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver/Heart</td>
<td>2.42 ± 0.75</td>
<td>3.52 ± 1.11</td>
</tr>
<tr>
<td>Liver/Mediastinum</td>
<td>3.35 ± 0.73</td>
<td>6.05 ± 1.53</td>
</tr>
<tr>
<td>Lung/Heart</td>
<td>1.19 ± 0.32</td>
<td>1.53 ± 0.65</td>
</tr>
<tr>
<td>Lung/Mediastinum</td>
<td>1.64 ± 0.30</td>
<td>2.58 ± 0.74</td>
</tr>
</tbody>
</table>

LEHR: low-energy high-resolution; ME: medium-energy.
determined by the early and late H/M ratios, the difference in $^{123}$I-mIBG WO between the two collimator types depends on the combination of all variables explaining the difference in both early and late H/M ratios between the ME and LEHR collimators. The large numbers of variables make the multivariate regression analyses for the difference between the two collimator types for $^{123}$I-mIBG WO less reliable and were therefore not assessed.
DISCUSSION

Cardiac sympathetic activity can be adequately assessed with $^{123}$I-mIBG scintigraphy. $^{123}$I-mIBG is a norepinephrine (NE) analogue that shares the same presynaptic uptake, storage and release mechanism as NE. The most commonly used semiquantitative measurements of myocardial $^{123}$I-mIBG uptake are the calculated early and late H/M ratios and $^{123}$I-mIBG WO derived from planar $^{123}$I-mIBG images. The early H/M ratio reflects the integrity of sympathetic nerve terminals. The late H/M ratio offers information about neuronal function resulting from uptake, storage and release. It has been suggested that $^{123}$I-mIBG WO reflects the neuronal integrity of the sympathetic tone/drive.11

Numerous single-centre studies have demonstrated that the late H/M ratio is an independent predictor of cardiac mortality and morbidity in CHF patients.3,5,9,19 However, when comparing studies, variability in the methods used to calculate the H/M ratio leads to substantial variation in the values obtained. This variation affects the clinical impact; that is, the H/M ratio may be wrongly calculated, resulting in incorrect risk classification. The major factor causing these variations in H/M ratio is the collimator choice. For example, the Japanese standard $^{123}$I-mIBG databases showed significant difference in healthy human H/M ratios: 2.39 ± 0.21 and 2.49 ± 0.25 for early and late imaging using an LE collimator and 2.76 ± 0.31 and 3.01 ± 0.35 using an ME or low-to-medium-energy collimator, respectively ($p < 0.0001$ for both early and late H/M ratios).20 The difference between collimator types can be explained by septal penetration of high-energy photons of $^{123}$I-mIBG. The septa of LE collimators are less thick than those of ME collimators, leading to increased septal penetration of high-energy photons (Figure 1). Methods for scatter correction in $^{123}$I-mIBG scintigraphy to improve the image quality and compare different collimator types have been described, but clinical use is very limited.14,21,22 In addition, correction for penetration may be essential for adequate comparison between collimators; however, these data are currently not available.

Phantom studies have demonstrated significant difference in H/M ratios between different collimator types.13,15,23 Specifications of collimators can vary both within the same manufacturer and among different manufacturers. This causes interinstitutional differences in the effect of septal penetration and consequently in estimations of H/M ratio. Therefore, Nakajima et al. developed a correction method using a phantom to provide comparable H/M ratio values between the different LE, low-to-medium-energy and ME-type collimators.23 However, studies on H/M ratio difference between different collimator types in humans with CHF are limited and contain a small number of patients. The present study is the first prospective study including more than 50 CHF patients using both LEHR and ME collimators for the acquisition of planar $^{123}$I-mIBG images.
In the current study, early and late H/M ratios derived from planar $^{123}$I-mIBG images using an ME collimator were significantly higher compared with the LEHR collimator-derived H/M ratios. These results are in line with previous phantom studies and comparable to small patient studies comparing different collimator types. Interestingly, there was a linear increase in difference with increasing H/M ratio. In planar LEHR collimator-acquired images, septal penetration of high-energy photons caused increased counts in both mediastinum and heart ROIs, leading to a regression to the mean when calculating H/M ratio. As the ME collimator has thicker septa leading to less septal penetration, the calculation of H/M ratio is less affected by a regression to the mean resulting in higher H/M ratio, and therefore most likely these H/M ratios more accurately reflect the real biodistribution of $^{123}$I-mIBG.

The ratios between the liver, lung and heart and mediastinum were higher when using an ME collimator compared with an LEHR collimator (Table 2). This confirms that mediastinum and heart ROIs are less affected by septal penetration of scattering of high-energy photons from the liver and lung when using an ME collimator. Multivariate regression analysis showed that 90% of the difference in early and late H/M ratios could be explained by scatter from the liver. In early acquisition, scatter from the lungs also contributes to differences in the early H/M ratio. However, scatter from the lungs did not contribute to the difference in late H/M ratios between collimator types. This can easily be explained by the biodistribution of $^{123}$I-mIBG, showing decreasing lung uptake over time. On the late images this results in a relatively higher contribution of scatter of high-energy photons and consequently septal penetration of the liver compared with the lungs.

The ADMIRE-HF was the first large multicentre study that showed that the late H/M ratio was an independent predictor of cardiac morbidity and mortality. In this study a predefined cutoff value for late H/M ratio of 1.6 was chosen using an LEHR collimator. Late H/M ratio less than 1.6 was associated with progression of heart failure, hospitalization, arrhythmia and mortality. Extrapolation of these results to institutions using ME collimators is unclear. Although Nakajima et al. have developed a correction method to translate H/M ratio derived with an LEHR collimator to an ME collimator value in a phantom, translating these findings to a clinical setting may be hampered by inter-individual and intra-individual variation of $^{123}$I-mIBG uptake in the liver and the lung.

As proposed by Flotats et al. standardization of acquisition is essential to compare $^{123}$I-mIBG scintigraphy results between different institutions and they recommended the use of ME collimators. This study underlines that the use of an ME collimator results in H/M ratios being less influenced by scatter from septal penetration. However, many institutions continue to use LEHR collimators because of availability and the relative inconvenience of changing collimators from study to study in daily practice.
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LIMITATION

The primary limitation of the present study is that the early and late acquisition with ME collimators was performed \(\sim15\) min after the acquisition with the LEHR collimator. As in time after injection \(^{123}\text{I}\)-mIBG uptake changes, this could have influenced the H/M ratio outcomes leading to underestimation of H/M ratio with the LEHR collimator. However, the difference in H/M ratios between the two collimator types is too large to be explained by the delay between the different acquisitions alone. Second, although collimators from two different vendors were used (Siemens and Philips), there was no difference in the impact that the LEHR and ME collimators from each vendor had on the H/M ratio (data not shown). Finally, this study only focussed on planar images, and therefore the influence of scatter due to septal penetration on the regional sympathetic innervation/activity as assessed by SPECT remains uncertain. This regional information appears to be of additional clinical value to the planar-derived parameters and should be assessed in future studies.

CONCLUSION

Early and late H/M ratios and subsequently \(^{123}\text{I}\)-mIBG WO derived from planar \(^{123}\text{I}\)-mIBG images are significantly lower when using an LEHR collimator compared with an ME collimator. This difference is caused by septal penetration of high-energy photons mainly from the liver and shows a linear increase with increasing H/M ratio. The thicker septa of the ME collimator reduce septal penetration and most likely result in a more realistic reflection of cardiac sympathetic activity. These results strengthen the importance of the recommendation to use ME collimators in semiquantitative \(^{123}\text{I}\)-mIBG studies.
REFERENCES


Chapter 2


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Chapter 3

A European myocardial $^{123}$I-\textit{m}IBG cross-calibration phantom study

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E Poel
K Nakajima
K Okuda
BL van Eck-Smit
GA Somsen
HJ Verberne
Chapter 3

ABSTRACT

Aim
Planar myocardial $^{123}$I-meta-iodobenzylguanidine ($^{123}$I-mIBG) scintigraphy is a highly reproducible technique. However, differences in collimator use is one of the most important factors that cause variation among institutions and studies in heart-to-mediastinum (H/M) ratio. Therefore, standardization among various gamma camera-collimator combinations is needed. Previously, a phantom has been developed to cross-calibrate different acquisition conditions in Japan. To further cross-calibration of European myocardial $^{123}$I-mIBG imaging, the aim of this study was to collect $^{123}$I-mIBG data for H/M ratios from common European gamma camera vendors.

Materials and methods
210 experiments were performed in 27 European institutions. Based on these experiments conversion coefficients for each gamma camera-collimator combination were calculated. An averaged conversion coefficient of 0.88 was used to calculate a standardized H/M ratio.

Results
On average LE collimator-derived H/M ratios were significantly lower compared to ME-collimator-derived H/M ratios. The mean conversion coefficients ranged from 0.553 to 0.605 for the LE collimator group and from 0.824 to 0.895 for the ME collimator group.

Conclusion
Clinically established H/M ratios can be converted into standardized H/M ratios by using cross-calibrated conversion coefficients. This standardization is important for identifying appropriate thresholds for adequate risk stratification. In addition this cross-calibration enables comparison between different national and international data.
INTRODUCTION

Cardiac $^{123}$I-mIBG scintigraphy, a non-invasive imaging technique to assess cardiac sympathetic activity, has been shown to be of clinical value, especially for the assessment of prognosis, in many cardiac diseases.\textsuperscript{1-5} The quantification method is essential to differentiate normal and abnormal cardiac sympathetic activity and to distinguish high and low risk groups. The heart-to-mediastinum (H/M) ratio is a simple method to correct for background and is highly reproducible with small inter- and intra-observer variation.\textsuperscript{6} However, standardization of acquisition and analysis is needed. The lack of standardization between different institutions is one of the factors that have hampered wide scale clinical implementation of cardiac $^{123}$I-mIBG scintigraphy. International efforts have been made to harmonize and standardize cardiac $^{123}$I-mIBG scintigraphy.\textsuperscript{7} These recommendations include proposals for patient preparation, administered dose of $^{123}$I-mIBG activity (MBq), scanning parameters, and analysis of the acquired data to obtain the most used semi-quantitative parameters (i.e. early and late H/M ratio and $^{123}$I-mIBG washout (WO)).

Collimator choice is one of the most important factors causing variation among institutions and studies.\textsuperscript{8,9} In addition to 159 keV photons $^{123}$I emits high-energy photons of 529 keV which penetrate the relatively thin septa of low energy (LE) collimators. This penetration leads to degradation of image quality and ultimately introduces variation in H/M ratios.\textsuperscript{10} Medium energy (ME) collimators have thicker septa and lower photon penetration compared to LE collimators and therefore have improved image quality and accuracy in myocardial $^{123}$I-mIBG imaging, however, at the expense of spatial resolution.\textsuperscript{11-13} Consequently the use of ME collimators is recommended for estimation the H/M ratios.\textsuperscript{7} However, LE collimators are still commonly applied for cardiac $^{123}$I-mIBG scintigraphy because of their wide availability.\textsuperscript{7} In addition, although the nomenclature of collimators is classified into 2 major groups of LE and ME collimators, various types of collimators have been developed depending on the clinical purpose. The variety in the collimator types used has hampered multicentre comparison of cardiac $^{123}$I-mIBG-derived parameters and single centre results could not easily be extrapolated to other institutions.\textsuperscript{14}

In Japan, a phantom for planar cardiac $^{123}$I-mIBG imaging has been developed to cross-calibrate different acquisition conditions.\textsuperscript{15} This phantom has been used to calculate conversion coefficients for different gamma camera-collimator combinations in Japan.\textsuperscript{16} With these conversion coefficients various conditions can be converted to standard H/M ratios. As an extension of this phantom study, the purpose of this study was to accumulate H/M ratios from common gamma camera vendors in Europe and compare these data with the data from Japan.
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MATERIAL AND METHODS

Phantom design and experiment
A light weight calibration phantom was used as previously described.\textsuperscript{17} 111 MBq \textsuperscript{123}I was mixed with 4,450 ml water to fill the phantom. Since all organ parts were connected as one compartment, no radionuclide concentration adjustment was required for each organ separately. A 3-cm acrylic plate was placed over the phantom to simulate human body attenuation, when imaging was performed. The 256 × 256 matrix images were acquired from the anterior and posterior views for 5 minutes, comparable to clinical planar cardiac \textsuperscript{123}I-mIBG imaging (Figure 1). The energy window was centred at 159 keV with a 15-20\% window. The experiments were performed using 210 conditions in 27 European institutions (see “Appendix” for list of all participating institutions).

Figure 1. Examples of planar \textsuperscript{123}I-mIBG images of the phantom in anterior (left panels) and posterior (right panels) view with Symbia system (Siemens, Erlangen, Germany). Note the difference in image quality due to septal penetration or scatter between low energy and medium energy collimators. LEHR = low energy high resolution; MELP = medium energy low penetration.
Cross-calibration phantom study

Mathematical reference value of H/M ratio

All 123I-mIBG phantom images were acquired in each participating institution. Data were anonymised and were sent to the Kanazawa University in Japan for central analysis. H/M ratios were mathematically calculated, assuming a linear attenuation coefficient (μ) of 123I for water as 0.147 cm\(^{-1}\). The standard equation for attenuation was used (i.e. exponential of -μx, where x stands for the thickness of the attenuating material). The mathematical calculated reference H/M ratio was corrected for attenuation, while Compton scatter and septal penetration of gamma rays were not included. The reference H/M ratios determined by the structure of the phantom were 2.60 and 3.50 (respectively anterior and posterior acquisition). Instead of the original phantom used in the Japanese studies, a new light-weight phantom was used in the European study. In the latter phantom, although the dimensions of the phantom were identical, some acrylic parts were made hollow to fill with non-radioactive water. Minor differences in reference values derived from the light-weight phantom were adjusted to obtain identical results compared to the original phantom.

Cross calibration

In this study two H/M ratios (anterior and posterior acquisitions) from each institution were plotted against the reference values (Figure 2). A linear regression equation was calculated using the formula:

\[
y - 1 = K \cdot (x - 1)
\]

(* denotes multiplication), in which the line always passes on the coordinate (1,1). The coefficient \(K_i\) (i.e. slope of the regression line for each institution) was used to convert the institutional H/M ratios to the reference values (H/M ratio\(_{ref}\)). In the second step the H/M ratio\(_{ref}\) was converted to a standardized H/M ratio using the \(K_{std}\). This process can be summarized as:

\[
\text{Standardized H/M ratio - 1} = K_{std}/K_i \cdot \text{(institutional H/M ratio - 1)}.
\]

The \(K_{std}\) was 0.88, defined as the average K values for typical ME collimators. The rationale for this conversion to the common ME collimator type is based on the recommendation to use ME collimators.
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Statistical analysis
The data are shown as mean ± standard deviation. Differences among groups were examined by one-way analysis of variance and Student’s t test. The linear regression equation of the H/M ratios between two conditions was calculated by the least square method. The statistics software JMP (version 11, SAS Institute Inc., Cary, NC, USA) was used and mathematical calculation was based on Mathematica 10 (Wolfram Research Inc., Champaign, IL, USA).

RESULTS

210 $^{123}$I-mIBG phantom studies were performed in 27 institutions in Austria, Belgium, the Netherlands and the United Kingdom including camera vendors of Siemens ($n = 148$), GE ($n = 44$) and Philips ($n = 18$). Collimator types were divided into 2 groups: LE and ME. The LE group included low energy high resolution (LEHR), general purpose (LEGP) and all-purpose (LEAP) collimators. The ME group included low-medium-energy general purpose (LMEGP), general purpose (MEGP) and low penetration (MELP) collimators.
Cross-calibration phantom study

**Figure 3.** Individual data points and box-whisker plots of H/M ratios using phantoms with the reference H/M ratio of 2.60 (panel A) and 3.50 (panel B). Green lines denote mean values. The box plot shows median and the 1st and 3rd quartile, and the ends of the whiskers are ± 1.5 *(interquartile range).*

**Figure 4.** Conversion coefficients to the reference values for the LE and ME collimator groups. Data points and box-whisker plots are also shown. Green lines denote mean values. The box plot shows median and the 1st and 3rd quartile, and the ends of the whiskers are ± 1.5 *(interquartile range).*
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H/M ratio measured in two phantom conditions
Overall the LE-collimator group showed lower H/M ratios compared with the ME-collimator group. For the phantom H/M ratio of 2.60, the LE-collimator \((n = 113)\) and ME-collimator \((n = 97)\) derived H/M ratios were 1.932 \(\pm 0.056\) and 2.685 \(\pm 0.088\), respectively, \((p < 0.0001)\). Similarly for the phantom H/M ratio of 3.50, the LE-collimator and ME-collimator derived H/M ratios were 2.281 \(\pm 0.074\) and 3.354 \(\pm 0.124\), respectively, \((p < 0.0001)\) (Figure 3).

Conversion coefficients determined by 2 data points
The conversion coefficients to the reference value are summarized according to the main collimator names: 3 LE subgroups and 3 ME subgroups. (Table 1) The average conversion coefficients were 0.553 for LEHR, 0.605 for LEAP, 0.570 for LEGP, 0.824 for LMEGP, and 0.882 for MEGP, and the highest was 0.895 for MELP types. When the conversion coefficients were divided into a LE and a ME group, the average values were respectively 0.556 \(\pm 0.021\) and 0.880 \(\pm 0.036\) \((p < 0.0001)\) (Figure 4).

Comparison between European and Japanese conversion coefficients
Overall there were no significant differences when the European conversion coefficients of LEHR, LEAP, LMEGP and MEGP collimators were compared with the Japanese conversion coefficients (Table 1). Only the conversion coefficients for LEGP and MELP collimators differed significantly \((p < 0.0001)\). However when the conversion coefficients for MEGP and MELP were combined the difference was no longer statistically significant. In contrast when the conversion coefficients for LEGP and LEAP were combined the statistical significant difference persisted between the European and Japanese data.

DISCUSSION

These are the results of the first European myocardial \(^{123}\)I-mIBG cross-calibration phantom study to calculate conversion coefficients for specific individual gamma camera-collimator combinations. The cross-calibration allowed for a conversion of institutional H/M ratios to standardized H/M ratios. These conversion coefficients will facilitate multicentre comparison of myocardial \(^{123}\)I-mIBG results and enables the extrapolation of the outcome of single- and multicentre studies to other institutions.

The design of collimator septa and apertures have influence on septal penetration and Compton scatter. ME collimators, as recommended by the EANM Cardiovascular Committee\(^7\), have thicker septa and lower penetration compared to LE collimators. The difference in collimator types is therefore one of the most important factors that affects variation in H/M ratios. It has been shown that H/M ratios derived from LE collimators are significantly lower compared to those from ME collimators.\(^8,17\) This has been confirmed by the previous
Cross-calibration phantom study

Table 1. Conversion coefficient of collimators: European vs. Japanese studies.

<table>
<thead>
<tr>
<th></th>
<th>Europe</th>
<th></th>
<th>Japan</th>
<th></th>
<th>p values between Europe and Japan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
<td>Mean</td>
</tr>
<tr>
<td>LEHR</td>
<td>103</td>
<td>0.553</td>
<td>0.018</td>
<td>73</td>
<td>0.552</td>
</tr>
<tr>
<td>LEGP+LEAP group **</td>
<td>10</td>
<td>0.591</td>
<td>0.024</td>
<td>25</td>
<td>0.648</td>
</tr>
<tr>
<td>LEGP</td>
<td>4</td>
<td>0.570</td>
<td>0.011</td>
<td>17</td>
<td>0.654</td>
</tr>
<tr>
<td>LEAP</td>
<td>6</td>
<td>0.605</td>
<td>0.020</td>
<td>2</td>
<td>0.624</td>
</tr>
<tr>
<td>LMEGP</td>
<td>16</td>
<td>0.824</td>
<td>0.035</td>
<td>46</td>
<td>0.829</td>
</tr>
<tr>
<td>MEGP+MELP group</td>
<td>81</td>
<td>0.891</td>
<td>0.025</td>
<td>53</td>
<td>0.895</td>
</tr>
<tr>
<td>MEGP ***</td>
<td>28</td>
<td>0.882</td>
<td>0.017</td>
<td>40</td>
<td>0.878</td>
</tr>
<tr>
<td>MELP</td>
<td>53</td>
<td>0.895</td>
<td>0.027</td>
<td>13</td>
<td>0.950</td>
</tr>
</tbody>
</table>

* Data from J Nucl Cardiol 2014; 21: 970-978
** LE general-all-purpose collimator is included in Japanese study
*** MEGP, ME general-all purpose, and ME collimators are included in Japanese study

Cross-calibration phantom study in Japan showing significant underestimation of H/M ratios derived from LE collimators.\(^6\) As H/M ratios help differentiating high-risk and low-risk groups this could have clinical implications. However, after correction to standardized H/M ratios, LE and ME collimators showed comparable values.\(^6\)

The present study shows that the conversion coefficients of the LE collimators are lower compared to the ME collimators which is in line with previous phantom studies in Japan (Table 1). The conversion coefficients for most LE and ME collimator sub-groups did not show any statistical differences between Europe and Japan. However, there was a significant difference in the LEGP and MELP sub-group.

There are several factors that could explain variation in the LEGP and MELP sub-group between the European and Japanese institutions. Most likely the small number of LEGP and MELP collimators may have resulted in a limited statistical power. In addition, these differences may be explained by small differences in the phantom used. In contrast to the original designed phantom used in Japan, a light-weight phantom was used for the current European study. After careful examining both cross-calibration phantoms by CT scanner (Symbia T6, Siemens. Erlangen, Germany) under the same conditions (120 mAs and 130 kV), there was a small difference of < 1 mm of the \(^{123}\)I-mIBG compartment between the conventional and light-weight types. Furthermore, one could expect minor differences between Japanese and European camera combinations due to differences in the design of the collimator septa and apertures and gamma camera crystals. However, we have confirmed with the manufacturers that both collimators and gamma
cameras used are manufactured identical for Europe and Japan. The differences between LEAP and MELP may also be explained by small variations in acquisition. In Europe and Japan both energy windows of 15% and 20% have been used according to local protocol. The acquisition time ranged from 3 to 10 minutes in Japan and was 5 minutes in Europe. The distance from collimator to phantom was the same in Europe and Japan. Moreover, although $^{123}$I was manufactured by different companies in Japan (FUJIFILM RI Pharma, Tokyo, Japan) and Europe (GE Healthcare, Eindhoven, The Netherlands), both products showed no contamination of other isotopes. Finally, there might be a difference between used acrylics of the original phantom and water in some compartments of the light-weight phantom. Although, both water and the used acrylics have an almost identical decay coefficient with a very similar scatter pattern this minor difference in phantom design may explain the found differences in coefficient values. In summary although there is no variation between Europe and Japan for most LE and ME groups, there is a minimal difference in the LEAP and MELP collimator group. As shown there is a variety of possible explanations for this small difference. However, except for the relative small number of experiments, the above mentioned factors are true for all comparisons between the European and Japanese data. Hence, if valid, these factors would have caused also differences between the other collimators groups. Therefore this difference is most likely driven for the largest part by the relative small number of experiments. Of course variation in phantom design, variation in energy windows and variation in acquisition time may have also contributed.

Standardization of H/M ratios has impact on patient management. Most importantly standardization of H/M ratio allows for the development of a universal prognostic threshold. This could be established by reanalyzing databases from a number of $^{123}$I-mIBG studies previously published. In addition future multicentre studies should aim for the use of a standardized H/M ratio, overcoming the impact of gamma camera and collimator differences. Finally, to further stress the importance of standardized H/M ratios, standardized values are essential in risk models for cardiac mortality.18

Our study has some limitations. Compared to the Japanese cross-calibration study we only used 2 (2.60 and 3.50) instead of 4 (1.35, 1.80, 2.60 and 3.50) references H/M ratios. However, conversion coefficients from 2 data points were nearly identical to those 4 data points.16 In addition, this cross-calibration method only corrects for high-energy photons coming from liver and lungs, which are the most important contributors of counts overestimation in the mediastinum and heart.8 However, this method does not correct for high-energy photons coming other organs like kidney and bladder.

In conclusion, differences in gamma camera-collimator combinations can be corrected to standardized ME collimator values with the use of a cross-calibration phantom. This method can readily be applied, reducing variation in outcome measures and thereby further the clinical role of myocardial $^{123}$I-mIBG scintigraphy.
APPENDIX

Participating institutions included (alphabetical order):
Academic Medical Center (Amsterdam, the Netherlands),
Amphia ziekenhuis (Breda, the Netherlands),
AZ Groeninge (Kortrijk, Belgium),
AZ Maria Middelares (Gent, Belgium),
AZ Sint Jan (Brugge, Belgium),
Diakonessenhuis (Utrecht, the Netherlands),
Erasmus Medical Center (Rotterdam, the Netherlands),
Gelderse Vallei (Ede, the Netherlands),
Groene Hart ziekenhuis (Gouda, the Netherlands),
Jeroen Bosch Ziekenhuis (Den Bosch, the Netherlands),
Leids University Medical Center (Leiden, the Netherlands),
Medical Center Leeuwarden (Leeuwarden, the Netherlands),
Onze Lieve Vrouw (Aalst, Belgium),
OLVG oost (Amsterdam, the Netherlands),
OLVG west (Amsterdam, the Netherlands),
Noord West ziekenhuisgroep (Alkmaar, the Netherlands),
Sint Augustinus (Wilrijk, Belgium),
Spaarne Gasthuis (Hoofddorp, the Netherlands),
Spaarne Gasthuis (Haarlem, the Netherlands),
University Hospital of North Durham (Durham, United Kingdom),
University Medical Center Groningen (Groningen, the Netherlands),
University Medical Center Utrecht (Utrecht, the Netherlands),
UZ Antwerpen (Edegem, Belgium),
UZ Brussel (Brussel, Belgium),
UZ Leuven (Leuven, Belgium),
Wilhelminenspital (Vienna, Austria),
Zuyderland Medical Center (Heerlen, the Netherlands)
Chapter 3

REFERENCES


Chapter 4

Impact of a predefined mediastinal ROI on inter-observer variability of planar \(^{123}\)I-\(m\)IBG heart-to-mediastinum ratio

DO Verschure
V Bongers
PJ Hagen
GA Somsen
BL van Eck-Smit
HJ Verberne
Chapter 4

ABSTRACT

Aim
Purpose of this study was to assess the impact of mediastinal region of interest (ROI) definition on intra- and inter-observer variability in relation to collimator type.

Materials and methods
Thirty-five subjects with CHF (80% men, mean age 66 ± 9 years, NYHA 2.4 ± 0.5, LVEF 29 ± 8.4%) were enrolled. 15 minutes and 4 hours post-injection (p.i.) of $^{123}$I-mIBG, planar images were sequentially acquired with low energy high energy (LEHR) and medium energy (ME) collimators. In the first analysis, observer-defined mediastinal ROI was used. In the second analysis, a predefined mediastinal ROI was used. Intra- and inter-observer variability of late H/M ratio was assessed using Lin’s concordance coefficient (LCC).

Results
There was substantial agreement between all three observers using predefined mediastinum ROI. LCCs for LEHR were 0.98, 0.96, and 0.95, for ME 0.98, 0.97, and 0.97. However, observer-defined mediastinal ROI resulted in poor-moderate agreement. LCCs for LEHR were 0.82, 0.94, and 0.70, for ME 0.77, 0.91, and 0.80. Intra-observer analysis using predefined mediastinal ROI showed substantial agreement. LCC was 0.97 for LEHR and 0.96 for ME.

Conclusion
Predefined mediastinal ROI results in low intra- and inter-observer variability of late H/M ratio and is, therefore, to be preferred over observer-defined mediastinal ROI. Intra- and inter-observer variability of late H/M ratio is not influenced by collimator choice.
INTRODUCTION

In patients with chronic heart failure (CHF) compensation mechanisms like the sympathetic nervous system and the renin-aldosterone-angiotensin system (RAAS) are activated. Initially, increased sympathetic stimulation compensates for impaired myocardial function, but long-term stimulation has detrimental effects on myocardial structure and function causing remodeling of the left ventricle. Increased myocardial sympathetic activity increases exocytosis of norepinephrine (NE) from in the presynaptic vesicles. In addition, the NE re-uptake via NE transporter (also called uptake-1) in the sympathetic terminal nerve axons is decreased. This results in increased NE concentration in the synaptic cleft.

Meta-iodobenzylguanine (mIBG) is a NE analog that shares the same presynaptic uptake, storage, and release mechanism as NE. Radiolabeling of mIBG with $^{123}$I allows assessment of the sympathetic system. In 1981, the potential use of $^{123}$I-mIBG for cardiac imaging was suggested. Especially since $^{123}$I-mIBG became available, myocardial $^{123}$I-mIBG scintigraphy has been increasingly used, mainly in Europe and Japan. And just recently, the Food and Drugs Administration has given the approval for myocardial $^{123}$I-mIBG scintigraphy in the United States of America.

The most commonly used semi-quantitative measurements of myocardial $^{123}$I-mIBG uptake are the calculated heart-to-mediastinum (H/M) ratio and $^{123}$I-mIBG washout (WO), determined from planar $^{123}$I-mIBG images. The early H/M ratio reflects the integrity of sympathetic nerve terminals. The late H/M ratio offers information about neuronal function resulting from uptake, storage, and release. The WO reflects neuronal integrity of sympathetic tone/drive. In patients with CHF, a low late H/M ratio has been shown to be an independent predictor for ventricular arrhythmia, appropriated ICD therapy, sudden cardiac death, and mortality. Additionally, an increased WO has been associated with an adverse prognosis.

Almost all reports about $^{123}$I-mIBG images include the late H/M ratio. However, the methods used to obtain these parameters show substantial variation and have not been standardized yet. This variation can be caused by acquisition parameters: collimator choice, acquisition time and duration, and post-processing analysis: size, shape, and location of the cardiac and mediastinal region of interest (ROI). Somsen et al. has demonstrated that the inter- and intra-individual variability is low using myocardial ROI including left ventricle cavity. Veltman et al. demonstrated a low intra- and inter-observer variability of H/M ratio on planar $^{123}$I-mIBG images using a fixed rectangular mediastinal ROI. In both studies, $^{123}$I-mIBG images were assessed with low energy high resolution (LEHR) collimators. However, there are studies that indicate better results with respect to variability and accuracy of myocardial parameters using medium energy (ME) collimators. To our best knowledge, there are no studies that
have compared the use of LEHR and ME collimators on the intra- and inter-observer variability. Therefore, the objective of this present study was to assess the impact of mediastinal ROI definition on intra- and inter-observer variability in relation to collimator type used for $^{123}$I-$m$IBG image acquisition.

Study Population
The study population consisted of patients with CHF with New York Heart Association (NYHA) functional class II-III/IV and an impaired left ventricle ejection fraction (LVEF) of < 35% who were referred for $^{123}$I-$m$IBG myocardial scintigraphy to the Department of Nuclear Medicine of the Diakonessenhuis, Utrecht, The Netherlands. The Diakonessenhuis is a larger teaching hospital with a large regional adherence area. All patients were individually optimally treated according to the ESC guidelines for acute and chronic heart failure.12

Data acquisition
All subjects were pre-treated with 250 mg oral sodium perchlorate to block uptake of free $^{123}$I by the thyroid gland 30 minutes before intravenous (IV) injection of 185 MBq $^{123}$I-$m$IBG (AdreView™, GE Healthcare, Eindhoven, The Netherlands). $^{123}$I-$m$IBG planar images were acquired with the subject in supine position. 15 minutes (early images) and 4 hours (late images) after IV injection of $^{123}$I-$m$IBG 10-minute planar images were acquired from an anterior thoracic view (256 × 256 matrix). All images were acquired with a 15% energy window centered at the 159 keV photopeak of $^{123}$I. Images were acquired using a dual-headed gamma camera (Philips skylight, Eindhoven, The Netherlands). Per time-point (i.e., early and late) an acquisition with a LEHR collimator was made directly followed by an acquisition with a ME collimator (Figure 1). By mounting a LEHR on one head of the gamma camera and the ME collimator on the second gamma camera head, it was possible to quickly switch between two different acquisitions. After the LEHR acquisition, the camera rotated so that the ME collimator was in the same anterior position as the earlier made LEHR acquisition while the subject was lying in the same supine position.

Planar $^{123}$I-$m$IBG images analysis
One experienced (V.B.) and one inexperienced (P.J.H.) nuclear medicine physicians from the same centre (Diakonessenhuis) and one experienced nuclear medicine researcher from the Academic Medical Center (D.O.V.) were asked to analyse the planar $^{123}$I-$m$IBG images using post-processing software on a workstation. All observers were blinded from patient data. The H/M ratios were calculated from the planar $^{123}$I-$m$IBG images using a ROI over the heart and the upper part of the mediastinum. The cardiac ROI was manually drawn over the myocardium including the left ventricular cavity. The mediastinal ROI with a rectangular shape was placed on the upper part of the mediastinum. The H/M ratio was calculated by dividing the mean count density in the cardiac ROI by the mean count density in the mediastinal ROI.9
Statistical analysis

All continuous variables are expressed as mean ± standard deviation. The intra- and inter-
observer variability of the early and late H/M ratio were assessed using Lin’s concordance 
coefficient (LCC) and the Bland-Altman analysis (expressed as mean difference between 
observers and 95% limits of agreement). First, the result for the LEHR and ME collimator 
were analysed separately based on absolute numbers. It was expected that the mean 
early and late H/M ratio using ME collimator would most likely be higher compared to 
the LEHR collimator obtained ratios. This would make a direct comparison between 
the collimator types difficult. Therefore, to allow for a better comparison between 
the LEHR and ME Bland-Altman analysis obtained results a relative mean difference was 
calculated, i.e., the mean difference between two observers was divided by the mean 
H/M ratio of two observers. This enables a better comparison between the ME and LEHR 
collimator obtained results. For clinically relevant agreement, the following criteria were 
used: LCC values < 0.90, 0.90 - 0.95, 0.95 - 0.99, and > 0.99 were considered poor, 
moderate, substantial, and almost perfect, respectively.13 All statistical analyses were 
performed using SPSS software package, version 20.0 (SPSS, Chicago, IL).
Chapter 4

RESULTS

Patient population
A total of 35 patients (80% men, mean age 65 ± 7.8 years) were included in the study. The baseline characteristics of the patient population are shown in Table 1. The mean NYHA functional class was 2.4 ± 0.5 and the mean LVEF was 29 ± 8.4%. Of the 35 patients, 83% had ischaemic cardiomyopathy. Medication used consisted of beta-blockers (87% of patients), angiotensin-converting enzyme inhibitors (ACE-I) or angiotensin II receptor blockers (ARB) (94% of patients), lipid-lowering agents (77% of patients), and loop diuretics (69% of patients).

Inter-observer variability of planar \(^{123}\text{I-mIBG}\) imaging
The mean early and late H/M ratio of all three readers using observer-dependent mediastinal ROI and predefined fixed mediastinal ROI are shown in Table 2. In general, these values are in line with the population of CHF patients studied.

LCC for inter-observer variability of early and late H/M ratio determined on planar \(^{123}\text{I-mIBG}\) images using LEHR and ME collimators with observer-defined (non-fixed) and fixed mediastinal ROI are shown in Table 3. An observer-defined mediastinal ROI resulted in a high inter-observer variability between all three readers, with a poor agreement for both early and late H/M ratio, independent of collimator choice. A predefined fixed mediastinal ROI improved the inter-observer agreement between all three observers. The inter-observer agreement for the early H/M ratio was poor to moderate when using a LEHR collimator. The observer agreement increased to moderate/substantial when using a ME collimator. The inter-observer agreement for the late H/M ratio was substantial for both the LEHR and the ME collimator.

The Bland-Altman analysis showed that the absolute mean difference (95% limits of agreement) in late H/M ratio with a fixed mediastinal ROI using LEHR collimator between all three observers was small, -0.02 (-0.06 to 0.02), -0.04 (-0.13 to 0.05), and -0.05 (-0.14 to 0.04), OBS1 vs OBS2, OBS1 vs OBS3, and OBS2 vs OBS3, respectively (Table 4). The Bland-Altman analysis for the ME collimator showed a small absolute mean difference (95% limits of agreement) of -0.01 (-0.20 to 0.18), 0.06 (-0.12 to 0.24), and 0.05 (-0.15 to 0.25), OBS1 vs OBS2, OBS1 vs OBS3, and OBS2 vs OBS3, respectively (Table 4).

The Bland-Altman analysis of the relative mean difference (95% limits of agreement) in late H/M ratio with a fixed mediastinal ROI using LEHR collimator was slightly smaller compared with ME collimator use, but not clinical relevant. The Bland-Altman plots of the inter-observer relative mean difference for both LEHR and ME collimators are shown in Figure 2. It shows neither bias nor trend between differences in late H/M ratio.
Table 1. Patient characteristics of study population (n=35)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male), n (%)</td>
<td>28 (80)</td>
</tr>
<tr>
<td>Age, mean ± SD (year)</td>
<td>65 ± 7.8</td>
</tr>
<tr>
<td><strong>Heart failure characteristics, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Ischaemic cardiomyopathy</td>
<td>29 (83)</td>
</tr>
<tr>
<td>Non-ischaemic cardiomyopathy</td>
<td>6 (17)</td>
</tr>
<tr>
<td>NYHA functional class, mean±SD</td>
<td>2.4 ± 0.5</td>
</tr>
<tr>
<td>LVEF, mean ± SD (%)</td>
<td>29 ± 8.4</td>
</tr>
<tr>
<td><strong>Heart rhythm, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Sinus rhythm</td>
<td>29 (83)</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>6 (17)</td>
</tr>
<tr>
<td><strong>Clinical cardiovascular risk factors, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Family history</td>
<td>18 (54)</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>8 (23)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>20 (57)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>19 (54)</td>
</tr>
<tr>
<td><strong>Medication, n (%)</strong></td>
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</tr>
<tr>
<td>Beta-blocker</td>
<td>31 (87)</td>
</tr>
<tr>
<td>ACE-I or ARB</td>
<td>33 (94)</td>
</tr>
<tr>
<td>Calciumchannel blocker</td>
<td>3 (9)</td>
</tr>
<tr>
<td>Lanoxin</td>
<td>4 (11)</td>
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<tr>
<td>Loop diuretic</td>
<td>24 (69)</td>
</tr>
<tr>
<td>Lipid-lowering agent</td>
<td>27 (77)</td>
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</table>
Table 2. Mean early and late H/M ratios determined on planar $^{123}$I-mIBG images of all observers using LEHR and ME collimators with observer defined (non-fixed) and fixed mediastinal region of interest (ROI).

<table>
<thead>
<tr>
<th></th>
<th>OBS1</th>
<th>OBS2</th>
<th>OBS3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean early H/M ratio</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LEHR collimator</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-fixed mediastinal ROI</td>
<td>1.40 ± 0.19</td>
<td>1.51 ± 0.21</td>
<td>1.60 ± 0.25</td>
</tr>
<tr>
<td>Fixed mediastinal ROI</td>
<td>1.50 ± 0.20</td>
<td>1.53 ± 0.22</td>
<td>1.58 ± 0.21</td>
</tr>
<tr>
<td>ME collimator</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-fixed mediastinal ROI</td>
<td>1.87 ± 0.42</td>
<td>2.11 ± 0.47</td>
<td>2.09 ± 0.47</td>
</tr>
<tr>
<td>Fixed mediastinal ROI</td>
<td>2.09 ± 0.45</td>
<td>2.09 ± 0.43</td>
<td>2.08 ± 0.42</td>
</tr>
<tr>
<td><strong>Mean late H/M ratio</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LEHR collimator</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-fixed mediastinal ROI</td>
<td>1.30 ± 0.18</td>
<td>1.40 ± 0.21</td>
<td>1.43 ± 0.22</td>
</tr>
<tr>
<td>Fixed mediastinal ROI</td>
<td>1.39 ± 0.22</td>
<td>1.40 ± 0.21</td>
<td>1.44 ± 0.21</td>
</tr>
<tr>
<td>ME collimator</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-fixed mediastinal ROI</td>
<td>1.65 ± 0.39</td>
<td>1.87 ± 0.48</td>
<td>1.79 ± 0.48</td>
</tr>
<tr>
<td>Fixed mediastinal ROI</td>
<td>1.86 ± 0.48</td>
<td>1.87 ± 0.46</td>
<td>1.81 ± 0.46</td>
</tr>
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</table>

OBS 1 = P.J.H., OBS 2 = V.B., OBS 3 = D.O.V.
Table 3. Lin’s Concordance Coefficient (LCC) for inter-observer variability of early and late H/M ratio determined on planar $^{123}$I-mIBG images using LEHR and ME collimator with observer defined (non-fixed) and mediastinal ROI.

<table>
<thead>
<tr>
<th></th>
<th>OBS 1 vs. OBS 2</th>
<th>OBS 2 vs. OBS 3</th>
<th>OBS 1 vs. OBS 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LEHR collimator</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analysis with non-fixed mediastinal ROI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCC Early H/M ratio</td>
<td>0.689 (0.506 – 0.811)</td>
<td>0.760 (0.597 – 0863)</td>
<td>0.484 (0.289 – 0.641)</td>
</tr>
<tr>
<td>LCC Late H/M ratio</td>
<td>0.818 (0.703 – 0.891)</td>
<td>0.940 (0.886 – 0.968)</td>
<td>0.703 (0.544 – 0.813)</td>
</tr>
<tr>
<td>Analysis with fixed mediastinal ROI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCC Early H/M ratio</td>
<td>0.915 (0.843 – 0.955)</td>
<td>0.918 (0.849 – 0.957)</td>
<td>0.850 (0.742 – 0.915)</td>
</tr>
<tr>
<td>LCC Late H/M ratio</td>
<td>0.977 (0.956 – 0.988)</td>
<td>0.962 (0.930 – 0.980)</td>
<td>0.946 (0.903 – 0.970)</td>
</tr>
<tr>
<td><strong>ME collimator</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analysis with non-fixed mediastinal ROI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCC Early H/M ratio</td>
<td>0.694 (0.513 – 0.816)</td>
<td>0.854 (0.730 – 0.923)</td>
<td>0.581 (0.347 – 0.747)</td>
</tr>
<tr>
<td>LCC Late H/M ratio</td>
<td>0.766 (0.629 – 0.858)</td>
<td>0.907 (0.831 – 0.949)</td>
<td>0.796 (0.646 – 0.886)</td>
</tr>
<tr>
<td>Analysis with fixed mediastinal ROI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCC Early H/M ratio</td>
<td>0.955 (0.915 – 0.977)</td>
<td>0.931 (0.869 – 0.965)</td>
<td>0.899 (0.811 – 0.948)</td>
</tr>
<tr>
<td>LCC Late H/M ratio</td>
<td>0.980 (0.961 – 0.989)</td>
<td>0.973 (0.949 – 0.986)</td>
<td>0.971 (0.945 – 0.985)</td>
</tr>
</tbody>
</table>

OBS 1 = P.J.H., OBS 2 = V.B., OBS 3 = D.O.V.
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Figure 2. Inter-observer variability of the late H/M ratio with fixed mediastinal ROI determined on planar $^{123}$I-MIBG images using LEHR collimator (left panel) and ME collimator (right panel). (OBS 1 = P.J.H., OBS 2 = V.B., OBS 3 = D.O.V.)
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Figure 3. Intra-observer variability (first vs. second measurement) of the late H/M ratio with fixed mediastinal ROI determined on planar 123I-mIBG images using LEHR collimator (left panel) and ME collimator (right panel).

Table 4. The inter-observer variability of late H/M ratios determined on planar 123I-mIBG images using LEHR (left panel) and ME (right panel) collimators.

<table>
<thead>
<tr>
<th></th>
<th>LEHR collimator</th>
<th>ME collimator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-fixed mediastinal ROI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBS1 vs OBS2</td>
<td>-0.10 (-0.26 – 0.06)</td>
<td>OBS1 vs OBS2 -0.23 (-0.77 – 0.21)</td>
</tr>
<tr>
<td>OBS2 vs OBS3</td>
<td>-0.03 (-0.26 – 0.10)</td>
<td>OBS2 vs OBS3 -0.10 (-0.24 – 0.44)</td>
</tr>
<tr>
<td>Fixed mediastinal ROI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBS1 vs OBS2</td>
<td>-0.13 (-0.35 – 0.09)</td>
<td>OBS1 vs OBS3 -0.13 (-0.60 – 0.34)</td>
</tr>
<tr>
<td>OBS2 vs OBS3</td>
<td>-0.02 (-0.06 – 0.02)</td>
<td>OBS2 vs OBS3 -0.01 (-0.20 – 0.18)</td>
</tr>
<tr>
<td>OBS1 vs OBS3</td>
<td>-0.05 (-0.14 – 0.04)</td>
<td>OBS1 vs OBS3 0.05 (-0.15 – 0.25)</td>
</tr>
</tbody>
</table>

The absolute mean differences (95 % limits of agreement) in the inter-observer analysis using non-fixed and fixed mediastinal ROI. OBS 1 = P.J.H., OBS 2 = V.B., OBS 3 = D.O.V.

Intra-observer variability of planar 123I-mIBG imaging
The intra-observer analysis using a predefined fixed mediastinal ROI showed a substantial agreement. The LCCs for the late H/M ratio using LEHR collimator was 0.97 and for the ME collimator 0.96, respectively. The intra-observer analysis showed a small absolute mean difference (95% limits of agreement) of -0.01 (-0.12 to 0.10) using LEHR collimator and -0.04 (-0.27 to 0.19) using medium collimator. The Bland-Altman plots of the intra-observer relative mean difference for both LEHR and ME collimators are shown in Figure 3.
DISCUSSION

This study showed that inter-observer variability of both early and late H/M ratios on planar $^{123}$I-mlBG images is largely influenced by definition of the mediastinal ROI. There was a substantial inter- and intra-observer reproducibility of late H/M ratio on planar $^{123}$I-mlBG images using a predefined fixed mediastinal ROI. However, an observer-defined mediastinal ROI negatively influenced the inter-observer reproducibility resulting in a poor inter-observer agreement. The choice of collimator had no influence on inter-observer agreement of the late H/M ratio. However, the inter-observer agreement of the early H/M ratio improved when using a ME collimator.

In general, our findings are comparable with the publication of Veltman et al. They reported a low variability of early and late H/M ratio determined on planar $^{123}$I-mlBG myocardial scintigraphy in CHF patients (age 64.5 ± 8.7 years, LVEF 26 ± 7.4, ischaemic CMP 63%) using a fixed mediastinal ROI. The inter-observer analysis showed an intraclass correlation coefficients (ICC) for late H/M ratio of 0.98 (0.97 - 0.99). However, this study was performed with only a LEHR collimator. The current study demonstrates that measurement of the late H/M ratio on planar $^{123}$I-mlBG myocardial scintigraphy is reliable and independent of collimator choice.

It has been demonstrated that late H/M ratio is a good prognostic indicator independent of other clinical used parameters such as left ventricular ejection fraction (LVEF). Therefore, $^{123}$I-mlBG myocardial scintigraphy could assist in a more individualized medical therapy in patients with CHF. Although the prognostic value of $^{123}$I-mlBG myocardial scintigraphy has been demonstrated, the technique has not been fully implemented in the clinical arena. This might be explained by the fact that there is considerable variation in the methods to obtain H/M ratios. This variation can be caused by acquisition parameters such as collimator choice, acquisition time, and duration. Inter-observer variation may also depend on the size and location of the cardiac and mediastinal ROI. Therefore, standardization of acquisition and post-processing analysis as proposed by Flotats et al. is essential. Improved standardization of cardiac $^{123}$I-mlBG imaging protocols would contribute to increased clinical applicability of $^{123}$I-mlBG scintigraphy.

The mediastinal ROI on planar cardiac $^{123}$I-mlBG scintigraphy reflects non-specific mediastinal activity. This region is regarded as a good reference for the qualification of cardiac sympathetic activity because it has less scatter from other organs and the low sympathetic activity in the mediastinum. Our study shows that the size and location of cardiac and mediastinal ROI can contribute to the variation of H/M ratio significantly. A rectangular mediastinal ROI is recommended. The size of the mediastinal ROI depends on the matrix size (128 × 128 or 256 × 256) and varies in the literature. A smaller mediastinal ROI leads to a more precise estimation of non-specific mediastinal uptake and is less influenced by non-specific scatter from uptake in the...
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lungs. Therefore, in theory a relatively small ROI would lead to a more appropriate estimation of the non-specific mediastinal uptake (i.e., lower mediastinal count density). However, the disadvantage of a smaller ROI is that it is more susceptible to sample variability in low-count regions such as the mediastinum. The use of a smaller ROI requires, therefore, a more precise and preferably predefined placement, based on “anatomical” landmarks. Our study showed that the size and positioning of the mediastinal ROI varies when observer dependent and that these differences resulted in variation of H/M ratios between the observers. Using a predefined fixed rectangular mediastinal ROI minimized this variation and resulted in an increased reproducibility and decreased variation between observers.

In this study, all three reviewers scored a lower early and late H/M ratio derived for the planar 123I-mIBG images assessed with the LEHR collimator compared to the ME collimator (Table 2). A collimator is a pivotal part of a gamma camera and constitutes of a leaden slate with holes and septa. These septa allow only those photons traveling parallel to the septa to pass through and be recorded by the crystal. Photons not traveling parallel to the septa are filtered out by the septa and therefore the thickness of the collimator septa is a major determinant of photon-penetration and image quality. In general, LEHR collimators are widely available and frequently used for 123I-mIBG imaging. However, in addition to the main photopeak of 159 keV 123I has a low-abundance of high-energy photons (approximately 3% with a photon energy of 529 keV). These high-energy photons penetrate the LEHR septa, resulting in a decreased image quality, especially in the presence of high count sources in the direct vicinity such as the liver and lungs. It has been described that this scatter affects the H/M ratios substantially. The scatter issue can be solved by using a ME collimator. In general, these collimators have thicker septa and are therefore better equipped to stop the high-energy 123I photons. Indeed, the decrease in scatter provides higher values of the H/M ratios for ME compared to LEHR collimators. However, the question whether these ME collimator obtained higher H/M ratios are a better reflection of true myocardial sympathetic activity remains to be answered. In addition, ME collimators are not as widely available as LEHR collimators, hampering standardized implementation of this solution.

The inter-observer variability of the early and late H/M ratio is largely affected by size and place of mediastinal ROI and less by the choice of collimator type. Compared to the LEHR collimator, the ME collimator slightly increased the inter-observer agreement for late H/M ratio from moderate to substantial (Table 3). An explanation would be the lack of scattering of high-energy photons from liver and lungs using the ME collimator resulting in better contrast between mediastinal/myocardium and liver/lung. In turn this increased contrast could improve the accuracy and reduce variation in drawing and placing of mediastinal and cardiac ROI and thereby reduce the variation in the late H/M ratio.
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To allow for a direct comparison between the LEHR and ME collimator derived results the mean difference between the observers was divided by the mean difference between of two observers by the mean H/M ratio of two observers. Interestingly, the Bland-Altman plots of this “corrected” relative mean difference between all three observers showed a greater variation using ME collimator compared to LEHR collimator (Figure 2). This is probably explained by the fact that the mediastinal and myocardial ROI when using a LEHR collimator has higher total counts as a result of scattering of high-energy photons from liver and lungs compared to ME collimator use. ROI’s with lower total counts will have more variability compared with ROI’s with high total counts. This effect would result in a regression to the mean on the LEHR obtained images and could explain the difference in variation between LEHR and ME collimator use.

LIMITATION

The primary limitation of the present study is the relatively small number of subjects included. However, as already stated our results are in line with the publication of Veltman et al.11 It seems therefore that our conclusions with regard to the mediastinal ROI definition are valid. Second, in this study only the intra and inter-observer variability on planar $^{123}$I-mIBG images was assessed. The intra- and inter-observer variability analyses on myocardial $^{123}$I-mIBG SPECT were not performed in this current study. The SPECT images give information on the regional sympathetic innervation/activity. This regional information appears to be of additional clinical value to the planar-derived parameters. For a proper understanding of these SPECT images, the variability of myocardial $^{123}$I-mIBG SPECT imaging needs to be assessed in future studies.

CONCLUSION

Inter-observer variability of both early and late H/M ratios on planar myocardial $^{123}$I-mIBG images is largely dependent on the definition of the mediastinal ROI. A predefined fixed mediastinal ROI showed a substantial agreement between observers, independent of collimator choice. In addition, compared to LEHR collimated acquisitions the ME collimated acquisitions resulted in higher H/M ratios. However, the choice of collimator type did not have a large impact on intra- and inter-observer variability.
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REFERENCES


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Polymorphism of SLC6A2 gene does not influence outcome of myocardial $^{123}\text{I-}m\text{IBG}$ scintigraphy in patients with chronic heart failure

DO Verschure
F Baas
BL van Eck-Smit
GA Somsen
HJ Verberne
Chapter 5

ABSTRACT

Aim
The norepinephrine transporter (NET), encoded by SLC6A2, is responsible for presynaptic NE-reuptake. $^{123}$I-mIBG is clinically used to evaluate cardiac sympathetic function. However, it is unknown if polymorphism of SLC6A2 influences cardiac sympathetic activity as assessed with $^{123}$I-mIBG. Therefore, we studied the influence of SLC6A2 SNPs on myocardial $^{123}$I-mIBG parameters in CHF.

Materials and Methods
Forty-nine adults with stable CHF (age 66.5 ± 8.1 years, LVEF 22.3 ± 6.4) were enrolled. Fifteen minutes (early) and 4 hours (late) after administration of $^{123}$I-mIBG planar images were acquired. The H/M ratio was calculated from the manually drawn ROI over the left ventricle and a fixed mediastinal ROI. Fourteen exons of the SLC6A2 gene were analyzed from whole blood samples.

Results
We found 6 different SLC6A2 SNPs, although none were functional. LVEF was the only independent predictor for early (adjusted $R^2 = 0.063$, $p = 0.045$) and late H/M ratio (adjusted $R^2 = 0.116$, $p = 0.010$). NT-proBNP was the only independent predictor for $^{123}$I-mIBG WO (adjusted $R^2 = 0.074$, $p = 0.032$). SLC6A2 SNPs were not associated with any myocardial $^{123}$I-mIBG-derived parameter.

Conclusion
In this specific CHF population, polymorphism of SLC6A2 gene was not associated with any $^{123}$I-mIBG-derived parameters.
INTRODUCTION

Norepinephrine (NE) is the neurotransmitter of the cardiac sympathetic system and is stored in vesicles in the presynaptic nerve terminals. On the basis of tissue NE content, the heart is characterized by dense sympathetic innervation with a gradient from atria to base of the heart and from base to apex of the ventricles. Only a small amount of the released NE in the synaptic cleft is available to stimulate the post-synaptic β-adrenergic receptors (β-AR) on the myocytes. Most of the NE undergoes reuptake into the nerve terminals via uptake-1 mechanism. This transport system, i.e. norepinephrine transporter (NET), is sodium- and chloride-dependent and responsible for approximately 70 – 90% of the NE re-uptake from the sympathetic cleft. Genetic or acquired defects of the NET could affect the NE homeostasis and cause alterations in synaptic NE levels with consequently alterations in β-AR stimulation. The NET is a member of solute carrier family 6 (SLC6A2) and is encoded by the SLC6A2 gene located on human chromosome 16q12.2 This gene is encoded by 16 exons which span 45 kb from the start to the stop codon. Single-nucleotide polymorphisms (SNPs) of the SLC6A2 gene which result in amino acid substitutions have been reported. Many of these variations were derived from specific psychiatric and cardiovascular phenotypes and only a limited number have been examined for alterations in function.6-9 In a familial form of idiopathic postural orthostatic tachycardia syndrome (POTS) a SNP of the SLC6A2 gene in exon 9 that resulted in loss of function of the NET was associated with increased NE plasma levels and increased heart rate.10,11 The cardiac sympathetic system is one of the neurohormonal compensation mechanisms that plays an important role in the pathogenesis of chronic heart failure (CHF). Patients with CHF have increased cardiac sympathetic activity with increased exocytosis of NE from the presynaptic vesicles, as well as increased plasma and urinary levels of NE concomitant with the severity of left ventricular dysfunction. In addition, the NE re-uptake via the NET is decreased resulting in elevated synaptic levels of NE. Initially, β-adrenergic receptor stimulation by increased NE levels helps to compensate for impaired myocardial function, but long-term NE excess has detrimental effects on myocardial structure and gives rise to a down regulation of post-synaptic β-adrenergic receptors. This down regulation leads to left ventricle remodeling and poor prognosis.

123I-mIBG, a NE analog, shares the same presynaptic uptake, storage and release mechanisms as NE. Radiolabeling of mIBG with 123I allows imaging with gamma cameras. Myocardial 123I-mIBG scintigraphy is a reliable non-invasive imaging technique to assess cardiac sympathetic activity and has been shown to be of clinical value, especially for the assessment of prognosis, in many cardiac diseases. However, there are several factors that influence the cardiac 123I-mIBG-derived parameters (e.g. choice of collimator and acquisition duration). It is conceivable that polymorphisms of the SLC6A2 gene might also influence these cardiac 123I-mIBG-derived parameters. Therefore, the aim of this study was to investigate the relation between polymorphisms of the SLC6A2 gene and presynaptic NE uptake in CHF patients as assessed with myocardial 123I-mIBG scintigraphy.
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MATERIAL AND METHODS

Subjects
Subjects with stable CHF eligible for implantable cardioverter device (ICD) implantation for primary prevention of sudden cardiac death, who were referred for $^{123}$I-$m$IBG scintigraphy to the department of nuclear medicine of the Academic Medical Center, in the period December 2010 - September 2015, were asked to participate. The principal study inclusion criteria were both ischaemic and non-ischaemic heart failure patients with New York Heart Association (NYHA) functional class II or III and LVEF < 35% as assessed with echocardiography. All subjects were treated with optimal medical therapy according to the European heart failure guidelines including, beta-blockers and angiotensin-converting-enzyme inhibitors (ACE-I) or angiotensin II receptor blockers (ARB) and when necessary loop diuretics. Exclusion for participation was pregnancy or intolerance for iodine. The study was approved by the local institutional review board and conducted according to the principles of the International Conference on Harmonization–Good Clinical Practice.

Genotyping
The deoxyribonucleic acid (DNA) of the subjects was extracted from whole-blood samples using standard protocols. In total 14 exons of the SLC6A2 gene were analysed by Sanger sequencing using BigDye terminator chemistry on a 3730XL capillary sequencer. Sequence traces were analysed in Codoncode Aligner software with the reference sequence NM: 001172504.1. Analysis was performed by an experienced observer blinded to patient data. The sequence variants were analysed for predicted effect on splicing using the Alamut software suite (Interactive Biosystems, France).

$^{123}$I-$m$IBG scintigraphy acquisition and analysis
To block uptake of free $^{123}$I by the thyroid gland, subjects were pre-treated with 250 mg oral potassium iodide 30 min before intravenous (IV) injection of 185 MBq $^{123}$I-$m$IBG. Fifteen minutes (early acquisition) and 4 hours (late acquisition) after administration of $^{123}$I-$m$IBG, 10-min planar images were acquired with the subjects in supine position using a medium energy collimator.

All planar $^{123}$I-$m$IBG images were analysed by one experienced observer (D.O.V.) blinded to patient data. Heart-to-mediastinum (H/M) ratios were calculated from the $^{123}$I-$m$IBG images using a region of interest (ROI) over the heart and the upper part of the mediastinum. The cardiac ROI was manually drawn over the myocardium including the left ventricular cavity. A fixed rectangular mediastinal ROI was placed on the upper part of the mediastinum. The location of the mediastinal ROI was determined in relation to the lung apex, the lower boundary of the upper mediastinum, and the midline between the lungs. The H/M ratio was calculated by dividing the mean count...
density in the cardiac ROI by the mean count density in the mediastinal ROI. The $^{123}$I-mIBG washout (WO) was calculated using the early and late H/M ratio with the following formula:

$$WO = \left( \frac{\text{early H/M ratio} - \text{late H/M ratio}}{\text{early H/M ratio}} \right) \times 100$$

The H/M ratio reflects presynaptic uptake of $^{123}$I-mIBG. The early H/M ratio reflects predominantly the integrity of sympathetic nerve terminals (i.e. number of functioning nerve terminals and intact uptake-1 mechanism). The late H/M ratio offers predominantly information about neuronal function resulting from uptake, storage and release. The $^{123}$I-mIBG WO reflects predominantly neuronal integrity of sympathetic tone/adrenergic drive.

**Statistical Analysis**

All continuous variables are expressed as a mean ± standard deviation. After demonstrating a normal distribution of variables, between-group comparisons were performed by using independent-sample t-tests. Differences between groups for continuous data were compared using analysis of variance (ANOVA). Multivariate regression analysis was performed to determine independent predictors of $^{123}$I-mIBG outcomes. Haplotype, genotype (the combination of 2 haplotypes), LVEF, N-terminal pro B-type Natriuretic Peptide (NT-proBNP) and functional class NYHA were used as explanatory variables. The overall goodness-of-fit for each model was expressed as the adjusted $R^2$. A p-value < 0.05 was considered to indicate a statistically significant difference. Statistical analyses were performed with SPSS, release 22.0 for Windows (SPSS Inc., Chicago, IL, USA 2003).
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Table 1. Baseline characteristics CHF patients

<table>
<thead>
<tr>
<th></th>
<th>Total ($n = 49$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>66 ± 8</td>
</tr>
<tr>
<td><strong>Sex, male (%)</strong></td>
<td>39 (80)</td>
</tr>
<tr>
<td><strong>Body mass index (kg/m²)</strong></td>
<td>27.5 ± 4.4</td>
</tr>
<tr>
<td><strong>LVEF (%)</strong></td>
<td>27.5 ± 4.4</td>
</tr>
<tr>
<td><strong>Heart rate (beats/min)</strong></td>
<td>76 ± 15</td>
</tr>
<tr>
<td><strong>Systolic BP (mmHg)</strong></td>
<td>127 ± 18</td>
</tr>
<tr>
<td><strong>Diastolic BP (mmHg)</strong></td>
<td>77 ± 11</td>
</tr>
<tr>
<td><strong>NYHA functional class</strong></td>
<td></td>
</tr>
<tr>
<td>II (%)</td>
<td>36 (73)</td>
</tr>
<tr>
<td>III (%)</td>
<td>13 (27)</td>
</tr>
<tr>
<td><strong>Etiology heart failure</strong></td>
<td></td>
</tr>
<tr>
<td>Ischemic (%)</td>
<td>28 (57)</td>
</tr>
<tr>
<td>Non-ischemic (%)</td>
<td>21 (43)</td>
</tr>
<tr>
<td><strong>Medical history</strong></td>
<td></td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>23 (47)</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>13 (27)</td>
</tr>
<tr>
<td><strong>Laboratory results</strong></td>
<td></td>
</tr>
<tr>
<td>NT-pro BNP (ng/L)</td>
<td>2109 ± 3169</td>
</tr>
<tr>
<td>$^{123}$I-mIBG scintigraphy</td>
<td></td>
</tr>
<tr>
<td>Early H/M ratio</td>
<td>2.11 ± 0.39</td>
</tr>
<tr>
<td>Late H/M ratio</td>
<td>1.81 ± 0.39</td>
</tr>
<tr>
<td>$^{123}$I-mIBG WO</td>
<td>13.8 ± 11.3</td>
</tr>
</tbody>
</table>

RESULTS

Subjects
Table 1 shows the characteristics of the study population. A total of 49 CHF subjects (80% men) were enrolled with a mean age of 66 ± 8 years and a mean LVEF of 22.3 ± 6.4%. The mean early H/M ratio was 2.11 ± 0.39, late H/M ratio was 1.81 ± 0.39 and $^{123}$I-mIBG WO was 13.8% ± 11.2%. 
SLC6A2 polymorphism and 123I-mIBG scintigraphy

**Genotyping**

Analysis of the SLC6A2 gene showed 6 different SNPs in 47 subjects (in 2 subjects no SNPs were found): c.1148-13A>C (rs5568), c.1287G>A p.Thr429Thr (rs5569), c.1389+9G>A (rs998424), c.1590+23T>C (rs1800887), c.1830+66C>T (rs2242447), c.1831-122T>A (rs6499773) (Figure 1). Only SNP rs5569 was located in an exon and was synonymous. All other SNPs were located in a non-coding area. None of the SNPs were functional (i.e., causing a change in amino acid or affecting splicing). In this study population ten different haplotypes could be constructed from the 6 founded SNPs (Figure 2) resulting in 22 different genotypes. The alleles of two SNPs rs5568 and rs2242447 showed linkage disequilibrium. Another fixed inherited combination of the SNPs is rs5569, rs998424 and rs2242447 showing high linkage disequilibrium.

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**Figure 1.** Relative contribution (%) of the six different single-nucleotide polymorphisms (SNPs) of the SLC6A2 gene in the study population (n = 49).

**Figure 2.** Relative contribution of haplotypes in 49 CHF patients including two times 49 alleles (n = 98). Ref. = reference allele without any SNPs; 1 = rs5568; rs2242447; 2 = rs5568; rs5569; rs998424; rs2242447; 3 = rs5569; rs998424; rs1800887; rs6499773; 4 = rs5569; rs998424; rs2242447; 5 = rs5569; rs1800887; rs2242447; 6 = rs1800887; 7 = rs1800887; rs6499773; 8 = rs2242447; 9 = rs6499773
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Table 2. Multivariate analysis of possible independent predictors of early H/M ratio, late H/M ratio and $^{123}$I-mIBG WO ($n = 49$).

<table>
<thead>
<tr>
<th>$^{123}$I-mIBG parameters</th>
<th>Independent predictor</th>
<th>Adjusted $R^2$</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early H/M ratio</td>
<td>LVEF</td>
<td>0.063</td>
<td>0.045</td>
</tr>
<tr>
<td>Late H/M ratio</td>
<td>LVEF</td>
<td>0.116</td>
<td>0.010</td>
</tr>
<tr>
<td>$^{123}$I-mIBG WO</td>
<td>NT-proBNP</td>
<td>0.074</td>
<td>0.032</td>
</tr>
</tbody>
</table>

Multivariate regression analysis

Multivariate regression analysis using haplotype, genotype, LVEF, NT-proBNP and functional NYHA class did not show any relation of haplotype or genotype with early and late H/M ratios nor $^{123}$I-mIBG WO. LVEF was the only independent predictor of early H/M ratio (adjusted $R^2 = 0.063$, $p = 0.045$) and late H/M ratio (adjusted $R^2 = 0.116$, $p = 0.010$). (Table 2) In addition, NT-proBNP was the only independent predictor for $^{123}$I-mIBG WO (adjusted $R^2 = 0.074$, $p = 0.032$).

DISCUSSION

To the best of our knowledge, this is the first time the relationship between SLC6A2 polymorphism and cardiac sympathetic activity has been studied. Although 6 SNPs of the SLC6A2 gene were found in this study, there was no relationship between these SNPs and cardiac sympathetic activity as assessed with $^{123}$I-mIBG.

The ME-collimator derived mean early and late H/M ratio in this CHF population were lower compared to ME-collimator derived mean early and late H/M ratio in healthy subjects. Recently, corrected mean values for ME-collimator derived early and late H/M ratio in healthy subjects have been reported (3.1 [2.2 - 4.0] and 3.3 [2.2 - 4.4], respectively). Compared to other CHF populations $^{123}$I-mIBG WO was relatively low. In part, this may be related to differences in WO calculation. However is may also be a reflection of the stable condition and adequate medical treatment of our patients.

Functional missense mutation in the SLC6A2 gene ( Ala457Pro) resulting in only 2% of the NET activity with consequently increase in NE plasma levels has been reported in a familial form of POTS. In addition, inhibition of NE uptake with atomoxetine worsens the symptom burden in subjects with POTS suggesting the important role of NE uptake in this syndrome. In essential hypertension myocardial NE uptake is impaired. Although hypertension is multifactorial it is conceivable that functional SLC6A2 SNPs affect blood pressure. SNPs of SLC6A2 have been identified and, only rs168924 was
SLC6A2 polymorphism and $^{123}$I-mIBG scintigraphy

associated with the incidence of essential hypertension. The discovery of the linkage with SLC6A2 gene mutations in POTS and hypertension resulting in decreased NE uptake activity suggests that a faulty NET may lead to an impaired cardiac $^{123}$I-mIBG uptake. Interestingly, there are differences between different organs in NE spillover. In general, the myocardial NE re-uptake is very efficient and only 2-3% of the systemic NE spillover (i.e. plasma) can be attributed to myocardial origin. As NE re-uptake mainly depends on NET, these data suggest that the myocardial SLC6A2 (i.e. NET) expression/activity level is higher compared to other tissues.

We assumed that polymorphism of the SLC6A2 gene could influence the NE uptake and consequently explain variation in the $^{123}$I-mIBG-derived parameters. In this CHF population there were 6 SNPs. Although most of these SNPs occur frequently (Table 3), none of these SNPs caused a change in amino acid or affect splicing. Therefore it was not surprising that variation in early H/M ratio, late H/M ratio or $^{123}$I-mIBG WO could not be explained by the different haplotypes.

LVEF and NT-proBNP were moderately, but significantly related to $^{123}$I-mIBG-derived parameters. It has been shown that BNP modulates autonomic nervous function by inhibiting cardiac sympathetic activity in CHF. As in CHF, prolonged increased cardiac sympathetic activity has a detrimental effect on the contractility of the myocardium and thereby negatively influences the LVEF.

Our study has some limitations. The sample size of the study is relatively small and may have resulted in a limited number of different haplotypes and statistical powers. In addition, the SNPs identified in our study were not functional (i.e. no change in amino acid). Therefore the effect of functional SNPs of the SLC6A2 gene to cardiac sympathetic activity assessed by $^{123}$I-mIBG scintigraphy remains unanswered.

The results of this study suggests that SNPs of SLC6A2 at non-slices sites do not affect the $^{123}$I-mIBG uptake. Consequently polymorphism of SLC6A2 is not a confounder of the myocardial $^{123}$I-mIBG-derived parameters in this population. However, extrapolation of these findings to the overall CHF population should be done with care.

Table 3. Frequency (%) worldwide and in Europe of the 6 SNPs founded in our study population.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Worldwide</th>
<th>Europe</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs5568</td>
<td>74.8</td>
<td>64.4</td>
</tr>
<tr>
<td>rs5569</td>
<td>76.7</td>
<td>64.0</td>
</tr>
<tr>
<td>rs998424</td>
<td>77.1</td>
<td>64.0</td>
</tr>
<tr>
<td>rs1800887</td>
<td>71.0</td>
<td>78.4</td>
</tr>
<tr>
<td>rs2242447</td>
<td>52.3</td>
<td>31.2</td>
</tr>
<tr>
<td>rs6499773</td>
<td>80.8</td>
<td>85.1</td>
</tr>
</tbody>
</table>
Chapter 5

In conclusion, the results of this study showed that in this specific CHF population the variation in $^{123}$I-mIBG-derived parameters cannot be explained by polymorphism of the SLC6A2 gene.

**New knowledge gained**

The current data suggest that functional polymorphism of the SLC6A2 gene seems less common in our CHF population compared to patients with hypertension or POTS. In addition, $^{123}$I-mIBG-derived parameters are more related to common prognostic parameters such as LVEF and NT-proBNP, than polymorphism of the SLC6A2 gene.
SLC6A2 polymorphism and 123I-mIBG scintigraphy

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Vascular time-activity variation in patients undergoing $^{123}\text{I}$-mIBG myocardial scintigraphy: implications for quantification of cardiac and mediastinal uptake

HJ Verberne
DO Verschure
GA Somsen
BL van Eck-Smit
AF Jacobson
Chapter 6

ABSTRACT

Aim
For the quantification of cardiac $^{123}$I-meta-iodobenzylguanidine ($^{123}$I-mIBG) uptake, the mediastinum is commonly used as a reference region reflecting nonspecific background activity. However, variations in the quantity of vascular structures in the mediastinum and the rate of renal clearance of $^{123}$I-mIBG from the blood pool may contribute to increased inter-individual variation in uptake. This study examined the relationship between changes in heart (H) and mediastinal (M) counts and the change in vascular $^{123}$I-mIBG activity, including the effect of renal function.

Material and methods
Fifty-one subjects with ischaemic heart disease underwent early (15 min) and late (4 h) anterior planar images of the chest following injection of $^{123}$I-mIBG. Vascular $^{123}$I-mIBG activity was determined from venous blood samples obtained at 2 min, 15 min, 35 min, and 4 h post-injection. From the vascular clearance curve of each subject, the mean blood counts/min per ml at the time of each acquisition and the slope of the clearance curve were determined. Renal function was expressed as the estimated creatinine clearance (e-CC) and the estimated glomerular filtration rate (e-GFR). Relations between H and M region of interest (ROI) counts/pixel, vascular activity, and renal function were then examined using linear regression.

Results
Changes in ROI activity ratios between early and late planar images could not be explained by blood activity, the slope of the vascular clearance curves, or estimates of renal function. At most 3% of the variation in image counts could be explained by changes in vascular activity ($p = 0.104$). The e-CC and e-GFR could at best explain approximately 1.5% of the variation in the slopes of the vascular clearance curve ($p = 0.194$).

Conclusion
The change in measured H and M counts between early and late planar $^{123}$I-mIBG images is unrelated to intravascular levels of the radiopharmaceutical. This suggests that changes in M counts are primarily due to decrease in soft tissue activity and scatter from the adjacent lungs.
INTRODUCTION

The myocardial sympathetic nervous system is activated in patients with heart failure (HF) and this activation has been shown to be associated with increased mortality.¹ Cardiac sympathetic hyperactivity can be scintigraphically visualized by ¹²³I-meta-iodobenzylguanidine (mIBG), a radiolabeled analogue of noradrenalin. This noninvasive technique has been demonstrated to be a powerful prognostic marker in HF patients.²⁻⁴ In myocardial ¹²³I-mIBG imaging, the most commonly used quantitative parameters are the heart-to-mediastinum (H/M) ratio and ¹²³I-mIBG washout (WO) determined from planar images. The H/M ratio is a measure of specific to nonspecific uptake, while the ¹²³I-mIBG WO is a measure of neuronal integrity. A basic assumption associated with use of the mediastinum as a reference for H/M ratio and ¹²³I-mIBG WO calculations is that the counts in this region represent nonspecific binding of the radioligand.

One potential confounder for assessment of nonspecific ¹²³I-mIBG background activity is residual tracer in the blood. Since the mediastinum contains a relatively large volume of vascular structures, intravascular ¹²³I-mIBG activity may contribute to total mediastinal activity which may influence the quantification of the H/M ratio. Since the clearance rate of ¹²³I-mIBG from the blood is largely dependent on renal function⁵⁻⁶, and renal dysfunction is often present in HF patients, differences in the rate of vascular clearance may also contribute to increased inter-individual variation in uptake quantification.⁷⁻⁸

The objective of this study was to assess the magnitude of the influence of residual vascular ¹²³I-mIBG activity on image quantification by examining the relationship between changes in vascular activity and changes in heart (H) and mediastinal (M) activity between early and late planar ¹²³I-mIBG images.

MATERIALS AND METHODS

As part of a prospective multicentre trial⁹, 51 subjects (48 male, 3 female; mean age 65.2) with ischaemic heart disease (history of ≥ 1 myocardial infarction) underwent myocardial ¹²³I-mIBG imaging. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by each institution’s human research committee. All subjects signed informed consent before performance of any study procedure.

Imaging procedures
All subjects received 370 MBq (10 mCi; ± 10%) of ¹²³I-mIBG (AdreView, GE Healthcare) and underwent 10-min planar images of the anterior thorax at 15 min (early; e) and 4
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h (late; l) post-injection (p.i.). The images were acquired with a 20% energy window centered at 159 keV, low-energy high-resolution (LEHR) collimation, and stored in a 128 × 128 matrix. All digital image files were sent to an Imaging Core Laboratory (ICL) for evaluation and analysis. Image analysis An experienced nuclear medicine technologist processed all the planar images to determine the H and M count densities. The heart region of interest (ROI) was drawn manually to include both ventricles and any atrial activity that was clearly visible. A 7 × 7 pixel square mediastinal ROI was drawn in the upper mediastinum, using the apices of the lungs as anatomic landmarks.

Activity per ROI (mean counts/pixel) was corrected for decay to the time of injection and expressed as activity in the myocardium and mediastinum at 15 min and 4 h p.i. (H_e, H_l, H/H_e, M_e, M_l, and M/M_e). In addition, commonly used semiquantitative 123I-mIBG myocardial parameters were assessed to better describe the clinical condition of the subjects. Early (15 min p.i.) and late (4 h p.i.) H/M ratios were calculated as the ratios of the mean counts per pixel in the two ROIs. All images and ROIs were reviewed by three independent readers, and a single aggregate H/M ratio was derived for each image, either the value accepted by at least two readers, or if this criterion was not satisfied, the average H/M ratio for all readers. In addition, myocardial 123I-mIBG WO was calculated as:

\[ WO = \left\{ \frac{\text{early H/M ratio} - \text{late H/M ratio}}{\text{early H/M ratio}} \right\} \times 100 \]

Vascular activity

Blood samples (2 ml) were taken at 2 min, 15 min, 35 min, and 4 h p.i. Subsequently, 1-ml aliquots were counted in a well counter (energy peak at 159 keV with a 15% energy window). Activity (counts/min) was corrected for decay to the time of injection. For each patient a vascular clearance curve was plotted. Figure 1 shows a typical example with two distinct phases, an accelerated clearance followed by a slower clearance. The slopes of the two phases of the clearance curves were determined according to biexponential curve fitting [accelerated phase (S_f) and the slower phase (S_s), expressed as cpm/ml per min].

Based upon the vascular clearance curve, the mean activity (cpm/ml) was calculated for the time intervals during which the two planar images were acquired (15 – 25 min and 3 h 50 min – 4 h p.i.: V_e and V_l). The ratio of V_e and V_l and the ratio between the slopes of the accelerated phase (S_f) and the slower phase (S_s) of the blood clearance were then calculated.
Vascular time-activity in $^{123}$I-mIBG scintigraphy

\[ \text{Fast slope} = \frac{(C_2 - C_1)}{(T_2 - T_1)} \]

Figure 1. In this typical example of a blood activity clearance curve, there is a clear distinction between a more accelerated phase and a slower phase. The slopes of both the faster ($S_f$) and slower ($S_s$) phases were calculated as illustrated in the figure.

Determination of renal function

Serum concentrations of creatinine were determined from blood samples obtained as part of screening evaluations performed within 7 days prior to $^{123}$I-mIBG imaging. Analyses were performed at a central laboratory, with reference ranges of $75 - 111 \mu$mol/l for men and $53 - 106 \mu$mol/l for women. Renal function was estimated using two methods. Estimated creatinine clearance (e-CC) was calculated (in ml/min) using the Cockcroft-Gault equation\textsuperscript{10}:

\[
e - CC = \frac{(140 - \text{[age (years)]} \times \text{[weight (kg)]})}{\text{[serum creatinine (\mumol/L)]}} \times (1.04 \text{ for females and } 1.23 \text{ for male})
\]

Estimated glomerular filtration rate (e-GFR) was calculated using the abbreviated Modification of Diet in Renal Disease (MDRD) equation\textsuperscript{11}:

\[
e - \text{GFR} = 32788 \times \text{[serum creatinine (\mumol/L)]}^{1.154} \times \text{[age (years)]}^{0.203} \times [0.742 \text{ for females}] \times [1.212 \text{ for blacks}]
\]

e-GFR was expressed per 1.73 m\textsuperscript{2} of body surface area (ml/min per 1.73 m\textsuperscript{2}). According to the guidelines for identification, management, and referral of adults with chronic kidney disease, patients were stratified as having impaired kidney function [e-CC or e-GFR $< 60 \text{ ml/min(per 1.73 m}^2)$] or normal function [e-CC or e-GFR $\geq 60 \text{ ml/min(per 1.73 m}^2)$].\textsuperscript{12}
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Statistical analysis

Linear regression was used to examine the relationships between the vascular activity ratio ($V_l/V_e$), the slope of the vascular clearance curve ($S_f/S_s$), and the scintigraphically determined activity in the myocardium and mediastinum at 15 min and 4 h p.i. ($H_e$, $H_l$, $H_e/H_l$, $M_e$, $M_l$, and $M_e/M_l$). The overall goodness-of-fit was expressed as the adjusted $R^2$. The $F$ test was used to assess whether the analysis explained a significant proportion of the variability. A $p < 0.05$ was considered to indicate a statistically significant difference. A significant adjusted $R^2$ would indicate that variation in the scintigraphically determined parameters could be explained by a percentage (adjusted $R^2$) of change in vascular activity. All statistical analyses were performed with SPSS (SPSS for Windows, version 17.0.2, SPSS Inc., Chicago, IL, USA).

RESULTS

Demographic and cardiac medical history information for the 51 subjects included in the study is summarized in Table 1. The majority of patients was male, had New York Heart Association (NYHA) class II HF, and had left ventricular ejection fraction (LVEF) < 40%. Eight patients had no history of HF. The majority of subjects were on a combination of beta-adrenergic receptor blockers and angiotensin-converting enzyme inhibitors (ACE-I) or angiotensin II receptor blockers (ARB) (Table 1).

Estimates of renal function showed a substantial variation (Table 1). In 26 (51%) patients creatinine levels were above the normal limit (25 men and 1 woman). Calculated e-CC was < 60 ml/min in 19 (38%) patients and the calculated e-GFR was < 60 mL/min/1.73 m² in 24 (47%) patients.

There was a considerable range in the initial (i.e., fast) part of the $^{123}$I-mIBG vascular clearance curves (Figure 2). Despite this individual variation, the mean $S_f$ was more than 60 times steeper compared to the mean $S_s$ (Table 2). Mean intravascular activity (cpm/ml) decreased approximately 65% between 15 min and 4 h p.i. (Table 2). The mean scintigraphically determined myocardial and mediastinal activity (counts/min) decreased approximately 37 and 33%, respectively, between 15 min and 4 h p.i. (Table 3).
Table 1. Baseline characteristics.

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>n = 51</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>48/3 (94/6%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.3 ± 9.1 [42 - 84]</td>
</tr>
<tr>
<td>NYHA (I/II/III)</td>
<td>4/28/11 (6/55/22%)</td>
</tr>
<tr>
<td>ACC/AHA Heart Failure class (B/C)</td>
<td>15/36 (29/71%)</td>
</tr>
<tr>
<td>LVEF</td>
<td>33 ± 8 [15 - 48]</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>45 (88%)</td>
</tr>
<tr>
<td>ACE-I / ARB</td>
<td>46 (90%)</td>
</tr>
<tr>
<td>Aldactone</td>
<td>16 (32%)</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>10 (20%)</td>
</tr>
<tr>
<td>Ca channel blocker</td>
<td>12 (24%)</td>
</tr>
</tbody>
</table>

Estimates of renal function

<table>
<thead>
<tr>
<th>Creatinine (μmol/L)</th>
<th>113.4 ± 42.4 [59 - 296]</th>
</tr>
</thead>
<tbody>
<tr>
<td>e-CC (mL/min)</td>
<td>74.8 ± 29.2 [19.9 - 156.9]</td>
</tr>
<tr>
<td>e-GFR (mL/min/1.73m²)</td>
<td>66.2 ± 21.4 [19.1 - 125.6]</td>
</tr>
</tbody>
</table>

Semi-quantitative $^{123}$I-mIBG parameters

<table>
<thead>
<tr>
<th>Early H/M ratio</th>
<th>1.51 ± 0.25 [0.60 - 1.99]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late H/M ratio</td>
<td>1.44 ± 0.18 [1.05 - 1.96]</td>
</tr>
</tbody>
</table>

$^{123}$I-mIBG WO (%) 8.2 ± 6.8 [5.3 - 10.8]

Data are expressed as mean ± SD and [range] or as absolute numbers and (percentage). NYHA: New York Heart Association functional classification of heart failure; LVEF: left ventricular ejection fraction; ACC/AHA Heart failure class: American College of Cardiology and American Heart Association classification of heart failure; ACE-I: angiotensin-converting enzyme inhibitor; ARB: angiotensin II receptor blocker; e-CC: estimated creatinine clearance calculated using the Cockcroft-Gault equation; e-GFR: estimated glomerular filtration rate calculated using the abbreviated Modification of Diet in Renal Disease (MDRD) equation.
Variation in any of the scintigraphic parameters ($M_e$, $M_l$, $M_e/M_l$, $H_e$, $H_l$, and $H_e/H_l$) could not be explained by intravascular activity (Table 4). At most the counts in the blood at 15 min ($V_e$) could explain approximately 3% of the variation in the mediastinal counts at 15 min ($M_e$) ($p = 0.120$). Variation of the scintigraphic parameters could also not be explained by the slope of the clearance curves (Table 5). The slope of the clearance curves could at best explain up to 3% of the variation in the mediastinal counts ($M_e$ vs $S_f$ and $M_l$ vs $S_s$, $p = 0.105$ and $p = 0.100$, respectively).

The variability in the change of intravascular activity ($S_r$, $S_s$, and $S_r/S_s$) could not be explained by either estimate of renal function (e-CC or e-GFR) (Table 6). The e-CC could at best explain approximately 1.5% of the variation in the fast slope of the vascular clearance curve (e-CC vs $S_r$, $p = 0.194$).

Figure 2. Scatter plot showing the blood activity in relation to time for all subjects.
Vascular time-activity in ¹²³I-mIBG scintigraphy

Table 2. Slope of blood activity clearance curve (biexponential curve fitting).

<table>
<thead>
<tr>
<th></th>
<th>Fast part</th>
<th>Slow part</th>
<th>Ratio fast vs. slow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope of clearance</td>
<td>-0.166 ± 0.083</td>
<td>-0.003 ± 0.001</td>
<td>63.11 ± 45.00</td>
</tr>
<tr>
<td>curve (counts/min)</td>
<td>(-0.353 – -0.034)</td>
<td>(-0.007 – -0.001)</td>
<td>(13.71 – 227.12)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD and range.

Table 3. Intravascular and imaging parameters

<table>
<thead>
<tr>
<th></th>
<th>Early (15 minutes)</th>
<th>Late (240 minutes)</th>
<th>Ratio late vs. early</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravascular activity</td>
<td>93379 ± 65841</td>
<td>31702 ± 23298</td>
<td>0.35 ± 0.11</td>
</tr>
<tr>
<td>(counts/min/ml)</td>
<td>(10074 – 322169)</td>
<td>(3568 – 162250)</td>
<td>(0.13 – 0.65)</td>
</tr>
<tr>
<td>Myocardial activity</td>
<td>97 ± 41</td>
<td>61 ± 27</td>
<td>0.63 ± 0.08</td>
</tr>
<tr>
<td>(counts/min)</td>
<td>(28 – 185)</td>
<td>(17 – 132)</td>
<td>(0.47 – 1.05)</td>
</tr>
<tr>
<td>Mediastinal activity</td>
<td>64 ± 28</td>
<td>43 ± 20</td>
<td>0.67 ± 0.09</td>
</tr>
<tr>
<td>(counts/min)</td>
<td>(21 – 115)</td>
<td>(13 – 82)</td>
<td>(0.48 – 1.15)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD and range.

Table 4. Variability of scintigraphic parameters in relation to intravascular activity (linear regression).

<table>
<thead>
<tr>
<th></th>
<th>Constant</th>
<th>Stand error c</th>
<th>Coefficient b</th>
<th>Stand error b</th>
<th>Adjusted R²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>M_e vs V_e</td>
<td>564.2</td>
<td>64.8</td>
<td>0.001</td>
<td>0.001</td>
<td>0.028</td>
<td>0.120</td>
</tr>
<tr>
<td>M_l vs V_l</td>
<td>430.2</td>
<td>45.8</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.905</td>
</tr>
<tr>
<td>M_l/M_e vs V_l/V_e</td>
<td>0.67</td>
<td>0.05</td>
<td>0.014</td>
<td>0.122</td>
<td>0.006</td>
<td>0.912</td>
</tr>
<tr>
<td>H_l vs V_l</td>
<td>878.4</td>
<td>97.3</td>
<td>0.001</td>
<td>0.001</td>
<td>0.014</td>
<td>0.191</td>
</tr>
<tr>
<td>H_e vs V_e</td>
<td>602.8</td>
<td>63.2</td>
<td>0.001</td>
<td>0.002</td>
<td>0.001</td>
<td>0.822</td>
</tr>
<tr>
<td>H/H_e vs V/V_e</td>
<td>0.59</td>
<td>0.04</td>
<td>0.126</td>
<td>0.109</td>
<td>0.01</td>
<td>0.253</td>
</tr>
</tbody>
</table>

SE: standard error, M_e: scintigraphically determined mediastinal counts 15 min p.i. (early), M_l: scintigraphically determined mediastinal counts 4 h p.i. (late), H_e: scintigraphically determined myocardial counts 15 min p.i. (early), H_l: scintigraphically determined myocardial counts 4 h p.i. (late), V_e: intravascular radioactivity 15 min p.i. (early), V_l: intravascular radioactivity 4 h p.i. (late)
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Table 5. Variability of scintigraphic parameters in relation to the slopes of the blood activity clearance curve (linear regression)

<table>
<thead>
<tr>
<th></th>
<th>Constant</th>
<th>Standard error</th>
<th>Coefficient b</th>
<th>Standard error</th>
<th>Adjusted R²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>M₂ vs S₁</td>
<td>532.9</td>
<td>83.3</td>
<td>-738.779</td>
<td>447.576</td>
<td>0.033</td>
<td>0.105</td>
</tr>
<tr>
<td>M₁ vs S₂</td>
<td>542.0</td>
<td>69.4</td>
<td>33361</td>
<td>19957</td>
<td>0.032</td>
<td>0.100</td>
</tr>
<tr>
<td>M₁/M₂ vs S₂/S₁</td>
<td>0.670</td>
<td>0.022</td>
<td>4.44 10⁻⁵</td>
<td>0.022</td>
<td>0.001</td>
<td>0.875</td>
</tr>
<tr>
<td>H₁ vs S₁</td>
<td>-0.127</td>
<td>0.031</td>
<td>-3.99 10⁻⁵</td>
<td>-0.192</td>
<td>0.018</td>
<td>0.172</td>
</tr>
<tr>
<td>H₁ vs S₂</td>
<td>0.004</td>
<td>0.000</td>
<td>8.98 10⁻⁵</td>
<td>0.000</td>
<td>0.016</td>
<td>0.177</td>
</tr>
<tr>
<td>H₁/H₂ vs S₂/S₁</td>
<td>0.631</td>
<td>0.020</td>
<td>-2.21 10⁻⁵</td>
<td>0.000</td>
<td>0.001</td>
<td>0.999</td>
</tr>
</tbody>
</table>

SE standard error, M₁ scintigraphically determined mediastinal counts 15 min p.i. (early), M₂ scintigraphically determined mediastinal counts 4 h p.i. (late), H₁ scintigraphically determined myocardial counts 15 min p.i. (early), H₂ scintigraphically determined myocardial counts 4 h p.i. (late), S₁ slope of the accelerated (fast: f) part of the blood activity clearance curve, S₂ slope of the slower (s) part of blood activity clearance curve.

Table 6. Variability of estimates of renal function in relation to the slopes of the blood activity clearance curve (linear regression).

<table>
<thead>
<tr>
<th></th>
<th>Constant</th>
<th>Standard error</th>
<th>Coefficient b</th>
<th>Standard error</th>
<th>Adjusted R²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>e-CC vs S₁</td>
<td>62.9</td>
<td>8.4</td>
<td>-59.3</td>
<td>45.1</td>
<td>0.014</td>
<td>0.194</td>
</tr>
<tr>
<td>e-CC vs S₂</td>
<td>63.6</td>
<td>10.3</td>
<td>-3470.1</td>
<td>2953.7</td>
<td>0.007</td>
<td>0.245</td>
</tr>
<tr>
<td>e-CC vs S₂/S₁</td>
<td>69.9</td>
<td>6.5</td>
<td>0.052</td>
<td>0.084</td>
<td>-0.012</td>
<td>0.536</td>
</tr>
<tr>
<td>e-GFR vs S₁</td>
<td>63.5</td>
<td>6.3</td>
<td>-11.0</td>
<td>33.8</td>
<td>-0.017</td>
<td>0.746</td>
</tr>
<tr>
<td>e-GFR vs S₂</td>
<td>63.2</td>
<td>7.6</td>
<td>-947.9</td>
<td>2158.8</td>
<td>-0.015</td>
<td>0.666</td>
</tr>
<tr>
<td>e-GFR vs S₂/S₁</td>
<td>65.4</td>
<td>4.8</td>
<td>-0.002</td>
<td>0.62</td>
<td>-0.019</td>
<td>0.970</td>
</tr>
</tbody>
</table>

SE standard error, e-CC estimated creatinine clearance based on Cockcroft-Gault equation, e-GFR estimated glomerular filtration rate according to the abbreviated Modification of Diet in Renal Disease (MDRD) equation, S₁ slope of the accelerated (fast: f) part of the blood activity clearance curve, S₂ slope of the slower (s) part of blood activity clearance curve.
DISCUSSION

Most in vivo scintigraphic quantification of regional neurotransmitter activity or receptor density involves comparison of uptake in a target ROI to that in a reference region of nonspecific uptake/binding. For assessment of sympathetic myocardial activity with 123I-mIBG scintigraphy, use of the mediastinum as a reference region is based on the assumption that there is a negligible amount of specific uptake of 123I-mIBG in this region. In addition, presence of 123I-mIBG in the blood pool is also assumed to be insignificant. The present results demonstrate that changes in blood pool/vascular 123I-mIBG activity do not significantly correlate with changes in heart and mediastinum activity between early and late planar images. This is consistent with rapid clearance of 123I-mIBG from the blood and uptake into organs (such as the heart) by means of the norepinephrine transporter.

As intravascular activity appears to play no role in the variation of the counts measured in the mediastinum, those counts likely reflect a combination of mediastinal tissue activity and scatter from 123I-mIBG in adjacent organs (e.g., liver, lungs). This shows that for routine quantification, the mediastinum is acceptable as a reference region. However, these findings do not confirm that the mediastinum represents a background region with only nonspecific uptake/binding. To test this hypothesis would require a study where the specific uptake of 123I-mIBG via the presynaptic norepinephrine transporter (i.e., uptake-1) was blocked and compared to a similar study without blocking. To our knowledge this type of study has not been performed in humans.

In subjects with normal renal function, intravenously administered 123I-mIBG is almost exclusively excreted via the kidneys within 24 h after injection, with approximately 35% of administered 123I-mIBG already excreted by 6 h. There are complex interactions between sympathetic regulation of renal function and cardiac function. For example, increased sympathetic activity reduces the filtration fraction. In addition, renal dysfunction is often present in HF patients, which may further increase the inter-individual variation of blood pool clearance. Moreover, as a reduced GFR is associated with a reduced blood clearance of 123I-mIBG, the excretion of 123I-mIBG is not only dependent on filtration but also by tubular secretion. In the present study, although approximately 40% of the patients had decreased renal function, differences in the rate of renal excretion did not contribute to variability in the mediastinal and myocardial 123I-mIBG uptake. Therefore, within the typical time frame of 123I-mIBG cardiac imaging (up to 4 h after injection), 123I-mIBG mediastinal and myocardial activity determinations appear independent of the rate of blood clearance via the kidneys.

Radioactivity as measured in the blood samples may be in part explained by 123I not bound to MIBG and this may have influenced our results. However, according to specifications of the manufacturer the radiochemical purity of the 123I-mIBG used prior
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to injection is always more than 97% and in clinical practice the fraction of free $^{123}$I does not exceed 2%. Secondly, after intravenous administration, $^{123}$I-mIBG in humans is very stable and not subject to in vivo deiodination.\(^7\) In addition, the clearance rate of both $^{123}$I-mIBG and free $^{123}$I from the blood pool is rapid and is almost exclusively dependent on renal excretion. Therefore, the impact of free $^{123}$I on the present results should be negligible.

In conclusion, in patients with ischaemic heart disease, due to rapid clearance of $^{123}$I-mIBG from the blood, the heart and mediastinal count densities measured on planar $^{123}$I-mIBG images at 15 min and 4 h are not affected by residual vascular activity. In addition, this observation holds for the broad range of renal function commonly encountered in heart disease patients. Therefore, no correction for blood pool activity or renal function is needed for the calculation of myocardial $^{123}$I-mIBG WO. However, the clinical appropriateness of using mediastinal activity as a background correction in such calculations, which depend on the assumption that there is no specific uptake/binding in this region, remains to be demonstrated.
REFERENCES


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Renal function in relation to cardiac $^{123}$I-$m$IBG scintigraphy in patients with chronic heart failure

DO Verschure
GA Somsen
BL van Eck-Smit
HJ Verberne
Chapter 7

ABSTRACT

Aim
The aim of this study was to explore if estimates of renal function could explain variability of $^{123}$I-meta-iodobenzylguanidine ($^{123}$I-mIBG) assessed myocardial sympathetic activity. Furthermore, estimates of renal function were compared to $^{123}$I-mIBG as predictors of cardiac death in chronic heart failure (CHF).

Materials and method
Semi-quantitative parameters of $^{123}$I-mIBG myocardial uptake and washout (WO) were calculated using early and late heart/mediastinum (H/M) ratio and $^{123}$I-mIBG WO. Renal function was calculated as estimated Creatinine Clearance (e-CC) and as estimated Glomerular Filtration Rate (e-GFR).

Results
Thirty-nine patients with CHF (24 males; age: $64.4 \pm 10.5$ years; NYHA II/III/IV: 17/20/2; LVEF: $24.0 \pm 11.5\%$) were studied. Variability in any of the semi-quantitative $^{123}$I-mIBG myocardial parameters could not be explained by e-CC or e-GFR. During follow-up (60 ± 37 months) there were 6 cardiac deaths. Cox proportional hazard regression analysis showed that late H/M ratio was the only independent predictor for cardiac death (Chi-square 3.2, regression coefficient: $-4.095$; standard error: 2.063; hazard ratio: 0.17 [95% CI: 0.000 – 0.950]). Addition of estimates of renal function did not significantly change the Chi-square of the model.

Conclusion
Semi-quantitative $^{123}$I-mIBG myocardial parameters are independent of estimates of renal function. In addition, cardiac sympathetic innervation assessed by $^{123}$I-mIBG scintigraphy seems to be superior to renal function in the prediction of cardiac death in CHF patients.
INTRODUCTION

The myocardial sympathetic nervous system is activated in patients with chronic heart failure (CHF) and has been shown to be associated with increased mortality. Cardiac sympathetic innervation can be scintigraphically visualized by $^{123}$I-meta-iodobenzylguanidine ($^{123}$I-mIBG), a radiolabelled analog of noradrenalin and has been shown to be a powerful prognostic marker in patients with CHF. In addition to $^{123}$I-mIBG there are many other prognostic markers in patients with CHF. Estimates of renal function for example, as measured by creatinine clearance and glomerular filtration rate (GFR), have been associated with mortality and morbidity in CHF. Interestingly in patients with chronic renal failure myocardial washout (WO) of $^{123}$I-mIBG, as a measure of increased myocardial sympathetic activity, has been shown to be increased. However, there is limited data on a direct comparison of the respective prognostic predictive value of sympathetic hyperactivity and renal dysfunction. Major clinical trials aimed to assess the prognostic value of $^{123}$I-mIBG have often excluded patients with substantial renal failure, further limiting the amount of prognostic information comparing these two variables. Furthermore, there are complex interactions between sympathetic regulation of renal function and cardiac function. For example increased sympathetic activity reduces the renal filtration fraction and a reduced GFR is associated with a reduced blood clearance of $^{123}$I-mIBG. In a recent study it was shown that differences in the rate of renal excretion did not contribute to variability in the mediastinal and myocardial $^{123}$I-mIBG uptake. However, whether this reduced blood clearance of $^{123}$I-mIBG has any impact on the semi-quantitative myocardial parameters is unknown. Therefore, the purpose of this study was twofold: 1) to explore if estimates of renal function could explain variability of $^{123}$I-mIBG assessed myocardial sympathetic activity and 2) to compare the prognostic value of estimates of renal function and myocardial $^{123}$I-mIBG assessed myocardial sympathetic activity in patients with CHF.

MATERIAL AND METHODS

The study was designed to re-evaluate the results of $^{123}$I-mIBG imaging studies and renal function in patients with CHF prior to 1st of November 2006 in relation to cardiac events. Requirements for inclusion of subjects in this “retrospective” study were: availability of the original digital $^{123}$I-mIBG image files; availability of serum creatinine measurements within 1 month before $^{123}$I-mIBG scintigraphy. Between January 1, 1996 and October 31, 2006, 39 CHF patients visiting the outpatient heart failure clinic met these requirements. Renal function was estimated using the serum creatinine based Cockcroft-Gault equation (estimated Creatinine Clearance: e-CC) and the abbreviated
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MDRD equation (estimated Glomerular Filtration Rate: e-GFR). Dutch national law does not require local ethics committee approval for retrospective studies. The study complies with the Declaration of Helsinki.

CHF severity was clinically evaluated according to the New York Heart Association (NYHA) classification at the time of imaging. The census date for follow-up was set at the 1st of November 2008 (at least 24 months follow-up). The mean follow-up after 

Measures of serum creatinine

Serum concentrations of creatinine were determined according to routine hospital procedure. Reference levels for creatinine were 75-110 µmol/L for men and 65-95 µmol/L for women, respectively.

Renal function

Renal function was determined by e-CC using the Cockcroft-Gault equation and expressed as mL/min:

\[
\text{e-GFR} = \frac{140 \times (\text{age} \times \text{weight})}{(\text{serum creatinine}) \times (\text{ratio}) \times (1.04 \text{ for females and 1.23 for male})}
\]

The e-GFR was calculated using the abbreviated MDRD equation:

\[
\text{e-GFR} = 32788 \times [\text{serum creatinine (µmol/L)}]^{1.054} \times [\text{age (years)}]^{0.203} \\
\times [0.742 \text{ for females}] \times [1.212 \text{ for blacks}]
\]

e-GFR was expressed per 1.73 m² of body surface area (mL/min/1.73 m²). According to the guidelines for identification, management and referral of adults with chronic kidney disease, patients were stratified to an impaired kidney function (e-CC or e-GFR < 60 mL/min/1.73 m²) and those with a normal e-CC or e-GFR (i.e. ≥ 60 mL/min/1.73 m²).

\[123^I\text{-mIBG: acquisition and semi-quantitative analysis}\]

Patients underwent myocardial scintigraphy to determine 

\[123^I\text{-mIBG} \text{ uptake reflecting neural norepinephrine reuptake and retention. To block thyroid uptake of free } 123^I, \text{ all patients received 100 mg potassium iodide orally, one hour prior to the injection of } 123^I\text{-mIBG. After a subsequent resting period of at least 30 minutes, patients were injected intravenously with approximately 185 MBq (5 mCi) of } 123^I\text{-mIBG (GE Healthcare, Eindhoven, the Netherlands). Fifteen minutes (early imaging) and 4 h (delayed imaging) after MIBG administration, a 10-min planar anterior image of the thorax was acquired using a dual-head gamma camera (e-cam, Siemens, Hoffman Estate, Illinois, USA). A 20% energy window was centred on the 159 keV photopeak of } 123^I. \text{ Images were acquired using a medium energy collimator and stored in } 128 \times 128 \text{ matrix.}\]
Renal function and $^{123}$I-mIBG scintigraphy

An experienced nuclear medicine technologist processed all planar images on a workstation (HERMES Medical Solutions, Stockholm, Sweden). The analysis of the myocardial scintigraphy data was performed blind to clinical status and estimates of renal function. $^{123}$I-mIBG myocardial activity was measured using a manually drawn region of interest (ROI) around the LV. The positioning of the fixed mediastinal ROI was standardized in relation to the lung apex, the lower boundary of the upper mediastinum, and the midline between the lungs. To evaluate $^{123}$I-mIBG myocardial uptake the Heart/Mediastinum (H/M) ratio was calculated from the early (early H/M ratio) and delayed images (late H/M ratio). Myocardial $^{123}$I-mIBG WO was defined as the percentage of change in activity from the early and delayed images:

$$WO = \frac{(\text{early } H/M \text{ ratio} - \text{late } H/M \text{ ratio})}{\text{early } H/M \text{ ratio}} \times 100$$

Follow-up

The primary outcome was defined as cardiac death during follow-up (aggregated from: death due to acute pulmonary oedema, progressive heart failure, myocardial infarction or ventricular arrhythmia). The secondary outcome was defined as potentially lethal ventricular arrhythmias during follow-up: documented episode of spontaneous sustained ventricular tachycardia (> 30 s) ventricular tachyarrhythmia, resuscitated cardiac arrest, or appropriate ICD therapy (anti-tachycardia pacing or defibrillation).

Long-term follow-up data were obtained from at least one of three sources: visit to the outpatient clinic; review of the patient’s hospital records; personal communication with the patient’s physician. An experienced cardiologist reviewed source documents to confirm occurrence of events. The cardiologist was blinded for both the estimates of renal function and the $^{123}$I-mIBG scintigraphic data.

Statistical analysis

Mean values were tested for differences using the unpaired t-test. Linear regression was used to examine the relationship between the estimates of renal function (e-CC and e-GFR) and the $^{123}$I-mIBG scintigraphic data (i.e. early H/M ratio, late H/M ratio and $^{123}$I-mIBG WO). The overall goodness-of-fit was expressed as the adjusted $R^2$. The F-test was used to assess whether the model explained a significant proportion of the variability. A significant adjusted $R^2$ would indicate that variation in the scintigraphically determined parameters could be explained by a percentage (adjusted $R^2$) of change in estimates of renal function. Multivariate Cox proportional hazard regression analysis was used to investigate the relation between survival and the following parameters: age, gender, several CHF variables, estimates of renal function and the $^{123}$I-mIBG scintigraphic data. First, several CHF variables (left ventricular ejection fraction (LVEF), NYHA class, QRS duration) and $^{123}$I-mIBG semi-quantitative myocardial parameters were included in the model followed by estimates of renal function and the $^{123}$I-mIBG scintigraphic data.
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(i.e. early H/M ratio, late H/M ratio and $^{123}$I-mIBG WO) were entered into the model according a stepwise forward likelihood ratio based method. Secondly, the possible additional value of renal function (e-CC and e-GFR) was determined. These data were added to the first model according the enter method (forced addition to the model). Chi-square, Cox proportional hazard regression coefficient (coefficient B) and exponent (exponent B) were used to describe the model and relative contribution of the parameters to the model. Exponent B is the predicted change in hazard for a unit increase in the predictor (i.e. hazard ratio). A p-value < 0.05 was considered to indicate statistical significance. All statistical analyses were performed with SPSS (SPSS for Windows, version 16.0, SPSS Inc, Chicago, II, USA).

RESULTS

Thirty-nine patients with CHF were included in this study; all patients had stable CHF. Baseline characteristics are described in Table 1. Twenty-three patients (59%) had ischaemia related CHF and sixteen patients had non-ischaemic CHF. Patients with ischaemia related CHF had a lower LVEF compared to those with non-ischaemic CHF ($p = 0.034$). The majority was male (62%) with a mean age of 64.4 ± 10.5 years. At baseline 94.9% of patients were treated with loop diuretics, 82.1% were on angiotensin converting enzyme (ACE) inhibitor or angiotensin II receptor blocker (ARB), and 46.2% were on beta-blockers.
Renal function and ¹²³I-mIBG scintigraphy

Table 1. Patient characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Overall (n = 39)</th>
<th>Ischaemic (n = 23)</th>
<th>Non-ischaemic (n = 16)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64 ± 11</td>
<td>66 ± 10</td>
<td>61 ± 11</td>
<td>0.962</td>
</tr>
<tr>
<td>Female/Male</td>
<td>15/24</td>
<td>6/17</td>
<td>7/9</td>
<td>0.057</td>
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<tr>
<td>LVEF (%)</td>
<td>24.0 ± 11.5</td>
<td>20.7 ± 8.6</td>
<td>28.6 ± 13.6</td>
<td>0.034</td>
</tr>
<tr>
<td>NYHA class</td>
<td></td>
<td></td>
<td></td>
<td>0.351</td>
</tr>
<tr>
<td>II</td>
<td>17</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>20</td>
<td>11</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>&lt;0.001</td>
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<tr>
<td>CABG</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>0.008</td>
</tr>
<tr>
<td>PCI</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0.078</td>
</tr>
<tr>
<td>Hypertension</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>0.428</td>
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<tr>
<td>Diabetes Mellitus</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>0.727</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Beta-blocker</td>
<td>18</td>
<td>10</td>
<td>8</td>
<td>0.688</td>
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<tr>
<td>ACE-I</td>
<td>29</td>
<td>17</td>
<td>12</td>
<td>0.939</td>
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<tr>
<td>ARB</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0.778</td>
</tr>
<tr>
<td>CCB</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0.778</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>13</td>
<td>9</td>
<td>4</td>
<td>0.357</td>
</tr>
<tr>
<td>Digoxine</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td>0.312</td>
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<tr>
<td>Loop diuretic</td>
<td>37</td>
<td>22</td>
<td>8</td>
<td>0.791</td>
</tr>
<tr>
<td>ECG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QRS duration (msec)</td>
<td>163 ± 43</td>
<td>167 ± 36</td>
<td>158 ± 54</td>
<td>0.564</td>
</tr>
<tr>
<td>LBBB</td>
<td>32</td>
<td>21</td>
<td>11</td>
<td>0.116</td>
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<tr>
<td>RBBB</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.418</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>0.700</td>
</tr>
</tbody>
</table>

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Table 2. Estimates of renal function and $^{123}$I-mIBG-derived parameters.

<table>
<thead>
<tr>
<th></th>
<th>Overall ($n = 39$)</th>
<th>Ischaemic ($n = 23$)</th>
<th>Non-ischaemic ($n = 16$)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Renal function</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e-CC</td>
<td>65.7 ± 33.1</td>
<td>58.1 ± 27.5</td>
<td>78.6 ± 38.6</td>
<td>0.076</td>
</tr>
<tr>
<td>e-GFR</td>
<td>60.0 ± 25.5</td>
<td>55.1 ± 26.6</td>
<td>67.1 ± 22.7</td>
<td>0.153</td>
</tr>
<tr>
<td><strong>$^{123}$I-mIBG-derived parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early H/M ratio</td>
<td>1.61 ± 0.46</td>
<td>1.51 ± 0.32</td>
<td>1.75 ± 0.58</td>
<td>0.108</td>
</tr>
<tr>
<td>Late H/M ratio</td>
<td>1.43 ± 0.38</td>
<td>1.36 ± 0.26</td>
<td>1.54 ± 0.49</td>
<td>0.139</td>
</tr>
<tr>
<td>$^{123}$I-mIBG WO</td>
<td>10.1 ± 10.4</td>
<td>9.21 ± 10.1</td>
<td>11.4 ± 11.0</td>
<td>0.528</td>
</tr>
</tbody>
</table>

e-CC: estimated Creatinine Clearance; e-GFR: estimated Glomerular Filtration Rate; H/M ratio: heart-to-mediastinum ratio; WO: washout. See for other abbreviations Table 1.

Table 3. Normal vs. abnormal estimates of kidney function in relation to $^{123}$I-mIBG-derived parameters.

<table>
<thead>
<tr>
<th></th>
<th>&lt;60 mL/min ($n = 17$)</th>
<th>≥60 mL/min ($n = 18$)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>e-CC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early H/M ratio</td>
<td>1.45 ± 0.36</td>
<td>1.74 ± 0.49</td>
<td>0.490</td>
</tr>
<tr>
<td>Late H/M ratio</td>
<td>1.29 ± 0.29</td>
<td>1.54 ± 0.41</td>
<td>0.370</td>
</tr>
<tr>
<td>$^{123}$I-mIBG WO</td>
<td>9.9 ± 11.1</td>
<td>10.3 ± 10.0</td>
<td>0.915</td>
</tr>
<tr>
<td><strong>e-GFR</strong></td>
<td>&lt; 60 mL/min/1.73 m$^2$ ($n = 23$)</td>
<td>≥ 60 mL/min/1.73 m$^2$ ($n = 16$)</td>
<td>$p$-value</td>
</tr>
<tr>
<td>Early H/M ratio</td>
<td>1.57 ± 0.42</td>
<td>1.67 ± 0.51</td>
<td>0.492</td>
</tr>
<tr>
<td>Late H/M ratio</td>
<td>1.38 ± 0.39</td>
<td>1.51 ± 0.36</td>
<td>0.309</td>
</tr>
<tr>
<td>$^{123}$I-mIBG WO</td>
<td>11.2 ± 12.2</td>
<td>8.5 ± 7.0</td>
<td>0.432</td>
</tr>
</tbody>
</table>

See for abbreviations Table 1 and Table 2.
Renal function and 123I-mIBG scintigraphy

The mean early H/M ratio was 1.61 ± 0.46, the mean late H/M ratio was 1.43 ± 0.38 and the mean 123I-mIBG WO was 10.1 ± 10.4% (Table 2). There was no difference in the 123I-mIBG semi-quantitative parameters or in the e-CC and e-GFR between ischaemic and non-ischaemic related CHF.

There were 17 patients with an impaired renal function based on e-CC (39.5 ± 10.5 mL/min, range 17-56 mL/min) and 23 with an impaired renal function based on e-GFR (42.0 ± 11.3 mL/min/1.73 m², range 17-59 mL/min/1.73 m²). Patients with a decreased e-CC or a decreased e-GFR did not differ in 123I-mIBG semi-quantitative parameters compared with patients with a normal e-CC or normal e-GFR (Table 3).

The variability in any of the 123I-mIBG semi-quantitative parameters could not be explained by either e-CC or e-GFR (Table 4). Estimates of renal function could at best explain approximately 3% of the variability of the 123I-mIBG semi-quantitative parameters (p = 0.851).

Cox proportional hazard regression analysis showed that late H/M ratio was the only independent predictor for cardiac death (Chi-square 3.2, coefficient B: -4.095; standard error: 2.063; hazard ratio: 0.17, 95%CI: 0.000 - 0.950). Forced addition of estimates of renal function did not significantly change the Chi-square of the model (Figure 1A).

### Table 4. Variability of the estimates of renal function in relation to 123I-mIBG-derived parameters.

<table>
<thead>
<tr>
<th></th>
<th>Constant</th>
<th>Stand error c</th>
<th>Coefficient b</th>
<th>Stand error b</th>
<th>Adjusted R²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>e-CC vs. early H/M ratio</td>
<td>49.3</td>
<td>21.2</td>
<td>10.6</td>
<td>13.1</td>
<td>-0.011</td>
<td>0.428</td>
</tr>
<tr>
<td>e-CC vs. late H/M ratio</td>
<td>40.4</td>
<td>24.0</td>
<td>18.2</td>
<td>16.8</td>
<td>0.005</td>
<td>0.285</td>
</tr>
<tr>
<td>e-CC vs. 123I-mIBG WO</td>
<td>66.8</td>
<td>8.0</td>
<td>-0.1</td>
<td>0.6</td>
<td>-0.029</td>
<td>0.851</td>
</tr>
<tr>
<td>e-GFR vs. early H/M ratio</td>
<td>59.1</td>
<td>15.4</td>
<td>0.6</td>
<td>9.2</td>
<td>-0.027</td>
<td>0.948</td>
</tr>
<tr>
<td>e-GFR vs. late H/M ratio</td>
<td>50.4</td>
<td>16.3</td>
<td>6.7</td>
<td>11.0</td>
<td>-0.017</td>
<td>0.546</td>
</tr>
<tr>
<td>e-GFR vs. 123I-mIBG WO</td>
<td>64.8</td>
<td>5.7</td>
<td>-0.5</td>
<td>0.4</td>
<td>0.011</td>
<td>0.240</td>
</tr>
</tbody>
</table>

See for abbreviations Table 1 and Table 2.

### 123I-mIBG and estimates of kidney function

The mean early H/M ratio was 1.61 ± 0.46, the mean late H/M ratio was 1.43 ± 0.38 and the mean 123I-mIBG WO was 10.1 ± 10.4% (Table 2). There was no difference in the 123I-mIBG semi-quantitative parameters or in the e-CC and e-GFR between ischaemic and non-ischaemic related CHF.

There were 17 patients with an impaired renal function based on e-CC (39.5 ± 10.5 mL/min, range 17-56 mL/min) and 23 with an impaired renal function based on e-GFR (42.0 ± 11.3 mL/min/1.73 m², range 17-59 mL/min/1.73 m²). Patients with a decreased e-CC or a decreased e-GFR did not differ in 123I-mIBG semi-quantitative parameters compared with patients with a normal e-CC or normal e-GFR (Table 3).

The variability in any of the 123I-mIBG semi-quantitative parameters could not be explained by either e-CC or e-GFR (Table 4). Estimates of renal function could at best explain approximately 3% of the variability of the 123I-mIBG semi-quantitative parameters (p = 0.851).

### Cardiac death

During follow-up 6 of the 39 (15.4%) patients had a cardiac death; mean interval after 123I-mIBG scintigraphy to cardiac death was 22 months with a range from 4 to 54 months. All 6 patients died as a result of severe progressive heart failure. Characteristics of patient with cardiac death and survivors are described in Table 5. The cardiac deaths were more likely to have a non-ischaemic aetiology of heart failure (p = 0.022). There was a statistically not significant trend towards lower e-CC and e-GFR values for patients with cardiac death compared to survivors (e-CC 53.4 ± 20.9 vs. 67.8 ± 34.5, p = 0.375; e-GFR 49.1 ± 15.7 vs. 62.0 ± 26.6, p = 0.259, respectively).

Cox proportional hazard regression analysis showed that late H/M ratio was the only independent predictor for cardiac death (Chi-square 3.2, coefficient B: -4.095; standard error: 2.063; hazard ratio: 0.17, 95%CI: 0.000 - 0.950). Forced addition of estimates of renal function did not significantly change the Chi-square of the model (Figure 1A).
Chapter 7

Figure 1. (A) Model predicting cardiac death: late H/M ratio enters the model first (Chi-square = 3.2). The addition of renal function did not significantly change the model (Chi-square for the model including e-CC = 4.1 and for the model including e-GFR = 4.0, respectively). (B) Model predicting potentially lethal arrhythmia: QRS duration is the only significant contributor to the model (Chi-square = 8.5). The addition of renal function did not significantly change the model (Chi-square for the model including e-CC = 8.7 and for the model including e-GFR = 9.1, respectively).
Potentially lethal ventricular arrhythmia

Nine patients developed potentially lethal ventricular arrhythmia: 5 had sustained ventricular tachycardia, 1 patient was resuscitated from a cardiac arrest and 3 patients had an appropriate ICD therapy (i.e. anti-tachycardia pacing). None of these arrhythmias resulted in sudden cardiac death.

Cox proportional hazard regression analysis showed that QRS duration was the only independent predictor for a potentially lethal ventricular arrhythmia (Chi-square 8.5, coefficient B: 0.028; standard error: 0.010; hazard ratio: 1.028, 95%CI: 1.021-1.049). Forced addition of estimates of renal function did not significantly change the Chi-square of the model (Figure 1B). None of the $^{123}$I-mIBG semi-quantitative parameters was predictive for a potentially lethal ventricular arrhythmia.

Table 5. Characteristics of cardiac deaths compared to survivors.

<table>
<thead>
<tr>
<th></th>
<th>Cardiac Death (n = 6)</th>
<th>Survivor (n = 33)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64 ± 14</td>
<td>64 ± 10</td>
<td>0.990</td>
</tr>
<tr>
<td>Female/Male</td>
<td>2/4</td>
<td>13/20</td>
<td>0.786</td>
</tr>
<tr>
<td>NYHA class</td>
<td></td>
<td></td>
<td>0.529</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>4</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Aetiology heart failure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischaemic/non-ischaemic</td>
<td>1/5</td>
<td>22/11</td>
<td>0.022</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>20.8 ± 10.9</td>
<td>24.6 ± 11.7</td>
<td>0.467</td>
</tr>
<tr>
<td>ECG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QRS duration (msec)</td>
<td>175 ± 66</td>
<td>161 ± 38</td>
<td>0.471</td>
</tr>
<tr>
<td>LBBB</td>
<td>5</td>
<td>27</td>
<td>0.647</td>
</tr>
<tr>
<td>Renal function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e-CC</td>
<td>53.4 ± 20.9</td>
<td>67.8 ± 34.5</td>
<td>0.375</td>
</tr>
<tr>
<td>e-GFR</td>
<td>49.1 ± 15.7</td>
<td>62.0 ± 26.6</td>
<td>0.259</td>
</tr>
<tr>
<td>$^{123}$I-mIBG-derived parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early H/M ratio</td>
<td>1.57 ± 0.36</td>
<td>1.62 ± 0.47</td>
<td>0.839</td>
</tr>
<tr>
<td>Late H/M ratio</td>
<td>1.34 ± 0.30</td>
<td>1.45 ± 0.39</td>
<td>0.512</td>
</tr>
<tr>
<td>$^{123}$I-mIBG WO</td>
<td>14.2 ± 12.7</td>
<td>9.4 ± 9.9</td>
<td>0.302</td>
</tr>
</tbody>
</table>

See for abbreviations Table 1 and Table 2.
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DISCUSSION

Semi-quantitative $^{123}$I-$m$IBG myocardial parameters are independent of estimates of renal function. In addition, cardiac sympathetic innervation assessed by $^{123}$I-$m$IBG scintigraphy seems to be superior to renal function in the prediction of prognosis in CHF patients.

Renal function and $^{123}$I-$m$IBG

In subjects with a normal kidney function, intravenous administrated $^{123}$I-$m$IBG is almost exclusively excreted via the kidneys within 24 hours after injection with approximately 35% of administered $^{123}$I-$m$IBG already excreted by 6 hours.\textsuperscript{17,18} As a reduced GFR is associated with a reduced blood clearance of $^{123}$I-$m$IBG, the excretion of $^{123}$I-$m$IBG is not only dependent on filtration but also by tubular secretion.\textsuperscript{10} In short kidney function is essential for the clearance of $^{123}$I-$m$IBG and may therefore influence scintigraphic outcome. However, the results of our study show that the variability in the semi-quantitative $^{123}$I-$m$IBG myocardial parameters cannot be explained by estimates of renal function. Therefore within the time-frame of $^{123}$I-$m$IBG cardiac imaging (up to 4 hours after injection), the semi-quantitative $^{123}$I-$m$IBG myocardial parameters are independent of renal function. These findings are in line with a recent publication showing that differences in the rate of renal excretion did not contribute to variability in mediastinal and myocardial counts between early and late planar $^{123}$I-$m$IBG images.\textsuperscript{11} This is eminent for clinical practice as renal dysfunction is often present in CHF patients.\textsuperscript{19,20}

Renal function, $^{123}$I-$m$IBG and prognosis in CHF

Renal dysfunction is not only often present in patients with CHF, the serum creatinine-based estimates of renal function have been shown to be independently related to mortality.\textsuperscript{21-25} In addition the sympathetic nervous system is one of the neurohormonal compensation mechanisms that plays an important role in the pathogenesis of CHF. Activation of this cardiac sympathetic system causes down regulation and desensitization of cardiac beta-adrenoreceptors and modification in the post synaptic signal transduction which contributes to arrhythmia development, progression of heart failure and ultimately cardiac death. Our results confirm previous findings that increased cardiac sympathetic activity assessed by $^{123}$I-$m$IBG scintigraphy is related to mortality.\textsuperscript{1,2,26}

However, there is limited data on a direct comparison of the respective prognostic predictive value of sympathetic innervation and renal dysfunction. To our knowledge only Furuhashi et al. studied this specific subject.\textsuperscript{7} In patients with CHF and a preserved GFR ($\geq$ 60 mL/min/1.73m$^2$) Cox proportional hazard regression analysis showed that late H/M ratio was the only independent predictor of cardiac death. However, the study
lacked statistical power to perform Cox proportional hazard regression analysis in the patient group with an impaired renal function (GFR < 60 mL/min/1.73m²).

The lack of additional prognostic value of renal function in our study might be explained by several different but probably interacting factors. First, the aetiology of CHF differs between different studies. In studies with a larger number of patients with ischaemia related cardiomyopathy, a higher predictive value of renal function was found. This might be explained by concomitant peripheral vascular disease and secondary nephrosclerosis. Our patient cohort was not large enough to allow for adequate subgroup analysis and therefore concomitant peripheral vascular disease remains a theoretical explanation for the found discrepancies. Secondly, the differences between our results and the findings of others may be related to the prevalence of reduced kidney function. However, even in patients with increased serum creatinine levels (> 2.5 mg/dl or > 220 μmol/L, approximately 3% of the study population), Opasisch et al. were not able to identify renal function as a prognostic indicator. Approximately 47% of our study population had at least a moderate impairment of renal function (i.e. e-CC or e-GFR < 60 ml/min (/1.73m²)). This prevalence is slightly lower compared to the majority of published data. Prevalence of renal dysfunction does therefore not explain the absence of renal function as a prognostic indicator.

LIMITATIONS AND CLINICAL IMPLICATIONS

The main limitation of this study is the small number of patients collected over an extended period of time when therapeutic guidelines were changing. This is reflected by the fact that the majority of included patients is relatively undertreated according to the current guidelines. Furthermore the mortality rate seems to be relatively low (i.e. 15%). However, the mortality rate is in line with the mortality rate as reported by other publications. Furuhashi et al. reported a mortality rate of 11% during a mean follow-up period of 33.7 months and the cardiac mortality rate of the ADMIRE-HF study (6% during a median follow-up period of 17 months). The extrapolation of the prognostic predictive value of our study is probably influenced by these factors. The prognostic findings of our study should therefore be considered as preliminary. However, remains that the aforementioned factors have no impact on the finding that semi-quantitative ¹²³I-mIBG myocardial parameters are independent of estimates of renal function.
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CONCLUSION

Semi-quantitative $^{123}$I-mIBG myocardial parameters are independent of estimates of renal function. Although the findings on the prognostic predictive value of this study should be considered as preliminary, the observations suggest that cardiac sympathetic innervation assessed by $^{123}$I-mIBG scintigraphy is superior in the prediction of prognosis in patients with CHF to estimates of renal (dys-) function. This finding might be clinically relevant as creatinine clearance is less costly to assess than $^{123}$I-mIBG.
Renal function and $^{123}$I-mIBG scintigraphy

REFERENCES


PART II

Prognostic value of cardiac $^{123}\text{I}-m\text{IBG}$ scintigraphy
For what endpoint does myocardial $^{123}$I-mIBG scintigraphy have the greatest prognostic value in patients with chronic heart failure? Results of a pooled individual patient data meta-analysis

DO Verschure
CE Veltman
A Manrique
GA Somsen
M Koutelou
A Katsikis
D Agostini
MC Gerson
BL van Eck-Smit
AJ Scholte
AF Jacobson
HJ Verberne
ABSTRACT

Aim
The purpose of this study was to determine the most appropriate prognostic endpoint for myocardial $^{123}$I-meta-iodobenzylguanidine ($^{123}$I-mIBG) scintigraphy in patients with chronic heart failure (CHF) based on aggregate results from multiple studies published in the past decade.

Methods and results
Original individual late (3 – 5 h) heart/mediastinum (H/M) ratio data of 636 CHF patients were retrieved from six studies from Europe and the USA. All-cause mortality, cardiac mortality, arrhythmic events, and heart transplantation were investigated to determine which provided the strongest prognostic significance for the mIBG imaging data. The majority of patients was male (78%), had a decreased left ventricular ejection fraction (31.1 ± 12.5%), and a mean late H/M ratio of 1.67 ± 0.47. During follow-up (mean 36.9 ± 20.1 months), there were 83 deaths, 67 cardiac deaths, 33 arrhythmic events, and 56 heart transplants. In univariate regression analysis, late H/M ratio was a significant predictor of all event categories, but lowest hazard ratios (HRs) were for the composite endpoint of any event (HR = 0.30, 95% CI 0.19 – 0.46), all-cause (HR = 0.29, 95% CI 0.16 – 0.53), and cardiac mortality (HR = 0.28, 95% CI 0.14 – 0.55). In multivariate analysis, late H/M ratio was an independent predictor for all event categories, except for arrhythmias.

Conclusion
This pooled individual patient data meta-analysis showed that, in CHF patients, the late H/M ratio is not only useful as a dichotomous predictor of events (high vs. low risk), but also has prognostic implication over the full range of the outcome value for all event categories except arrhythmias.
123I-mIBG scintigraphy for risk assessment in CHF

INTRODUCTION

Despite the numerous single-centre studies demonstrating the prognostic value of myocardial 123I-meta-iodobenzylguanidine (mIBG) imaging in chronic heart failure (CHF) patients, clinical use of this procedure remains limited.1-4 One potential reason for the limited clinical impact of many publications is that the different ways in which increased risk was characterized could not be directly related to patient management considerations. In addition, most of the studies analysed the results using a dichotomous division of patients into low and high cardiac uptake groups, without adequate standardization of the thresholds chosen for division of the populations.5 Cardiac uptake of 123I-mIBG was only rarely analysed as a continuous quantitative variable, making it difficult to assess the full scope of the prognostic potential of 123I-mIBG.6,7 In addition, there was a lack of consistency in defining the population in whom 123I-mIBG should be used and the endpoints most likely to influence therapeutic decisions.

The purpose of this study was to examine the relative performance of cardiac 123I-mIBG imaging results as a prognostic marker for different endpoints in order to determine the endpoint for which this imaging had the greatest power. The basis for this new analysis was the aggregate results from individual patient data from multiple studies published in the past decade.

METHODS

Study selection

Eligibility criteria

Published studies were eligible if survival was analysed in patients with heart failure stratified by the late heart to mediastinal (H/M) ratio as a parameter of 123I-mIBG myocardial uptake. The primary outcomes of interest were all-cause mortality, cardiac death, non-fatal arrhythmic events [i.e. sustained ventricular tachycardia, resuscitated cardiac arrest, and implantable cardioverter defibrillator (ICD) activations], cardiac transplantation, and a composite endpoint of any of the listed events.

Search strategy

A computer-assisted search was performed on the medical databases MEDLINE (January 2000 to January 2012), PubMed (January 2000 to January 2012), EMBASE (January 2000 to January 2012), the Cochrane Controlled Trial Register, and the Cochrane Database of Systematic Reviews (from their inception to January 2012). We used the following previously described highly sensitive search and adapted strategy5,8 (((MIBG* [WORD] OR metaiodobenzylguanidine [WORD]) AND (heart
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[WORD] AND failure [WORD]) AND (incidence [MESH] OR mortality [MESH] OR followup studies [MESH] OR mortality [SH] OR prognosis* [WORD] OR predict* [WORD] OR course [WORD])). The publications were restricted to those originating from Europe or the USA without any language restrictions. In addition, data from the ADHERE-HF trial were not accessible for the current analysis.

Selection procedure

All publications matching the eligibility criteria were retrieved. In the case of overlapping and duplicated datasets, care was taken to include only the most recent or most complete dataset. The primary responsible authors of the selected articles were contacted to determine whether the data used in the original publications still existed, and whether they were willing and able to share individual subject results for the combined pooled meta-analysis.

Definition of endpoints

All-cause mortality data were extracted from the long-term survival information collected as part of the original published research. The time to death was defined as the number of days from the date of $^{123}$I-mIBG administration until the date of death. For reasons of consistency, the follow-up was truncated at 60 months. Cardiac death was a component of the endpoint of all-cause mortality. Based on information collected during the course of the original studies, all reported deaths were categorized as either cardiac, non-cardiac, or unknown. Cardiac mortality included sudden cardiac death, deaths as a result of progressive heart failure or acute myocardial infarction, and other deaths for which complications involving the heart were a central factor. All other deaths were categorized as non-cardiac if a primary cause such as malignancy or infection was known or unknown if there was no information concerning the circumstances of the death.

Arrhythmic events included any of the following documented occurrences: resuscitated cardiac arrest, appropriate ICD discharge (antitachycardia pacing or defibrillation), sustained ventricular tachycardia of > 30 s duration and a heart rate > 100 bpm. The rhythm must also have been poorly tolerated (associated with hypotension and collapse) and/or have required an intervention (intravenous medications, antitachycardia pacing, and direct current shock) to terminate. Cardiac transplantation performed for any indication was recorded.

Statistical analysis

Cox’s proportional hazard regression analysis was used to investigate the relationship between several possible patient-related explanatory variables [age, gender, late H/M ratio, left ventricular ejection fraction (LVEF), aetiology of CHF, and baseline New York Heart Association (NYHA) functional class] and the different endpoints: all-cause mortality, cardiac death, arrhythmic events, cardiac transplantation, and the composite endpoint. In the case of multiple events, only the first event was used for analysis. Each individual parameter was entered in the Cox’s proportional hazard regression analysis,
based on forward likelihood ratio, if \( p < 0.05 \) and removed from the analysis, if \( p > 0.10 \). The \( \chi^2 \) test, Cox’s proportional hazard regression coefficient (coefficient \( B \)), and exponent (exponent \( B \)) were used to describe the model and relative contribution of the parameters to the model. Exponent \( B \) can, therefore, be considered to be the predicted change in hazard for a unit change in the predictor, i.e. hazard ratio (HR). A \( p \)-value of \(< 0.05 \) was considered statistically significant. All statistical analyses were performed with the SPSS software (SPSS for Windows, version 20.0; SPSS, Inc., Chicago, IL, USA).

RESULTS

Study selection
Full reports or abstracts from 129 references of papers yielded eight studies that fulfilled the inclusion criteria of our pooled individual patient data meta-analysis. The primary responsible authors of these eight studies were contacted and six agreed to share their individual patient data, all but one of the datasets were generated in Europe.9-14 The individual data of 601 subjects could be retrieved from local databases and aggregated into one database. Compared with the total number of subjects mentioned in the original publications, 35 additional subjects could be added to the aggregated database. As part of an ongoing registry of CHF patients undergoing \( ^{123}\text{I}-\text{mIBG} \), these subjects all came from one centre and were added to the local database after the original publication.11 These 35 additional subjects were not included in any other previous publication. In addition, further follow-up data collected after the original publication were submitted for subjects in one previous aggregated study.10 Therefore, 636 subjects were eligible for the aggregated analysis. Figure 1 shows the progress through the selection of studies eligible for the pooled individual patient data meta-analysis.

Datasets
The types of data available for all patients were: demographics, medical history, medication usage, CHF etiology, LVEF, late H/M ratio, and follow-up data. The majority of datasets was lacking complete information on other parameters (e.g. biochemistry and renal function data), thus requiring exclusion of these variables from the multivariate analyses. All eligible studies reported that, to block uptake of free \( ^{123}\text{I} \) by the thyroid gland, patients were pre-treated with either a form of saturated solution of potassium iodide or perchlorate prior to the injection of \( ^{123}\text{I}-\text{mIBG} \). In all eligible studies, patients were intravenously injected with 185 MBq (5 mCi) of \( ^{123}\text{I}-\text{mIBG} \). Anterior planar images of the chest were acquired in almost all eligible studies at 3 - 4 h (‘late’) post-injection of \( ^{123}\text{I}-\text{mIBG} \). In all studies, the myocardial region of interest (ROI) was drawn manually. A square or rectangular mediastinal ROI was drawn in the upper mediastinum where the size of the mediastinal ROI changed with matrix size. All eligible studies reported that the H/M ratio was calculated as the ratio of the counts/pixel in the two ROIs.
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Reports retrieved
n = 129

Excluded reports (n = 121):
not originated from Europe or USA: 62
review and case reports: 35
ADMIRE-HF related: 4
Internal Conversion Devices (ICD): 7
Miscellaneous: 13
hypertension, diabetes mellitus,
acquisition related reports

Reports fulfilling inclusion criteria
n = 8

Authors willing to share their individual patient data
n = 6

Figure 1. Selection of studies eligible for pooled individual patient data meta-analysis. One hundred and twenty-one reports were excluded: 62 reports not originating from Europe or the USA, 35 review and case reports, 4 reports related to ADMIRE-HF, 7 studies on ICD, and a group of 13 studies with miscellaneous subjects ranging from hypertension, diabetes mellitus to acquisition-related reports. No studies with overlapping and/or duplicated datasets were found. Non-electronic search (contact with authors and hand searching) did not result in additional (unpublished) studies that fulfilled the eligibility criteria.
Table 1. Patient characteristics.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56 ± 12</td>
<td>Range: 21-87</td>
</tr>
<tr>
<td>Female/Male</td>
<td>137/499</td>
<td>22% vs. 78%</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.1 ± 4.8</td>
<td>Range: 16 – 45.8</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>31.1 ± 12.5</td>
<td>Range: 7 – 47</td>
</tr>
<tr>
<td>NYHA class</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>33</td>
<td>5.2%</td>
</tr>
<tr>
<td>II</td>
<td>300</td>
<td>47.2%</td>
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<tr>
<td>III</td>
<td>278</td>
<td>43.7%</td>
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<tr>
<td>IV</td>
<td>25</td>
<td>3.9%</td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischaemic vs. non-ischaemic</td>
<td>264/372</td>
<td>42% vs. 58%</td>
</tr>
<tr>
<td>Hypertension</td>
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<td></td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
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<td></td>
</tr>
<tr>
<td>Medication</td>
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<td></td>
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<tr>
<td>Beta blockers</td>
<td>420</td>
<td></td>
</tr>
<tr>
<td>ACE-I/ARB</td>
<td>519</td>
<td></td>
</tr>
<tr>
<td>Aldosterone antagonist</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>Amiodarone</td>
<td>102</td>
<td></td>
</tr>
</tbody>
</table>


Subject characteristics
The majority of the 636 subjects (599 from Europe and 37 from the USA) was male, had non-ischaemic CHF and a decreased LVEF, and was on beta-blockers, aldosterone antagonists, and either angiotensin-converting enzyme inhibitors (ACE-I) or angiotensin II receptor blockers (ARB) (Table 1).

Follow-up and events
During follow-up, truncated at 60 months (mean 36.9 ± 20.1 months with a range of 1 – 60 months), 159 patients had 172 events: 83 deaths of which 67 were cardiac deaths, 33 arrhythmic events, and 56 cardiac transplantations. The majority of subjects experienced one event and only 13 subjects had a second event. All these 13 subjects eventually died: six subjects had a cardiac transplantation prior to death and the remaining seven subjects had arrhythmias prior to death.
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Figure 2. Kaplan–Meier (K–M) survival curves classified by quintiles of late H/M ratio. The K–M survival curve for the combined endpoint without any relation to the late H/M ratio shows an overall cumulative increase of events over time (A). When the K–M survival curves for the combined event are
plotted in relation to the late H/M ratio (expressed in quintiles), there is a clear association showing that with lower late H/M ratio the risk of events increase (B). This holds also true for all-cause mortality (C) and cardiac mortality (D) and to lesser extent for arrhythmias (E) and cardiac transplantation (F).
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123I-mIBG parameters
In all the subjects, the late H/M ratio was available. The mean late H/M ratio was 1.67 ± 0.47 (first quintile ≤ 1.32; second to fourth quintile 1.33 – 1.97, and fifth quintile ≥ 1.98). Figure 2 shows the Kaplan–Meier curves for the late H/M ratio (in quintiles) in relation to the events. For all defined endpoints, decreasing late H/M ratio was associated with increased event risk. This association was strongest for mortality (both all cause and cardiac) and weakest for arrhythmic events.

Univariate Cox’s regression analysis
Table 2 presents the result of the univariate Cox regression analyses. The late H/M ratio was a significant predictor for all event categories, but the highest χ² and the lowest HR were for the composite endpoint of any event (HR = 0.30, 95% CI: 0.19 – 0.46). The late H/M ratio had similar power as a predictor of all-cause mortality, cardiac mortality, or cardiac transplantation (Table 2). For the prediction of arrhythmic events, the late H/M ratio had the lowest χ² and least powerful HR.

Multivariate Cox’s regression analysis
For all event categories, except for arrhythmias, late H/M ratio was an independent predictor (Tables 3-7): i.e. a lower late H/M ratio was associated with a higher risk of events.

For the composite endpoint of any event, late H/M ratio was an independent predictor. Gender (i.e. females associated with a lower risk), LVEF (i.e. lower LVEF associated with a higher risk), and NYHA functional class (i.e. higher NYHA associated with a higher risk) were also identified as independent predictors (Table 3).

The independent predictors of all-cause mortality and cardiac mortality were late H/M ratio and LVEF (i.e. lower LVEF was associated with a higher risk), whereas for all-cause mortality age (i.e. higher age was associated with higher risk) was also identified as an independent predictor (Tables 4 and 5).

For cardiac transplantation, the independent predictors were late H/M ratio, age (i.e. higher age was associated with a lower risk), LVEF (i.e. lower LVEF was associated with a higher risk), and baseline NYHA functional class (i.e. higher NYHA class was associated with a higher risk) (Table 6).

Arrhythmias could independently be predicted by age (i.e. higher age was associated with a higher risk) and baseline NYHA functional class (i.e. higher NYHA class was associated with a lower risk) (Table 7).

Aetiology of heart failure (i.e. ischaemic vs. non-ischaemic) was not an independent predictor in the multivariate analyses for any of the outcome events.
Table 2. Univariate analysis for late H/M ratio as a predictor of events at 60-month follow-up.

<table>
<thead>
<tr>
<th>Events</th>
<th>( \chi^2 )</th>
<th>HR (95% CI)</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any event</td>
<td>36.86</td>
<td>0.30 (0.19 – 0.46)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>20.14</td>
<td>0.29 (0.16 – 0.53)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Cardiac transplant</td>
<td>17.22</td>
<td>0.22 (0.10 – 0.49)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Cardiac mortality</td>
<td>17.13</td>
<td>0.28 (0.14 – 0.55)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>10.00</td>
<td>0.33 (0.16 – 0.67)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Outcome of univariate analyses is ordered based on \( \chi^2 \) scoring. Any event = combined end-point; \( \chi^2 \) = chi-square; HR = hazard ratio; 95% CI = 95% confidence interval.

Table 3. Multivariate analysis for predictors of the combined endpoint of any event at 60-month follow-up.

<table>
<thead>
<tr>
<th>Any event</th>
<th>( \chi^2 ) of the model</th>
<th>HR (95% CI)</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>67.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late H/M ratio</td>
<td></td>
<td>0.43 (0.27 – 0.67)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td>0.55 (0.36 – 0.85)</td>
<td>0.006</td>
</tr>
<tr>
<td>LVEF</td>
<td></td>
<td>0.97 (0.95 – 0.99)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>NYHA</td>
<td></td>
<td>1.34 (1.04 – 1.72)</td>
<td>0.023</td>
</tr>
</tbody>
</table>

Outcome of multivariate analysis is ordered based on HR scores. \( \chi^2 \) = chi-square; HR = hazard ratio; 95% CI = 95% confidence interval. LVEF: left ventricular ejection fraction, NYHA: New York Heart Association functional classification. Parameters used as possible explanatory variables: age, gender, late H/M ratio, LVEF, aetiology of CHF and NYHA class.

Table 4. Multivariate analysis for predictors of all-cause mortality at 60-month follow-up.

<table>
<thead>
<tr>
<th>All-cause mortality</th>
<th>( \chi^2 ) of the model</th>
<th>HR (95% CI)</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>33.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late H/M ratio</td>
<td></td>
<td>0.50 (0.26 – 0.96)</td>
<td>0.038</td>
</tr>
<tr>
<td>LVEF</td>
<td></td>
<td>0.97 (0.95 – 0.99)</td>
<td>0.005</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td>1.02 (1.00 – 1.04)</td>
<td>0.025</td>
</tr>
</tbody>
</table>

Outcome of multivariate analysis is ordered based on HR scores. \( \chi^2 \) = chi-square; HR = hazard ratio; 95% CI = 95% confidence interval. LVEF: left ventricular ejection fraction. Parameters used as possible explanatory variables: age, gender, late H/M ratio, LVEF, aetiology of CHF and NYHA class.
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Table 5. Multivariate analysis for predictors of cardiac mortality at 60-month follow-up.

<table>
<thead>
<tr>
<th>Cardiac mortality</th>
<th>$\chi^2$ of the model</th>
<th>HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late H/M ratio</td>
<td>24.94</td>
<td>0.40 (0.20 – 0.82)</td>
<td>0.012</td>
</tr>
<tr>
<td>LVEF</td>
<td>0.97 (0.94 – 0.99)</td>
<td>0.007</td>
<td></td>
</tr>
</tbody>
</table>

Outcome of multivariate analysis is ordered based on HR scores. $\chi^2 = \chi$-square; HR = hazard ratio; 95% CI = 95% confidence interval. LVEF: left ventricular ejection fraction. Parameters used as possible explanatory variables: age, gender, late H/M ratio, LVEF, aetiology of CHF and NYHA class.

Table 6. Multivariate analysis for predictors of cardiac transplantations at 60-month follow-up.

<table>
<thead>
<tr>
<th>Cardiac transplantation</th>
<th>$\chi^2$ of the model</th>
<th>HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late H/M ratio</td>
<td>72.56</td>
<td>0.34 (0.14 – 0.79)</td>
<td>0.012</td>
</tr>
<tr>
<td>Age</td>
<td>0.95 (0.93 – 0.97)</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>LVEF</td>
<td>0.95 (0.92 – 0.98)</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>NYHA</td>
<td>2.66 (1.73 – 4.08)</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Outcome of multivariate analysis is ordered based on HR scores. $\chi^2 = \chi$-square; HR = hazard ratio; 95% CI = 95% confidence interval. LVEF: left ventricular ejection fraction, NYHA: New York Heart Association functional classification. Parameters used as possible explanatory variables: age, gender, late H/M ratio, LVEF, aetiology of CHF and NYHA class.

Table 7. Multivariate analysis for predictors of arrhythmias at 60-month follow-up.

<table>
<thead>
<tr>
<th>Arrhythmias</th>
<th>$\chi^2$ of the model</th>
<th>HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NYHA</td>
<td>10.10</td>
<td>0.57 (0.33 – 0.98)</td>
<td>0.04</td>
</tr>
<tr>
<td>Age</td>
<td>1.04 (1.01 – 1.07)</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

Outcome of multivariate analysis is ordered based on HR scores. $\chi^2 = \chi$-square; HR = hazard ratio; 95% CI = 95% confidence interval. NYHA: New York Heart Association functional classification. Parameters used as possible explanatory variables: age, gender, late H/M ratio, LVEF, aetiology of CHF and NYHA class.
DISCUSSION

This study demonstrates the independent prognostic value of increased cardiac sympathetic activity as assessed by the late H/M ratio as a measure of $^{123}$I-$m$IBG myocardial uptake when used as a continuous parameter by aggregating individual data of patients with HF from multiple single-centre cohort studies. While the late H/M ratio can be effectively used as a dichotomous or categorical predictor of events (high vs. low risk), the present results confirm that the risk of events is continuously associated with the late H/M ratio, with prognostic implication over the full range of this parameter.

In heart failure, abnormal activity of the sympathetic nervous system has been shown to be of pathophysiological importance. Increased neuronal release of norepinephrine (NE) in response to a deterioration of cardiac function is accompanied by decreased presynaptic NE reuptake due to down-regulation of the cardiac NE transporter. If prolonged, this leads to a reactive desensitization of the myocardial beta-adrenergic receptors in the synaptic cleft, further exacerbating ventricular dysfunction.

$m$IBG is a radiolabelled NE analogue that allows for the visualization and quantification of myocardial sympathetic innervation. $m$IBG shares the same uptake, storage, and release mechanisms as NE, but is not metabolized. The quantified myocardial $^{123}$I-$m$IBG parameters have proved to be of prognostic value in CHF. The meta-analyses of Verberne et al. and Kuwabara et al. showed that HF patients with abnormal myocardial $^{123}$I-$m$IBG parameters have a significantly worse prognosis compared with those with relatively preserved myocardial $^{123}$I-$m$IBG parameters (i.e. late H/M ratio and $^{123}$I-$m$IBG myocardial washout). The ADMIRE-HF trial demonstrated for the first time in a large prospective study that the late H/M ratio, especially in concert with LVEF and B-type natriuretic peptide (BNP), was a strong independent predictor of prognosis in HF patients. The recent publication by Nakata et al. confirmed, by pooled analyses of independent cohort studies from Japan, the long-term prognostic value of cardiac $^{123}$I-$m$IBG uptake in patients with CHF independently of other markers, such as NYHA functional class, BNP, and LVEF. However, only in the most recent studies have $^{123}$I-$m$IBG parameters been analysed consistently as continuous variables. In the majority of earlier studies, cardiac uptake of $^{123}$I-$m$IBG was either dichotomized to differentiate high-risk from low-risk populations (ADMIRE-HF) or subdivided into more pre-specified risk groups (i.e. low, intermediate, and high risks). These categorizations made it difficult to assess the full scope of the prognostic potential of $^{123}$I-$m$IBG. The results of this study using the late H/M ratio as a continuous parameter give further support to the prognostic role of cardiac $^{123}$I-$m$IBG in patients with CHF.

The two available meta-analyses on the prognostic role of $^{123}$I-$m$IBG in CHF were based on published aggregate imaging and outcome results. However, this study, like the recent study by Nakata et al., involved collection of the individual data records.
for individual patients with CHF. This enabled detailed analyses of the full combined population and the merged data from the participating sites provided consistent evidence of efficacy for cardiac $^{123}$I-mIBG imaging equivalent to that typically obtained from a multicentre prospective trial.

The less robust performance of the planar late H/M ratio for prediction of arrhythmic events than for cardiac or all-cause death has been observed in other studies. There are several possible explanations for this finding. The relationship between occurrence of arrhythmic events and late H/M ratio as a measure of global cardiac innervation status is non-linear (highest incidence in patients with modest reduction in uptake) and as a result reduces the effectiveness of the Cox proportional hazard regression analysis. This non-linear relationship was also observed in the present dataset, with the highest incidence of arrhythmic events in patients with a late H/M ratio of $1.40 – 1.59$ (15/147; 10.2%). The use of planar rather than SPECT quantitation of innervation status also likely contributes to the poorer performance of the imaging results, given previous demonstrations of the relationship between regional $^{123}$I-mIBG defect extent and susceptibility to occurrence of arrhythmic events. The use of tomographic techniques to better predict arrhythmic events is further corroborated by the publication of Fallavollita et al., focusing on regional abnormalities using PET. So, while cardiac $^{123}$I-mIBG imaging has potential for improving assessment of arrhythmic risk in CHF patients, realizing this potential will require greater use of SPECT with appropriate methods for relative or preferably absolute uptake quantification.

As aetiology of heart failure (i.e. ischaemic vs. non-ischaemic) was not an independent predictor in the multivariate analyses for any of the outcome events, this already suggested that in time the events and possible explanatory variables were relatively more or less equally divided over both groups. In addition, the number of patients and their outcome events in the two subgroups (i.e. ischaemic and non-ischaemic) was not sufficient to support meaningful multivariate analyses for the individual event categories.

One factor that has constrained acceptance of cardiac $^{123}$I-mIBG imaging as a clinical patient management tool in heart failure has been the variability of technical aspects of the procedure. Although most publications have included the late H/M ratio as the measure of myocardial uptake, the methods used to obtain this parameter have varied. For example, variation in collimator selection and the impact of administered activity, acquisition time, and duration have been shown to influence the final results. With the publication of the proposal for standardization of $^{123}$I-mIBG cardiac sympathetic imaging by the European Association of Nuclear Medicine (EANM), these variations will hopefully be limited in the future. However, as the majority of studies included in the present analysis was performed before the publication of this standardization proposal, the impact of variation in outcome related to the aforementioned parameters cannot be assessed.
The lack of consensus on how to extrapolate the available $^{123}$I-mIBG data into clinical practice is reflected in the absence of $^{123}$I-mIBG in the majority of the current guidelines regarding heart failure except in Japan. The Japanese Circulation Society guidelines for nuclear cardiology list the use of $^{123}$I-mIBG for the evaluation of severity and prognosis of heart failure as Class I recommendation (general agreement of effectiveness and usefulness) based on level B evidence (verified by two or more multicentre randomized intervention trials on fewer than 400 patients, well-designed comparative studies, or large-scale cohort studies). As the amount of high-quality data continues to accumulate, it is likely that $^{123}$I-mIBG imaging will eventually be incorporated into both Europe and the USA HF guidelines. However, the prerequisite for this is that future studies need to be of high quality and with sufficient numbers of patients to allow for adequate and statistically reliable analyses.

**LIMITATIONS**

The two most limiting factors of this study are that data from Japan were excluded, and that ADMIRE-HF data were not available. The exclusion of data from Japan was primarily related to the difference in the numerical range of the published H/M ratio results, compared with data from Europe or the USA. Published late H/M ratio values in control subjects from Japan are higher compared with similar data from Europe and the USA ($2.42 \pm 0.30$ vs. $1.93 \pm 0.16$). These differences cannot be explained by differences in baseline characteristics of the control subjects, but are probably related to variations in technique, especially variation in types of collimators. While the exclusion of Japanese data may have limited the statistical power of this study, the results of this study are nevertheless similar to those of Nakata et al. With regard to ADMIRE-HF, even if these data had been available, their addition would likely have skewed the aggregate results towards shorter follow-up, given the median 17 months in that study compared with 37 months in the present analyses.

In contrast to the ADMIRE-HF study and the Nakata publication, this study had no access to BNP and/or N-terminal prohormone BNP (NT-proBNP) data. We can therefore not exclude the possible prognostic role BNP or NT-proBNP might have played in our analyses. However, in both the recent Nakata publication and in the ADMIRE publication, late H/M ratio remained significant in analyses including BNP.
CONCLUSION

This meta-analysis, using the individual data of 636 CHF patients predominantly from Europe, showed the intermediate to long-term (i.e. 5 years) prognostic value of cardiac sympathetic activity as assessed with cardiac $^{123}$I-mIBG-derived late H/M ratio. The continuous numeric late H/M ratio has prognostic implication over the full numeric range of the parameter, with greatest strength as a predictor of mortality. In the present retrospective analyses, the weakest performance of the planar H/M ratio was for prediction of arrhythmias. In the future, use of the late H/M ratio by cardiologists for individual patient-risk assessment or choice of therapeutic interventions will depend on improvements in the technical consistency of clinical $^{123}$I-mIBG examinations, and prospective generation of data documenting a positive effect of this procedure in clinically relevant situations.
REFERENCES


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Myocardial $^{123}$I-mIBG scintigraphy in relation to markers of inflammation and long-term clinical outcome in patients with stable chronic heart failure

DO Verschure
R Lutter
BL van Eck-Smit
GA Somsen
HJ Verberne
ABSTRACT

Aim
Chronic heart failure (CHF) results in both increased cardiac sympathetic activity and myocardial inflammation. The aim of this study was to identify the relationship between severity of heart failure (i.e. NT-proBNP and LVEF), cardiac sympathetic activity (123I-mIBG scintigraphy) and measures of inflammation in subjects with stable, optimally treated CHF. In addition, the predictive value for cardiac events (i.e. ventricular arrhythmia, progression of CHF and cardiac death) of 123I-mIBG parameters and these inflammatory markers was evaluated.

Materials and methods
Fifty-five CHF patients (age 66.3±8.0 years, 78% male, LVEF 22.4±6.3) referred for cardiac 123I-mIBG imaging were included. At 15 minutes (early) and 4 hours (late) after i.v. administration of 123I-mIBG (185 MBq), planar images were acquired. Early heart-to-mediastinum (H/M) ratio, late H/M ratio and 123I-mIBG washout (WO) were calculated. NT-proBNP and markers of inflammation (i.e. C-reactive protein (CRP), IL-1β, IL-6, IL-10, IL-12p40, tumor necrosis factor-α (TNF-α), soluble (s)E-selectin, myeloperoxidase (MPO), plasminogen activator inhibitor-1 (PAI-1), tPA, tumor necrosis factor receptor (TNFR) 1 and 2 and interferon (IFN) α and β) were measured in blood plasma samples, taken just before 123I-mIBG administration.

Results
Mean early H/M ratio was 2.12 ± 0.39, late H/M ratio was 1.84 ± 0.40 and 123I-mIBG WO was 13.0 ± 10.9. LVEF was the only independent predictor of late H/M ratio (adjusted R² = 0.100, p = 0.011). NT-proBNP was an independent predictor of 123I-mIBG WO (adjusted R² = 0.090, p = 0.015). CRP, IL12p40, TNF-α, sE-selectin, MPO, PAI-1, tPA and TNFR2 were not related to late H/M ratio and 123I-mIBG WO.

During a median follow-up of 34 months (2-58 months), 13 patients experienced a cardiac event (ventricular arrhythmia (4), progression of CHF (4) and cardiac death (5)). Univariate Cox regression analysis showed that the risk of a cardiac event was associated with CRP (HR 1.047 [1.013-1.081]), NT-proBNP (HR 1.141 [1.011-1.288]), MPO (HR 0.998 [0.996-1.000]) and late H/M ratio (HR 0.182 [0.035-0.946]). Multivariate Cox regression analysis showed that only CRP, NT-proBNP, MPO and IL-12p40 were predictors of a cardiac event.

Conclusion
Inflammation and cardiac sympathetic activity seem not to be related in stable CHF patients. This is corroborated by the finding that they both provide prognostic information in this specific CHF population. The current findings should be regarded as insightful but preliminary.
INTRODUCTION

Chronic heart failure (CHF) is a complex syndrome characterized by increased activity of the sympathetic nervous system, increased NT-proBNP levels and increased pro-inflammatory cytokines in plasma and myocardial tissue. Inflammation plays an important role in the pathogenesis and progression of many forms of heart failure (HF). With progression of CHF, the plasma levels of pro-inflammatory cytokines increase. In addition, cardiac sympathetic hyperactivity is associated with progression of CHF. However, there is only limited information on the relation between the sympathetic nervous activity and the inflammatory status in CHF patients.

Cardiac sympathetic activity can non-invasively be assessed with cardiac $^{123}$I-meta-iodobenzylguanidine ($^{123}$I-mIBG). mIBG is a norepinephrine (NE) analog that shares the same presynaptic uptake, storage and release mechanism as NE. Radiolabeling of mIBG with $^{123}$I allows imaging with gamma cameras. The heart-to-mediastinum (H/M) ratio reflects presynaptic myocardial uptake of $^{123}$I-mIBG. The early H/M ratio reflects predominantly the integrity of sympathetic nerve terminals (i.e. number of functioning nerve terminals and intact uptake-1 mechanism). The late H/M ratio offers predominantly information about neuronal function resulting from uptake, storage and release. The myocardial $^{123}$I-mIBG washout (WO) reflects predominantly neuronal integrity of sympathetic tone/adrenergic drive. The late H/M ratio and $^{123}$I-mIBG WO have been shown to be of clinical value, especially for the assessment of prognosis, in many cardiac diseases. Decreased late H/M ratio and increased $^{123}$I-mIBG WO are associated with poor outcome in subjects with CHF.

The aim of this study was to identify the relationship between severity of HF (i.e. NT-proBNP and LVEF), cardiac sympathetic activity assessed with $^{123}$I-mIBG scintigraphy and measures of inflammation in patients with stable CHF. In addition, we evaluated the prognostic value of these inflammatory markers and myocardial $^{123}$I-mIBG-derived parameters (i.e. late H/M ratio and $^{123}$I-mIBG WO).

MATERIAL AND METHODS

Subjects

Patients with stable CHF (New York Heart Association (NYHA) class II to III) who were referred for $^{123}$I-mIBG scintigraphy in their work-up for primary ICD implantation between 2010 and 2015 to the department of Nuclear Medicine of the Academic Medical Center in Amsterdam were enrolled. The inclusion criteria were as follows: stable heart failure (i.e. no myocardial infarction, hospitalization or progression of heart failure) at least 3 months before inclusion and treatment with optimal medical therapy.
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according to the European HF guidelines including beta-blockers and angiotensin-converting-enzyme inhibitors (ACE-I) or angiotensin II receptor blockers (ARB) and if necessary loop diuretics. Exclusion for participation was pregnancy or intolerance for iodine. All subjects provided written informed consent. The study was approved by the local institutional review board and conducted according to the principles of the International Conference on Harmonization–Good Clinical Practice.

123I-mIBG scintigraphy acquisition and analysis

All patients continued their HF medication prior to 123I-mIBG scintigraphy. To block uptake of free 123I by the thyroid gland, subjects were pretreated with 250 mg oral potassium iodide 30 min before intravenous (IV) injection of 185 MBq 123I-mIBG (GE Healthcare, Eindhoven, the Netherlands). Fifteen minutes (early acquisition) and 4 hours (late acquisition) after administration of 123I-mIBG, 10-min planar images were acquired with the subjects in supine position using a gamma camera equipped with a medium energy (ME) collimator.

All planar 123I-mIBG images were analysed by one experienced observer (D.O.V.) blinded to patient data. H/M ratios were calculated from the 123I-mIBG images using a region-of-interest (ROI) over the heart and the upper part of the mediastinum. The H/M ratio was calculated by dividing the mean count density in the cardiac ROI by the mean count density in the mediastinal ROI. The 123I-mIBG WO was calculated as follows:

$$\text{WO} = \left( \frac{\text{early H/M ratio} - \text{late H/M ratio}}{\text{early H/M ratio}} \right) \times 100$$

Markers of inflammation

Before administration of 185 MBq 123I-mIBG, 2 x 4.0 ml of venous blood was collected into ethylenediaminetetraacetic acid (EDTA)-containing tubes. Immediately after collection of the required blood volume, one EDTA tube was analysed for CRP and NT-proBNP by standard procedures. The second EDTA tube was mixed with the sampled blood and immediately cooled on ice. Within 30 min of collection, the tubes were centrifuged at 1700 g for 10 min. The plasma was transferred to cryovals and stored in upright position at -70 °C till analysis. All samples were analysed at the end of the study for IL-1β, IL-6, IL-8, IL-10, IL-12p40, TNF-α, sE-selectin, MPO, PAI-1, tPA, TNFR1 and TNFR2 and INF α and β by luminex. Samples were measured with ProCarta reagents (eBioscience) following the supplier’s protocol and read on a Bioplex 200 (BioRad).

Clinical follow-up and event adjudication.

Follow-up was based on telephone interviews (D.O.V.) and medical records. All subjects received standard clinical care and were followed up until: 1) subject death was confirmed; 2) the trial was terminated. The Clinical Adjudication Committee
reviewed data from case report forms and source documents to confirm the occurrence of CEs, specifically: 1) HF progression: increase in symptomatic status from NYHA functional class II to III or IV, or increase from NYHA class III to class I or 2) potentially life-threatening arrhythmic event, including documented episode of spontaneous sustained (30 s) ventricular tachyarrhythmia, resuscitated cardiac arrest, or appropriate ICD therapy (antitachycardia pacing or defibrillation); or 3) cardiac death (further classified as due to HF progression, sudden cardiac death [SCD]).

**Statistical Analysis**
All continuous variables are expressed as mean ± standard deviation. After demonstrating a normal distribution of variables, between-group comparisons were performed using independent-sample *t*-tests. Non-detectable levels of biomarkers were treated as zeroes for the analysis. Continuous data were compared using analysis of variance (ANOVA). The association between \(^{123}\text{I}-\text{mIBG}\) outcomes and biomarkers was assessed using Pearson's correlation coefficient (2-tailed). Multivariate linear regression analysis was performed to determine independent predictors of \(^{123}\text{I}-\text{mIBG}\) parameters. The overall goodness-of-fit for each model was expressed as the adjusted \(R^2\). The F-test was used to assess whether a model explained a significant proportion of the variability. A *p*-value < 0.05 was considered to indicate a statistical significance. Univariate and multivariate Cox proportional hazards regression analysis was used to assess independent predictors of cardiac events. ROC analysis was used to determine the optimal cut-off value (i.e. highest product of sensitivity and specificity) for predictors of CEs. Statistical analyses were performed with SPSS, release 22.0 for Windows (SPSS Inc., Chicago, IL, USA 2003).
Table 1. Baseline clinical characteristics of CHF patients.

<table>
<thead>
<tr>
<th></th>
<th>All (n = 55)</th>
<th>Cardiac event (n = 13)</th>
<th>No cardiac event (n = 42)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66.3 ± 8.0</td>
<td>66.4±7.0</td>
<td>66.2±8.4</td>
<td>0.118</td>
</tr>
<tr>
<td>Male (%)</td>
<td>43 (78)</td>
<td>10 (77)</td>
<td>33 (79)</td>
<td>0.809</td>
</tr>
<tr>
<td>Ischaemic (%)</td>
<td>33 (60)</td>
<td>8 (62)</td>
<td>25 (60)</td>
<td>0.790</td>
</tr>
<tr>
<td>Non-ischaemic (%)</td>
<td>22 (40)</td>
<td>5 (38)</td>
<td>17 (40)</td>
<td></td>
</tr>
<tr>
<td>Systolic (mmHg)</td>
<td>125 ± 17</td>
<td>126 ± 14</td>
<td>125 ± 19</td>
<td>0.223</td>
</tr>
<tr>
<td>Diastolic (mmHg)</td>
<td>76 ± 10</td>
<td>77±8</td>
<td>75±11</td>
<td>0.148</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>22.4 ± 6.3</td>
<td>23.9 ± 6.6</td>
<td>22.1 ± 6.2</td>
<td>0.491</td>
</tr>
<tr>
<td>NYHA</td>
<td></td>
<td></td>
<td></td>
<td>0.051</td>
</tr>
<tr>
<td>Class II (%)</td>
<td>41 (75)</td>
<td>8 (62)</td>
<td>33 (79)</td>
<td></td>
</tr>
<tr>
<td>Class III (%)</td>
<td>14 (25)</td>
<td>5 (38)</td>
<td>9 (21)</td>
<td></td>
</tr>
<tr>
<td>Betablockers (%)</td>
<td>43 (78)</td>
<td>9 (69)</td>
<td>34 (81)</td>
<td>0.118</td>
</tr>
<tr>
<td>ACE-I/ARBS(%)</td>
<td>50 (91)</td>
<td>13 (100)</td>
<td>37 (88)</td>
<td>0.004</td>
</tr>
<tr>
<td>MRAs (%)</td>
<td>18 (33)</td>
<td>5 (38)</td>
<td>13 (31)</td>
<td>0.560</td>
</tr>
<tr>
<td>Loop diuretics (%)</td>
<td>38 (69)</td>
<td>10 (77)</td>
<td>28 (67)</td>
<td>0.122</td>
</tr>
<tr>
<td>Statines (%)</td>
<td>38 (69)</td>
<td>9 (69)</td>
<td>29 (69)</td>
<td>0.980</td>
</tr>
<tr>
<td>Aspirin (%)</td>
<td>32 (58)</td>
<td>6 (46)</td>
<td>26 (62)</td>
<td>0.448</td>
</tr>
<tr>
<td><strong>123I-mIBG parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early H/M ratio</td>
<td>2.12 ± 0.39</td>
<td>2.00 ± 0.28</td>
<td>2.16 ± 0.42</td>
<td>0.140</td>
</tr>
<tr>
<td>Late H/M ratio</td>
<td>1.84 ± 0.40</td>
<td>1.66 ± 0.28</td>
<td>1.90 ± 0.42</td>
<td>0.137</td>
</tr>
<tr>
<td><strong>123I-mIBG WO</strong></td>
<td>13.0 ± 10.9</td>
<td>19.8 ± 10.6</td>
<td>11.8 ± 10.8</td>
<td>0.897</td>
</tr>
</tbody>
</table>
RESULTS

In total 252 patients with CHF were screened for enrollment. However the majority of the subjects did not meet the inclusion criteria of at least 3 months stable heart failure, already participate in other studies or refused to give informed consent. Baseline patient characteristics including 123I-mIBG-derived parameters are presented in Table 1. The study population comprised 55 stable CHF patients (43 male and 12 female) with a mean age of 66.3 ± 8.0 years. Forty-one (75%) patients were in NYHA class II and 14 (25%) we in NYHA class III. The mean LVEF was 22.4 ± 6.3% and 60% of the patients had ischaemic heart disease. The mean early H/M ratio was 2.12 ± 0.39, the late H/M ratio was 1.84 ± 0.40 and the mean 123I-mIBG WO was 13.0 ± 10.9. Most of the plasma levels of IL-1β, IL-6, IL-8, IL-10, TNFR1, IFN-α and IFN-β were below the detection limit. Table 2 shows only biomarkers with levels above lower limit of detection in all patients.

Association between 123I-mIBG and markers of inflammation

The LVEF, NYHA functional class, NT-proBNP, CRP, TNFα, TNFR2, sE-selectin, IL12p40, MPO, PAI-1 and tPA were used as explanatory variables of late H/M ratio and 123I-mIBG WO. Late H/M ratio was significantly associated with LVEF (R = 0.342, p = 0.011) and NT-proBNP (R = -0.272, p = 0.045). 123I-mIBG WO was also significantly associated with LVEF (R = -0.286, p = 0.034), NYHA (R = 0.281, p = 0.038) and NT-proBNP (R = 0.325, p = 0.015). Multivariate regression analysis using both biomarkers and clinical parameters (i.e. LVEF, NYHA functional class) showed LVEF as the only independent predictor of late H/M ratio (adjusted R² = 0.100, p = 0.011). NT-proBNP was the only independent parameter associated with 123I-mIBG WO (adjusted R² = 0.090, p = 0.015) (Table 3).

Table 2. Biomarkers of CHF patients.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>All (n = 55)</th>
<th>Cardiac event (n = 13)</th>
<th>No cardiac event (n = 42)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/L)</td>
<td>5.44 ± 11.07</td>
<td>9.4 ± 20.3</td>
<td>4.2 ± 5.9</td>
<td>0.001</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>6.00 ± 2.41</td>
<td>5.56 ± 1.41</td>
<td>6.12 ± 2.65</td>
<td>0.483</td>
</tr>
<tr>
<td>sE-selectin (ng/ml)</td>
<td>26.33 ± 10.67</td>
<td>28.91 ± 10.83</td>
<td>25.54 ± 10.62</td>
<td>0.800</td>
</tr>
<tr>
<td>IL-12p40 (pg/ml)</td>
<td>7.51 ± 5.58</td>
<td>9.68 ± 6.81</td>
<td>6.83 ± 5.04</td>
<td>0.154</td>
</tr>
<tr>
<td>MPO (ng/ml)</td>
<td>72.60 ± 56.44</td>
<td>52.05 ± 32.31</td>
<td>78.96 ± 60.95</td>
<td>0.018</td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>8.50 ± 1.69</td>
<td>8.50 ± 2.04</td>
<td>8.51 ± 1.60</td>
<td>0.355</td>
</tr>
<tr>
<td>tPA (ng/ml)</td>
<td>1.56 ± 0.68</td>
<td>1.73 ± 0.83</td>
<td>1.51 ± 0.64</td>
<td>0.294</td>
</tr>
<tr>
<td>TNFR2 (pg/ml)</td>
<td>174 ± 57</td>
<td>191 ± 50</td>
<td>168 ± 58</td>
<td>0.602</td>
</tr>
<tr>
<td>NT-proBNP (ng/L)</td>
<td>1974 ± 3026</td>
<td>3280 ± 5402</td>
<td>1570 ± 1675</td>
<td>0.000</td>
</tr>
</tbody>
</table>
None of the patients were lost during a median follow-up of 34 months (2 - 58 months). Thirteen patients (24%) experienced a first CE: progression of HF ($n = 4$), arrhythmic event with appropriate ICD therapy ($n = 4$) and cardiac death ($n = 5$; four subjects due to sudden cardiac death (SCD) and one due to progression of HF). In addition, one patient had a non-cardiac death. There was a significant difference in plasma levels of NT-proBNP, CRP and MPO between patients with and without CEs (Table 2). However, there was no significant difference in late H/M ratio and $^{123}$I-mIBG WO between both groups. Univariate Cox regression analysis showed that the risk of a CE was associated with CRP, NT-proBNP, MPO and late H/M ratio (Table 4). Multivariate Cox regression analysis showed that CRP, MPO, IL-12p40 and NT-proBNP were independent predictors of a CE. CRP, MPO, IL12p40 and NT-proBNP did not show any mutual relationship with each other (data not shown). Figure 1 shows cumulative event curves for the late H/M ratio and CRP. ROC analysis showed that the optimal cut-off values for the late H/M ratio and CRP were 1.68 and 1.85 mg/l, respectively. These cut-off values resulted in a moderate discriminative power: AUC for late H/M ratio 0.69 and for CRP 0.64, respectively.

### Predictors of cardiac events

None of the patients were lost during a median follow-up of 34 months (2 - 58 months). Thirteen patients (24%) experienced a first CE: progression of HF ($n = 4$), arrhythmic event with appropriate ICD therapy ($n = 4$) and cardiac death ($n = 5$; four subjects due to sudden cardiac death (SCD) and one due to progression of HF). In addition, one patient had a non-cardiac death. There was a significant difference in plasma levels of NT-proBNP, CRP and MPO between patients with and without CEs (Table 2). However, there was no significant difference in late H/M ratio and $^{123}$I-mIBG WO between both groups. Univariate Cox regression analysis showed that the risk of a CE was associated with CRP, NT-proBNP, MPO and late H/M ratio (Table 4). Multivariate Cox regression analysis showed that CRP, MPO, IL-12p40 and NT-proBNP were independent predictors of a CE. CRP, MPO, IL12p40 and NT-proBNP did not show any mutual relationship with each other (data not shown). Figure 1 shows cumulative event curves for the late H/M ratio and CRP. ROC analysis showed that the optimal cut-off values for the late H/M ratio and CRP were 1.68 and 1.85 mg/l, respectively. These cut-off values resulted in a moderate discriminative power: AUC for late H/M ratio 0.69 and for CRP 0.64, respectively.

### Table 3. Multivariate regression analysis to determine independent predictors for late H/M ratio (upper panel) and $^{123}$I-mIBG WO (lower panel).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient $b$</th>
<th>Standard error $b$</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>1.351</td>
<td>0.193</td>
<td></td>
</tr>
<tr>
<td>LVEF</td>
<td>0.022</td>
<td>0.008</td>
<td>0.011</td>
</tr>
<tr>
<td>Goodness-to-fit of the model</td>
<td>Adjusted R$^2$</td>
<td>$p$-value</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.100</td>
<td>0.011</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient $b$</th>
<th>Standard error $b$</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>10.667</td>
<td>1.678</td>
<td></td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>0.001</td>
<td>0.000</td>
<td>0.015</td>
</tr>
<tr>
<td>Goodness-to-fit of the model</td>
<td>Adjusted R$^2$</td>
<td>$p$-value</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.090</td>
<td>0.015</td>
<td></td>
</tr>
</tbody>
</table>
Cardiac sympathetic activity and inflammation in CHF

Figure 1. Examples of cumulative event curves for different parameters. A. Comparing CHF patients with late H/M ratio < 1.68 versus > 1.68 ($p = 0.019$). B. Comparing CHF patients with CRP < 1.85 mg/L versus CRP > 1.85 mg/L ($p = 0.032$).
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Table 4. Univariate and multivariate Cox regression analysis for cardiac events.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.047 (1.013-1.081)</td>
<td>0.000</td>
</tr>
<tr>
<td>NT-proBNP (ng/L)</td>
<td>1.141 (1.011-1.288)</td>
<td>0.023</td>
</tr>
<tr>
<td>IL-12p40 (pg/ml)</td>
<td>1.072 (0.980-1.173)</td>
<td>0.123</td>
</tr>
<tr>
<td>MPO (ng/ml)</td>
<td>0.985 (0.971-0.999)</td>
<td>0.030</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>1.033 (0.949-1.123)</td>
<td>0.452</td>
</tr>
<tr>
<td>NYHA</td>
<td>2.096 (0.683-6.426)</td>
<td>0.186</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>0.787 (0.508-1.219)</td>
<td>0.302</td>
</tr>
<tr>
<td>sE-selectin (ng/ml)</td>
<td>1.009 (0.958-1.063)</td>
<td>0.730</td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>0.892 (0.626-1.271)</td>
<td>0.528</td>
</tr>
<tr>
<td>tPA (ng/ml)</td>
<td>1.059 (0.468-2.396)</td>
<td>0.890</td>
</tr>
<tr>
<td>TNFR2 (pg/ml)</td>
<td>1.002 (0.992-1.011)</td>
<td>0.743</td>
</tr>
<tr>
<td>Early H/M ratio</td>
<td>0.323 (0.065-1.600)</td>
<td>0.166</td>
</tr>
<tr>
<td>Late H/M ratio</td>
<td>0.182 (0.035-0.946)</td>
<td>0.042</td>
</tr>
<tr>
<td>$^{123}$I-mIBG WO</td>
<td>1.045 (0.987-1.106)</td>
<td>0.136</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The findings of this study show that in this specific, optimally treated and stable, CHF population general markers of inflammation were not associated with increased cardiac sympathetic activity assessed with $^{123}$I-mIBG. In addition, the cardiac sympathetic activity was associated with severity of heart failure (i.e. NT-proBNP and LVEF). In line with the lack of association between myocardial $^{123}$I-mIBG parameters and markers of inflammation, both were related to the prognosis of this specific CHF population.

Irrespective of the etiology of CHF, pro-inflammatory cytokines are implicated in the progression of CHF.\cite{4,15,16} In CHF patients increased levels of TNF-α are associated with impaired LV function and consequently increased mortality.\cite{17,18} Although the exact mechanism of TNF-α in relation to CHF remains to be elucidated, it has been reported that TNF-α induces β-adrenergic receptor (βAR) desensitization.\cite{19} This phenomenon identifies a cross-talk between TNF-α and βAR function that at least in part contributes to a potential further reduction in cardiac contractility in CHF. In addition, increased levels of IL-6 have been reported in CHF patients and are correlated to plasma levels of NE (R = 0.839, p < 0.0001).\cite{20} In our study plasma levels of IL-1β, IL-6, IL-10,
IFN-α, IFN-β and TNFR1 were below the detection limit. We consider it unlikely that our procedure failed to detect these cytokines as we took great care to process the blood samples quickly and limit activation. In addition, the earlier spike experiments for these cytokines yielded good recoveries and the internal standards were correct.

A possible explanation for these undetectable levels could be the treatment with statins (hydroxymethylglutaryl-CoA reductase inhibitors), aspirin, ACE-I, ARBs, mineralocorticoid receptor antagonists (MRAs) and beta-blockers. Statins have pleiotropic benefits independent of cholesterol levels including anti-inflammatory effects and it has been suggested that statins might reduce the production of TNF-α, IL-1, and IL-6. In addition aspirin, ACE-I/ARBs, MRAs and beta-blockers have been shown to decrease plasma levels of cytokines. Consequently, the use of these drugs could have influenced the plasma levels of cytokines in our population. In addition, these findings may suggest that our stable CHF patients were optimally treated.

In line with others, we showed increased cardiac sympathetic activity (i.e. decreased late H/M ratio and increased $^{123}$I-mIBG WO) in a stable CHF population. However, in contrast to previous studies with IDCM, our study did not show a significant correlation between the most important markers of inflammation (i.e. TNF-α, IL-1β and IL-6) and cardiac sympathetic activity. IL-1β and IL-6 levels were below the lower limit of quantification, whereas TNF-α was detectable, but did not show a correlation. In conclusion, in this population of stable, optimally treated CHF markers of inflammation were subordinate to the more frequently used markers of prognosis in CHF (i.e. NT-proBNP, LVEF, NYHA class) in relation to sympathetic activity.

LVEF and NT-proBNP were moderately, but significantly related to late H/M ratio. In addition, LVEF, NT-proBNP and NYHA class were moderately related to $^{123}$I-mIBG WO. Recently, it has been shown that BNP modulates autonomic nervous function by inhibiting cardiac sympathetic activity in CHF. As in CHF prolonged increased cardiac sympathetic activity has a detrimental effect on the contractility of the myocardium, this influences the LVEF. This is in line with the found negative association between LVEF and $^{123}$I-mIBG WO.

**Predictor of cardiac events**

Increased cardiac sympathetic activity occurs early in the CHF disease process. Initially, βAR stimulation by the increased NE levels helps to compensate for impaired myocardial function, but long-term NE excess has detrimental effects on myocardial structure and gives rise to a down regulation of post-synaptic βARs. This down regulation leads to left ventricle remodeling and poor prognosis. In our study decreased late H/M ratio was associated with CEs (Figure 1A). This is line with two large meta-analyses and a large prospective multicentre trial. However, in our study not late H/M ratio, but CRP, MPO, IL12p40 and NT-proBNP were the only independent predictors of CEs.
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CRP is regarded a non-specific marker for acute inflammation. Several studies have examined CRP levels in coronary heart disease and have demonstrated its prognostic value.\textsuperscript{31,32} Interestingly, in our study, CRP levels were significantly higher in patients with an event compared to those without CEs (Figure 1B). Similar results were reported in IDCM patients.\textsuperscript{33} The past decade high-sensitive (hs)CRP has been introduced and is commonly used for cardiovascular risk stratification. Increased hsCRP is associated with increased risk for cardiovascular disease and mortality.\textsuperscript{34}

MPO, an enzyme derived from neutrophilic granulocytes reflects inflammation and plaque destabilization. In addition to a possible candidate biomarker to predict future adverse events in patients with acute coronary syndromes (ASC) and myocardial infarction\textsuperscript{35,36}, MPO has been associated with the severity of CHF.\textsuperscript{4} In our study the levels of MPO were elevated. Interestingly, activation of neutrophils leads to the release of preformed IL-8 and MPO. As we found no IL-8 in conjunction with MPO, we consider it unlikely that MPO was generated during processing of blood samples.\textsuperscript{37} In contrast to previous studies,\textsuperscript{35,36} we found a negative association between these elevated levels of MPO and CEs, suggesting a protective mechanism of MPO. However, due to the relatively limited sample size, this extraordinary finding should be interpreted with great care.

IL-12p40 is elevated in an early stage of atherosclerosis both at the mRNA and protein level.\textsuperscript{38} Elevated levels of IL-12p40 have also been reported in patients with coronary artery disease (CAD).\textsuperscript{39,40} Interestingly in a murine atherosclerosis study, aspirin reduced the levels of IL-12p40, suggesting the involvement of IL-12p40 on vascular inflammation.\textsuperscript{41} Our study showed a prognostic value of IL-12p40 in a stable CHF population. This association is most likely explained by the high percentage (i.e. 60\%) of CAD in our CHF population.

NT-proBNP is a widely used powerful predictor of clinical outcome and a better marker for efficacy of drug treatment in patients with HF than other biomarkers or clinical parameters. Its clinical use has been endorsed in clinical practice guideline.\textsuperscript{13} NT-proBNP was an independent predictor of CEs, in line with previous results showing almost exponentially raising risk with increasing levels of NT-proBNP.\textsuperscript{42}

Our study has several limitations. The major limitation of the study is the relative small number of patients included. This may have resulted in a limited statistical power. Therefore, the results of the study should be regarded as insightful but preliminary and larger studies are necessary to confirm our findings. Second, the study population was heterogeneous including ischaemic and non-ischaemic heart disease. Although markers of inflammation were elevated in both groups, it is conceivable that elevation of these markers is more pronounced in the presence of atherosclerosis (i.e. ischaemia). Third, the event rate was relatively low in this stable heart failure population, resulting in non-significant result for the individual endpoints. Therefore, a combination of the individual endpoints into a single endpoint was chosen. Finally treatment with statins, aspirin, ACE-I or ARBs, MRAs and beta-blockers may have influenced cytokine
Cardiac sympathetic activity and inflammation in CHF

production. However, treatment of CHF with these drugs is followed according to the international HF guidelines. Therefore, our results are an accurate reflection of this specific stable CHF population.

In conclusion, this study demonstrated that some markers of inflammation were undetectable most likely reflecting adequate medical treatment in clinically stable and optimally treated CHF patients. However, there is no association between detectable general markers of inflammation and cardiac sympathetic activity in this stable CHF population. This was corroborated by the fact that both markers of inflammation and cardiac sympathetic activity were prognostic indicators of CEs.

New knowledge gained
The results of this study support the notion that inflammation and cardiac sympathetic activity are both important markers reflecting the multifactorial aspects of heart failure progression.
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REFERENCES


Cardiac sympathetic activity and inflammation in CHF


Chapter 10

Cardiac $^{123}$I-mIBG scintigraphy predicts freedom of appropriate ICD therapy in stable chronic heart failure patients

DO Verschure
JR de Groot
S Mirzaei
O Gheysens
K Nakajima
BL van Eck-Smit
GA Somsen
HJ Verberne
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ABSTRACT

Aim
Chronic heart failure (CHF) is a life-threatening disease, partly due to sudden cardiac death (SCD). Implantable cardioverter defibrillators (ICD) for primary prevention of SCD have improved overall survival of CHF patients. However, a high percentage of patients never receives appropriate ICD therapy. This prospective multicentre study evaluated whether cardiac sympathetic activity assessed by planar ¹²³I-mIBG scintigraphy could optimize selection for ICD implantation.

Materials and Methods
135 stable CHF subjects (age 64.5 ± 9.3 years, 79% male, LVEF 25 ± 6%) referred for ICD implantation for primary prevention in 13 European institutions were enrolled. All subjects underwent planar and SPECT ¹²³I-mIBG scintigraphy. Planar images were acquired at 15 minutes (early) and 4 hours (late) after administration of ¹²³I-mIBG. Early and late heart-to-mediastinum (H/M) ratio, ¹²³I-mIBG washout (WO) and late summed scores were calculated. Cross-calibrated phantom study-data were used to correct for different gamma camera-collimator use. The primary endpoint was appropriate ICD therapy. The secondary endpoint was the combined endpoint of all first cardiac events: appropriate ICD therapy, progression of heart failure (HF) and cardiac death.

Results
During a median follow-up of 30 months (6 - 68 months), 24 subjects (17.8%) experienced a first cardiac event (appropriate ICD therapy [12], HF progression [6], cardiac death [6]). The combination of late H/M ratio (HR 0.461 [0.281 - 0.757]) and LVEF (HR 1.052 [1.021 - 1.084]) was significantly associated with freedom of appropriate ICD therapy (p < 0.001). Late H/M ratio was independently associated with the combined endpoint (HR 0.135 [0.035 - 0.517], p = 0.001).

Conclusion
Planar myocardial ¹²³I-mIBG-derived late H/M ratio and LVEF were associated with freedom of appropriate ICD therapy. In addition there was a significant association between late H/M ratio and the combined endpoint. Therefore, ¹²³I-mIBG scintigraphy seems to be able to optimize the selection of CHF subjects who might benefit from ICD implantation.
123I-mIBG scintigraphy helpful for prophylactic ICD implantation?

INTRODUCTION

Despite therapeutic improvements the prognosis of chronic heart failure (CHF) remains unfavorable partly due to sudden cardiac death (SCD). The introduction of implantable cardioverter defibrillators (ICD) has improved overall survival of CHF patients. Based on large randomized trials, current European guidelines recommend ICD implantation for primary prevention of fatal arrhythmias in subjects with a left ventricular ejection fraction (LVEF) < 35% and symptomatic CHF with NYHA class ≥ 2 under optimal pharmacological therapy. ICDs applied for primary or secondary prevention of SCD reduce the relative risk of death by 20%. However, the MADIT II (Second Multicenter Automated Defibrillator Implantation Trial) has shown that the absolute reduction of fatal events was only 5.6% (mortality was 19.8% in the control and 14.2% in the ICD group during a mean follow-up of 20 months). In addition, the SCD-HeFT (Sudden Cardiac Death in Heart Failure Trial) study showed that the annual number of ICD shocks was 7.1%. However, the number of inappropriate ICD shocks was 2.0%. The cumulative number of appropriate shocks increased to 21% in the 5th year post-implantation. Conversely, three years after ICD implantation for primary prevention, a remarkably high percentage (65%) of patients had never received appropriate ICD therapy. Moreover, the risk of malfunction, (post)operative complications and the relatively high cost of these devices urges for optimization of current ICD selection criteria for primary prevention.

Cardiac sympathetic activity can non-invasively be assessed with meta-iodobenzylguanidine (123I-mIBG). The past decades, myocardial 123I-mIBG scintigraphy has been shown to predict prognosis in CHF patients. A late heart-to-mediastinum (H/M) ratio < 1.6 has been suggested to be a predictor of ventricular arrhythmia. Furthermore, decreased 123I-mIBG uptake and increased wash-out (WO) are associated with increased incidence of SCD or appropriate ICD therapy. Most of these studies have been conducted in various populations, both with primary and secondary prevention of SCD. In addition, extrapolation of the obtained data is hampered by the fact that the data were not corrected for differences in gamma camera-collimators. Therefore, the aim of this prospective study was to evaluate whether increased cardiac sympathetic activity (i.e., cross-calibrated decreased H/M ratio, increased 123I-mIBG WO) assessed by planar and SPECT 123I-mIBG scintigraphy could identify high-risk CHF patients most likely to experience appropriate ICD therapy for primary prevention of SCD.
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METHODS

Thirteen institutions in Europe (see “Appendix” for list of all participating institutions) participated. The study was approved by the local institutional review boards and conducted according to the principles of the International Conference on Harmonization–Good Clinical Practice. All subjects provided written informed consent before participation. The study was registered on www.trialregister.nl (registration number NTR2735).

Design

All included patients underwent cardiac $^{123}$I-mIBG scintigraphy within 2 weeks prior to ICD implantation and were followed for the occurrence of the primary and secondary endpoints. The primary endpoint for this study was appropriate ICD therapy: ICD therapy to overcome potentially fatal ventricular arrhythmias: i.e. anti-tachycardia pacing (ATP) or shock triggered by ventricular tachycardia or fibrillation. The secondary endpoint was defined as the combined endpoint of all first cardiac events: appropriate ICD therapy, progression of heart failure (HF) and cardiac death.

Subjects

Patients with stable CHF (ischaemic or non-ischaemic) who were referred for ICD implantation for primary prevention of SCD were enrolled between July 2010 - October 2015. The inclusion criteria were: 1. LVEF < 35%, 2. New York Heart Association (NYHA) functional class II or III, 3. Pacemaker-naive, 4. Stable and treated with optimal medical therapy for at least 3 months according to the European HF guidelines. Exclusion criteria were: 1. History of defibrillation to treat a previous ventricular arrhythmic event, 2. History of acute myocardial infarction within the previous 30 days. As part of the workup for ICD implantation all subjects underwent complete clinical evaluation including echocardiography and blood sample analysis.

$^{123}$I-mIBG scintigraphy acquisition and analysis

To block uptake of free $^{123}$I by the thyroid gland, subjects were pretreated with 250 mg oral potassium iodide 30 min before intravenous (IV) injection of 185 MBq $^{123}$I-mIBG (Adreview®, GE, Healthcare). Fifteen minutes (early acquisition) and 4 hours (late acquisition) after administration of $^{123}$I-mIBG, 10-min planar images were acquired from an anterior thoracic view (256 × 256 matrix) with the subjects in supine position. A 20% window was centered at 159 KeV. Additional SPECT $^{123}$I-mIBG images, without attenuation correction, were acquired after the late planar acquisitions (128 × 128 matrix).

All $^{123}$I-mIBG data were anonymized and sent to the study coordinating centre (Academic Medical Center, Amsterdam, the Netherlands). Planar data were analysed by one experienced observer (D.O.V.) blinded to patient data using post-processing software. Heart-to-mediastinum (H/M) ratio was calculated from planar $^{123}$I-mIBG images using a manually drawn region-of-interest (ROI) over the heart and a fixed rectangular
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mediastinal ROI. To correct for differences in gamma camera-collimator combination, institutional early and late planar H/M ratios were converted to standardized values by using conversion coefficients from our previous 123I-mIBG cross-calibrated phantom study. The washout (WO) was defined by:

\[
WO = \left( \frac{\text{early H/M ratio} - \text{late H/M ratio}}{\text{early H/M ratio}} \right) \times 100
\]

All late SPECT 123I-mIBG images were analysed by two experienced observers (B.L.v.E.S. and H.J.V.) blinded to patient data according a previous published protocol. Summed scores (range 0–68) were derived by the standard 17-segment model and 5-point scoring method.

ICD implantation

After myocardial 123I-mIBG imaging transvenous or subcutaneous ICDs were implanted in the participating institutions. Testing of sensing, pacing and defibrillation thresholds was performed according to local protocols. In case of patient eligibility for cardiac resynchronization therapy (CRT), a combined CRT-D device was implanted. Settings for detection of ventricular tachycardias or fibrillation were at the discretion of the implanting physician.

Clinical follow-up and event adjudication

Follow-up was based on telephone interviews (D.O.V.) and medical records. All subjects received standard clinical care and were followed up until: 1. subjects death was confirmed by medical records of the general practitioner; 2. the trial was terminated (30th of April 2016). The Clinical Adjudication Committee, whose members were unaware of the scintigraphy data, reviewed all data from case record forms and source documents to confirm occurrence of cardiac events, specifically: 1. HF progression: increase in symptomatic status from NYHA functional class II to III or IV, or increase from NYHA class III to class IV; 2. potentially life-threatening arrhythmic event, including documented episode of spontaneous sustained (30 s) ventricular tachyarrhythmia, resuscitated cardiac arrest, or appropriate ICD therapy: ATP or defibrillation; or 3. cardiac death (further classified as due to terminal heart failure and SCD).

Comparison with a historical Japanese CHF cohort

To compare the mortality rate of our study population with other published data we used a risk model, based on a historical Japanese CHF cohort. This model estimates the 2-years mortality risk based on four variables (NYHA class, age, LVEF and standardized late H/M ratio). Using the median 2-year mortality of 9%, patients were divided into 2 groups: mortality rate < 9% and ≥ 9%. Since the Japanese risk model was made using data from 1990, the effect of ICD therapy was not included and
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| Table 1. Patients characteristics with subgroups appropriated ICD therapy, combined endpoint (CE) and no CE. |
|---|---|---|---|---|
| | All \((n = 135)\) | Appropriatie ICD discharge \((n = 12)\) | CE \((n = 24)\) | No CE \((n = 111)\) |
| Age (years) | 64.5 ± 9.3 | 65.8 ± 6.9 | 66.4 ± 7.1 | 64.1 ± 9.6 | 0.471 |
| Male (%) | 106 (79) | 10 (83) | 21 (88) | 85 (77) | 0.462 |
| Ischaemic heart disease (%) | 80 (59) | 9 (75) | 16 (67) | 64 (58) | 0.409 |
| NYHA functional class | | | | | 0.308 |
| II | 104 (77) | 8 (67) | 16 (67) | 88 (79) | |
| III | 31 (23) | 4 (33) | 8 (33) | 23 (21) | |
| LVEF (%) | 25.0 ± 6.2 | 26.2 ± 5.8 | 25.4 ± 6.3 | 25.0 ± 6.2 | 0.787 |
| BMI | 27.9 ± 4.7 | 28.1 ± 3.9 | 29.1 ± 6.0 | 27.7 ± 4.3 | 0.364 |
| Systolic blood pressure (mmHg) | 127 ± 19 | 129 ± 18 | 125 ± 16 | 128 ± 19 | 0.828 |
| Diastolic blood pressure (mmHg) | 73 ± 12 | 77 ± 12 | 76 ± 10 | 75 ± 12 | 0.852 |
| GRS time (msec) | 125 ± 21 | 125 ± 21 | 117 ± 18 | 132 ± 32 | 0.209 |
| ICD type | | | | | |
| Subcutaneous (%) | 7 (5) | 2 (17) | 2 (8) | 3 (3) | 0.065 |
| CRT (%) | 56 (41) | 3 (25) | 10 (42) | 45 (41) | 0.980 |
| Cardiovascular risk factors | | | | | |
| Diabetes (%) | 39 (29) | 5 (42) | 10 (42) | 29 (26) | 0.330 |
| Hypertension (%) | 76 (56) | 7 (58) | 13 (54) | 63 (57) | 0.965 |
| Dyslipidemia (%) | 56 (42) | 7 (58) | 12 (50) | 44 (40) | 0.465 |
| Smoking (%) | 34 (25) | 3 (25) | 11 (46) | 23 (21) | 0.633 |
| Medication | | | | | |
| Beta-blocker (%) | 112 (83) | 11 (92) | 18 (75) | 94 (85) | 0.379 |
| CCB (%) | 12 (9) | 25 (25) | 4 (17) | 8 (7) | 0.081 |
| ACE-I/ARB (%) | 126 (93) | 11 (92) | 22 (92) | 104 (94) | 0.917 |
| Loop diuretic (%) | 88 (65) | 10 (83) | 20 (83) | 68 (61) | 0.051 |
| MRA(%) | 64 (47) | 7 (58) | 13 (54) | 51 (46) | 0.594 |
| Asperin (%) | 64 (47) | 5 (42) | 10 (42) | 54 (49) | 0.770 |
123I-mIBG scintigraphy helpful for prophylactic ICD implantation?

Continuation table 1.

<table>
<thead>
<tr>
<th></th>
<th>All (n = 135)</th>
<th>Appropriate ICD discharge (n = 12)</th>
<th>CE (n = 24)</th>
<th>No CE (n = 111)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statine (%)</td>
<td>86 (64)</td>
<td>9 (75)</td>
<td>15 (63)</td>
<td>71 (64)</td>
<td>0.733</td>
</tr>
<tr>
<td>Planar 123I-mIBG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early H/M ratio</td>
<td>2.04 ± 0.39</td>
<td>2.09 ± 0.27</td>
<td>2.00 ± 0.26</td>
<td>2.06 ± 0.42</td>
<td>0.721</td>
</tr>
<tr>
<td>Late H/M ratio</td>
<td>1.79 ± 0.38</td>
<td>1.74 ± 0.20</td>
<td>1.66 ± 0.24</td>
<td>1.82 ± 0.40</td>
<td>0.131</td>
</tr>
<tr>
<td>123I-mIBG WO</td>
<td>12.4 ± 9.2</td>
<td>16.2 ± 8.0</td>
<td>16.6 ± 9.2</td>
<td>11.5 ± 8.9</td>
<td>0.017*</td>
</tr>
<tr>
<td>Late SPECT 123I-mIBG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summed score</td>
<td>39.4 ± 15.5</td>
<td>34.9 ± 13.1</td>
<td>37.4 ± 14.7</td>
<td>39.8 ± 15.7</td>
<td>0.516</td>
</tr>
<tr>
<td>Predicted 2-year mortality</td>
<td>12.4 ± 9.4</td>
<td>14.3 ± 9.5</td>
<td>16.1 ± 11.0</td>
<td>11.6 ± 11.0</td>
<td>0.036</td>
</tr>
<tr>
<td>risk (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = difference between CE and no CE, p = 0.035. NYHA: New York Heart Association, LVEF: left ventricular ejection fraction; BMI: body mass index; ICD: implantable cardioverter defibrillator, CRT: cardiac resynchronization therapy; CCB: calcium channel blocker; ACE-I: angiotensin converting enzyme-inhibitor; ARB: angiotensin II receptor blockers; MRA: mineral receptor blocker.

therefore appropriate ICD therapy was scored as a fatal event. As this mortality risk model assumes complete 2-year follow-up, patients who had a follow-up < 2 years and were alive were excluded for this analysis.

STATISTICAL ANALYSIS

All continuous variables are expressed as mean ± standard deviation. Difference between groups for continuous data we compared using analysis of variance (ANOVA) with post-hoc Bonferroni. Efficacy analysis used univariate and multivariate Cox proportional regression hazards models using age, NYHA class, LVEF, early and late H/M ratio, 123I-mIBG WO and SPECT summed score as variables. Forward elimination determined the combination of variables that most influenced the time-over-event model. The overall goodness-of-fit for each model was expressed as the adjusted $R^2$. The F-test was used to assess whether a model explained a significant proportion of the variability. A $p$-value <0.05 was considered to indicate a statistically significant difference. Statistical analyses were performed with SPSS, release 22.0 (SPSS Inc., Chicago, IL, USA 2003).
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Figure 1. Frequency of combined endpoint per tertile late H/M ratio.

Figure 2. Kaplan Meier curves of appropriated ICD therapy in patients with (blue line) and without (red line) ischaemic heart disease.
RESULTS

Subjects
A total of 135 stable CHF patients (79% men, age 64.5 ± 9.3) were enrolled. Baseline characteristics of the study population are shown in Table 1. Almost 60% of the patients had ischaemic heart disease. Mean NYHA class was 2.2 ± 0.4 and mean LVEF was 25.0 ± 6.2%. The mean early H/M ratio was 2.05 ± 0.39, the late H/M ratio was 1.79 ± 0.38 and 123I-mIBG WO was 12.4 ± 9.2%. The mean SPECT summed score was 39.4 ± 15.5.

Predictor of appropriate ICD therapy and cardiac events
Follow-up was complete for all 135 patients with a median follow-up of 30 months (6 - 68 months). In total 34 patients experienced 43 events during the course of the study. Death from all causes occurred in 15 patients (11.1%). Of these, 9 were cardiac deaths (6.7%), of which 5 were SCD and 4 due to terminal heart failure. Seventeen patients (12.6%) received ICD therapy of whom 12 patients received appropriate therapy (i.e., ATP in 8 patients and ICD shocks in 4 patients). Furthermore 6 patients had progression of HF.

For the analysis of the primary and secondary endpoint 24 patients (17.8%) experienced a first cardiac event during the course of the study: appropriate ICD therapy (n = 12), progression of CHF (n = 6), cardiac death (n = 6), consisting of SCD (n = 5) and terminal heart failure (n = 1) (Figure 1). Kaplan Meier curves for appropriate ICD therapy in patients with and without ischaemic heart disease was not significantly different (Figure 2).

Six patients (4.4%) experienced more than one cardiac event. One patient experienced inappropriate ICD therapy more than once. Pocket revision due to hematoma post-implantation was needed in 2 patients and ICD replacement due to end of life of the battery was performed in 3 patients.

A total of 98 patients had complete follow-up at 2 years. There was agreement between the estimated and the actual events (2 vs. 3 in the group with risk of < 9% and 10 vs. 8 in the group with risk of ≥ 9%, between estimated and actual events, respectively) (Table 2). Thereby validating the mortality rate in our study population.

There was an overlap in late H/M ratio between the patients with and without appropriate ICD therapy (Figure 3). Appropriate ICD therapy exclusively occurred in patients with late H/M ratios between 1.40 and 2.10. Figure 4 shows Kaplan Meier curves for appropriate ICD therapy for each tertile of late H/M ratio. Multivariate Cox regression analysis showed no independent predictors for appropriate ICD therapy. In univariate Cox regression analysis decreased late H/M ratio (HR 0.134 [0.035 - 0.515], p = 0.001) and increased WO (HR 1.062 [1.014 - 1.112], p = 0.010) were associated with the occurrence of the combined endpoint. Multivariate Cox regression analysis showed that only decreased late H/M ratio remained significant as an independent predictor of the combined endpoint (Table 3).
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Table 2. Two-year events compared with number of events estimated by 2-year risk model.

<table>
<thead>
<tr>
<th></th>
<th>Appropriate ICD discharge</th>
<th>Sudden cardiac death</th>
<th>End stage HF death</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-year risk &lt; 9% (n = 44)</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Estimated number of cardiac death by 2-year risk model</td>
<td>6.8%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>6.8%</td>
</tr>
<tr>
<td>2-year risk ≥ 9% (n = 54)</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Estimated number of cardiac death by 2-year risk model</td>
<td>7.4%</td>
<td>5.6%</td>
<td>1.9%</td>
<td>14.8%</td>
</tr>
</tbody>
</table>

Table 3. Multivariate Cox regression analysis for freedom of appropriate ICD therapy and combined endpoint of first cardiac events.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR (95% CI)</th>
<th>χ²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined endpoint</td>
<td>Late H/M ratio 0.135 (0.035-0.517)</td>
<td>10.136</td>
<td>0.001</td>
</tr>
<tr>
<td>Free of appropriate ICD therapy</td>
<td>LVEF 1.052 (1.021-1.084)</td>
<td>17.542</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Late H/M ratio 0.461 (0.281-0.757)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As there were no independent predictors for appropriate ICD therapy we performed a Cox regression analysis to look for clinical independent predictors of freedom of appropriate ICD therapy. Univariate Cox regression analysis showed that freedom of appropriate ICD therapy was associated with increased LVEF (HR 1.045 [1.015 - 1.077], \( p = 0.003 \)), decreased early H/M ratio (HR 0.504 [0.304 - 0.836], \( p = 0.006 \)) and decreased late H/M ratio (HR 0.533 [0.329 - 0.863], \( p = 0.009 \)). In multivariate Cox regression analysis only LVEF and late H/M ratio remained independent predictors of freedom of appropriate ICD therapy (Table 3).

DISCUSSION

The present study demonstrates that in stable CHF patients with prophylactic ICD implantation late H/M ratio was the only independent predictor for the combined end-
\textit{\textsuperscript{123}I-mIBG scintigraphy helpful for prophylactic ICD implantation?}

![Boxplot of late H/M ratios for patients with and without appropriate ICD therapy.](image1)

**Figure 3.** Boxplot of late H/M ratios for patients with and without appropriate ICD therapy ($p = 0.036$). Green lines are mean values. The box plot indicates median, 25%, and 75% quartile with whiskers for both ends.

![Kaplan Meier curves for appropriate ICD therapy with tertiles.](image2)

**Figure 4.** Kaplan Meier curves for appropriate ICD therapy with tertiles: H/M ratio < 1.40, H/M ratio 1.40 - 2.10, H/M ratio >2.10.
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point. None of the parameters tested was able to predict appropriate ICD therapy solely. However, freedom of appropriate ICD therapy was independently predicted by lower late H/M ratio and higher LVEF.

ICD implantation has become an integral component of contemporary HF management. Although the benefits of ICD implantation have been demonstrated in several randomized clinical trials, questions have been raised if the current patient selection criteria are adequate, as it is still unclear which patients will benefit from ICD implantation. In the MADIT II population only 35% of the patients received appropriate ICD therapy during 3 years of follow-up. Moreover, in about 15% of patients with coronary artery disease, SCD is the first manifestation of underlying cardiac disease.

Even though LVEF < 35% is most commonly used for risk stratification for ventricular arrhythmias, it does not adequately identify patients at risk for SCD. For example, arrhythmic deaths occur also in populations with normal LVEF, but these patients are not qualified for ICD implantation according to the current guidelines. Shah et al. have shown that cardiac $^{123}$I-mIBG imaging provides prognostic information across a broad spectrum of LVEFs.

In this contemporary study, modern ICD programming with longer detection times was used. This type of programming could explain the relative small number of ICD therapy in the current study compared to the results of the SCD-HeFT study.

In contrast to previous studies that demonstrated an increased risk of ventricular arrhythmia or appropriate ICD therapy with decreased late H/M ratio, the results of the present study showed that a decreased late H/M ratio was associated with freedom of appropriate ICD therapy (i.e. no ventricular arrhythmia). The results in the present study showed “bell-shape” curve for late H/M ratio in relation to appropriated ICD therapy (Figure 1). Patients with intermediate late H/M ratio (range 1.4 - 2.10) are more likely to have appropriate ICD therapy compared to patients with low and high late H/M ratios (Figure 4). In line with our findings Travin et al. concluded that the presumption of a monotonic increase in risk of an arrhythmic event with increasing $^{123}$I-mIBG SPECT defects may not be correct. This conclusion was based on the observation that in ischaemic CHF patients, those with intermediate defects on $^{123}$I-mIBG SPECT summed score appeared to be at the highest risk for cardiac events. Both findings of the present study and Travin et al. are new and may be useful to better identify patients who, most likely, benefit from ICD implantation.

In addition to global cardiac sympathetic hyperactivity assessed with $^{123}$I-mIBG scintigraphy there is evidence that in ischaemic HF regional innervation/perfusion mismatch with a larger defect size on $^{123}$I-mIBG SPECT than on myocardial perfusion imaging SPECT, predispose to ventricular arrhythmias. In a large prospective study in 116 CHF patients, eligible for ICD implantation for both primary and secondary prevention of SCD, $^{123}$I-mIBG SPECT was shown to be an independent predictor of appropriate ICD therapy and cardiac death. The cumulative incidence of appropriate
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ICD therapy during 3 years of follow-up was significantly higher when a relatively large \(^{123}\text{I}-\text{mIBG}\) SPECT defect (median summed score \(\geq 26\)) was present. Similar results have been shown in a prospective PET study using \(^{11}\text{C}\)-hydroxyephedrine for assessing sympathetic activity in ischaemic heart disease \((n = 204)\), in which the innervation defect size predicted cause-specific mortality from SCD independently of LVEF and infarct volume.\(^{25}\)

In the present study the defect size of \(^{123}\text{I}-\text{mIBG}\) SPECT was large \((39.4 \pm 15.5)\). However, there was no significant difference between patients with and without appropriated ICD therapy. Consequently, \(^{123}\text{I}-\text{mIBG}\) SPECT had no additional predictive value to planar \(^{123}\text{I}-\text{mIBG}\)-derived parameters (i.e. late H/M ratio and \(^{123}\text{I}-\text{mIBG}\) WO). One of the possible explanations for these findings could be the inclusion of patients with both ischaemic and non-ischaemic HF, whereas in innervation/perfusion mismatch studies only subjects with ischaemic HF were included. However, the cause of HF (ischaemic and non-ischaemic) was not associated with appropriate ICD therapy.

For the secondary endpoint, decreased late H/M ratio was associated with increased risk for combined endpoint independent of LVEF and NYHA functional class. This outcome is in line with previous studies that demonstrated the prognostic value of \(^{123}\text{I}-\text{mIBG}\) scintigraphy derived parameters in CHF.\(^{26,27}\) In addition, a meta-analysis has shown similar results, but the majority of included studies were single centre.\(^6\) The large multicentre ADMIRE-HF reported that decreased late H/M ratio was associated with the composite endpoint of HF progression, ventricular tachyarrhythmia and death, independent of LVEF and brain natriuretic peptide (BNP).\(^7\) A predefined late H/M ratio > 1.6 was associated with an incidence of 1% cardiac death per year while the annual cardiac mortality in a cohort with late H/M level < 1.2 was almost 10 times higher (9.6%).

The majority of patients who die of ventricular arrhythmia have structural heart disease, predominately coronary artery disease. Although the exact pathophysiology of ventricular arrhythmias is still a matter of debate, it has been recognized that myocardial ischaemia and scar tissue may serve as substrate for ventricular arrhythmias. Areas with slow conduction may facilitate the development of reentrant tachycardia.\(^{28}\) Cardiac sympathetic hyperactivity is also an important factor in the genesis of potential lethal ventricular arrhythmias in patients with impaired LVEF. In these patients rhythm abnormalities develop in relation to enhanced automaticity, triggered automaticity, and reentrant mechanisms. These mechanisms are enhanced by release of NE. In addition non-uniform denervated myocardium in infarct zones can be hypersensitive to NE.\(^{29}\) Especially the border zone of infarct tissue with viable myocardial tissue is predisposed to develop reentrant circuits. This mechanism is most likely triggered by the fact that sympathetic nerve fibers are more susceptible to ischaemia than myocytes, thereby causing a disbalance between still viable but partly denervated and normal myocardium.\(^{30,31}\) This disbalance in myocardial sympathetic innervation may create a myocardial substrate particularly vulnerable to arrhythmia and arrhythmic
The results of our study, showing a higher rate of appropriated ICD therapy in ischaemic HF to non-ischaemic HF, are in line with this hypothesis (Figure 2).

Recently, the DANISH trial has shown that prophylactic ICD implantation in patients with symptomatic non-ischaemic systolic heart failure was not associated with improved survival compared with usual clinical care. However, SCD occurred less often in the ICD group compared to the control group (HR 0.50 [0.31 - 0.82], p = 0.005). Most likely the outcome of this study will change the indications for prophylactic ICD implantation in non-ischaemic CHF patients. Some non-ischaemic CHF patients remain at an increased risk for SCD. In line with this finding, our study showed that despite optimal medical therapy patients without ischaemic heart disease still received appropriated ICD therapy. Future studies will be needed to confirm whether myocardial \textsuperscript{123}I-mIBG scintigraphy is helpful in specifically identifying non-ischaemic CHF patients with an increased SCD risk.

Based on the results of previous studies, it has been suggested that screening of guideline eligible CHF patients selected for ICD with myocardial \textsuperscript{123}I-mIBG scintigraphy may be cost effective with respect to ICD implantation with minimal impact on survival. Incorporating myocardial \textsuperscript{123}I-mIBG scintigraphy into the assessment of CHF patients eligible for ICD implantation was associated with a 21% reduction in ICD utilization. Consequently, the number needed to screen to prevent 1 ICD implantation is 5. Screening with \textsuperscript{123}I-mIBG imaging will reduce the costs per patient with minimal losses of 0.001 and 0.040 life-years, respectively, over 2 and 10 years.

**Study limitations**

Although the current study clearly demonstrated that \textsuperscript{123}I-mIBG assessed cardiac sympathetic activity was predictive for the overall prognosis and appropriate ICD therapy, some limitations need to be considered. Most importantly, the relatively small event rate of appropriate ICD therapy may have resulted in a limited statistical power. Therefore, the results should be regarded as hypothesis-generating. In addition, the aetiology of HF in the enrolled patient population was heterogeneous including ischaemic and non-ischaemic HF. Therefore, additional studies are needed to establish the specific role of \textsuperscript{123}I-mIBG imaging in specific subpopulations. Furthermore, \textsuperscript{123}I-mIBG SPECT imaging in patients with severe LV dysfunction is frequently hampered by reduced myocardial tracer uptake, resulting in poor-quality tomographic images.

In conclusion, planar myocardial \textsuperscript{123}I-mIBG scintigraphy derived late H/M ratio and LVEF were associated with freedom of appropriate ICD discharge. In addition there was a significant association between late H/M ratio and the combined endpoint. Therefore, \textsuperscript{123}I-mIBG scintigraphy appears to be able to optimize the selection of CHF subjects who might benefit from an ICD implantation and therefore helps to constrain the HF related costs.
APPENDIX

Participating local investigators (alphabetical order of institutions) included:

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W. Jansen, Tergooi Ziekenhuis, Hilversum, the Netherlands,
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W. Mullens, Ziekenhuis Oost-Limburg, Genk, Belgium

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REFERENCES


123I-mIBG scintigraphy helpful for prophylactic ICD implantation?


Iodine-123-mIBG scintigraphy helpful for prophylactic ICD implantation?
PART III

Cardiac $^{123}$I-mIBG scintigraphy in patients other than heart failure
Cardiac sympathetic activity
in 22q11.2 deletion syndrome

DO Verschure
E Boot
TA van Amelsvoort
J Booij
BL van Eck-Smit
GA Somsen
HJ Verberne
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ABSTRACT

Aim
22q11.2 Deletion syndrome (22q11.2DS) affects catechol-O-methyl-transferase (COMT), which involves the degradation of norepinephrine (NE). Clinically, adults with 22q11.2DS are at increased risk for sudden unexpected death. Although the causes are likely multifactorial, increased cardiac sympathetic activity with subsequent fatal arrhythmia, due to increased levels of NE, should be considered as a possible mechanism predisposing to this premature death. The purpose of this study was to determine whether cardiac sympathetic activity is increased in 22q11.2DS, both at baseline and following an acute NE depletion with alpha-methyl-para-tyrosine (AMPT).

Materials en methods
Five adults with 22q11.2DS and five age- and sex-matched healthy controls underwent 2 sessions with either AMPT or placebo administration before [123I]-mIBG scintigraphy. Heart-to-mediastinum (H/M) ratios were determined from the images 15 minutes (early) and 4 hours (late) after administration of [123I]-mIBG and the [123I]-mIBG washout (WO) was calculated as an indicator of adrenergic drive.

Results
At baseline there were no significant differences in both early and late H/M ratio between 22q11.2DS and controls. However, there was a significant difference in [123I]-mIBG WO between 22q11.2DS and controls (-4.92 ± 2.8 and -10.44 ± 7.2, respectively; p = 0.027), but a “negative” [123I]-mIBG WO does not support an increased sympathetic drive. In addition there was a trend towards a higher late H/M ratio after AMPT administration compared to baseline which was more pronounced in 22q11.2DS.

Conclusion
This study for the first time suggests normal cardiac sympathetic activity in adults with 22q11.2DS assessed by [123I]-mIBG scintigraphy. Although there is a small difference in adrenergic drive compared to healthy subjects, this most likely does not explain the increased unexpected death rate in the 22q11.2 DS population.
INTRODUCTION

22q11.2 Deletion syndrome (22q11.2DS) is caused by a microdeletion on the long arm of chromosome 22 and affects approximately 1:2000 live births.\(^1\)\(^2\) This genetic condition has a highly variable clinical phenotype with amongst others congenital heart disease (CHD) and psychiatric disorders.\(^3\) The deleted region in 22q11.2DS spans more than 40 genes, one of which is the gene that encodes for catechol-O-methyl-transferase (COMT).\(^4\) This enzyme involves the degradation of catecholamines, including dopamine (DA) and norepinephrine (NE) (Figure 1). People with 22q11.2DS have haploinsufficiency of COMT, resulting in lower enzymatic activity\(^5\), which may result in abnormal catecholamine levels. Indeed, we showed that in 22q11.2DS subjects urinary DA concentrations are increased and urine and plasma levels of DA metabolites are decreased compared with healthy subjects.\(^6\) In addition, acute monoamine depletion paradigms using alpha-methyl-\(p\)-tyrosine (AMPT), a reversible inhibitor of the first and rate-limiting step in the biosynthesis of catecholamines (Figure 1), has been used successfully to assess endogenous brain DA \textit{in vivo}.\(^7\)

Clinically, individuals with 22q11.2DS who survive childhood have diminished life expectancy and have an increased risk of sudden unexpected death.\(^8\) Although the causes are likely multifactorial, hyperactivity of the cardiac sympathetic system is an important factor for the pathophysiology of fatal arrhythmias including enhance automaticity, triggered automaticity and reentrance.\(^9\) Therefore increased cardiac sympathetic activity, due to increased levels of NE, should be considered as a possible mechanism predisposing to premature death in 22q11.2DS. However, to the best of our knowledge, no data are available whether the cardiac sympathetic system is affected in adults with 22q11.2DS.

NE is a sympathetic neurotransmitter that stimulates the \(\beta\)-adrenoreceptors, which induces positive chronotropic and inotropic effects. \textit{Meta}-iodobenzylguanidine (mIBG), a NE analog, shares the same presynaptic uptake, storage and release mechanism as NE. Radiolabeling with \(^{123}\)I allows assessment of presynaptic \(^{123}\)I-mIBG uptake through the uptake-1 mechanism (i.e. NE transporter). This non-invasive technique has been extensively validated and shown to be of clinical value in many cardiac diseases.\(^10\)-\(^12\)

We hypothesized that, due to COMT haploinsufficiency, people with 22q11.2DS are exposed to increased cardiac levels of NE and thereby have an increased cardiac sympathetic activity. In addition, the effect of acute monoamine depletion by AMPT could give additional information about the cardiac NE metabolism. Therefore, the purpose of this study was to determine whether cardiac sympathetic activity assessed with \(^{123}\)I-mIBG scintigraphy, both at baseline and following an acute depletion challenge with AMPT, is different in adults with 22q11.2DS (without CHD) compared with healthy controls.
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Tyrosine → L-DOPA → Dopamine → Norepinephrine

TH
AMPT

MAO
COMT

DOPAC
3-MT
Normetanephrine
DHPG

COMT
MAO

HVA
MHPG

Figure 1. Catecholamine metabolism and the negative effect of alpha-methyl-para-tyrosine (AMPT), a reversible inhibitor of tyrosine hydroxylase (TH), on the biosynthesis of catecholamines. MAO, monoamine oxidase; COMT, catechol-O-methyl-transferase; DOPAC, 3,4-dihydroxyphenylacetic acid; 3-MT, 3-methoxytyramine; DHPG, dihydroxyphenylglycine; HVA, homovanillic acid; MHPG, 3-methoxy-4-hydroxyphenylglycol.

MATERIAL AND METHODS

Subjects
Adults with 22q11.2DS were recruited through the Dutch 22q11.2DS Family Association. For each 22q11.2DS subject, an age- and sex-matched healthy control was included. Inclusion criteria for all subjects were as follows: (1) no current or past psychiatric history, (2) no current or previous exposure to antipsychotics or stimulant medication; (3) no CHD, proven by echocardiography; and for 22q11.2DS subjects (4) a deletion at the 22q11.2 region as determined by fluorescent in-situ hybridization. All subjects provided written informed consent. The study was approved by the local institutional review board and conducted according to the principles of the International Conference on Harmonization–Good Clinical Practice.

Protocol
Subjects underwent 2 identical sessions separated by a 2 week interval in which they received either AMPT or placebo. Subjects were randomized and blinded to oral administration of AMPT or placebo. After administration of AMPT or placebo all subjects underwent $^{131}$I-mIBG scintigraphy and blood samples were drawn for analysis of prolactin (PRL) at baseline and after AMPT administration.

Questionnaires
To observe for extrapyramidal side-effects of AMPT, all subjects were assessed with the Simpson Angus Scale, Beck Anxiety Inventory and Subjective Well-being under Neuroleptic treatment scale at baseline and 8 h after the initial AMPT dose ($T_0$ and $T_p^{13-15}$).
Depletion regimen

The AMPT competition model is a well-accepted paradigm to assess endogenous brain DA. Due to the acute, but reversible, decrease in DA production induced by AMPT, the availability of 123I-iodobenzamide (123I-IBZM) to bind to free striatal DA D_{2/3}-receptors is increased. 123I-IBZM is a selective DA antagonist that binds to dopamine D_{2/3}-receptors. Comparing DA D_{2/3}-receptor binding at baseline and in the AMPT-induced DA depleted state provides an indirect assessment of endogenous brain DA. This paradigm is now used to assess the cardiac NE. The administration of AMPT induces not only a decrease in production of DA but also of NE (Figure 1). We hypothesized that acute, but reversible decrease in NE production induced by AMPT result in less competition between 123I-mIBG and NE resulting in increased 123I-mIBG uptake. Based on our experience in previous studies a total dose of 1500 mg AMPT (Pharmaceutics International Inc., United States of America) was administered over 4 h (Figure 2).

Prolactin

DA is the predominant hypothalamic inhibiting factor of PRL release in humans, and DA D_{3} receptor stimulation has inhibiting effects on PRL release in the anterior pituitary. 16 In addition, in sheep NE has inhibiting effects on PRL release. 17 Previously, PRL levels have been used as a proxy marker of the effectiveness of catecholamine depletion by AMPT. 18 Therefore, plasma concentrations of PRL were measured at baseline and at 4 and 6 h after the first AMPT administration (T_{0}, T_{4} and T_{6}, respectively).

123I-mIBG scintigraphy acquisition and analysis

To block uptake of free 123I by the thyroid gland, subjects were pretreated with 250 mg oral potassium iodide 30 min before intravenous (IV) injection of 185 MBq 123I-mIBG (T_{4.45}). Fifteen minutes (early images) (T_{5}) and 4 h (late images) (T_{8.45}) after administration of 123I-mIBG, 10-min planar images were acquired.
All planar $^{123}$I-mIBG images were analysed by one experienced observer (D.O.V.) blinded to patient data. Heart-to-mediastinum (H/M) ratios were calculated from the $^{123}$I-mIBG images using a region-of-interest (ROI) over the heart and the upper part of the mediastinum (Figure 3). The H/M ratio was calculated by dividing the mean count density in the cardiac ROI by the mean count density in the mediastinal ROI. $^{123}$I-mIBG washout (WO) was calculated as followed:

$$WO = \left( \frac{\text{early H/M ratio} - \text{late H/M ratio}}{\text{early H/M ratio}} \right) \times 100$$

The H/M ratio reflects presynaptic uptake of $^{123}$I-mIBG. The early H/M ratio reflects predominantly the integrity of sympathetic nerve terminals (i.e. number of functioning nerve terminals and intact uptake-1 mechanism). The late H/M ratio offers predominantly information about neuronal function resulting from uptake, storage and release. The $^{123}$I-mIBG WO reflects predominantly neuronal integrity of sympathetic tone/adrenergic drive.

Statistical Analysis
All continuous variables are expressed as a mean ± standard deviation. After demonstrating a normal distribution of variables, between-group comparisons were performed by using independent-sample t-tests and paired t-tests. Statistical analysis concerning PRL levels at different time points were performed using repeated-measure ANOVA with a posthoc Bonferroni correction. A two-tailed probability value lower than 0.05 was selected as an indicator of statistical significance. Statistical analyses were performed with SPSS, release 22.0 for Windows (SPSS Inc., Chicago, IL, USA 2003).

RESULTS

Demographic data
Five subjects with 22q11.2DS and five age- and sex-matched controls, aged 20 – 39 years completed the protocol. The age (mean ± SD) of the subjects was 28.6 ± 4.8 and 28.0 ± 7.9 years, respectively. There were 3 males and 2 females in both groups.

AMPT depletion
Only three subjects (2 subjects with 22q11.2DS and 1 control) reported feeling tired after AMPT administration, which resolved in the hours after the last AMPT intake. No serious adverse events were observed.
Cardiac sympathetic activity in 22q11.2DS

Figure 3. Example of post processing planar $^{123}$I-mIBG images of a 22q11.2DS subject (late H/M ratio = 2.97). The positioning of the mediastinum ROI (M) is determined in relation to the lung apex, the lower boundary of the upper mediastinum, and the midline between the lungs. The manually drawn cardiac ROI (H) is placed over the myocardium including the left ventricular cavity.

![Image of cardiac sympathetic activity](image.png)

Figure 4. Mean plasma prolactin (µg/L) levels following α-methyl-para-tyrosine (AMPT) administration in subjects with 22q11.2DS and controls. Both in 22q11.2DS and controls, administration of AMPT induced an increase in PRL, although this was only significant in the 22q11.2DS group.

![Box plots of prolactin levels](image.png)
Peripheral markers of prolactin

Baseline levels of PRL (T0) were not significantly different between 22q11.2DS subjects (14.6 ± 3.0 μg/l) and controls (14.3 ± 3.2 μg/l). In both groups there was an increase of mean PRL levels 4h (T4) after the first gift of AMPT (Figure 4). The levels of PRL subsequently decreased at T6. Compared with baseline there was no significant difference between both groups in mean PRL levels at T4 and T6. The change of PRL levels from baseline was only significant in the 22q11.2DS group.

123I-mIBG scintigraphy

The results of the 123I-mIBG scintigraphy are shown in Table 1. At baseline and after depletion with AMPT there were no significant differences in early and late H/M ratio between the 22q11.2DS and the control group. However, there was a significant difference in 123I-mIBG WO at baseline between the 22q11.2DS and the control group (p = 0.027). AMPT did not significantly change early and late H/M ratio and 123I-mIBG WO compared to baseline in both groups. However, compared to baseline there was no significant difference in 123I-mIBG WO between the 22q11.2DS and the control group (p = 0.786).

Table 1. 123I-mIBG scintigraphy results at baseline and after depletion with AMPT and the difference between baseline and after depletion with AMPT.

<table>
<thead>
<tr>
<th></th>
<th>22q11 DS (n = 5)</th>
<th>Controls (n = 5)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early H/M ratio</td>
<td>2.67 ± 0.34</td>
<td>2.59 ± 0.33</td>
<td>0.652</td>
</tr>
<tr>
<td>Late H/M ratio</td>
<td>2.80 ± 0.37</td>
<td>2.85 ± 0.32</td>
<td>0.465</td>
</tr>
<tr>
<td>123I-mIBG WO</td>
<td>-4.92 ± 2.82</td>
<td>-10.4 ± 7.29</td>
<td></td>
</tr>
<tr>
<td>AMPT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early H/M ratio</td>
<td>2.67 ± 0.25</td>
<td>2.50 ± 0.40</td>
<td>0.266</td>
</tr>
<tr>
<td>Late H/M ratio</td>
<td>2.93 ± 0.40</td>
<td>2.90 ± 0.33</td>
<td>0.641</td>
</tr>
<tr>
<td>123I-mIBG WO</td>
<td>-9.94 ± 7.11</td>
<td>-16.75 ± 7.66</td>
<td>0.786</td>
</tr>
<tr>
<td>Difference baseline and AMPT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early H/M ratio</td>
<td>-0.00 ± 0.24</td>
<td>-0.09 ± 0.13</td>
<td>0.385</td>
</tr>
<tr>
<td>Late H/M ratio</td>
<td>0.14 ± 0.37</td>
<td>0.05 ± 0.23</td>
<td>0.463</td>
</tr>
<tr>
<td>123I-mIBG WO</td>
<td>-5.02 ± 5.35</td>
<td>-6.31 ± 5.48</td>
<td>0.970</td>
</tr>
</tbody>
</table>
DISCUSSION

This is the first study that investigated $^{123}$I-mIBG assessed cardiac sympathetic activity in adults with 22q11.2DS, a relatively common genetic condition associated with sudden unexpected death of unknown cause and impaired catecholamine turnover. In our study early and late H/M ratio in 22q11.2DS subjects were not different from healthy controls, and comparable with data published in other healthy (Japanese) subjects.\textsuperscript{20} In both groups late H/M ratio was higher compared to early H/M ratio. Consequently, $^{123}$I-mIBG WO was “negative” (i.e. indicative for increased $^{123}$I-mIBG myocardial uptake over time). The $^{123}$I-mIBG WO depends on the release, uptake and spillover of NE. In healthy subjects the myocardial NE release is on average > 20 times the myocardial spillover.\textsuperscript{21} In chronic heart failure (CHF) the presynaptic exocytosis of NE is increased and the uptake-1 mechanism, responsible for 90% of the NE re-uptake, is blocked resulting in increased spillover of NE. Therefore presynaptic levels of stored $^{123}$I-mIBG will decrease over time in CHF patients resulting in a lower late H/M ratio compared to early H/M ratio with consequently a “positive” $^{123}$I-mIBG WO. In healthy subjects the pre-synaptic NE release is limited and uptake-1 mechanism works properly resulting in accumulation of $^{123}$I-mIBG over time with higher late H/M ratio compared to early H/M ratio and consequently a “negative” $^{123}$I-mIBG WO.

Although there was no significant difference between 22q11.2DS subjects and healthy subjects in H/M ratio, the $^{123}$I-mIBG WO was significantly less negative in 22q11.2DS subjects compared to healthy subjects suggesting differences in adrenergic drive. However, the clinical effect of this difference in adrenergic drive is uncertain. As far as we know only increased $^{123}$I-mIBG WO (i.e. “positive” $^{123}$I-mIBG WO indicative for increased cardiac adrenergic drive) is associated with increased cardiac mortality and lethal arrhythmias.\textsuperscript{10,22} Therefore the results of our study suggest that cardiac sympathetic activity in adults with 22q11.2DS is probably not related to the incidence of unexpected death.

AMPT

In both groups PRL levels were increased shortly after the first administration of AMPT and subsequently decreased, indicative for the reversible tyrosine hydroxylase inhibition. However, in contrast to a previous study,\textsuperscript{6} there was no significant difference in PRL levels after administration of AMPT in the control group. In addition, there was no significant difference in PRL levels between 22q11.2DS subjects and controls. This could be explained by the relatively small sample size of the study.

Here we used, for the first time, the same AMPT paradigm used to assess changes in endogenous brain DA concentrations, to evaluate whether changes in endogenous peripheral NE concentrations may change the cardiac $^{123}$I-mIBG uptake. The present study showed a trend towards a higher late H/M ratio after acute depletion by AMPT.
compared to baseline. This rise in late H/M ratio was more pronounced in 22q11.2DS compared to healthy subjects. This is in line with the hypothesis that levels of peripheral endogenous NE in 22q11.2DS are increased compared to healthy subjects.

In contrast to the significant effect of AMPT to peripheral NE markers\(^6\), there was no significant effect at a group level on early and late H/M ratio and \(^{123}\text{I}-\text{mIBG WO}\). The lack of effect of AMPT on the scintigraphically assessed cardiac sympathetic activity may be explained by differences in NE spillover between different organs. In general the re-uptake of NE in the myocardium is very efficient and only contributes to 2-3% of the systemic NE spillover (i.e. plasma).\(^{23}\) Although there is a significant effect of AMPT on NE plasma levels, the myocardial NE re-uptake may be so efficient that the AMPT-induced changes cannot be visualized by myocardial \(^{123}\text{I}-\text{mIBG scintigraphy}\). Second, the used doses of AMPT may have been too low to induce a significant effect on the sympathetic activity. However, higher doses of AMPT may result in serious side-effects.\(^7\) Hypothetically a third explanation is that, to compensate for the decreased levels of NE, the uptake-1 mechanism is down regulated as in CHF, to increase the NE levels in the synaptic cleft and consequently diminish the effect of AMPT on \(^{123}\text{I}-\text{mIBG uptake}\). Finally, the timing between the start of administration of AMPT and the \(^{123}\text{I}-\text{mIBG scintigraphy}\) might be suboptimal. In the present study a time window of 5 h was chosen based on the time window of \(^{123}\text{I}-\text{IBZM SPECT imaging}\) and a significant decrease of NE (metabolites) levels 3 and 6 h after the start of administration of AMPT.\(^6\) It is speculative if a larger time window would have changed the outcomes of \(^{123}\text{I}-\text{mIBG scintigraphy}\). Nevertheless, the results tend to show that depletion with AMPT may be useful to investigate the cardiac sympathetic activity, but validations studies and larger trails are needed to demonstrate its effectiveness.

Our study has some limitations. First, the sample size of the study is relatively small and may have resulted in a limited statistical power. Second, although patients were randomly selected there is selection bias, as only those patients with 22q11.2 DS were enrolled who were physical and mental able to participate. Third, as a significant proportion of adults with 22q11.2DS have CHD and/or psychiatric disorders, and subjects with CHD and/or antipsychotic or psychostimulant medication were excluded, extrapolation of the current findings to the more general 22q11.2DS population is speculative. It may be of value to evaluate \(^{123}\text{I}-\text{mIBG uptake}\) in these subgroups of 22q11.2DS patients as well.

In conclusion, this study for the first time suggests a normal \(^{123}\text{I}-\text{mIBG cardiac sympathetic activity}\) in adults with 22q11.2DS. Although there is a small difference in adrenergic drive compared with healthy subjects, this most likely does not explain the increased unexpected death rate in the 22q11.2 DS population. Larger studies are necessary to confirm this hypothesis. The current data should be regarded as insightful but preliminary and extrapolation to the overall 22q11.2DS population should be done with great care.
REFERENCES


Chapter 11


Chapter 12

Tako-tsubo cardiomyopathy: how to understand possible pathophysiological mechanism and the role of \( ^{123}\text{I}-\text{mIBG} \) imaging

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HJ Verberne
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ABSTRACT

Tako-tsubo cardiomyopathy (TCM) is an increasingly recognized clinical syndrome characterized by acute reversible apical ventricular dysfunction, commonly preceded by exposure to severe physical or emotional stress. In this review we give a short overview on clinical presentation and treatment of TCM and discuss the possible pathophysiological mechanisms of TCM and the role of various non-invasive imaging modalities in TCM with a focus on the potential role of $^{123}$I-meta-iodobenzylguanidine (${\text{mIBG}}$) scintigraphy.

Currently, the dominating hypothesis on the pathophysiology of TCM postulates that high levels of the neurotransmitter epinephrine may trigger a change in intracellular signaling in ventricular myocytes. More specific, epinephrine stimulates G-protein coupled $\beta_2$ adrenergic receptors ($\beta_2\text{AR}$) which are located on ventricular myocytes. Normal levels of this neurotransmitter predominantly stimulate the intra-cellular G-protein, and induce a positive inotropic effect. However, with significant increasing levels of epinephrine, the predominance of stimulation is shifted from G-stimulating to the G-inhibitor protein coupling, which leads to a negative inotropic effect. Interestingly, this negative inotropic effect is the largest in the apical myocardium where the $\beta_2\text{AR}:\beta_1\text{AR}$ ratio is the highest within the heart. Echocardiography and ventriculography are essential to diagnose TCM, but new imaging tools are promising to diagnose TCM and to evaluate therapeutic efficacy. Cardiovascular magnetic resonance (CMR) can be used to differentiate TCM from other myocardial diseases, such as myocarditis. $^{123}$I-mIBG scintigraphy can be used to assess ventricular adrenergic activity and may guide optimization of individual (pharmacological) therapy.

These new insights into the possible pathophysiological mechanisms and novel diagnostic imaging modalities can be used as starting point for the development of international guidelines of TCM which may increase the awareness, and optimize the treatment of TCM.
INTRODUCTION

Tako-tsubo cardiomyopathy (TCM), also known as stress-induced cardiomyopathy, apical ballooning syndrome or broken heart syndrome was first described in Japan in 1990.¹ It is characterized by transient systolic dysfunction of apical and/or mid segments accompanied with ballooning of the segments. The clinical presentation can mimic acute myocardial infarction, in the absence of obstructive coronary artery disease. The Japanese phrase ‘tako tsubo’ can be translated in English as ‘octopus pot’, a fishing jar with a narrow neck and wide base used to trap an octopus. This description reflects the visual appearance of the heart on left ventriculography. Although the first report was published in 1990 it lasted several years to recognize this phenomenon in Europe and the United States of America.¹ ⁴ In 2006, the American Heart Association incorporated TCM into its classification of cardiomyopathies as a primary acquired cardiomyopathy.⁵ Subsequently many publications have discussed on possible pathophysiological mechanisms of TCM, the diagnostic workup using multimodality imaging techniques, and therapeutic options. Currently, it can be anticipated that TCM is still under-diagnosed due to lack of awareness and knowledge of diagnostic possibilities. However, well established imaging techniques, such as cardiovascular magnetic resonance (CMR) and ¹²³I-meta-iodobenzylguanidine (¹²³I-mIBG) scintigraphy are promising imaging modalities to diagnose TCM. To increase the awareness of TCM, this review will discuss new insights into possible pathophysiological mechanisms of TCM and the impact that these new insights may have on therapeutic and diagnostic strategies.

Diagnostic criteria

Although after the first publications TCM is increasingly recognized, there is no consensus or guideline on the diagnostic criteria for TCM. However, Prasad et al. proposed that the diagnosis of TCM requires all of the following criteria: 1. Transient hypokinesis, akinesis, or dyskinesis in the mid and apical segments of the left ventricle; regional wall motion abnormalities that extend beyond a single epicardial vascular distribution; and frequently but not always preceded by a stressful trigger; 2. The absence of obstructive coronary disease or angiographic evidence of acute plaque rupture; 3. New ECG abnormalities (ST elevation and/or T-wave inversion) or modest elevation in cardiac troponin levels; and 4. The absence of pheochromocytoma and myocarditis.⁶

Prevalence

Some of the best available estimates on the prevalence of TCM come from small series of patients (7 to 16 patients per study) presenting with suspected acute coronary syndrome (ACS).⁷ ⁹ The prevalence of TCM in these studies ranged between 1.9 – 2.2 percent. In line with these data a recent meta-analysis showed that TCM accounted for 1.7 – 2.2 percent of cases presenting with suspected ACS.¹⁰ In a large registry of 3265
patients with troponin-positive ACS the prevalence of TCM was 1.2 percent. TCM is diagnosed in about 0.02 percent of all general hospitalizations in the United States of America, mostly in elderly women. Since it can be assumed that TCM is underdiagnosed, the true prevalence is higher.

Clinical features
TCM affects predominantly post-menopausal women and is usually preceded by exposure to physical or emotional stress (e.g., unexpected death in the family, abuse, exhausting work). Major symptoms of TCM are chest pain at rest, mimicking acute myocardial infarction (AMI), and dyspnea. Syncope or out-of-hospital cardiac arrest are rare. Acute complications occur in approximately 20 percent of patients with TCM and include cardiogenic shock, left sided heart failure, pulmonary edema, torsades de pointes, left ventricular thrombus formation or free wall rupture. Cardiogenic shock can be due to left ventricular failure or obstruction of the outflow tract of the left ventricle.

Electrocardiogram and biomarkers
The ECG often reveals ST-elevation (predominately precordial) during the acute phase, followed by T-wave inversion, QT-prolongation and sometimes Q-wave formation during the subacute phase. Differentiation between TCM and AMI using ECG may be difficult. However, compared with anterior myocardial infarction, reciprocal ST-segment depression is less likely. In addition, occasionally ST-elevation in the inferior leads is present. Cardiac markers, especially high-sensitivity troponin, are slightly elevated and normalize earlier in TCM as compared to AMI. It has been shown that in patients with TCM high-sensitive troponin I is more elevated at presentation compared to patients with STEMI. However, the maximum high-sensitive troponin I during follow-up was higher in patients with STEMI than patients with TCM. However, these differences in high-sensitive troponin I on group level are very small and therefore not useful to differentiate between STEMI and TCM for each individual patient. Furthermore, brain natriuretic peptide (BNP) or N-terminal pro-BNP are usually elevated as markers of ventricular dysfunction. However, these parameters are not specific for TCM and are not associated with a poor TCM prognosis.

Echocardiography and ventriculography
Transthoracic echocardiography or ventriculography during the acute phase may reveal left mid-ventricular dysfunction and apical akinesis or dyskinesis with apical ballooning. Importantly, most often wall motion abnormalities extent beyond the distribution of any single coronary artery. Mean left ventricular ejection fraction (LVEF) ranges from 20 to 49 percent. LV basal hyperkinesis with left ventricular outflow tract (LVOT) obstruction may occur and may cause severe mitral regurgitation as result of systolic anterior motion (SAM) of the anterior mitral valve leaflet. In the acute phase some patients with TCM are in shock. Urgent echocardiography is necessary to differentiate between LVOT obstruction and severe left ventricle dysfunction.
Tako-tsubo cardiomyopathy

There is no accurate way to reliably discriminate between TCM and AMI using ECG and cardiac biomarkers. Coronary angiography is essential for the differentiation between TCM and AMI. In general significant coronary artery stenosis is absent in TCM.

**Treatment**

Generally, in the acute phase of TCM the patient is treated with commonly used medication for systolic heart failure: beta-blockers (BB), ACE-inhibitors (ACE-I) and or angiotensine II receptor blockers (ARB) and diuretics. When a thrombus in the left or right ventricle is present, anticoagulation should be prescribed for 6 months to prevent systemic embolization. In the acute phase, TCM can be accompanied by cardiogenic shock. Inotropic agents are contra-indicated when shock is caused by LVOT obstruction as they may aggravate the clinical condition: inotropic agents may lead to catecholamine excess and can induce or worsen the degree of LVOT obstruction. In addition, intra-aortic balloon pump counter-pulsation can be used in these patients to improve hemodynamics. If shock is due to LV dysfunction without LVOT obstruction, inotropic agents are indicated. After the acute phase BB and ACE-I/ARB should be initiated and continued until left ventricular function is normalized. However, in light of preventing a possible recurrence of TCM, triggered by persisting increased myocardial adrenergic activity, it can be considered to continue BB and ACE-I/ARB treatment.

**Prognosis**

In general TCM has a favorable prognosis.\(^{18}\) However in the United States of America the in-hospital mortality is 4.2 percent.\(^{19}\) Interestingly male patients showed a higher mortality rate than females (8.4% vs. 3.6%). In general, after the acute phase left ventricular function normalizes in four weeks.\(^{20}\) Some studies have reported recurrence of apical ballooning.\(^{18,21}\) In one study with 100 patients followed for 4.4 years recurrence of TCM was found in 10% of patients whereas 31% had episodes of chest pain without significant coronary artery disease.\(^{21}\) Prognostic parameters of TCM are not known.

**Pathophysiology**

The precise pathophysiological mechanism of TCM has not been completely elucidated. Emotional, psychological or physical stress is frequently, but not always present prior to the onset of TCM, and may thus trigger the onset of disease.\(^{18}\) It has been suggested that epinephrine-mediated myocardial stunning in TCM is related to multiple coronary artery spasm and impaired coronary microcirculation. However, since various ballooning patterns extend beyond the distribution of any single coronary artery, ischaemia due to epicardial spasm seems unlikely and would not explain the various ballooning patterns. Considerable evidence points to epinephrine as an important factor in the pathophysiology. In the acute phase of TCM, plasma epinephrine levels are more elevated compared with the acute phase of a myocardial infarction.\(^{20}\) In general, these elevated epinephrine levels normalize within a few days. This is in keeping with the fact that TCM-like abnormalities, like apical wall motion abnormalities and ECG
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changes are associated with epinephrine-secreting pheochromocytoma resulting in a “catecholamine storm”, but not with norepinephrine (NE)- and/or dopamine-secreting pheochromocytoma. However, it has been reported that accidental administration of epinephrine (including single intramuscular 1 mg dose from an epinephrine auto-injector) can result in TCM-like abnormalities.22 NE predominately stimulates β₁AR on ventricular myocytes leading to a positive inotropic response. This is the result of β₁AR coupling to the G stimulating (Gs) protein. Epinephrine also binds to β₂AR and activates the same intracellular Gs-protein, but has a higher affinity for β₂AR (Kd = 0.4 nM) than NE (Kd = 30 nM).23 The mechanism of regional wall motion difference between apex and base is thought to be due to a greater proportion of β₂AR relative to β₁AR in the apex, since higher concentration of adrenergic innervations at the base of the heart is counterbalanced by increased apical βAR response/sensitivity to epinephrine.26,27 The human heart has a higher β₂AR:β₁AR ratio in apical than in basal cardiomyocytes.24,28 It was shown that this higher apical β₂AR:β₁AR ratio results in an enhanced β₂AR-specific inotropic response of the apical as compared to the basal cardiomyocytes.28 This higher β₂AR:β₁AR ratio in the apex makes this part of the myocardium probably more vulnerable/sensitive to excessive epinephrine stimulation, which may explain the decreased apical and preserved basal wall motion in the acute phase of TCM.

The pathophysiological basis of TCM may be explained by a direct “toxic” effect of epinephrine on cardiomyocytes. This is supported by a recent study performed in rats, in which a high bolus of epinephrine, but not NE, resulted in a cardiomyopathy mimicking TCM.28 It has been demonstrated in animal studies that β₂AR, when exposed to high levels of epinephrine, shifts from positively inotropic Gs coupling to negative inotropic G-inhibitor (Gi) coupling.23,28 This process is described as ligand/stimulus directed-trafficking or biased agonism (Figure 1). This effect was not observed after equivalent high dose of NE. It is assumed that β₂AR has one binding site for NE and two binding sites for epinephrine.23 The affinity of epinephrine for these two different binding sites varies so that when the high binding sites are fully saturated with epinephrine then the low binding sites begin to form complexes with epinephrine. Binding of epinephrine to high-affinity sites triggers the Gs protein, whereas binding to the low-affinity site stimulates Gi protein (Figure 2).23 After the increased levels of epinephrine are cleared from the circulation, β₂AR shifts back from Gi to Gs protein coupling, enabling cardiomyocytes to recover their inotropic function. This would explain the reported recovery of ventricular function in TCM when epinephrine levels are normalized.

β₂AR coupling to Gi protein is reported to be cardioprotective and anti-apoptotic.29,30 Blocking β₂AR Gi signaling in animal models before exposure to increased epinephrine levels induced mortality due to cardiogenic shock and hypokinesia.28 This might be explained by the possible increased cardiotoxic effects of high epinephrine levels via uninhibited β₁AR-Gs and β₂AR-Gs signaling.
It was also reported that epinephrine-induced apical hypokinesis exacerbates after administration of βAR-blockers which activate Gi protein coupling.28 A few β-blockers are pure neutral antagonists, while most act as partial or inverse agonists, or show biased agonism for βAR.29 Propanolol has relatively high β2AR-Gi protein inverse agonistic properties that enhance and prolong the negative inotropic effect of epinephrine at apex and base. Carvedilol has been shown to have less β2AR-Gi protein inverse agonistic properties and consequently has little inotropic effects on the apex.
but converts the initial positive inotropic response to epinephrine at the base to a significantly negative inotropic response. Therefore carvedilol, at least theoretically, may be useful in the treatment of TCM with severe LVOT obstruction secondary to basal hypercontractility. In contrast, the β2AR-selective blocker bisoprolol reduced the positive inotropic effect at the base and had no effect on the apical myocytes. These findings suggest that treatment with βAR-blocker with more β2AR-blocking properties would be preferable. However, the above-described findings of βAR-blockers are mainly derived from animal experiments. Extrapolation of these findings to humans remains speculative. Although the possible mechanism of apical ballooning seems to be explained by the previously described effect of epinephrine, the question remains why not everyone who is exposed to emotional and physical stress develops TCM. We hypothesize two possibilities: patients with TCM have a higher release of epinephrine compared with persons without TCM and/or those with TCM are more sensitive to epinephrine due to higher density of β2AR and/or have another expression of Gs or Gi proteins.

TCM presents with typical apical ballooning, but there are reports that described reverse or inverted morphological patterns as a variant of this disease with involvement of the basal- and mid-ventricular segments and normal contractility of the apical segments. Since the use of CMR a few cases with right ventricle involvement have been reported. The mechanism of these different patterns is still unclear. It has been suggested that the variations in these regional wall motion abnormalities is mainly related to difference in the anatomic location of β2AR:β1AR ratio and/or polymorphism.

Sex Difference in TCM prevalence

There is a striking difference in the incidence of TCM in females as compared to males; about 90% of reported cases concern females. This could be explained by sex-related differences in adrenal medulla response to sudden high-intensity adrenergic stimulation and differences in the pharmacokinetics of epinephrine. In addition basal/resting epinephrine plasma levels are lower in women compared to men. This difference could reflect reduced basal release of epinephrine enabling the possibility for an increased sudden epinephrine response to stress. An increased sensitivity of the β1AR in women could favor the protective effects of β1AR-Gi protein signaling resulting in negative inotropism in the apical myocardium, the region with the highest density of β1AR. Perhaps men who lack this protective effect develop more acute cardiotoxicity mediated by β1AR-Gs protein signaling following high elevations in catecholamine levels, resulting in a fatal event rather than cardiomyopathy. This suggestion is supported by the increased in-hospital mortality of TCM in males compared with females (8.4% vs. 3.6%).

TCM predominantly affects postmenopausal women assuming that estrogens play a role in the aetiology of TCM. It is known that estrogens have cardioprotective effects against acute myocardial injury through a variety of complex mechanisms. Yet, it is unclear how the lack of cardioprotective estrogens in postmenopausal women increases the risk of TCM. One of the possible mechanisms is upregulation of myocardial contractility.
In line with this, myocardial $\beta_1$AR expression is upregulated in ovariectomized rats and this effect is reversed by estrogen replacement.  These findings suggest that estrogen may affect cardiac responses to sympathetic stimulation by altering the expression of myocardial $\beta_1$ARs. However, TCM is mainly related to $\beta_2$ARs and therefore, changes in $\beta_1$AR expression by estrogens cannot fully explain the increased incidence of TCM in post-menopausal women. Changes in immediate early gene (IEG) expression could be an alternative explanation.

In rodent models it has been demonstrated that stress activates IEG expression in the central nervous system and myocardium. These myocardial changes in IEG expression are mediated by activation of both $\alpha$- and $\beta$AR. It has been demonstrated that ovariectomized rats while subjected to immobilization stress have less IEG expression with estrogen supplementation compared to those without estrogen supplementation. This further underscores that estrogens have cardioprotective effects.

**Non-invasive imaging techniques**

For the diagnosis of TCM echocardiography is the imaging modality of first choice. It’s widely available, easy to perform at the bedside and it is non-ionizing. However with developments in CMR and nuclear imaging by mean of $^{123}$I-mIBG scintigraphy, it’s possible to distinguish TCM from other cardiac diseases and to evaluate the cardiac adrenergic activity. (Figure 3)
Chapter 12

Cardiovascular magnetic resonance

CMR is suited for evaluation of patients with TCM and can help differentiating TCM from myocarditis or myocardial infarction. In addition to the accurate visualization of regional wall motion abnormalities it enables quantification of right and left ventricular function and assessment of additional abnormalities like pericardial effusion, and ventricular thrombus. Compared to echocardiography CMR is an excellent non-invasive imaging technique to visualize right ventricle involvement or inverted TCM. CMR also provides markers for reversible injury such as edema, inflammation and irreversible injury, like necrosis and fibrosis. In contrast to myocardial infarction late gadolinium enhancement (LGE) as a marker for fibrosis has only been seen in 0 to 8% in case of TCM.\textsuperscript{34,39,40} This finding may help differentiate TCM from entities with similar clinical presentations such as myocarditis and myocardial infarction, i.e. myocardial infarction typically exhibits a subendocardial pattern of LGE while myocarditis usually displays a patchy subepicardial pattern.\textsuperscript{38} T2 weighted images can help to visualize edema.\textsuperscript{41} Global edema with high signal intensity (SI ratio of myocardium to skeletal muscle of 1.9 or higher) in the mid and apical myocardium confirms the diagnosis TCM, whereas a patchy signal is more compatible with myocarditis.\textsuperscript{34} Recently, a novel CMR method using T1 weighted mapping has been reported to assess acute myocardial edema.\textsuperscript{42} This non-contrast method seems promising as it has high diagnostic performance compared to T2 weighted CMR and is highly reproducible.

\textsuperscript{123}I-mIBG scintigraphy

Meta-iodobenzyguanidine (mIBG) is a NE analog that has the same presynaptic uptake, storage and release mechanism as NE. Radiolabeling of mIBG with \textsuperscript{123}I or \textsuperscript{131}I allows for imaging with gamma cameras. In 1980 the potential use of \textsuperscript{131}I-mIBG for cardiac imaging was suggested.\textsuperscript{43,44} The last decades, \textsuperscript{123}I-mIBG scintigraphy has been developed to evaluate cardiac adrenergic function and the usefulness of \textsuperscript{123}I-mIBG scintigraphy has been demonstrated in many cardiac diseases.\textsuperscript{45-47} In TCM \textsuperscript{123}I-mIBG scintigraphy reveals impaired apical myocardial uptake of \textsuperscript{123}I-mIBG on planar images (Figure 4).\textsuperscript{48,49} This is thought to be induced by increased adrenergic stimulation and consequently increased NE levels. Interestingly, the trigger of TCM is high release of epinephrine, but not NE. The impaired uptake of \textsuperscript{123}I-mIBG may be explained as follows: NE and epinephrine are both taken up from the synaptic cleft by the uptake-1 (i.e. NE transporter: NET) and uptake-2 (i.e. extraneuronal monoamine transporter: EMT) (Figure 5). It has been demonstrated that uptake of NE is inhibited in the presence of high levels of epinephrine.\textsuperscript{50} Therefore, in TCM decreased uptake of NE (i.e. \textsuperscript{123}I-mIBG) via uptake-1 could be explained as an indirect effect to high circulating levels of epinephrine.

Single Photon Emission Computed Tomography (SPECT) \textsuperscript{123}I-mIBG is important for regional evaluation of myocardial innervation in TCM. SPECT \textsuperscript{123}I-mIBG imaging demonstrated mainly decreased NE uptake of the myocardial apex.\textsuperscript{48} Interestingly, this pattern follows the increasing $\beta_2$AR/$\beta_1$AR ratio from the base to the apex. Apical
cardiomyocytes have been shown to express a higher density of $\beta_2$ARs and therefore a higher sensitivity to epinephrine compared to the basal cardiomyocytes, resulting in epinephrine-induced regional stunning. \(^{28}\) We assume that the hyperadrenergic state by high levels of epinephrine causes downregulation of $\beta_2$ARs. Alterations in the pre-synaptic signal transduction result in an impaired uptake-1 function in order to maintain high levels of catecholamines with effect of stimulating those $\beta_2$ARs that are still functional. This hypothesis is supported by studies showing that the presynaptic trace amine-associated receptor 1 (TAAR 1) in the brain is activated by monoaminergic neurotransmitters like NE, dopamine and serotonin. TAAR1 activation by these common biogenic amines can modulate monoaminergic transporters, including the dopamine, NE and serotonin transporter. \(^{51,52}\) It can be assumed that this not only occurs in the brain, but also in other organs such as the heart (Figure 5). This phenomenon may explain the impaired apical uptake of $^{123}$I-mIBG on SPECT images in patients with TCM.

Although left ventricular function and epinephrine levels are normalized after a few weeks, several case reports show persisting decreased $^{123}$I-mIBG uptake on SPECT images in the apical myocardium. \(^{48,49}\) The mechanism of this persisting regional impaired uptake of $^{123}$I-mIBG uptake is yet unclear. We assume that the increased apical density and sensitivity of the $\beta_2$AR to epinephrine causes a prolonged effect of downregulation of $\beta_2$AR and impaired uptake-1 function. This would maintain relatively higher levels of epinephrine and NE in the synaptic cleft and would in turn cause these receptors and transporters to recover more slowly compared to more basal located $\beta_2$ARs. In addition, the phenomenon of persisting decreased myocardial $^{123}$I-mIBG uptake may in part be explained by preexisting myocardial sympathetic denervation.

Of interest is whether especially the slow recovery of apical $^{123}$I-mIBG uptake may identify those patients who are at a higher risk for the recurrence of TCM. Therefore SPECT $^{123}$I-mIBG may guide optimization of individual (pharmacological) therapy to prevent recurrent TCM.

Figure 4. $^{123}$I-mIBG scintigraphy planar images in the acute phase of TCM. The early (A, 15 min post injection (p.i.)) and late (B, 4 hours p.i.) planar images show clearly absence of myocardial $^{123}$I-mIBG uptake. Due to the lack of myocardial $^{123}$I-mIBG uptake SPECT images could not reliably be reconstructed.
Figure 5. Schematic representation of the sympathetic synapse. Norepinephrine (NE) is synthesized within neurons by an enzymatic cascade. Dihydroxyphenylalanine (DOPA) is generated from tyrosine and subsequently converted to dopamine by DOPA decarboxylase. Dopamine is transported into storage vesicles by the energy-requiring vesicular monoamine transporter (VMAT). NE is synthesized by dopamine β-hydroxylase within these vesicles. Neuronal stimulation leads to NE release through fusion of vesicles with the neuronal membrane (exocytosis). Most NE undergoes reuptake into nerve terminals by the presynaptic NE transporter (uptake-1) and is re-stored in vesicles (following uptake by vesicular amine transporter 2 (VMAT2)) or is metabolized in cytosol dihydroxyphenylglycol (DHPG) by monoamine oxidase (MAO). Postsynaptic NE undergoes reuptake into the myocytes by the extraneuronal monoamine transporter (uptake-2). Presynaptic trace amine-associated receptor 1 (TAAR 1) can be activated by monoaminergic neurotransmitters like NE and epinephrine. TAAR1 activation can modulate uptake-1 resulting in decrease uptake of NE.
CONCLUSION

TCM is increasingly recognized as a separate clinical diagnosis. The diagnosis should particularly especially be considered in female patients with chest pain and/or unexplained heart failure. It is essential to exclude significant coronary artery stenosis by coronary angiography. Typical apical left ventricular ballooning is present on ventriculography and echocardiography. High levels of epinephrine and the subsequent bias agonism of β2ARs may play a pivotal role in the development of TCM. As predominantly postmenopausal women are mainly affected, estrogens may play a role. However, the exact mechanism is yet unclear and needs to be investigated. Another unanswered question is why not everyone with stress develops TCM. New imaging techniques such as CMR may help in differentiating TCM from myocarditis and myocardial infarction. In addition CMR can also visualize right ventricle involvement or inverted TCM. 123I-mIBG myocardial scintigraphy may assess the adrenergic state and may be useful for estimating prognosis and guiding (pharmacological) therapy. Animal studies suggest that treatment with a neutral antagonist like carvedilol would be preferable than an inverse agonist like propanolol, but this hypothesis has not been tested in humans. The prognosis after the acute phase of TCM is good, although recurrent TCM has been described.

Finally, there is a need to establish a registry for TCM patients to better understand its natural history and its true occurrence. This would help to better define the disease process and would in turn enable a better understanding of possible risk factors associated with the start of the disease but also helps in identifying risk factors associated with prognosis and recurrence of TCM. In addition randomized trials should be performed to evaluate therapeutic strategies to promote swift recovery of left ventricular function and prevent recurrence of TCM.
Chapter 12

REFERENCES


Chapter 12


Tako-tsubo cardiomyopathy


Chapter 12


Chapter 13

Summary, general discussion, future perspective and conclusions
Chapter 13

SUMMARY

The cardiac sympathetic system is one of the neurohormonal compensation mechanisms that plays an important role in the pathogenesis of chronic heart failure (CHF). Patients with CHF have increased cardiac sympathetic activity with increased exocytosis of norepinephrine (NE) from the presynaptic vesicles and impaired NE re-uptake via the norepinephrine transporter (NET) in the sympathetic terminal nerve axons. This results in increased levels of NE in the synaptic cleft. Initially, β-adrenergic receptor (AR) stimulation by increased synaptic NE levels helps to compensate for impaired myocardial function. However, long-term NE excess has detrimental effects on myocardial structure and gives rise to a down regulation and availability of post-synaptic β-AR. This leads to left ventricular remodeling and is associated with increased mortality and morbidity in CHF.

Cardiac sympathetic activity can non-invasively be visualized by nuclear medicine techniques. The most commonly used SPECT tracer is the NE analogue 123I-meta-iodobenzylguanidine (123I-mIBG). The most commonly used semi-quantitative measurements of myocardial 123I-mIBG uptake are the early heart-to-mediastinum (H/M) ratio, derived 15 minutes post injection (p.i.) of 123I-mIBG, late H/M ratio, derived 4 hours p.i. of 123I-mIBG and the 123I-mIBG washout (WO), calculated as the difference between early and late H/M ratio and expressed as a percentage of the early HM ratio.

Although a large number of studies on 123I-mIBG assessed cardiac sympathetic activity in CHF have been published, the lack of standardization between different institutions have hampered wide scale clinical implementation. In this thesis further standardization of cardiac 123I-mIBG scintigraphy is validated (part I). The prognostic value of cardiac 123I-mIBG scintigraphy in CHF was studied and thereby provided further evidence for clinical implementation of cardiac 123I-mIBG scintigraphy (part II). In addition the use of cardiac 123I-mIBG scintigraphy in populations other than CHF are discussed (part III).

PART I

A collimator is a pivotal part of a gamma camera and constitutes of a thick sheet of lead, with thousands of adjacent parallel holes through it and corresponding septa. These septa allow only those photons traveling parallel to the septa to pass through and be recorded by the crystal. Photons not traveling parallel to the septa are filtered out by the septa. In addition to the main photopeak of 159 keV 123I emits a low-abundance of high-energy photons. These high-energy photons leads to penetration of collimator septa and subsequent degrade the image quality and affects estimation of the H/M ratio. Therefore, the thickness of the collimator septa is a major determinant of this
Summary and discussion

High energy photon-penetration. Consequently, collimator choice is one of the most important factors causing variation in myocardial $^{123}$I-mlIBG-derived parameters among institutions and published scientific studies. In chapter 2, the influence of differences between collimators on the planar cardiac $^{123}$I-mlIBG-derived parameters is evaluated (i.e., early and late H/M ratio) in 53 patients with CHF. Parameters calculated using a low energy high resolution (LEHR) collimator were significantly lower compared with those from a medium energy (ME) collimator (late H/M ratio $1.41 \pm 0.18$ vs. $1.80 \pm 0.41$, $p < 0.001$). Interestingly, with increasing H/M ratio, the difference between the ratios increased in favour of the ME collimator. This difference could be explained by septal penetration of high-energy photons from both liver and lungs in mediastinum and myocardium, being the lowest when using the ME collimator. These results strengthen the importance of the recommendation to use ME collimators in cardiac $^{123}$I-mlIBG scintigraphy.

Although the H/M ratio is a simple method to correct for background, standardization of acquisition and analysis is needed. The lack of standardization between different institutions is one of the factors that have hampered the wide scale clinical implementation of planar cardiac $^{123}$I-mlIBG scintigraphy. As already mentioned, differences in collimators cause variation among institutions and studies. In chapter 3, the results of a European phantom cross-calibration study of planar cardiac $^{123}$I-mlIBG scintigraphy are described. Based on the results of this phantom study, a conversion coefficient for each gamma camera-collimator combination was calculated. With these conversion coefficients, various conditions can be converted to standardized H/M ratios. This cross-calibration enables a better comparison between institutions which is important for identifying appropriate thresholds for differentiating high and low risk heart failure patients.

In chapter 4, the impact of mediastinal ROI definition on intra- and inter-observer variability in relation to collimator type was assessed. An observer-defined mediastinal ROI was compared to a predefined mediastinal ROI. Substantial agreement was found between all 3 observers using predefined mediastinum ROI for both LEHR and ME collimator use. However, an observer-defined mediastinal ROI resulted in a poor to moderate agreement independent of collimator choice. In addition, intra-observer analysis using a predefined mediastinal ROI showed substantial agreement. Lin’s concordance coefficient did not differ significantly between LEHR and ME collimators (i.e., 0.97 and 0.96 respectively). A predefined mediastinal ROI is therefore to be preferred over observer defined mediastinal ROIs.

The NET, encoded by the solute carrier family 6 (SLC6A2), is responsible for reuptake of NE into the presynaptic nerve terminals and is a regulator of NE homeostasis. $^{123}$I-mlIBG shares the same presynaptic uptake, storage, and release mechanism as NE. Although polymorphism of the SLC6A2 gene has been reported, the effect on cardiac $^{123}$I-mlIBG uptake is unknown. In chapter 5, the influence of SLC6A2 single-
nucleotide polymorphisms (SNP) on $^{123}$I-mIBG parameters (i.e. early and late H/M ratio and $^{123}$I-mIBG washout) in 49 CHF patients eligible for ICD implantation for primary prevention was examined. Six different SLC6A2 SNPs were found, but none (of them) were functional. Consequently polymorphisms of the SLC6A2 gene in this study cohort were not associated with any $^{123}$I-mIBG myocardial parameter.

For the quantification of cardiac $^{123}$I-mIBG uptake, the mediastinum is used as a reference region reflecting nonspecific background activity. However, variations in the quantity of vascular structures in the mediastinum and renal clearance rate of $^{123}$I-mIBG from the blood pool may contribute to increased inter-individual variation in $^{123}$I-mIBG uptake. In chapter 6 the relationship between changes in H and M counts and the change in vascular $^{123}$I-mIBG activity, including the effect of renal function was examined. Changes in region of interest (ROI) activity ratios between early (15 minutes p.i.) and late (4 hours p.i.) planar images could not be explained by blood activity, vascular clearance, or renal function. The change in measured H and M counts between early and late planar $^{123}$I-mIBG images was unrelated to intravascular levels of the radiopharmaceutical. This suggests that changes in M counts are primarily due to decrease in soft tissue activity and scatter from the adjacent lungs.

$^{123}$I-mIBG is primarily cleared via the kidneys. In addition patients with CHF tend to have a reduced kidney function. It is therefore logical to assume that a reduced glomerular filtration rate (GFR) is associated with a reduced blood clearance of $^{123}$I-mIBG and thereby could influence the $^{123}$I-mIBG-derived parameters (i.e. early and late H/M ratio and $^{123}$I-mIBG WO). In chapter 7 it was explored whether renal function expressed as the estimated glomerular filtration rate (e-GFR) could explain variability of $^{123}$I-mIBG assessed myocardial sympathetic activity in a CHF population. In addition, renal function was compared to $^{123}$I-mIBG-derived parameters as predictors of cardiac death in CHF. $^{123}$I-mIBG-derived parameters were independent from renal function. Furthermore, $^{123}$I-mIBG assessed cardiac sympathetic activity was found to be superior to renal function in the prediction of prognosis in patients with CHF.

**PART II**

Although arrhythmia, lethal cardiac events and prognosis are multifactorial and have several determinants, cardiac $^{123}$I-mIBG scintigraphy alone and more likely in combination with other determinants may be able to better select CHF patients with increased risk. Chapter 8 provides a meta-analysis of original individual late H/M ratio data of 636 CHF patients retrieved from six studies from Europe and the United States of America. The use of $^{123}$I-mIBG parameters to predict all-cause mortality, cardiac mortality, arrhythmic events, and heart transplantation was investigated. Late H/M ratio was a significant predictor of all event categories, but lowest hazard ratios
(HRs) were for the composite endpoint of any event (HR 0.30 [0.19 – 0.46]), all-cause mortality (HR 0.29, [0.16 – 0.53]), cardiac transplant (HR0.22 [0.10 – 0.49]), cardiac mortality (HR 0.28 [0.14 – 0.55]), and arrhythmia (HR 0.33 [0.16–0.67]). In multivariate analysis, late H/M ratio was an independent predictor for all event categories, except for arrhythmias. Interestingly, in CHF patients, late H/M ratio is not only useful as a dichotomous predictor of events (high vs. low risk), but also has prognostic implication over the full range of the outcome value for all event categories except arrhythmias.

CHF results not only in an increased cardiac sympathetic activity but is also associated with increased myocardial inflammation. In chapter 9 the relationship between severity of heart failure, $^{123}$I-mIBG assessed cardiac sympathetic activity and measures of inflammation in 55 subjects with stable, optimally treated CHF was studied. In addition, the predictive value of $^{123}$I-mIBG parameters and markers of inflammation for cardiac events (i.e. appropriate ICD therapy, progression of CHF and cardiac death) was evaluated. Left ventricular ejection fraction (LVEF) was the only independent predictor of late H/M ratio and NT-proBNP was the only independent predictor of $^{123}$I-mIBG WO. CRP, IL12p40, TNF-α, sE-selectin, MPO, PAI-1, tPA and TNFR2 were not related to late H/M ratio or $^{123}$I-mIBG WO. During median follow-up of 34 months 13 patients experienced a cardiac event. These cardiac events were associated with CRP, NT-proBNP, MPO and late H/M. However, multivariate analysis showed only CRP, NT-proBNP, MPO and IL12p40 as predictors of cardiac events. Inflammation and cardiac sympathetic activity seem not to be related in stable, optimally treated CHF patients. This is corroborated by the finding that they both provide prognostic information in this specific stable CHF population.

Chapter 10 evaluates whether $^{123}$I-mIBG assessed cardiac sympathetic activity could identify high-risk CHF subjects most likely to experience appropriate ICD therapy. 135 subjects with stable, optimally treated CHF were enrolled in 13 centres in Europe. Conversion coefficients from our cross-calibration study were used to correct for the different gamma camera-collimator used. During follow-up 24 patients experienced a cardiac event (appropriate ICD therapy ($n = 12$), NYHA functional class progression ($n = 6$) and cardiac death ($n = 6$)). The late H/M and LVEF were associated with freedom of appropriate ICD therapy. Therefore $^{123}$I-mIBG assessment of cardiac sympathetic activity enables better identification of the prognosis in stable CHF patients and can be used for better selection of patients who benefit from ICD implantation and therefore helps to constrain the CHF related costs.

PART III

22q11.2 Deletion syndrome (DS) affects catechol-O-methyl-transferase (COMT), which involves the degradation of NE, the neurotransmitter of the cardiac sympathetic system.
Chapter 13

Adults with 22q11.2DS are clinical at increased risk for sudden unexpected death. Although the causes are likely multifactorial, increased cardiac sympathetic activity with subsequent fatal arrhythmia, due to increased levels of NE, should be considered as a possible mechanism. In chapter 11 it was determined whether $^{123}$I-mIBG scintigraphy assessed cardiac sympathetic activity is increased in 22q11.2DS, both at baseline and following an acute NE depletion with alpha-methyl-para-tyrosine (AMPT). For the first time normal cardiac sympathetic activity in adults with 22q11.2DS has been demonstrated. Although there was a small difference in adrenergic drive compared to healthy subjects, this most likely does not explain the increased unexpected death rate in the 22q11.2 DS population.

Tako-tsubo cardiomyopathy (TCM) is an increasingly recognized clinical syndrome characterized by acute reversible apical ventricular dysfunction, commonly preceded by exposure to severe physical or emotional stress. In chapter 12 an overview is given of the clinical presentation and treatment of TCM and all various non-invasive imaging techniques with a focus on the potential role of $^{123}$I-mIBG scintigraphy. In addition the possible pathophysiological mechanisms of TCM are discussed. Currently, the dominating hypothesis on the pathophysiology of TCM postulates that high levels of the neurotransmitter epinephrine may trigger a change in intracellular signaling in ventricular myocytes. With significant increasing levels of epinephrine, the predominance of stimulation is shifted from G-stimulating to the G-inhibitor protein coupling, which leads to a negative inotropic effect. Interestingly, this negative inotropic effect is the largest in the apical myocardium where the $\beta_2$AR: $\beta_1$AR ratio is the highest. It has been demonstrated that uptake of NE via the NET is inhibited in the presence of high levels of epinephrine. Therefore, in TCM, decreased uptake of $^{123}$I-mIBG via the NET could be explained as an indirect effect to high circulating levels of epinephrine. Therefore $^{123}$I-mIBG scintigraphy may guide optimization of individual (pharmacological) therapy in TCM. These new insights can be used as starting point for the development of international guidelines of TCM which may increase the awareness, and optimize the treatment of TCM.

CONCLUSION AND FUTURE PERSPECTIVES

Cardiac sympathetic activity can non-invasively be assessed by cardiac $^{123}$I-mIBG scintigraphy. However, the lack of standardisation of acquisition and post-acquisitions analysis have hampered comparison between different institutions and therefore wide scale clinical implementation. In this thesis the influence of several acquisition, post-acquisition and patient related parameters such as collimator choice, mediastinal ROI definition, polymorphism of the SLC6A2 gen, renal function and inflammation, on cardiac $^{123}$I-mIBG scintigraphy outcome were studied. The results showed that...
Summary and discussion

Standization and validation of these parameters in combination with cross-calibration of gamma camera-collimator results in a good reproducibility with a small inter- and intra-observer variation. Moreover, the standardized cardiac $^{123}$I-$m$IBG scintigraphy outcomes seem to have good prognostic value. Therefore, major objections for clinical implementation have been overcome. Furthermore, the use of standardized H/M ratios allows development of universal prognostic cut-off values (high vs. low risk). These cut-off values can be calculated by re-analysis of the data of previous published multicentre studies. Finally, it should be stressed that standardization of the H/M ratio is essential for the preparation of adequate risk models.

Despite therapeutic improvements the prognosis of CHF remains unfavorable in part due to sudden cardiac death (SCD). The introduction of implantable cardioverter defibrillators (ICD) has improved the overall survival of CHF. However, a high percentage of CHF with ICD implantation will never receive appropriate ICD therapy. Moreover, there is a risk of malfunction and operative complications of these devices. Furthermore, this therapeutic strategy has a great impact on the healthcare budgets. Therefore, it is essential to optimize the current selection criteria to improve patient selection for ICD implantation. This thesis showed that stable CHF patients with intermediate late H/M ratios are more likely to have appropriate ICD therapy compared to patients with low and high late H/M ratios. These findings are new and may be useful to better identify patients who, most likely, benefit from ICD implantation and therefore helps to constrain the CHF-related costs. Recently, the ADMIRE-ICD study has been started. This randomized, multicentre study will evaluate the prognostic value of planar $^{123}$I-$m$IBG scintigraphy in CHF. Subjects will be randomized to a standard care group receiving an ICD implementation for primary prevention of SCD or a $^{123}$I-$m$IBG scintigraphy group. Subjects in the $^{123}$I-$m$IBG scintigraphy group will receive an ICD for primary prevention of SCD based on a predefined late H/M ratio cut-off level of $\leq 1.6$. The primary endpoint is all-cause mortality and the secondary endpoint is combined endpoint of hospitalization, cardiac death and ventricular arrhythmia. The first results of this study are expected by the end of 2019. To improve the clinical impact of the ADMIRE-ICD study, the use of standardized H/M ratios is strongly recommended.

In conclusion, improving standardization and validation of cardiac $^{123}$I-$m$IBG scintigraphy will lead to a much more accepted application for individual CHF management. These findings will help to improve the quality of cardiac $^{123}$I-$m$IBG imaging and to facilitate appropriate use of cardiac $^{123}$I-$m$IBG imaging.
Chapter 14

Samenvatting, algemene discussie, perspectief en conclusies
SAMENVATTING

Het cardiale sympathische zenuwstelsel is een neuro hormonaal compensatiemechanisme dat een belangrijke rol speelt in de pathogenese van chronisch hartfalen (CHF). Patiënten met CHF hebben een verhoogde cardiale sympathische activiteit. De combinatie van een verhoogde afgifte van de neurotransmitter norepinephrine (NE) vanuit de presynaptische zenuwuiteinden en de verminderde NE heropname via de NE transporter resulteert in een verhoogde NE spiegel in de synaptische spleet. Om de verminderde linker ventrikelfunc te compenseren heeft deze verhoogde NE spiegel aanvankelijk een stimulerend effect op de postsynaptische β-adrenerge receptoren (AR). Echter, langdurige β-AR stimulatie door NE heeft nadelige effecten op de myocardiale structuur en leidt tot downregulatie en beschikbaarheid van de β-AR. Dit leidt tot remodelling van de linker ventrikel, waarbij de linker ventrikel kan gaan dilateren of hypertrofiëren. Bovendien is een verhoogde cardiale sympathische activiteit in patiënten met CHF geassocieerd met verhoogde morbiditeit en mortaliteit. Cardiale sympathische activiteit kan niet-invasief worden gemeten met behulp van het radiofarmacon $^{123}$I-meta-iodobenzylguanidine ($^{123}$I-mIBG), een NE analoog. De semi kwantitatieve cardiale $^{123}$I-mIBG parameters worden berekend als een verhouding tussen specifieke opname (het hart) en niet-specifieke opname (het mediastinum). De meest gebruikte semi-kwantitatieve cardiale $^{123}$I-mIBG parameters zijn de vroege hart-mediastinale (H/M) ratio, bepaald 15 minuten na toediening van $^{123}$I-mIBG, de late H/M ratio, bepaald 4 uur na toediening van $^{123}$I-mIBG en de cardiale $^{123}$I-mIBG uitwas, berekend als het verschil tussen de vroege H/M ratio en de late H/M ratio en uitgedrukt als een percentage van de vroege H/M ratio. Ondanks een groot aantal gepubliceerde studies over cardiale $^{123}$I-mIBG scintigrafie hebben methodologische en analytische variaties grootschalige klinische implementatie belemmerd. In dit proefschrift wordt verdere standaardisatie van cardiale $^{123}$I-mIBG scintigrafie gevalideerd (Deel I). Daarbij wordt de prognostische waarde van cardiale $^{123}$I-mIBG scintigrafie in patiënten met CHF geëvalueerd (Deel II). Tenslotte wordt het gebruik van cardiale $^{123}$I-mIBG scintigrafie in andere populaties dan CHF besproken (Deel III).

DEEL I

Een collimator is een essentieel onderdeel van een gammacamera en bestaat uit een loden plaat met een groot aantal parallelle openingen, gescheiden door tussenschotten (septa). Schuin invallende fotonen worden afgereemd/gestopt door het lood van de septa en alleen de recht invallende fotonen bereiken het kristal van de gammacamera. Naast 159 keV fotonen, vervalt $^{123}$I ook met hogere energetische fotonen. Deze hoger energetische fotonen kunnen de septa van de collimatorendoortrappen met als gevolg
Samenvatting en discussie

een verslechtering van de beeldkwaliteit en introductie van variatie in uitkomstmaten zoals de H/M ratio. Collimatorkeuze is daarom een van de belangrijkste factoren die kan leiden tot variatie in cardiale $^{123}$I-mIIBG parameters (H/M ratio en $^{123}$I-mIIBG uitwas).

In hoofdstuk 2 is het effect van de verschillende collimatoren onderzocht op de late H/M ratio. Een groep patiënten met CHF onderging $^{123}$I-mIIBG scintigrafie met zowel een low energy high resolution (LEHR) collimator als een medium energy (ME) collimator. De late H/M ratio’s met een LEHR collimator waren significant lager in vergelijking met die van een ME collimator (late H/M ratio 1.41 ± 0.18 vs. 1.80 ± 0.41, p < 0.001). Het verschil tussen de H/M ratio verkregen met de LEHR collimator en de H/M ratio verkregen met de ME collimator nam toe bij toename van de gemiddelde H/M ratio. Dit verschil kan worden verklaard door septumpenetratie van hoog energetische fotonen vanuit de lever en de longen die daarmee op zowel mediastinaal als op myocardiaal niveau de uitkomst beïnvloeden. De mate van septumpenetratie bleek het laagst bij ME collimatoren. Daarmee onderstrepen deze resultaten het belang van het gebruik van ME collimatoren voor cardiale $^{123}$I-mIIBG scintigrafie.

Het gebrek aan standaardisatie tussen verschillende instellingen is een van de factoren die de klinische implementatie van cardiale $^{123}$I-mIIBG scintigrafie heeft belemmerd. Zoals al in het vorige hoofdstuk aangetoond zijn verschillen in collimatoren oorzaak voor variatie in de H/M ratio. In hoofdstuk 3 zijn de resultaten beschreven van een Europese fantoomstudie voor de cross-kalibratie van cardiale $^{123}$I-mIIBG scintigrafie. Op basis van de resultaten van deze fantoomstudie zijn conversiecoëfficiënten voor een groot aantal gammacamera-collimator combinaties berekend. Deze conversiecoëfficiënten kunnen gebruikt worden om de H/M ratio’s van verschillende gammacamera-collimator combinaties om te rekenen naar een gestandaardiseerde H/M ratio. Daarmee zorgt deze cross-kalibratie voor een betere vergelijking van de H/M ratio’s tussen instellingen. Dit is essentieel voor het vaststellen van een universele H/M ratio om op een juiste wijze hoog- en laagrisicopatiënten met hartfalen te kunnen identificeren.

In hoofdstuk 4 is de invloed beschreven van de definitie en plaatsing van de mediastinale regio van interesse (ROI) op de intra- en interobserver variabiliteit in relatie tot collimator-type. Een door individuele observers gedefinieerde mediastinale ROI werd vergeleken met een vooraf gedefinieerde mediastinale ROI. De H/M ratio’s berekend met de vooraf gedefinieerde mediastinale ROI toonde een substantiële overeenkomst tussen de verschillende beoordelaars (inter-observer) ongeacht het type collimator dat werd gebruikt voor het verkrijgen van de beelden (LEHR versus ME collimatoren). Daarnaast, als dezelfde beoordelaar een tweede maal gevraagd werd de analyse te doen met een vooraf gedefinieerde mediastinale ROI (intra-observer), was er eveneens een aanzienlijke overeenkomst. Wederom was deze variatie onafhankelijk van het type collimator. Daarentegen zorgde de H/M ratio berekend met een door beoordelaars zelf gedefinieerde mediastinale ROI tot aanzienlijke variatie in de uitkomst tussen de beoordelaars. Deze variatie was onafhankelijk van het type
collimator dat werd gebruikt. Daarom heeft, voor cardiale $^{123}$I-mIBG scintigrafie, een vooraf gedefinieerde mediastinale ROI de voorkeur boven een door de beoordelaar gedefinieerde mediastinale ROI.

De NE transporter, gecodeerd door het “solute carrier familie 6 member 2” (SLC6A2) gen, is verantwoordelijk voor de heropname van NE in de presynaptische zenuwuiteinden en daarmee een belangrijke modulator van de synaptische NE homeostase. $^{123}$I-mIBG gebukt dezelfde presynaptische opname, opslag en afgifte mechanismes als NE. Hoewel polymorfisme van het SLC6A2 gen is gerapporteerd, is het effect op cardiale $^{123}$I-mIBG opname onbekend. In hoofdstuk 5 is de invloed van single-nucleotide polymorfisme van het SLC6A2 gen onderzocht op $^{123}$I-mIBG parameters in 49 patiënten met CHF. Zes verschillende single-nucleotide polymorfismen van het SLC6A2 gen werden gevonden, maar geen enkel bleek functioneel te zijn. Het is daarmee niet verwonderlijk dat polymorfisme van het SLC6A2 gen in deze studiepopulatie niet geassocieerd was met cardiale $^{123}$I-mIBG parameters.

Voor de kwantificering van cardiale $^{123}$I-mIBG opname wordt het mediastinum gebruikt als referentiegebied voor afspiegeling van aspecifieke/achtergrond activiteit. Variaties in de hoeveelheid vasculaire structuren in het mediastinum en de renale klaring van $^{123}$I-mIBG kunnen leiden tot interindividuele variatie in cardiale $^{123}$I-mIBG opname. In hoofdstuk 6 is de relatie tussen de veranderingen in myocardiale (H) en mediastinale (M) counts en de verandering in vasculaire $^{123}$I-mIBG concentraties onderzocht. Omdat $^{123}$I-mIBG voornamelijk wordt geklaard door de nieren is in deze studie ook de invloed van de nierfunctie op de H en M counts onderzocht. Veranderingen in gemeten H/M ratio’s tussen vroege (15 minuten) en late (4 uur) $^{123}$I-mIBG afbeeldingen kunnen niet worden verklaard door $^{123}$I-mIBG concentraties in het bloed, vasculaire klaring of nierfunctie. De verandering in gemeten H en M counts tussen vroeg en late $^{123}$I-mIBG afbeeldingen was niet gerelateerd aan de intravasculaire spiegels van het radiofarmacon. Dit suggereert dat verandering in mediastinale counts voornamelijk het gevolg is van afname van activiteit in de weke delen en verstrooiing van fotonen uit de aangrenzende longen.

Patiënten met CHF hebben vaak een verminderde nierfunctie. Daarnaast is de verminderde nierfunctie ook geassocieerd met een verhoogde kans op mortaliteit in deze patiëntengroep. Verder wordt $^{123}$I-mIBG voornamelijk door de nieren geklaard. Het is dus logisch om te veronderstellen dat een verlaagde glomerulaire filtratiesnelheid (GFR) geassocieerd is met een verminderde bloedklaring van $^{123}$I-mIBG en daardoor de cardiale $^{123}$I-mIBG parameters beïnvloedt. In hoofdstuk 7 is bestudeerd of de cardiale $^{123}$I-mIBG parameters in patiënten met CHF geassocieerd zijn met de nierfunctie. Daarnaast zijn nierfunctie en $^{123}$I-mIBG parameters onderzocht als voorspellers van cardiale dood. De $^{123}$I-mIBG parameters bleken onafhankelijk van de nierfunctie. Bovendien bleek cardiale $^{123}$I-mIBG scintigrafie een betere voorspeller van de prognose bij patiënten met CHF dan de nierfunctie.
Samenvatting en discussie

DEEL II

De prognose in CHF wordt het meest waarschijnlijk multifactorieel beïnvloed. Hierbij speelt de cardiale sympathische activiteit ook een rol. Het is dus aannemelijk dat cardiale sympathische activiteit zoals bepaald met $^{123}$I-mIBG scintigrafie, hetzij alleen, maar aannemelijker in combinatie met andere parameters, behulpzaam zou kunnen zijn bij de risicocategorisatie van CHF patiënten. **Hoofdstuk 8** beschrijft een meta-analyse van originele individuele late H/M ratio data van 636 patiënten met CHF afkomstig uit zes studies van Europa en de Verenigde Staten van Amerika. De late H/M ratio werd onderzocht als voorspeller van sterfte, cardiale sterfte, aritmieën en harttransplantatie. In deze meta-analyse was de late H/M ratio, zowel als dichotome als continue parameter, een onafhankelijke voorspeller voor alle eventcategorieën met uitzondering van aritmie. Deze uitkomst maakt dat de late H/M ratio niet alleen kan worden gebruikt als een dichotome voorspeller van events (hoog- vs. laagrisico), maar ook heeft de late H/M ratio prognostische waarde over het volledige bereik van uitkomstwaardes voor alle eventcategorieën, behalve aritmie.

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HF leidt niet alleen tot een verhoogde cardiale sympathische activiteit, maar is ook geassocieerd met verhoogde myocardiale inflammatie. **Hoofdstuk 9** beschrijft de relatie tussen de ernst van het hartfalen, cardiale sympathische activiteit gemeten middels cardiale $^{123}$I-mIBG scintigrafie en inflammatie in 55 stabiele en medicamenteus optimaal behandelde patiënten met CHF. Daarnaast is de voorspellende waarde van inflammatie en $^{123}$I-mIBG parameters onderzocht voor cardiale events (terechte ICD therapie, progressie van HF en cardiale sterfte). De linker ventrikelpijkeit fractie (LVEF) was de enige onafhankelijke voorspeller van de late H/M ratio en NT-proBNP was de enige onafhankelijke voorspeller van cardiale $^{123}$I-mIBG uitwas. Geen van de markers van inflammatie bleken geassocieerd te zijn met late H/M ratio of cardiale $^{123}$I-mIBG uitwas. Tijdens een mediane follow-up van 34 maanden kregen 13 patiënten een cardiaal event. Tijdens de mediane follow-up van 34 maanden kregen 13 patiënten een cardiaal event. Deze cardiale events bleken bij univariate analyse geassocieerd te zijn met het C-reactieve protein (CRP), NT-proBNP, MPO en late H/M ratio. Echter, bij multivariate analyse bleken alleen CRP, NT-proBNP, MPO en IL-12p40 voorspellers van cardiale events te zijn. Concluderend, in stabiele, optimaal behandelde patiënten met CHF, lijken inflammatie en cardiale sympathische activiteit niet geassocieerd te zijn. Dit wordt nog eens bevestigd doordat beiden prognostische waarde hebben in deze specifieke stabiele CHF populatie.

ICD implantatie ter primaire preventie van plotse hartdood is inmiddels een integraal onderdeel geworden van de behandeling van CHF. Deze ICD’s gaan echter in een groot deel van de patiënten met CHF nooit af. Daarnaast zijn ICD’s aanzienlijk kostbaar. Er lijkt dus een noodzaak om de huidige selectiecriteria voor ICD implantatie te optimaliseren. **Hoofdstuk 10** evalueert of cardiale sympathische activiteit, gemeten middels $^{123}$I-mIBG scintigrafie, kan helpen deze patiëntenselectie te optimaliseren. In een
Deel III

In het 22q11.2 deletie syndroom (DS) is de werking van catechol-O-methyltransferase (COMT) verminderd. Dit enzym reguleert de afbraak van NE. Volwassenen met 22q11.2DS blijken klinisch een verhoogd risico op een plotse dood te hebben. Hoewel de oorzaken waarschijnlijk multifactorieel zijn, kan een verhoogde cardiale sympathische activiteit met fatale aritmie vanwege verhoogde NE spiegels, worden beschouwd als een mogelijk oorzaak. In hoofdstuk 11 is bestudeerd of cardiale sympathische activiteit, gemeten middels 123I-mIBG scintigrafie, verhoogd is bij 22q11.2DS, zowel voor als na een acute NE depletie met behulp van alfa-methyl-para-tyrosine (AMPT). Volwassenen met 22q11.2DS blijken zowel voor als na AMPT toediening een normale cardiale sympathische activiteit te hebben ten opzichte van gezonde proefpersonen. Hoewel in vergelijking met gezonde proefpersonen er een klein verschil is in cardiale 123I-mIBG uitwas, is dit hoogstwaarschijnlijk niet verklarend voor het verhoogde risico op plotse dood in patiënten met het 22q11.2 DS.

Tako-tsubo cardiomyopathie (TCM) is een klinisch syndroom gekenmerkt door acute, reversibele disfunctie van de apex van het linker ventrikel, vaak voorafgegaan door blootstelling aan ernstige fysieke of emotionele stress. Hoofdstuk 12 beschrijft een overzicht van de klinische presentatie en behandeling van TCM. Ook de rol van verschillende niet-invasieve beeldvormende technieken wordt besproken met de focus op de mogelijke rol van cardiale 123I-mIBG scintigrafie. Daarnaast worden de mogelijke pathofysiologische mechanismen van TCM besproken. De meest geldende hypothese over de pathofysiologie van TCM is dat verhoogde plasma spiegels van de neurotransmitter epinefrine leiden tot een verandering van de intracellulaire signalering in myocyten in het myocard. Bij significante stijging van epinefrine spiegels in het bloed verschuift de stimulatie van het G-stimulerend naar het G-remmende eiwit, wat leidt tot

Chapter 14

Europese multicenter studie werden 135 stabiele patiënten met CHF, met een indicatie voor een ICD ter primaire preventie van plotse hartdood volgens de huidige richtlijnen, geïncludeerd. Conversiecoëfficiënten uit de eerder beschreven cross-kalibratiestudie werden gebruikt om te corrigeren voor de verschillende gammacamera-collimator combinaties. Tijdens een mediane follow-up van 30 maanden kregen 24 patiënten een cardiaal event (terechte ICD therapie \( n = 12 \), progressie van hartfalen \( n = 6 \) en cardiale sterfte \( n = 6 \)). De late H/M ratio en LVEF bleken geassocieerd te zijn met het uitblijven van terechte ICD therapie. Daarnaast bleek de late H/M ratio significant geassocieerd te zijn met het gecombineerde eindpunt van alle eerste cardiale events. Deze resultaten suggereren dat cardiale 123I-mIBG scintigrafie behulpzaam kan zijn bij een betere identificatie van patiënten die hoogstwaarschijnlijk profiteren van ICD implantatie.
Samenvatting en discussie

een negatief inotroop effect (verminderde contractiliteit van het myocard). Dit negatief inotrope effect is het grootst in de apex van de linker ventrikel waar de $\beta_2$AR:$\beta_1$AR ratio het hoogst is. Hoewel TCM geassocieerd is met verhoogde epinefrine spiegels blijkt dat met behulp van $^{123}$I-mIBG scintigrafie afwijkende adrenerge activiteit kan worden vastgesteld. Een mogelijke verklaring hiervoor is dat verhoogde epinefrine spiegels leiden tot verminderde werking van de norepinefrine transporter met als gevolg verminderde $^{123}$I-mIBG opname. Aangezien uitkomstmaten van $^{123}$I-mIBG scintigrafie belangrijke voorspellers zijn voor prognose in verschillende cardiale ziektes, zou $^{123}$I-mIBG scintigrafie behulpzaam kunnen zijn om individuele (farmacologische) therapie te optimaliseren bij patiënten met TCM. Deze nieuwe inzichten kunnen worden gebruikt als uitgangspunt voor de ontwikkeling van internationale richtlijnen voor TCM, ter bevordering van de behandeling van TCM.

CONCLUSIE EN TOEKOMSTPERSPECTIEVEN

Cardiale sympathische activiteit kan worden afgebeeld middels cardiale $^{123}$I-mIBG scintigrafie. Echter, cardiale $^{123}$I-mIBG scintigrafie kan door een groot aantal parameters worden beïnvloed. Verschillende parameters zoals collimatorkeuze, mediastinale ROI selectie, polymorfisme van het SLC6A2 gen, nierfunctie en inflammatie zijn in dit proefschrift bestudeerd. De resultaten van dit proefschrift tonen aan dat standaardisatie van een deel van deze parameters in combinatie met cross-kalibratie van gammacamera gebruik, leidt tot een goede reproduceerbaarheid met een beperkte inter- en intra-observer variatie. Bovendien lijken de uitkomstmaten van gestandaardiseerde cardiale $^{123}$I-mIBG scintigrafie een duidelijke prognostische waarde te hebben in patiënten met CHF. Daarmee lijken de belangrijke bezwaren die een verdere klinische implementatie tegenhielden te zijn weggenomen. Bovendien maakt het gebruik van gestandaardiseerde H/M ratio’s de ontwikkeling mogelijk van een universele prognostische afkapwaarde (hoog- versus laagrisico). Deze universele waarde kan worden vastgesteld door bijvoorbeeld de data van eerder gepubliceerde multicenter studies opnieuw te analyseren. Ten slotte dient te worden benadrukt dat gestandaardiseerde H/M ratio’s essentieel zijn voor het opstellen van risicomodellen.

ICD implantatie ter primaire preventie van plotse hartdood is inmiddels een integraal onderdeel geworden van de behandeling van patiënten met CHF. Echter, bij een groot deel van de patiënten met CHF blijkt de ICD nooit terechte therapie te geven. Het is dus noodzakelijk om de huidige selectiecriteria voor ICD implantatie te optimaliseren. Onlangs is daarom de ADMIRE-ICD studie gestart. Deze gerandomiseerde, multicenter studie onderzoekt de prognostische waarde van $^{123}$I-mIBG scintigrafie in patiënten met CHF. Patiënten zullen worden gerandomiseerd naar een groep die standaard een ICD implantaat ter primaire preventie van plotse hartdood krijgt of een $^{123}$I-mIBG groep,
die alleen een ICD voor primaire preventie van plotse hartdood krijgt op basis van een vooraf gedefinieerde late H/M ratio ≤ 1.6. Het primaire eindpunt is mortaliteit en het secundaire eindpunt is het gecombineerde eindpunt van hospitalisatie, hartdood en ventriculaire aritmie. De eerste resultaten van deze studie worden tegen het einde van 2019 verwacht. Om de klinische impact van deze studie te vergroten wordt, zoals in dit proefschrift wordt aangetoond, het gebruik van gestandaardiseerde H/M ratio sterk aanbevolen.

Concluderend leidt verbetering van standaardisatie en validatie van cardiale $^{123}$I-mIBG scintigrafie tot een veel meer geaccepteerde benadering voor individuele hartfalen behandeling. De bevindingen van dit proefschrift zullen helpen om de kwaliteit van de cardiale $^{123}$I-mIBG scintigrafie te verbeteren en de klinische implementatie van de cardiale $^{123}$I-mIBG scintigrafie te vergemakkelijken.
Appendix

Dankwoord
Curriculum Vitae
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DANKWOORD

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Dankwoord
CURRICULUM VITAE

Derk Otto Verschure was born in Schiedam, the Netherlands, on 4 December 1978. In 1997 he completed his secondary education (VWO) at the Bisschoppelijk College in Weert and began his study of medicine at the Academic Medical Center (AMC) of the University of Amsterdam. As part of his clinical rotations he spent two months at the department of internal medicine and cardiology of the Medical University of Southern Africa (Medusa). He successfully completed his medical studies at the AMC in 2005.

In the course of his preliminary studies, he developed a fascination with cardiology. Consequently, following his graduation, he worked as an intern in cardiology at the Meander hospital in Amersfoort. In 2007 he began his specialization in cardiology under the supervision of G.A. Somsen at the Onze Lieve Vrouwe Gasthuis (OLVG) in Amsterdam. From 2010 onwards, he combined this training with research towards a doctorate at the Nuclear Medicine Department of the AMC under the supervision of professor B.L.F. van Eck-Smit, H.J. Verberne and G.A. Somsen. The results of the research conducted during this PhD trajectory are described and analyzed in this thesis.

In 2014, after his specialization in cardiology, he started a fellowship in cardiovascular imaging at the Noordwest Ziekenhuisgroep, Alkmaar. Here he specialized in echocardiography, cardiac CT, and cardiac MRI. Currently he is working at the cardiology department of the Zaans Medical Center, Zaandam.

Derk Verschure is married to Sara Dorsman. They live in Amsterdam with their sons Olivier and Stijn.
Appendix

BIBLIOGRAPHY

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