DETECTION OF MYOCARDIAL ISCHEMIA USING REAL-TIME MYOCARDIAL CONTRAST ECHOCARDIOGRAPHY

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Hälsa och Samhälle

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To my lovely wife Cornelia and my wonderful children, Amadeus, Ofelia and Esmeralda
Without you I would never have kept my balance

CONTENTS

Preface

MY CONTRIBUTIONS TO THE INCLUDED PAPERS OF THIS THESIS

I planned the major part of the work and performed all echocardiographic examinations except a few examinations in paper I where I and a co-author alternated. SPECT examinations were carried out by expert staff at the department of clinical physiology. I have been responsible for all of the writing with helpful correspondence by my tutors and co-authors when needed.

NOT INCLUDED PAPERS TO WHICH I HAVE CONTRIBUTED DURING MY WORK OF THE PRESENT THESIS:

Borgquist R, **Gudmundsson P**, Winter R, Nilsson P, Willenheimer R. Erectile dysfunction in healthy subjects predicts reduced coronary flow velocity reserve. Int J Cardiol. 2006 Sep 20;112(2):166-70.

Gudmundsson P, Rydberg E, Winter R, Willenheimer R. Visually estimated ejection fraction is closely correlated with quantitative methods. Int J Cardiol. 2005 May 25;101(2):209-12.

Winter R, **Gudmundsson P**, Willenheimer R. Real-time perfusion adenosine stress echocardiography in the coronary care unit: a feasible bedside tool for predicting coronary artery stenosis in patients with acute coronary syndrome. Eur J Echocardiogr. 2005 Jan;6(1):31-40.

Rydberg E, **Gudmundsson P**, Kennedy L, Erhardt L, Willenheimer R. Left atrioventricular plane displacement but not left ventricular ejection fraction is influenced by the degree of aortic stenosis. Heart. 2004 Oct;90(10):1151-5.

Winter R, **Gudmundsson P**, Ericsson G, Willenheimer R. Correlation of the M-mode atrioventricular plane early diastolic downward slope and systolic parameters. Coupling of LV systolic and early diastolic function. Int J Cardiovasc Imaging. 2004 Apr;20(2):101-6

Rydberg E, Arlbrandt M, **Gudmundsson P**, Erhardt L, Willenheimer R. Left atrioventricular plane displacement predicts cardiac mortality in patients with chronic atrial fibrillation. Int J Cardiol. 2003 Sep;91(1):1-7.

Winter R, **Gudmundsson P**, Willenheimer R. Feasibility of noninvasive transthoracic echocardiography/Doppler measurement of coronary flow reserve in left anterior descending coronary artery in patients with acute coronary syndrome: a new technique tested in clinical practice. J Am Soc Echocardiogr. 2003 May;16(5):464-8.

ORIGINAL PAPERS

I. Real-time perfusion adenosine stress echocardiography versus myocardial perfusion adenosine scintigraphy for the detection of myocardial ischemia in patients with stable coronary artery disease. *Petri Gudmundsson, Reidar Winter, Magnus Dencker, Mariusz Kitlinski, Ola Thorsson, Lennart Ljunggren, Ronnie Willenheimer.* Clin Physiol Funct Imaging. 2006 Jan;26(1):32-8.

II. High-resolution grey scale or angio mode power modulation? Head to head comparisons of two modalities of real-time perfusion adenosine stress echocardiography with simultaneous SPECT. *Petri Gudmundsson, Kambiz Shahgaldi, Reidar Winter, Magnus Dencker, Mariusz Kitlinski, Ola Thorsson, Lennart Ljunggren, Ronnie Willenheimer.* Submitted.

III. Parametric quantification of myocardial ischemia using real-time perfusion adenosine stress echocardiography images, with SPECT as reference method. *Petri Gudmundsson, Kambiz Shahgaldi, Reidar Winter, Magnus Dencker, Mariusz Kitlinski, Ola Thorsson, Lennart Ljunggren, Ronnie Willenheimer.* Submitted.

IV. Quantitative detection of myocardial ischemia by real-time perfusion adenosine stress echocardiography. A comparison with SPECT. *Petri Gudmundsson, Kambiz Shahgaldi, Reidar Winter, Magnus Dencker, Mariusz Kitlinski, Ola Thorsson, Lennart Ljunggren, Ronnie Willenheimer.* Submitted.

ABBREVIATIONS

RTP	Real time perfusion
ASE	Adenosine stress echocardiography
RTP-ASE	Real time perfusion adenosine stress echocardiography
SPECT	99mTc-sestamibi single-photon emission computed tomography
DSE	Dobutamine atropine stress echocardiography
ECG	Electrocardiogram
CAD	Coronary artery disease
LAD	Left anterior descending coronary artery
LCx	Left circumflex coronary artery
RCA	Right coronary artery
RPD	Right posterior descending coronary artery
MI	Mechanical index
MCE	Myocardial contrast echocardiography
AM	Angio mode of power modulation
HR	High resolution grey scale of power modulation
A	Peak signal intensity
β	Myocardial blood flow velocity
Axβ	Myocardial blood flow
A-r	Peak signal intensity reserve
β-r	Myocardial blood flow velocity reserve
Axβ-r	Myocardial blood flow reserve
PPV	Positive predictive value
NPV	Negative predictive value
ROC	Receiver Operating Characteristic

ABSTRACT

Echocardiography is an ideal clinical method for obtaining information about morphology and function of the heart. Echocardiography is more accessible, mobile and inexpensive compared to other imaging techniques and has become the perhaps most used diagnostic method in cardiology during recent years. To assess myocardial ischemia, different types of stress echocardiography have been available, where mainly wall motion analysis at rest and stress has been used to evaluate the presence and extent of ischemia. During the last few years, second generation contrast agents have become clinically available. This has improved image quality in echocardiography, which, combined with new ultrasound technical developments, has made it possible to obtain echocardiographic images of myocardial perfusion. When this myocardial contrast echocardiography technique is carried out in real-time, as in the studies of this thesis, it is labelled real-time perfusion (RTP). RTP in combination of adenosine stress (RTP-ASE) has the potential to become a valuable clinical tool to evaluate myocardial ischemia. If proven as accurate as other clinically and scientifically accepted methods, such as 99mTc-sestamibi single-photon emission computed tomography (SPECT), RTP-ASE might become an alternative method. Compared to SPECT, it is more accessible, mobile, inexpensive, and without radiation, compared to dobutamine-atropine stress echocardiography (DSE) it is more tolerable and swifter, and it is more accurate than exercise ECG.

In all studies of this thesis, we performed RTP-ASE in patients with known or suspected stable coronary artery disease (CAD), admitted to adenosine SPECT evaluation. Adenosine was infused to provoke relative regional hypo-perfusion in ischemic myocardial territories. Using a

SONOS 5500 echocardiography machine, patients underwent RTP imaging during Sonovue® infusion, before and throughout the adenosine stress, also used for SPECT. RTP images were stored for later, blinded, off-line analysis. In studies III and IV, the commercially available software Qontrast® was used to generate parametric images of myocardial perfusion and quantitative values of perfusion replenishment from RTP-ASE image loops. Method of reference for the ischemia evaluation in the thesis was the presence or absence of reversible ischemia at SPECT. The left ventricular myocardium was divided into three territories corresponding to the distribution territories of the three main coronary arteries; left anterior descending (LAD), left circumflex (LCx) and right coronary artery (RCA).

In studies I and II, we investigated the feasibility of RTP-ASE for the detection of ischemia using visual interpretation of RTP-ASE loops acquired at rest and stress.

Study III was carried out to examine the value of quantitatively generated parametric perfusion images from RTP-ASE loops, in detecting myocardial ischemia.

In study IV, the usefulness of quantitative detection of myocardial ischemia from RTP-ASE loops was assessed. Data comparing quantitative measurements of perfusion replenishment from RTP-ASE images at rest and stress were used as markers of ischemia.

The results from the studies in this thesis suggest that visual evaluation of ischemia from RTP-ASE images, using angio-mode as well as high resolution grey scale mode, is accurate and feasible. It is therefore a clinically useful method in patients with known or suspected stable CAD. Quantification of ischemia or parametric imaging for ischemia evaluation using Qontrast®, are not yet suitable for clinical use, as judged by the findings of this thesis. However, since further technical development can be expected, quantitative assessment of myocardial perfusion may well be a clinically useful method in the near future.

INTRODUCTION

Echocardiography has become a widespread imaging technique for evaluating morphology and function of the heart and its components. An echocardiographic examination can be performed almost anywhere and anytime since it is a very mobile technique. Echocardiography is associated with an extremely low rate of hazardous events and is relatively inexpensive. Alternative imaging modalities, like X-ray, scintigraphy or magnetic resonance imaging, can sometimes provide better image quality, but are not nearly as mobile techniques. X-ray computer tomography and scintigraphy transmit radioactivity to the patient and most alternative techniques are in most cases more expensive than echocardiography. These are some of the reasons making echocardiography an appealing technique to use and to develop further for an even more widespread use in cardiac disease.

However, there are also limitations of the echocardiographic technique. One is that it demands experienced operators and interpreters in order to obtain both good enough image quality and correct interpretations. There is also, in some patients, low signal to noise ratio of the images, especially from a digital point of view, when post-processing or quantifying findings from the echocardiographic images. Differences in image quality between patients depend on patient composition and, due to high acoustic impedance differences, ribs and lungs might decrease image quality.

Basic echocardiographic principles

Echocardiography and ultrasound-based imaging techniques are based on the principle that, an ultrasound signal is partly or completely re-

flected when propagating from one tissue to a tissue with different acoustic impedance. The reflected ultrasound, the echo, from a specific point is then detected at the source where it was originally sent out. The time delay between sending out the ultrasound and receiving the echo is proportional to the distance the signal has travelled and, thus, the distance from the source to the point of reflection can be calculated and displayed. The more of the signal that is reflected, the less is continuing further into the tissue and, finally, all of the energy of the ultrasound beam is reflected or absorbed as energy in the tissue. All echoes from different depths of the tissue can be displayed at the corresponding depth of a screen and by sending multiple ultrasound beams simultaneously, a two-dimensional image can be generated. By repeating the procedure, multiple frames can be generated every second and, thus, be presented as moving images of, for example, the heart. Higher signal power (mechanical index [MI]) increases image quality, but at the same time the energy transmitted into the tissue increases. However, using MI less than 1.5 is considered to be harmless in adult humans. Increasing MI to higher levels becomes less beneficial since the amount of reflected echo power is proportional to the power of the transmitted signal. Therefore, proportionally more signal power will be reflected as near field reflections and will not contribute as much in the far field as wanted.

Echocardiography in Myocardial ischemia

Echocardiography can be used to evaluate myocardial ischemia in patients with known and suspected coronary artery disease (CAD), using different ways to provoke ischemia in the myocardium. These include physical exercise, dobutamine and dipyridamole infusion, and pacemaker stimulation to increase heart rate (Pozzoli, Fioretti et al. 1991; Marwick, Willemart et al. 1993; Olmos, Dakik et al. 1998; Parodi, Picano et al. 1999; Smart, Bhatia et al. 2000; Biagini, Schinkel et al. 2005; Bombardini, Agrusta et al. 2005).

In low risk patients with suspected myocardial ischemia, evaluation of ischemia is generally recommended for optimal care and treatment (1997; Erhardt, Herlitz et al. 2002). Ischemia can be assessed by exercise ECG, single-photon emission computed tomography (SPECT) or dobutamine atropine stress echocardiography (DSE), where the two latter are well established and more accurate methods (Schinkel, Bax et al. 2003; Sozzi, Elhendy

et al. 2003; Picano 2004; Underwood, Anagnostopoulos et al. 2004), although more expensive.

DSE uses wall motion evaluation to detect myocardial ischemia. This is possible since myocardial wall motion during maximum stress increases in normal and decreases in ischemic segments of the myocardium. DSE is however quite strenuous for the patient, because of a relatively long examination time with high heart rate, with or without chest pain.

Adenosine stress echocardiography (ASE) can also be used for ischemia evaluation, but demands evaluation of myocardial perfusion to reach similar accuracy for detecting ischemia and can not solely rely on wall motion assessment (Marwick, Willemart et al. 1993; Takeishi, Chiba et al. 1994; Lafitte, Matsugata et al. 2001). Therefore, the best approach in ASE is to investigate myocardial perfusion instead of or in combination with wall motion assessment. This would also bring the ischemia evaluation closer to the origin of ischemia according to the ischemic cascade (Figure 1) (Nesto and Kowalchuk 1987), which is likely to be beneficial to the detection of myocardial ischemia. However, visualisation of myocardial perfusion by

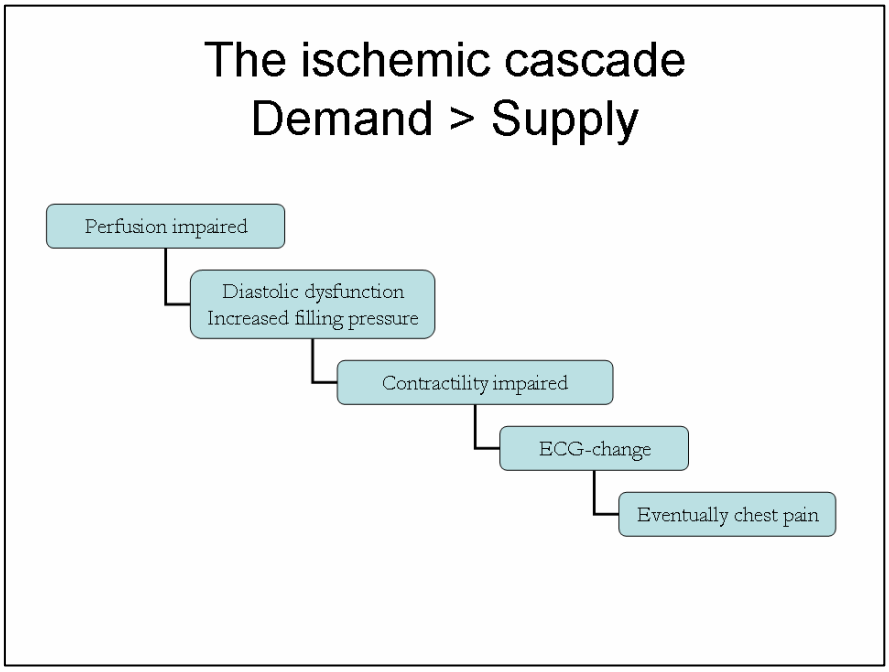


Figure 1. *The ischemic cascade.*

echocardiography has only been possible during the last few years. This was first achieved by administering echocardiographic contrast agents directly into the aortic root and (Kemper, O'Boyle et al. 1983), recently, by intravenous injection of second generation contrast agents (Kaul 1990; Becher, Tiemann et al. 1997; Becher and Burns 2000; Heinle, Noblin et al. 2000; Agati, Funaro et al. 2001; Senior, Villanueva et al. 2004; Janardhanan, Dwivedi et al. 2005).

Echocardiographic contrast agents

The components of blood, including erythrocytes, are very poor reflectors of ultrasound. Therefore, it has long been impossible to measure the presence or absence of blood in the ventricular myocardium. It became possible to overcome this problem when second generation contrast agents were developed. After intravenous injection, second generation micro bubbles survive the passage through the lung capillaries and can enter the left ventricle. Consequently, they can also enter the coronary arteries and the myocardial vascular bed. The micro bubbles are encapsulated, gas-filled (air or high-molecular-weight gas) micro spheres with a protein or phospholipid shell. They are 2-8 µm in diameter, i.e. as small as or smaller than erythrocytes, and can therefore follow the blood anywhere the erythrocytes go.

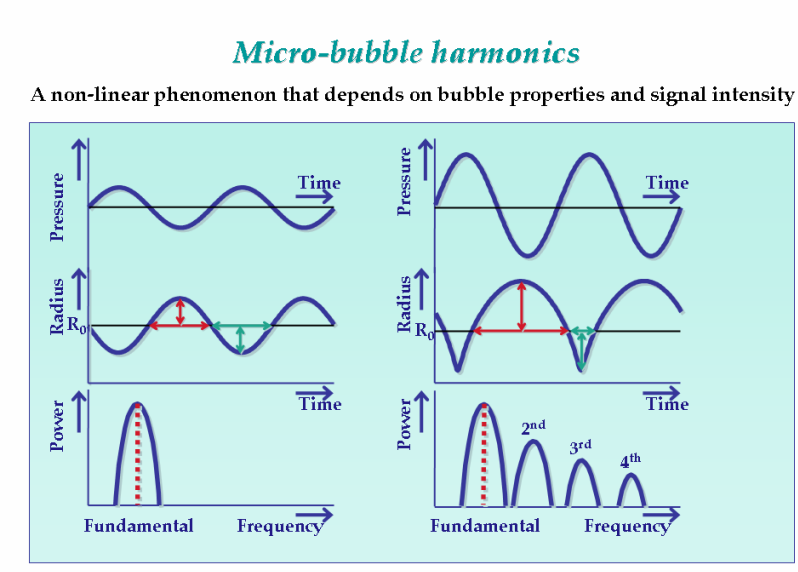


Figure 2. *Micro-bubbles harmonics.*

The micro-bubbles have specific characteristics making them ideal to detect by ultrasound examination. First of all, they reflect ultrasound very well and are, therefore, usually very easy to detect in an ultrasound image. When they are exposed to ultrasound above an amplitude corresponding to a MI between 0.1 and 1, they begin to vibrate (resonate) in a way that generates overtones of the original frequency of the reflected ultrasound signal (second and third harmonics) (figures 2 and 3). If the ultrasound system is programmed to detect these overtones, technical solutions make it possible to differentiate between signals from the tissue and the contrast agent and, consequently, between myocardium and contrast in the myocardium, i.e. myocardial perfusion. However, when the micro-bubbles are exposed to a higher MI than approximately 1, they implode and give rise to one strong harmonic signal. Although this signal is very

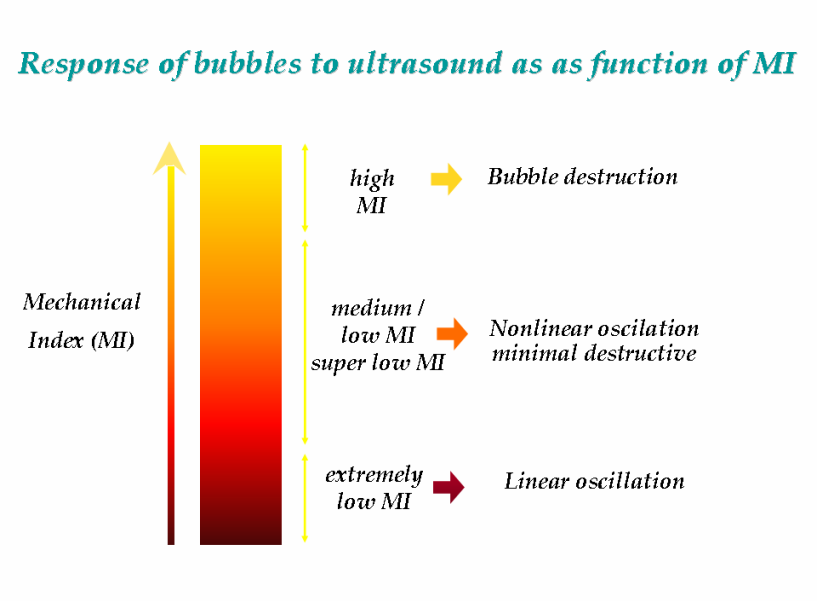


Figure 3. *Micro-bubble response to different levels of mechanical index (MI)*

easy to detect, each bubble will only produce one signal since it is destroyed when generating the signal. This second high MI destructive technique demands intermittent imaging of one frame every second, or up to every 8th heartbeat, if assessment of myocardial perfusion is the objective. The high MI perfusion technique is quite difficult, since the image

view has to be contained without the possibility to see the actual image between the image frames. Furthermore, the high MI technique does not allow for evaluation of wall motion, which might be valuable during a stress test.

If the MI is kept low, between 0.1-0.2, the micro bubbles still generate non-linear echoes, which enables differentiation from tissue echoes. Since the bubbles are not destroyed, perfusion replenishment and/or wall motion evaluation is possible. This low MI technique is in this thesis called real-time perfusion (RTP).

Sonovue® is the second generation contrast agent used in the studies of this thesis. The Sonovue® micro-bubbles consist of a phospholipid monolayer which encapsulate the inert gas sulphur-hexafluoride (SF_6). These bubbles have a mean diameter of 2.5 μm and 90 % of the bubbles are smaller than 8 μm.

There are various techniques for RTP, which differ between manufacturers, but they all try to differentiate between tissue and contrast and display contrast and, thus, perfusion continuously, without destroying the micro-bubbles.

Contrast agents and safety

Echocardiography is generally an extremely safe method for cardiac examination. However, when a contrast agent is added to the examination there is also some added risk to the patient. The contrast agent Sonovue® showed very good safety in early studies. Nevertheless, after three fatal adverse events in connection with administration of Sonovue®, the safety was recently questioned (Morel, Schwieger et al. 2000; de Groot, van Zwieten-Boot et al. 2004; Dijkmans, Visser et al. 2005). The fatal cases could have been coincidental, since the patients were in poor clinical condition before Sonovue® was given, but the events have resulted in contra-indications for Sonovue® in high-risk patients such as patients with acute coronary syndrome. However, contra-indications do not concern patients with stable CAD, such as those included in the studies performed within this thesis.

Myocardial Contrast Echocardiography (MCE) in myocardial ischemia

For several reasons, MCE is a promising technique in the context of evaluating myocardial ischemia. It allows the use of several stressors (Kaul, Senior et al. 1997; Lafitte, Matsugata et al. 2001; Olszowska, Kostkiewicz et al. 2003; Mulvagh 2004; Picano 2004; Senior, Lepper et al. 2004; Tsutsui, Xie et al. 2005; Jeetley, Hickman et al. 2006), it can be performed at almost any place anytime since it is a very mobile technique, it is associated with a very low risk of hazardous events, and it is reasonably inexpensive. Therefore, it is worthwhile to further investigate the ability of MCE techniques to detect ischemia.

The various MCE techniques can be roughly classified into two types: (1) The intermittent imaging technique using high MI; and (2) the RTP technique using a low MI. In other studies, both techniques have shown promising results as regards their ability to detect myocardial ischemia (Becher, Tiemann et al. 1997; Kaul, Senior et al. 1997; Heinle, Noblin et al. 2000; Mor-Avi, Caiani et al. 2001; Ronderos, Boskis et al. 2002; Mulvagh 2004; Tsutsui, Xie et al. 2005; Winter, Gudmundsson et al. 2005; Jeetley, Hickman et al. 2006; Korosoglou, Dubart et al. 2006). The principal marker of myocardial ischemia is the reduction of perfusion during stress, as compared with perfusion at rest. Both rest and stress images are based on the replenishment of myocardial perfusion during up to ten heartbeats of myocardial perfusion, following destructive frames of high MI, i.e. above 1.0. Replenishment at stress should be complete after two heartbeats, compared to approximately four heartbeats at rest (Becher and Burns 2000).

Intermittent imaging is possibly a more contrast specific technique. Since it uses high MI, it processes ultrasound signals with higher amplitudes and, therefore, probably higher signal to noise ratio. However, as mentioned above, it is a more demanding technique to carry out and it cannot be directly combined with wall motion analysis, which might be of value when comparing stress and rest images. RTP has the benefit of being in real time, which makes the technique somewhat easier to perform. In addition, by using the same RTP images it can be combined with wall motion evaluation. RTP generates many frames which can be a benefit but also a disadvantage. When interpreting many frames, it may be easier to discard a few frames with poor image quality, which might be beneficial. However, it could also provoke a choice between frames

with signs of ischemia and frames without signs of ischemia, which may make the interpretation somewhat more subjective. The RTP technique should be superior for quantification, because it produces more frames with perfusion information compared to intermittent imaging, which increases the statistical power of the calculations. Inversely, RTP has the disadvantage that it shows less contrast specificity, since the power of the ultrasound signals is lower and, consequently, the signal to noise ratio becomes lower. Furthermore the continuous imaging might, regardless of the low MI, still disrupt micro bubbles close to the transducer. This will for example happen if the focal zone is placed in the deeper part of the image sector, because the ultrasound beams then overlap each other laterally, which leads to locally higher MI in the near field of the sector.

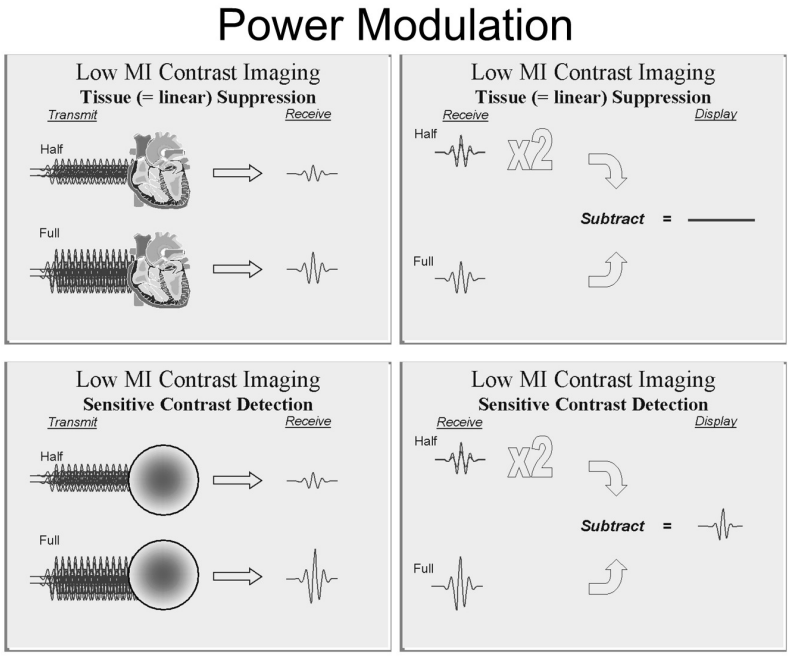


Figure 4. *Principles of RTP power modulation technique.*
RTP, real-time perfusion.

Power Modulation

The RTP technique used in all studies in this thesis is the Power Modulation technique. It is based on the principle that the echoes reflected

from contrast bubbles are different from those reflected from tissue, allowing these two types of echoes to be accurately differentiated and displayed.

Two different ultrasound signals are sent out and their echoes are compared. The first signal (S1) has low amplitude and the second signal (S2) has the double amplitude of S1, but still with a low MI (<0.2). The echoes generated by the reflection of S1 are multiplied by two and are, thereafter, subtracted by the received echoes from S2. If the received echo from S1 comes from tissue, it will result in zero amplitude after subtraction by the echo from S2. However, if the received echo from S1 comes from contrast bubbles, it will result in a preserved amount of amplitude after multiplication and subtraction by the S2 echo, since the contrast bubble, due to its specific ultrasound characteristics, has altered the amplitude of S1 (see also figure 4).

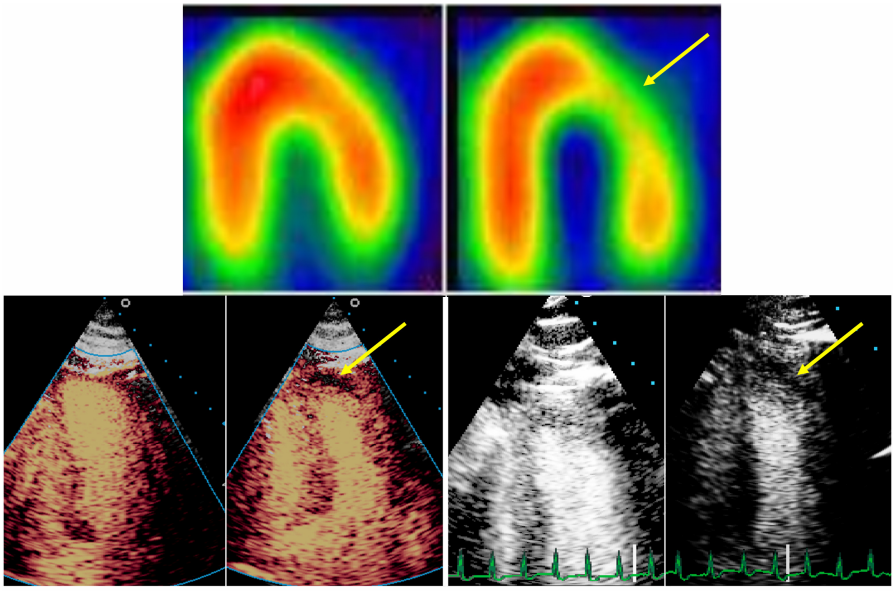


Figure 5. *Patient with apical, anterior perfusion defect at stress (arrows), not present at rest (corresponding left image) in AM (left), HR (right) and SPECT (top).*

AM, angio-mode; HR, high resolution grey-scale; SPECT, 99mTc-sestamibi single-photon emission computed tomography.

Two types of power modulation are available; the angio-mode (AM) and the high resolution grey scale (HR). Both are based on the same technique, but, as indicated by the name, HR has a higher pixel resolution compared to AM. On the other hand, AM uses coloured pixels to display contrast in the image, which might be easier to detect in a visual analysis compared to the grey scale contrast presentation used for HR. HR suppresses tissue totally and only displays echoes that power modulation identifies as echoes from contrast.

An example of an anterior perfusion defect at stress compared to rest, for AM, HR and SPECT, is demonstrated in figure 5.

From Visual to Parametric and Quantitative MCE.

Visual

Traditionally, echocardiographic perfusion images have been visually evaluated (Becher, Tiemann et al. 1997; Porter, Xie et al. 2001; Winter, Gudmundsson et al. 2005; Korosoglou, Dubart et al. 2006). In the setting of myocardial ischemia evaluation it has been done by comparing perfusion images at stress with baseline images. If perfusion visually diminishes at stress, that part of the myocardium is considered ischemic.

Parametric, Quantitative, Qontrast®

Visual assessment of RTP-ASE images is subjective and, consequently, user dependent. Therefore, various software programs are being developed for quantification of myocardial perfusion by contrast echocardiography. The technical development of these programs is difficult, since echocardiographic images often are of relatively poor quality, from a digital point of view, and since both heart and patient movement has to be dealt with in the most possible automated manner. Studies for quantification of myocardial perfusion, by various methods, have generally shown good accuracy, but a relatively high number of non-interpretable myocardial segments due to poor image quality (Korosoglou, da Silva et al. 2004; Yu, Skyba et al. 2004; Agati, Tonti et al. 2005; Moir, Haluska et al. 2005; Vogel, Indermuhle et al. 2005; Malm, Frigstad et al. 2006). Furthermore, echocardiographic myocardial perfusion quantification is still quite time consuming compared to visual assessment of perfusion or wall motion.

Quantitative techniques have shown promising results in animal experiments (Lafitte, Higashiyama et al. 2002; Agati, Tonti et al. 2004) and in hu-

mans (Bekeredjian, Hilbel et al. 2003; Korosoglou, da Silva et al. 2004; Peltier, Vancraeynest et al. 2004; Malm, Frigstad et al. 2006). However, there are few studies from clinical settings and most of these have been done with different software. If a quantitative echocardiographic technique demonstrated equivalent results to SPECT in detecting myocardial ischemia, it could be an alternative method, more available and without radiation compared to SPECT, more tolerable and swifter than DSE, and more accurate than exercise ECG.

Qontrast® (AMID®, Roma, Italy; Bracco™, Milano, Italy) is a recently developed and commercially available software, with algorithms that automatically follow the left myocardium contours throughout the cardiac cycle and throughout the replenishment period of the RTP image loop. Since Qontrast® can provide parametric images of myocardial perfusion, resembling those generated from SPECT, it has the potential to become an user-friendly, available and objective semi-quantitative tool for myocardial ischemia evaluation by RTP-ASE in these patients; parametric images are easier to understand and differences in perfusion between rest and stress could be more easily detected than in moving RTP replenishment loops. Few studies have investigated the value of parametric images from myocardial contrast stress echocardiograms for the evaluation of myocardial ischemia (Hansen, Bekeredjian et al. 2004; Yu, Skyba et al. 2004; Toledo, Jacobs et al. 2006), and so far, to our knowledge, no study has used Qontrast®.

Qontrast® may also provide a practical way to quantify myocardial perfusion by contrast echocardiography, and has shown promising initial results in both animals and patients with acute myocardial infarction (Agati, Tonti et al. 2004; Agati, Tonti et al. 2005). However, it has not yet been investigated in patients with suspected stable myocardial ischemia.

In this thesis

The first step in this thesis was to test the ability of visual interpretation of RTP-ASE to correctly detect myocardial ischemia, as compared with SPECT, since visual interpretation is the easiest way to interpret RTP images and demands no extra plug-in software. Furthermore, when the first studies of this thesis were performed, quantitative tools were not available at our research laboratory, which was another reason to begin with visual evaluation.

In study I the only RTP-technique accessible was AM. When HR was developed and made available on the Sonos 5500 system, the natural sequel was to compare this new technique with the existing one, which gave rise to study II.

Visual evaluation has always been questioned due to its subjective nature, and rightfully so, since visual ways of interpreting are difficult to pass on from teacher to pupils without drift and misunderstandings. However, the echocardiographic technique is generally largely dependent on visual analysis, not the least when acquiring the images, and so far visual analysis may be reliable enough. Nevertheless, the possibility of quantifying perfusion in echocardiography is warranted and would, if showing good results, take the MCE techniques to a higher status and bring about more widespread use in clinical practise.

Quantification of perfusion from RTP images is, so far, a quite susceptible and time-consuming operation. This is partly due to the, from a digitally point of view, low signal to noise ratio and heart movement in the ultrasound images, which demands manual tracings and compensations necessary. If this could be avoided by any quantitative method or software it would make it more useful in clinical practice where time often is an issue. Since Qontrast® uses automatic methods for parametric imaging, we hypothesized that this software could provide a feasible and swift tool for accurate detection of myocardial ischemia. This lead to study III, in which parametric images of RTP at rest and stress, generated through Qontrast®, were the base of ischemia interpretation.

Study III still contains a visual and, therefore, subjective component, although diminished through quantitative processing in Qontrast®, namely the visual evaluation of different grades of colours at stress compared to rest. To test if Qontrast® could adequately quantify perfusion, compared to SPECT and compared to visual interpretation like in study I, we carried out study IV. This way of using Qontrast® is more time-consuming than the ischemia evaluation used in study III, and thereby less clinically appealing. However, since it is purely quantitative it is less questionable and, if results were to be favourable enough, study IV could potentially prove Qontrast® to be comparable to SPECT in detecting myocardial ischemia. Furthermore, positive results in study IV could, if similar, confirm results in study III.

Method of reference

SPECT was chosen as method of reference in this thesis since it, similar to RTP-ASE, evaluates perfusion. However, data indicates that RTP might be more sensitive to myocardial ischemia than SPECT due to its higher spatial resolution (Tiemann, Ghanem et al. 2001; Hagendorff, Pfeiffer et al. 2003; Senior, Lepper et al. 2004). Therefore, SPECT might not be the ideal gold standard. In work performed by other groups, MCE methods have been compared with other scintigraphic methods, such as Thallium (Oraby, Hays et al. 2002; Bekeredjian, Hilbel et al. 2003), as well as with coronary angiography (Moir, Haluska et al. 2005; Winter, Gudmundsson et al. 2005; Malm, Frigstad et al. 2006) and even with some combinations of both SPECT and coronary angiography (Olszowska, Kostkiewicz et al. 2003; Jeetley, Hickman et al. 2006; Korosoglou, Dubart et al. 2006; Malm, Frigstad et al. 2006). Coronary angiography might have added information that could possibly have contributed to explain differences in the results between RTP-ASE and SPECT. However, coronary angiography was not possible to perform within the present study protocol, mostly due to ethical reasons, and still, SPECT is at least theoretically more appealing as method of reference since evaluation of myocardial perfusion is the objective of the investigations.

Furthermore SPECT has shown excellent prognostic value (Underwood, Anagnostopoulos et al. 2004) and if RTP-ASE were to show equivalent results, it could be an alternative method, more available and without radiation compared to SPECT, more tolerable and swifter than DSE and more accurate than exercise ECG.

Considering its safety, feasibility and accessibility, RTP-ASE has the potential of becoming the method of choice to confirm or discard myocardial ischemia, if proven sufficiently accurate compared to SPECT. Therefore, it was felt that it would be worthwhile to investigate RTP-ASE further in this context.

AIMS

The aims of this thesis were

I. To prospectively investigate the ability of RTP-ASE with visual estimation to detect myocardial ischemia, in comparison with 99mTc-sestamibi SPECT, in unselected patients referred to SPECT due to suspected or known stable myocardial ischemia.

II. To compare the two power modulation techniques, AM and HR, during RTP-ASE, for the detection of myocardial ischemia, as compared with 99mTc-sestamibi SPECT, in unselected patients referred to SPECT due to suspected or known stable myocardial ischemia.

III. To examine the value of RTP-ASE with Qontrast®-generated parametric images for the evaluation of myocardial ischemia, using SPECT as reference, in unselected patients referred to SPECT due to suspected or known stable myocardial ischemia.

IV. To examine if RTP-ASE with Qontrast® quantification can be used to correctly evaluate myocardial ischemia, as compared with visual evaluation of ischemia by RTP-ASE, as well as with SPECT, in unselected patients referred to SPECT due to suspected or known stable myocardial ischemia.

MATERIAL AND METHODS

Patients

All patients in studies I-IV were randomly included patients admitted to adenosine SPECT evaluation of known or suspected stable coronary artery disease. They were all prospectively asked to participate in the studies and written informed consent was obtained from all participating patients. The institutional ethics committee of Lund University, Sweden, approved the studies.

Some patients are included in all studies, but only studies III and IV comprise the exact same cohort. One patient in study I chose not to participate. In study I, one of the remaining 34 patients had non-interpretable echocardiography images, both regarding wall motion and perfusion, and was therefore excluded from the comparison with SPECT.

In study II, one of the 51 included patients had non-interpretable echocardiography images, both regarding wall motion and perfusion, and was therefore excluded from the study.

Two of the 69 patients eligible for inclusion in studies III and IV were excluded since they had visually non-interpretable echocardiography images.

Baseline characteristics of the patients in study I-IV are presented in Table 1.

Echocardiographic equipment

The echocardiographic equipment used in all four studies was a Sonos 5500 (Philips, Andover, MA, USA) with S3 probe. RTP using the power modulation AM was used in all studies and in study II power modulation

HR was also used. Both power modulation RTP software are commercially available as an option in the Sonos 5500 system.

Table 1. *Characteristics of patients in studies I-IV.*

	Study I (n=33)	Study II (n=50)	Study III and IV (n=67)
Age (years)	66 (±11)	70 (±8)	68 (±10)
Male (sex)	33 %	36 %	33 %
LVEF at rest	52 (±12) %	55 (±9) %	54 (±11) %
Previous AMI	42 %	40 %	40 %
Previous PCI	18 %	22 %	19 %
Previous CABG	15 %	16 %	13 %
History of heart failure	21 %	12 %	13 %
History of hypertension	36 %	54 %	48 %
Previous valvular surgery	0 %	0 %	0 %
Beta-blocker	54 %	54 %	57 %
ACE inhibitor use	33 %	28 %	28 %
ARB use	9 %	14 %	12 %
Nitro-glycerine use (short acting)	54 %	60 %	57 %
Nitrate use (long acting)	27 %	28 %	25 %
Diuretic use	21 %	29 %	27 %
Calcium blocker use	6 %	24 %	18 %
Sinus rhythm	94 %	92 %	93 %
Dilated left ventricle	21 %	10 %	13 %
Dilated left atrium	41 % (n=22)	31 % (n=29)	33 % (n=43)
Significant valvular disease	19 % (n=21)	6 % (n=31)	7 % (n=43)
Regional WMA/PD at rest	52 %	60 %	60 %

LVEF, left ventricular ejection fraction; AMI, acute myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; ACE, angiotensin converting enzyme; ARB, angiotensin-receptor blocker; WMA, wall motion abnormality; PD, perfusion defect.

Study protocol

Real-time perfusion adenosine stress echocardiography

Patients in all studies were examined in a left lateral recumbent position. The second-generation contrast agent Sonovue® was infused in the left decubital vein using an infusion pump dedicated for this purpose (VueJect® Esaote, Genova, Italy; Bracco™, Milano, Italy), which automatically rotates the syringe to prevent sedimentation. The infusion rate of Sonovue was set between 1.0 and 1.3 ml/min (Becher and Burns 2000). Adenosine and echo contrast were infused in the same peripheral venous catheter, using a separate infusion pump through a three-way tap.

Adenosine was given at an infusion rate of 100 µg/kg/min during one minute, after which the infusion rate was increased to 140 µg/kg/min.

Infusion of adenosine in studies III and IV had minor but significant effects on both heart rate and blood pressure, where heart rate increased from 72 ± 14 to 82 ± 14 ($p<0.001$), systolic blood pressure fell from 133 ± 20 to 127 ± 20 ($p<0.001$) and pulse-pressure product increased from 9.65 ± 2.32 k to 10.44 ± 2.57 k ($p<0.001$). Only minor side-effects occurred and no stress-test had to be interrupted due to side-effects. At SPECT 18 patients (27%) were ischemic with a total of 19 ischemic territories, 10 left anterior descending (LAD) territories, 5 left circumflex (LCx) territories and 4 right coronary artery (RCA) territories.

All patients underwent RTP imaging (mechanical index=0.1) during infusion of echo contrast, at rest and after a minimum of one minute of hyperaemia during adenosine stress (at 140 µg/kg/min). Image acquisition was started after at least one minute of Sonovue® infusion. RTP image loops containing 8-10 heartbeats were collected from the parasternal long-axis and apical four- and two-chamber views, respectively. At the beginning of each loop a destruction impulse of 10 high mechanical index frames (mechanical index=1.5) was given to destroy all contrast micro bubbles in the myocardium (Bahlmann, McQuillan et al. 2002).

In all studies, during RTP AM, the AM gain was set at between 60 and 70%, depending on what was suitable for the individual patient as judged by a visual on-line assessment, and 2D greyscale gain was set at zero. During RTP HR (only in study II) the greyscale gain was set between 90 and 95%, depending on what was suitable for the individual patient, as judged by a visual on-line assessment. Focus was set close to the base of the left ventricle. All images were stored digitally for later off-line analysis.

Real-time perfusion interpretation

All of the different interpretations were made at different time-points blinded to the results of any other interpretation.

Visual RTP-ASE interpretation

Image interpretation was performed off line, analyzing myocardial perfusion and wall motion by RTP-ASE, using the EnConcert Image Diagno-

sis Application (Philips, Andover, Massachusetts). A separate analysis exclusively of perfusion was also made for both AM and HR to estimate the value of sole perfusion analysis.

Each segment was attributed to one of the three main coronary vessel areas of interest; the LAD; the LCx; and the RCA (Figure 8). Myocardial ischemia was visually evaluated comparing rest and stress images, using both perfusion and wall motion analysis in a complementary manner. A visually detected perfusion defect during stress was used as the principal marker of ischemia. Thus, a myocardial segment was considered ischemic if perfusion was impaired in the stress images, compared to the rest images (Lafitte, Matsugata et al. 2001). Perfusion defects were analysed at the earliest four beats following the destruction impulse at rest and after two beats at peak stress.

Wall motion was used in addition to reveal perfusion defect artefacts at rest and to evaluate segments with suspected perfusion artefacts at stress (A detailed description of perfusion artefact assessment and wall motion analysis is given later, in a separate section). Since perfusion can be decreased without a decrease in wall motion in ASE, the use of solitary wall motion analysis in segments with perfusion artefacts might decrease the sensitivity with regard to ischemia. However, this complementary use of wall motion analysis increases the number of interpretable segments without negatively affecting specificity (Winter, Gudmundsson et al. 2005).

Parametric RTP-ASE evaluation

Parametric images from rest and adenosine stress were generated from contrast replenishment RTP loops using Qontrast® as follows: Two points were manually placed in the left ventricular cavity of the perfusion images. The first point was placed in the centre of the cavity where the apex "half-circle" ends, i.e. approximately two thirds from the base of ventricle, where it was always inside the cavity (never in the myocardium) during the complete loop. The point was placed in a cavity area that was fully opacified directly after the destruction impulse in the beginning of the loop, as well as throughout the entire RTP-loop, since this formed the basis of the maximum image contrast intensity reference-point. Any isolated frames not fulfilling these criteria were excluded from analysis. The second point was placed at the base of the ventricle, enabling the software to automatically outline the complete left ventricle, including both

cavity and myocardium, with dotted "M-mode" lines crossing perpendicular through the myocardial wall (Figure 6).

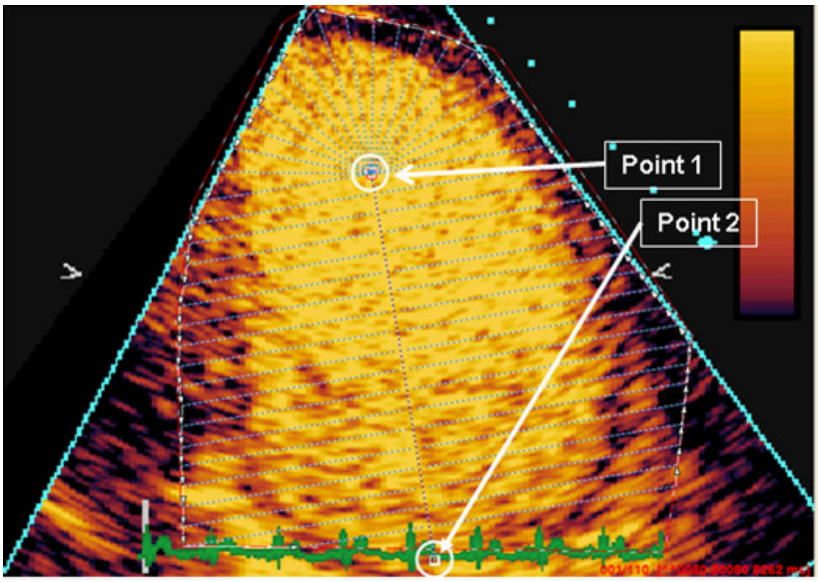


Figure 6. *Manually placed points to enable an automatic outline of the complete left ventricle, including both cavity and myocardium, before automated Qontrast® perfusion analysis.*

The first frame was selected to be the one directly after destruction impulse frames. Automatic perfusion analysis was then started. Qontrast® uses an advanced image processing technique that allows identification of the dynamic image sequence coherence in a space-time domain. The technique enables tracking of the myocardial pixel movement throughout the cardiac cycle and the entire RTP-loop. This increases the accuracy of the perfusion evaluation compared to triggered imaging, due to the higher number of quantifiable frames. Three parametric images were then automatically generated from the perfusion analysis, displaying either the peak signal intensity (A), myocardial blood flow velocity (β) or myocardial blood flow (Axβ). These were generated for each pixel, from the replenishment curve of each pixel, according to the standard curve $A=A(1-e^{-\beta t})$. Each of the parametric images could be displayed using a different number of colours (4, 8 or full), where more colours corresponded to

more levels between zero and 100% with regard to A, β or Axβ. Examples of the different parametric images are shown in Figure 7.

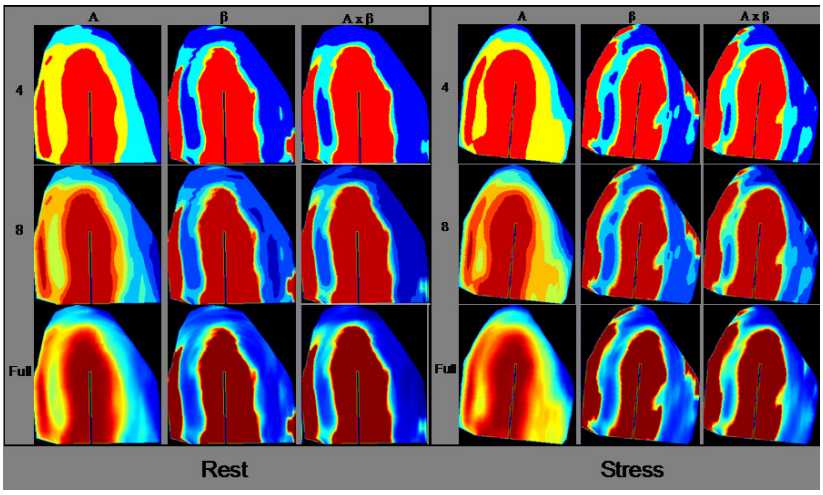


Figure 7. *Parametric images of A, β, Axβ in different map segmentations, 4, 8 and full number of colours. Example with images at rest and stress. A, peak signal intensity; β, myocardial blood flow velocity; Axβ, myocardial blood flow.*

In the parametric images, the left ventricle was divided into 17 segments (Cerqueira, Weissman et al. 2002). Each segment was attributed to one coronary territory of interest, corresponding to the LAD, LCx and RCA arteries (Figure 8). Images at rest were compared with those at stress to evaluate ischemia. The respective map segmentations (4, 8, full) were evaluated at different time-points, blinded to the result of other map segmentations and to the result of the SPECT examination. Parametric images of A, β and Axβ for the same map segmentation were evaluated at the same time.

For each segment and each parametric parameter (A, β and Axβ), differences between rest and stress were given a value between one and five: 1) a large negative colour difference, i.e. an area change >1/3 of the segment; 2) a small negative difference, i.e. an area change <1/3 of the segment; 3) no difference; 4) a small positive difference, i.e. an area change <1/3 of the segment; and 5) a large positive difference, i.e. an area change

>1/3 of the segment. Accordingly, the lower the score, the more ischemic was the segment.

Distribution territories of the three main coronary arteries

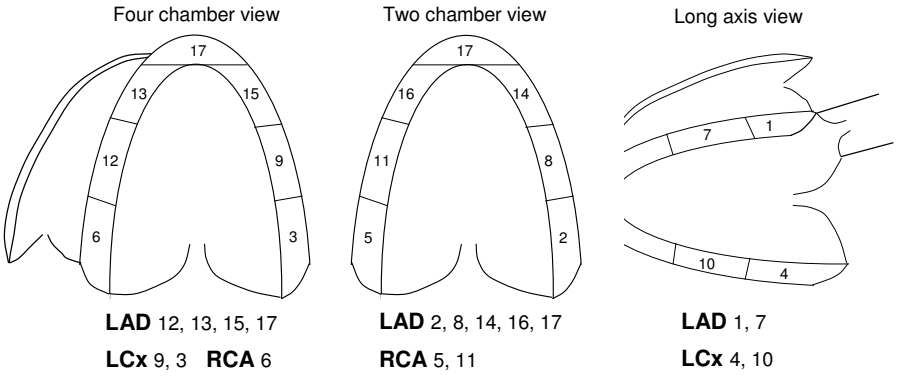


Figure 8. *Distribution territories of the three main coronary arteries in a 17 segment model. Left anterior descending (LAD), left circumflex (LCx) and right coronary artery (RCA).*

Three different types of analyses were made from the parametric ischemic scores, for each of the map segmentations:

1. WCS – "Worst case scenario": The lowest score of a variable (i.e. A, β or Axβ) in any segment belonging to a coronary distribution territory (i.e. LAD, LCx or RCA), was considered the correct score for that territory.
2. SUM – "Sum of worst case scenario". To reduce the individual value of A, β and Axβ, their respective WCS scores were added together. This new score could consequently have a value between 3 and 15. This summation procedure decreases the effect on the ischemia evaluation of artefacts originating from one specific parametric variable.
3. SoftSUM – "Soft case scenario summation". Since echocardiography is known to have higher spatial resolution than SPECT, which could lead to false positive rulings of ischemia by RTP-ASE, a third analysis was made to diminish the influence of a single segment score. Within a certain coronary territory, the mean

of all segments' A, β, Axβ-summations was calculated. The SoftSUM could, accordingly, also have a score between 3 and 15.

Quantitative RTP-ASE analysis

The pure quantitative analysis used in study IV was carried out by tracing parametric images that were generated in the same fashion as in study III, from RTP images in apical four- and two-chamber and parasternal long-axis view, at rest and stress, respectively.

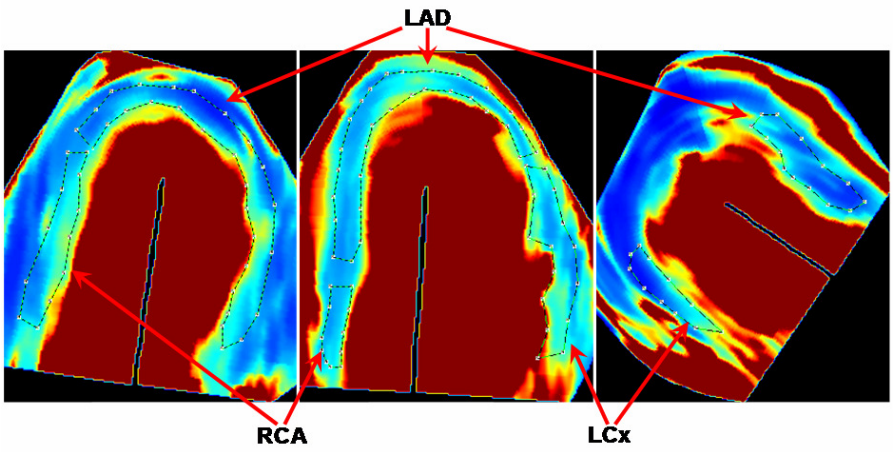


Figure 9. *Tracings of coronary territories of interest in four-chamber (middle), two-chamber (left) and long-axis (right) views. LAD, left anterior descending; LCx, left circumflex; RCA, right coronary artery.*

To acquire quantitative values of A, β and Axβ, region of interests were manually traced both at rest and stress, corresponding to the distribution territories of the three main coronary arteries; LAD, LCx and RCA (figure 9). Since earlier studies indicated that β is the most sensitive quantitative parameter (Moir, Haluska et al. 2005; Malm, Frigstad et al. 2006), special care was taken that the tracing would align correctly in the parametric β image, avoiding red areas, which correspond to contrast in the left ventricular cavity or could be perfusion artefacts origin from main coronary arteries. Red areas in the β image could also be caused from mathematically generated high β-values due to very low A-values, which predominantly could occurs in rest images where the A-values can be low for physiological reasons.

Comparing A, β and Axβ values at rest and stress (hyperaemia), the corresponding reserve values (A-r; β-r and Axβ-r) were derived by dividing the stress value with the matching rest value, thus resembling invasive measurement of coronary flow reserve. Accordingly, this generated three A-r, β-r, and Axβ-r values from the LAD territory, originating from the three different echocardiographic views (from four- and two-chamber, and long-axis views), two reserve values each from the LCx territory (from four-chamber and long-axis views) and two from the RCA territory (from four- and two-chamber views). The lowest A-r, β-r and Axβ-r value from any of the views corresponding to a coronary territory was selected for the ischemia comparison with SPECT, since the lowest reserve value should originate from the most ischemic or the least perfused territory.

Perfusion artefacts and non-interpretable territories

In all studies, a visual judgment of the RTP-loops was done with regard to perfusion artefacts. In studies III and IV, there was also a judgment of the parametric images, which could result in additional non-interpretable territories.

Artefacts from visual judgment of RTP-loops.

Wall motion was used to reveal perfusion defect artefacts at rest and to evaluate segments with suspected perfusion artefacts at stress. Since wall motion should not be normal if a segment has a true perfusion defect at rest, a perfusion defect at rest was considered to be an artefact when wall motion was normal in that segment. A perfusion defect at peak stress was considered to be an artefact if there was a suspicion of a perfusion artefact, such as lateral or anterior shadowing from ribs or lungs, or basal segments shadowed by contrast. In such segments, the ischemic evaluation was based on wall motion analysis alone. If wall motion decreased at stress compared to rest images, the segment was considered ischemic. Since perfusion can be decreased without a decrease in wall motion in ASE, the use of solitary wall motion analysis in segments with perfusion artefacts might decrease the sensitivity with regard to ischemia. However, this complementary use of wall motion analysis increases the number of interpretable segments without negatively affecting specificity (Winter, Gudmundsson et al. 2005).

Non-interpretable territories by judgment of Qontrast® parametric images.

In the quantitative analysis, territories were considered non-interpretable using the Qontrast® software due to low parametric image quality, which made it too difficult to differentiate the left ventricular myocardium from the cavity. A coronary territory could also be considered non-interpretable if the manual placement of cavity points in Qontrast® could not be placed as predefined in the left ventricular cavity.

SPECT

The rest and stress studies were performed using a 2-day protocol, starting with injection of 600 MBq 99mTc-tetrofosmin at stress. The stress examination was performed simultaneous with the RTP-ASE examination. Normal findings at stress were not followed by a rest study. Pathological stress studies were followed by a rest study with injection of 800 MBq 99mTc-tetrofosmin. A five-minute adenosine infusion protocol was used. Starting the infusion with 100 µg/ml/min of adenosine for 1 minute, the dose was then increased to 140 µg/ml/min for two minutes before injecting 99mTc-tetrofosmin. Infusion of adenosine was continued for 2 min after the injection of 99mTc-tetrofosmin. The scintigraphic data were acquired one hour after the end of the stress test, using continuous SPECT over 180 degree elliptical rotation from the 45 degree right anterior oblique position, with a dual-head gamma camera (Siemens AG Medical Solutions, Erlangen, Germany). Low energy high-resolution collimator and a zoom factor of 1.0 were used. We obtained 64 projections in a 128x128 matrix, with an acquisition time of 20 s per projection. Tomographic reconstruction and calculation of short axis slice images were performed using Siemens software. A two-dimensional Butterworth pre-reconstruction filter was used with critical frequency of 0.35, order 5. For each patient, the same sets of short axis slices were then processed with an automatic software package (4D-MSPECT) on a Siemens e.soft workstation. The software package defined apex and base and generated, coronal, longitudinal, sagital tomographic slices as well as polar maps with schematic map of the territories of the main coronary arteries used for scoring. Radiotracer uptake of the vascular segments were scored visually and stress images were compared with rest images

regarding ischemia and infarct. The specialist in nuclear medicine who performed the scoring was blinded to the results of the RTP analysis.

STATISTICS

All statistical analyses were performed using The SPSS® (Version 12.0.1 or 14.0, Chicago, IL, USA) statistical program.

Power calculations in all studies were based on a sensitivity and specificity between 80 and 90 % of the methods used. We assumed a sensitivity and specificity of 85% in the study. With 30 patients we would have a 95% confidence interval of ± 13 %, with 50 patients ± 10 % and with 100 patients ± 7% around sensitivity and specificity.

Method of reference for the ischemia evaluation in the studies was the presence or absence of reversible ischemia at the SPECT examination. Results are expressed as mean ± SD and as percent. $P<0.05$ denoted statistical significance.

Calculations of sensitivity and specificity, positive and negative predictive values (PPV, NPV), as well as accuracy and Kappa values in the three predefined distribution areas of the three main coronary vessels were performed in all studies.

In study II, the chi-squared test was used to assess differences between AM and HR.

In studies III and IV, receiver operating characteristic (ROC) curves were used to examine and compare predictive ability of different parametric variables, by calculating sensitivity, specificity, accuracy and area under the curve.

In study IV, the unpaired t-test was used to test for differences between patients. For intra-assay variability of quantitative measurements of A and β, coefficient of variation was used.

SUMMARY OF RESULTS

Paper I

Of 102 coronary territories assessed, 99 (97%) were considered interpretable. These 99 territories were analysed both using SPECT and RTP. The overall level of agreement between RTP-ASE and SPECT in detecting ischemia was 92% in all segments with a Kappa value of 0.67. The level of agreement was 97 % in LCx, 91 % in RPD and 88 % in LAD segments. Accuracy, sensitivity, specificity, predictive values and Kappa values for the detection of myocardial ischemia of RTP-ASE compared to SPECT are shown in Table 2.

Table 2. *Accuracy, positive (PPV) and negative (NPV) predictive values, sensitivity, specificity and Kappa values of RTP-ASE using SPECT as method of reference.*

	Any territory (n=99)	LAD (n=33)	LCx (n=33)	RPD (n=33)
Accuracy (%)	92	88	97	91
PPV (%)	63	63	83	0
NPV (%)	98	96	100	97
Sensitivity (%)	83	83	100	0
Specificity (%)	93	89	96	94
Kappa	0.67***	0.64***	0.89***	-0.042 ns

*LAD, left anterior descending coronary artery; LCx, left circumflex artery; RPD, right posterior descending coronary artery, ***=p<0.001, ns=not significant.*

Perfusion artefacts were present in 35 (35%) segments at rest and in 24 (24%) segments at peak. Of the 59 segments with artefacts, 34 (58%) were located in basal parts of the left ventricle in apical views, and 23 (68%) of these 34 basal segments had artefacts at rest.

Wall motion was non-decisive with regard to ischemia evaluation in a segment with a perfusion artefact at rest if the perfusion at stress was normal, since normal perfusion at stress is not consistent with ischemia.

Results regarding perfusion artefacts are summarised in Table 3. In 26 (44%) of the segments with perfusion artefacts, wall motion was decisive for the ischemia evaluation; two at rest and 24 at peak. Wall motion was decisive in two segments at rest, since perfusion defects in these two segments were observed both at rest and during hyperaemia. The perfusion defect at rest was considered to be an artefact due to normal wall motion at rest, since wall motion should not be normal if a segment has a true perfusion defect at rest. Hence, when perfusion and/or wall motion was impaired during stress the segment was judged ischemic despite unchanged impaired perfusion. The ischemic evaluation was in accordance with SPECT in 25 (96%) of these 26 segments. In 23 (88%) of the 26 segments, wall motion correctly acquitted segments from ischemia.

Table 3. *Perfusion artefacts characteristics.*

	Rest	Stress	Total
Basal	23	11	34
Overall	35	24	59
WMA decisive	2	24	26
Accurate	2	23	25

WMA, wall motion analysis

On a patient basis (n=33), the ischemic evaluation with RTP-ASE showed an accuracy of 91% with sensitivity, specificity, PPV and NPV of 78%, 96%, 88% and 92%, respectively.

Paper II

Of 150 coronary areas assessed, all were considered interpretable and were analysed both using SPECT and RTP with AM and HR. The overall level of agreement between RTP-ASE and SPECT in detecting ischemia was 93% for AM and 96% for HR. The chi-squared test for difference between AM and HR in correctly judging ischemia was borderline-significant (p=0.08). The Kappa values were 0.67 for AM and 0.75 for HR (p<0.001). Accuracy, sensitivity, specificity, predictive values and Kappa values for the detection of myocardial ischemia of RTP-ASE AM and HR compared to SPECT are shown in Table 4. HR generally showed somewhat higher values for kappa, accuracy, positive prediction and specificity, whereas AM showed higher sensitivity values. The NPVs were similar. The same comparative values for the sole perfusion interpretation concerning AM and HR compared to SPECT are displayed in Table 5, demonstrating differences similar to the combined perfusion and wall motion analysis. Accuracy and Kappa values for the agreement between AM and HR are shown in Table 6, indicating lesser agreement between AM and HR than for their respective comparison with SPECT.

Table 4. *RTP-ASE AM versus HR with combined perfusion and wall motion analysis. Accuracy, PPV, NPV sensitivity, specificity and Kappa of RTP-ASE using SPECT as method of reference.*

		All CA (n=150)	Patient (n=50)	LAD (n=50)	LCx (n=50)	RPD (n=50)
AM	Accuracy (%)	93	88	88	94	98
AM	PPV (%)	57	71	54	50	100
AM	NPV (%)	99	97	100	100	98
AM	Sensitivity (%)	92	92	100	100	75
AM	Specificity (%)	93	87	86	94	100
AM	Kappa	0.67***	0.72***	0.63***	0.64***	0.85***
HR	Accuracy (%)	96	92	90	96	100
HR	PPV (%)	77	91	67	67	100
HR	NPV (%)	98	92	93	98	100
HR	Sensitivity (%)	77	78	57	67	100
HR	Specificity (%)	98	97	95	98	100
HR	Kappa	0.75***	0.78***	0.56***	0.65***	1.00***

*AM, angio-mode; HR, high resolution; PPV, positive predictive value; NPV, negative predictive value; CA, coronary area; LAD, left anterior descending coronary artery; LCx, left circumflex artery; RPD, right posterior descending coronary artery, ***=p<0.001.*

Table 5. *RTP-ASE AM versus HR using solitary perfusion analysis. Accuracy, PPV, NPV, sensitivity, specificity and Kappa of RTP-ASE using SPECT as method of reference.*

		Any CA (n=134)	Patient (n=39)	LAD (n=44)	LCx (n=42)	RPD (n=48)
AM	Accuracy (%)	89	85	81	92	94
AM	PPV(%)	48	71	43	50	60
AM	NPV (%)	99	96	100	100	98
AM	Sensitivity (%)	92	92	100	100	75
AM	Specificity (%)	89	81	78	91	96
AM	Kappa	0.58***	0.68***	0.50***	0.63***	0.63***
HR	Accuracy (%)	97	95	93	98	100
HR	PPV (%)	80	90	67	100	100
HR	NPV (%)	98	97	97	98	100
HR	Sensitivity (%)	80	90	80	50	100
HR	Specificity (%)	98	97	95	100	100
HR	Kappa	0.78***	0.87***	0.69***	0.66***	1.00***

*AM, angio-mode; HR, high resolution; PPV, positive predictive value; NPV, negative predictive value; CA, coronary area; LAD, left anterior descending coronary artery; LCx, left circumflex artery; RPD, right posterior descending coronary artery, ***=p<0.001.*

Table 6. *Agreement between RTP-ASE AM and HR, using combined perfusion and wall motion analysis, and using solitary perfusion analysis.*

	Any CA (n=150)	Patient (n=50)	LAD (n=50)	LCx (n=50)	RPD (n=50)
RTP + WM					
Agreement (%)	92	80	82	94	98
Kappa	0.61***	0.51***	0.43**	0.64***	0.85***
Sole RTP					
Agreement (%)	88	77	78	91	94
Kappa	0.47***	0.52**	0.43**	0.37**	0.54***

*AM, angio-mode; HR, high resolution; CA, coronary area; LAD, left anterior descending coronary artery; LCx, left circumflex artery; RPD, right posterior descending coronary artery; RTP, real time perfusion; WM, wall motion; **=p<0.01; ***=p<0.001.*

In Table 7, the numbers of non-interpretable coronary areas are presented. In the sole perfusion analysis the numbers include coronary areas considered to be perfusion artefacts. There is evidence of similar loss of interpretable coronary areas for both AM and HR.

Table 7. *Non-interpretable coronary areas for RTP-ASE AM and HR, with combined perfusion and wall motion analysis, and solitary perfusion analysis.*

		Any CA (n=150)	Patient (n=50)	LAD (n=50)	LCx (n=50)	RPD (n=50)
AM	RTP + WM (%)	0	0	0	0	0
AM	Sole RTP (%)	13.3	22.0	16.0	24.0	0
HR	RTP + WM (%)	0	0	0	0	0
HR	Sole RTP (%)	10.7	22.0	12.0	16.0	4.0

AM, angio-mode; HR, high resolution; CA, coronary area; LAD, left anterior descending coronary artery; LCx, left circumflex artery; RPD, right posterior descending coronary artery; RTP, real time perfusion; WM, wall motion.

Paper III

Of the 201 coronary distribution territories, 31 (15%) could not be analysed by echocardiography due to perfusion artefacts.

The results of the ischemia analysis based on the Qontrast®-generated parametric images are summarised in tables 8, 9 and 10. Cut-off values were chosen to obtain the most optimal sensitivity and specificity. Accuracy, sensitivity and specificity were only calculated for variables showing significant area under the curve. Of the different parametric variables, β and Axβ showed the highest values for area under the curve, kappa and accuracy. Significant results were found when all coronary territories were analysed and predominantly in the LAD territory.

Table 8. *Worst case scenario. Results for the respective map segmentation and the different parametric values, A, peak signal intensity, β, myocardial blood flow velocity and Axβ, myocardial blood flow.*

MS	PV	Coronary Territory	Sens (%)	Spec (%)	Acc (%)	PPV (%)	NPV (%)	kappa	AUC
4	A	All	84	47	80	24	94	0.22**	0.66 *
4	A	LAD	80	67	78	40	93	0.37**	0.77 *
4	A	LCx							0.45 NS
4	A	RCA							0.60 NS
4	β	All	71	82	72	24	97	0.25***	0.80 ***
4	β	LAD	62	100	59	29	100	0.26**	0.81 **
4	β	LCx							0.73 NS
4	β	RCA							0.78 NS
4	Axβ	All	77	71	75	25	96	0.25***	0.77 ***
4	Axβ	LAD	62	89	65	31	97	0.28**	0.77 *
4	Axβ	LCx							0.73 NS
4	Axβ	RCA							0.74 NS
8	A	All	44	82	82	21	92	0.14 NS	0.67 *
8	A	LAD	79	67	77	38	93	0.35**	0.76 *
8	A	LCx							0.56 NS
8	A	RCA							0.52 NS
8	β	All	73	71	73	23	96	0.23***	0.75 **
8	β	LAD	83	67	80	43	93	0.41**	0.77 *
8	β	LCx	83	75	82	27	98	0.32**	0.83 *
8	β	RCA							0.60 NS
8	Axβ	All	79	65	78	26	95	0.26***	0.76 ***
8	Axβ	LAD	72	89	75	38	97	0.40**	0.83 **
8	Axβ	LCx							0.76 NS
8	Axβ	RCA							0.61 NS
F	A	All							0.64 NS
F	A	LAD	90	44	82	44	90	0.34*	0.74 *
F	A	LCx							0.43 NS
F	A	RCA							0.59 NS
F	β	All							0.59 NS
F	β	LAD							0.54 NS
F	β	LCx							0.60 NS
F	β	RCA							0.61 NS
F	Axβ	All							0.62 NS
F	Axβ	LAD							0.59 NS
F	Axβ	LCx							0.45 NS
F	Axβ	RCA							0.69 NS

*MS, map segmentation; PV, parametric value; F, Full; Sens, sensitivity; Spec, specificity; Acc, accuracy; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; *=p<0.05; **=p<0.01; ***=p<0.001; NS, not significant. Grey tone represent NS.*

Table 9. *SUM worst case scenario. Results for the respective map segmentations.*

MS	Coronary Territory	Sens (%)	Spec (%)	Acc (%)	PPV (%)	NPV (%)	kappa	AUC
4	All	75	71	75	24	96	0.25***	0.78***
4	LAD	76	78	81	39	94	0.38**	0.84 **
4	LCx							0.66 NS
4	RCA							0.76 NS
8	All	77	77	77	27	94	0.30***	0.78***
8	LAD	77	89	79	42	97	0.45***	0.84 **
8	LCx	81	75	80	25	97	0.29*	0.81 *
8	RCA							0.58 NS
F	All	69	59	68	20	94	0.13*	0.66 *
F	LAD							0.70 NS
F	LCx							0.52 NS
F	RCA							0.63 NS

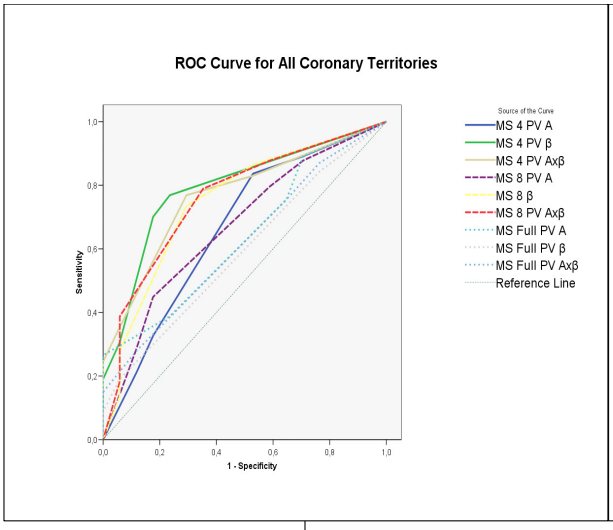
*MS, map segmentation; F, Full; Sens, sensitivity; Spec, specificity; Acc, accuracy; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; *=p<0.05; **=p<0.01; ***=p<0.001; NS, not significant. Grey tone represent NS.*

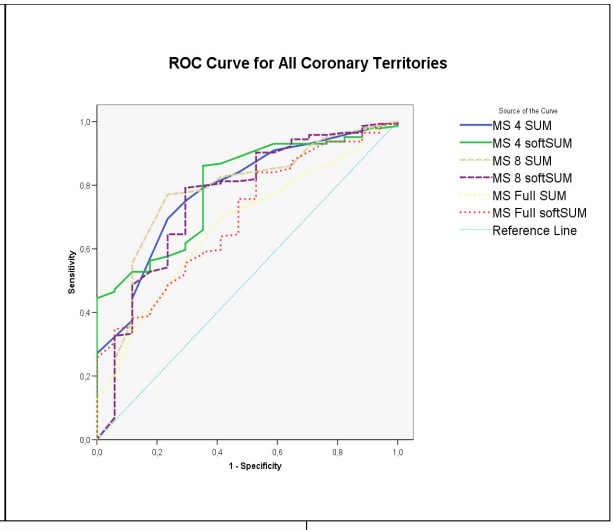
Table 10. *Soft case scenario. Results for the respective map segmentations.*

MS	Coronary Territory	Sens (%)	Spec (%)	Acc (%)	PPV (%)	NPV (%)	kappa	AUC
4	All	86	65	84	34	96	0.37***	0.80***
4	LAD	91	78	89	64	95	0.63***	0.85 **
4	LCx							0.66 NS
4	RCA	56	100	58	13	100	0.13*	0.80 *
8	All	78	71	77	27	96	0.28***	0.75 **
8	LAD	87	67	83	50	93	0.47**	0.82 **
8	LCx							0.78 NS
8	RCA							0.61 NS
F	All	83	53	80	24	93	0.21**	0.69 **
F	LAD	81	56	77	36	91	0.30*	0.75 *
F	LCx							0.67 NS
F	RCA							0.63 NS

*MS, map segmentation; F, Full; Sens, sensitivity; Spec, specificity; Acc, accuracy; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; *=p<0.05; **=p<0.01; ***=p<0.001; NS, not significant. Grey tone represent NS.*

ROC curves and bars for the area under the curve for the different variables are shown in Figure 10, where cut-points for different values can be appreciated, such as the level of sensitivity with preserved 100 % specificity.





a

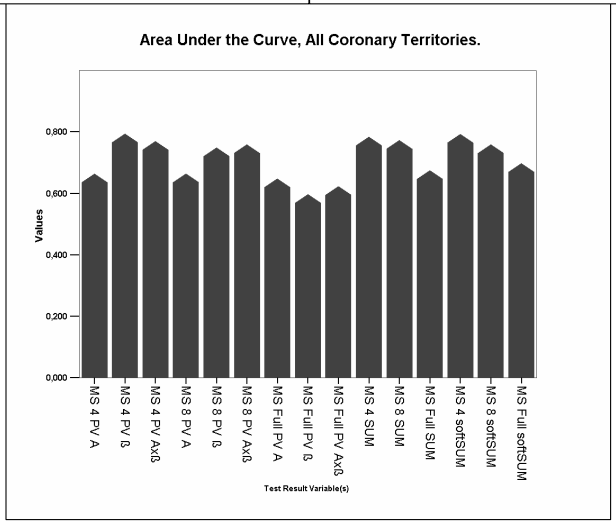
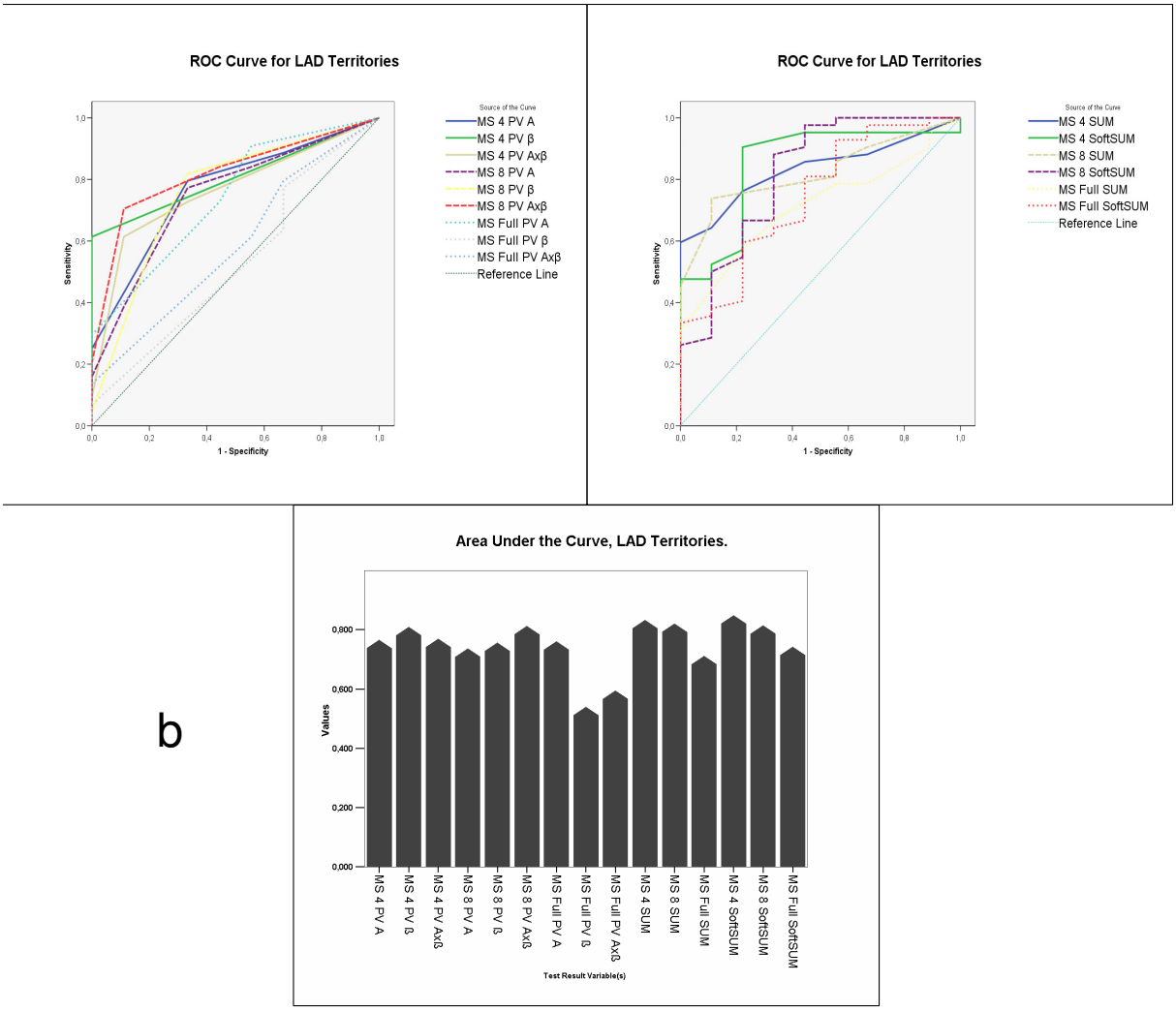
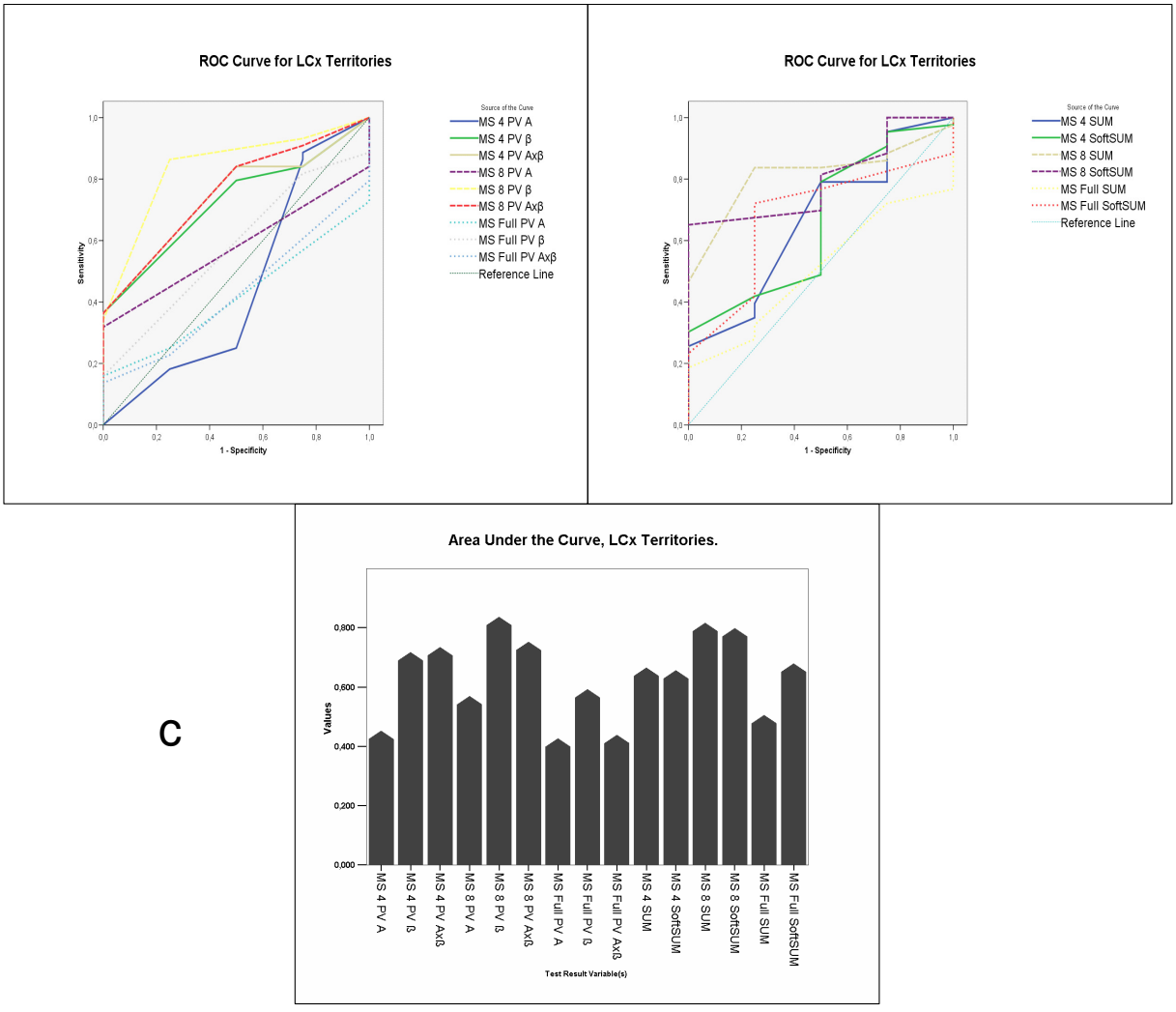


Figure 10 a. *ROC and tables of area under the curve for A, β, Axβ, SUM and SoftSUM in the different map segmentations (MS), i.e. 4, 8 and full colours. a) All coronary territories.*
A, peak signal intensity; β, myocardial blood flow velocity; Axβ, myocardial blood flow; PV, parametric value; SUM, sum of worst case scenario; softSUM, soft case scenario summation.



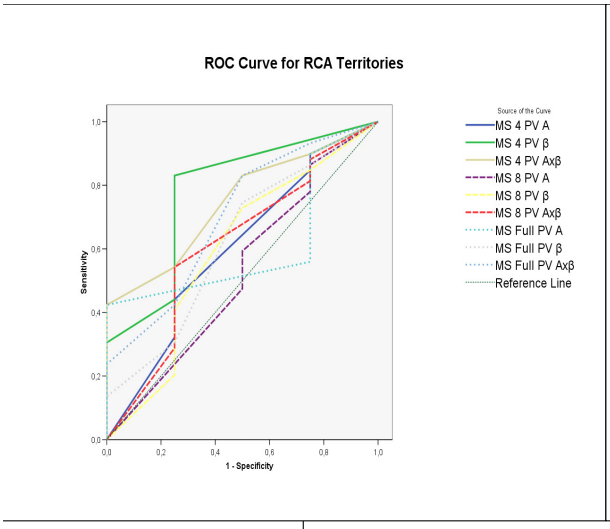
b

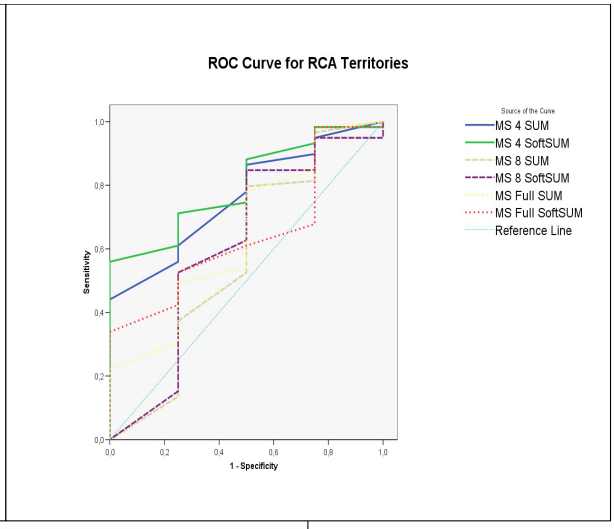
Figure 10 b. *ROC and tables of area under the curve for A, β, Axβ, SUM and SoftSUM in the different map segmentations (MS), i.e. 4, 8 and full colours. b) LAD territory.*
LAD, left anterior descending; A, peak signal intensity; β, myocardial blood flow velocity; Axβ, myocardial blood flow; PV, parametric value; SUM, sum of worst case scenario; softSUM, soft case scenario summation.



C

Figure 10 c. *ROC and tables of area under the curve for A, β, Axβ, SUM and SoftSUM in the different map segmentations (MS), i.e. 4, 8 and full colours. c) LCx territory.*
LCx, left circumflex; A, peak signal intensity; β, myocardial blood flow velocity; Axβ, myocardial blood flow; PV, parametric value; SUM, sum of worst case scenario; softSUM, soft case scenario summation.





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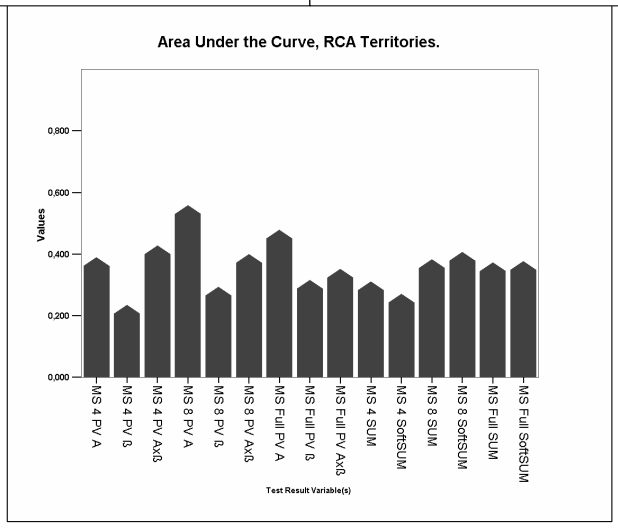


Figure 10 d. *ROC and tables of area under the curve for A, β, Axβ, SUM and SoftSUM in the different map segmentations (MS), i.e. 4, 8 and full colours. d) RCA territory.*
RCA, right coronary artery; A, peak signal intensity; β, myocardial blood flow velocity; Axβ, myocardial blood flow; PV, parametric value; SUM, sum of worst case scenario; softSUM, soft case scenario summation.

Paper IV

Of the 201 coronary distribution territories, 28 (14%) could not be analysed due to perfusion artefacts according to the visual perfusion analysis. These territories were still evaluated in the visual perfusion analysis with combined wall motion evaluation, since wall motion still could be analysed in these territories. In the quantitative analysis two more territories were considered non-interpretable using the Qontrast® software due to low parametric image quality, which made it too difficult to differentiate the left ventricular myocardium from the cavity. A summary of non-interpretable territories is presented in Table 11.

Table 11*. Non interpretable coronary territories for Qontrast® quantification and visual interpretation with complementary wall motion analysis (Vis 1) and sole perfusion interpretation (Vis 2).*

	All Territories (n=201)	LAD (n=67)	LCx (n=67)	RPD (n=67)	Patient (n=67)
Qontrast® (%)	30 (15)	12 (18)	16 (24)	2 (3)	15 (22)
Vis 1 (%)	0	0	0	0	0
Vis 2 (%)	28 (14)	11 (16)	16 (24)	1 (1)	22 (33)

In Table 12 the different results from both quantitative and visual interpretations are summarised. Quantitative reserve parameters β-r and Axβ-r showed significant area under the curve and kappa in all coronary territories. All quantitative reserve parameters expressed significant kappa values in the LAD coronary territory. Both visual analyses demonstrated higher kappa values and accuracy than any quantitative parameter.

Table 12. *Results for the respective quantitative variables and visual interpretations (QV), A-reserve, (A-r), β-reserve, (β-r) and Axβ-reserve (Axβ-r), visual RTP-ASE interpretation with complementary wall motion (Vis 1) and with sole perfusion interpretation (Vis 2).*

QV	Coronary Territory	Acc (%)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	kappa	AUC
A	All	43	39	75	11	94	NS	0.529 NS
A	LAD	67	66	75	27	94	0.237 *	0.705 NS
A	LCx	29	28	50	6	87	NS	0.229 NS
A	RCA	32	28	100	8	100	NS	0.518 NS
β	All	80	83	56	25	95	0.249 ***	0.678 *
β	LAD	82	83	75	43	95	0.442 **	0.773 *
β	LCx	82	94	25	25	94	NS	0.590 NS
β	RCA	72	74	50	11	96	NS	0.666 NS
Axβ	All	75	77	63	22	95	0.213 **	0.665 *
Axβ	LAD	82	83	75	43	95	0.442 **	0.818 **
Axβ	LCx	90	96	25	33	94	NS	0.404 NS
Axβ	RCA	58	57	75	10	97	NS	0.680 NS
A	Patientwise	35	11	100	29	11	NS	NA
β	Patientwise	60	53	79	38	87	0.233 *	NA
Axβ	Patientwise	47	38	73	31	79	NS	NA
Vis 1	All	93	90	93	59	99	0.67 ***	NA
Vis 1	LAD	87	90	86	53	98	0.59 ***	NA
Vis 1	LCx	94	100	94	56	100	0.68 ***	NA
Vis 1	RCA	96	75	100	100	98	0.85 ***	NA
Vis 1	Patientwise	90	89	90	76	96	0.75 ***	NA
Vis 2	All	92	94	92	55	99	0.67 ***	NA
Vis 2	LAD	86	100	83	53	100	0.61 ***	NA
Vis 2	LCx	94	100	94	57	100	0.68 ***	NA
Vis 2	RCA	95	75	97	60	98	0.64 ***	NA
Vis 2	Patientwise	89	91	88	71	97	0.73 ***	NA

*Sens, sensitivity; Spec, specificity; Acc, accuracy; AUC, area under the curve; *=p<0.05; **=p<0.01; ***=p<0.001; NS, not significant; NA, not applicable. Grey tone indicates not significant or not applicable.*

From the ROC curves (Figure 11) area under the curve is visualised and levels of sensitivity at different specificity values can be estimated. Despite the non-significant area under the curve for all reserve variables in the LCx and RCA territories, there is a notable level of sensitivity at preserved 100 % specificity for β-r in both territories, and for Axβ-r in the RCA territory.

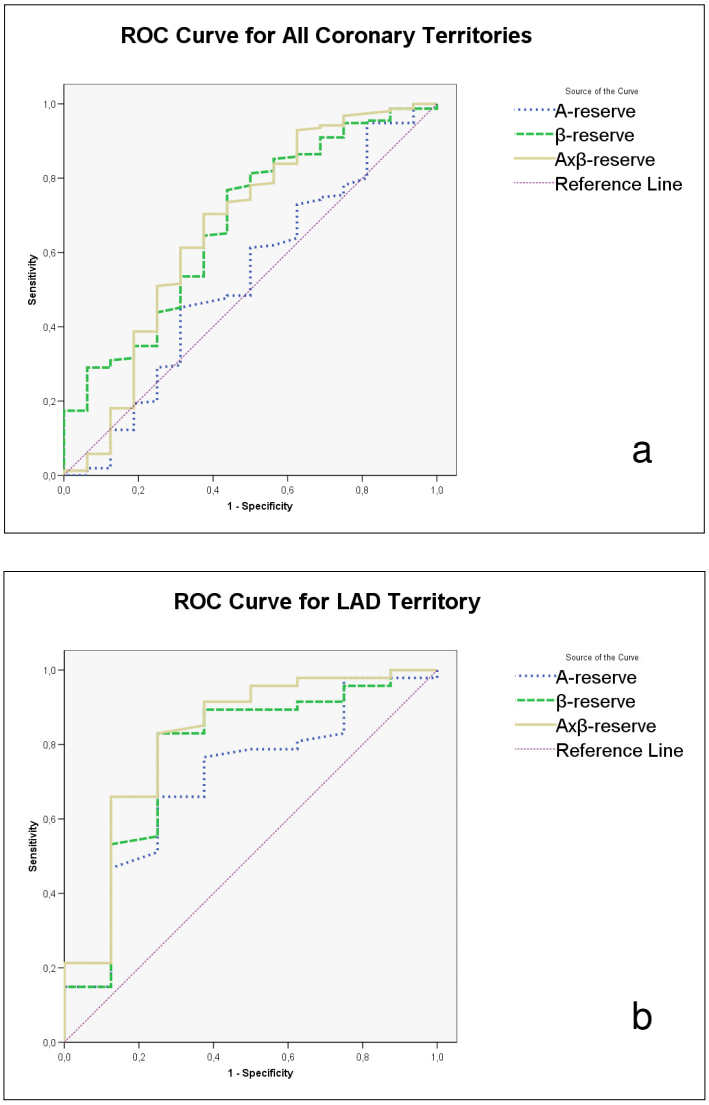
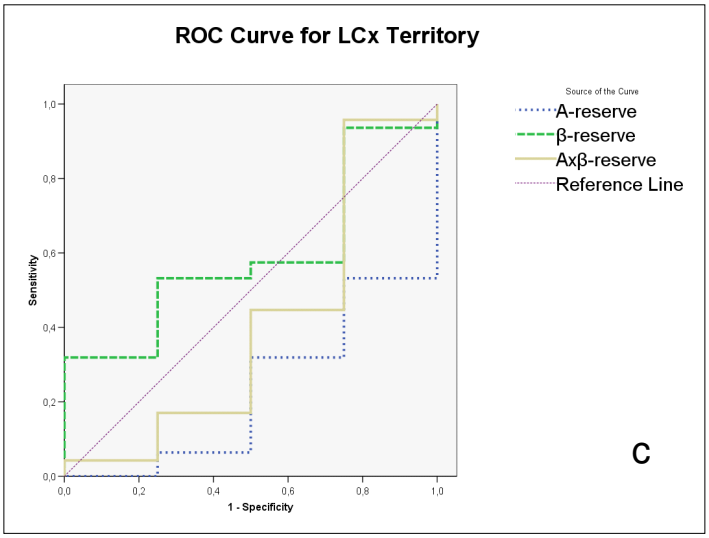


Figure 11 a-b. *Receiver operator characteristics curves of the quantitative perfusion measurements A-, β- and Axβ- reserve in all coronary territories (a), LAD territory (b), as compared with ischemia at SPECT.*
LAD, left anterior descending; A-r, peak signal intensity reserve; β-r, myocardial blood flow velocity reserve; Axβ-r, myocardial blood flow reserve.



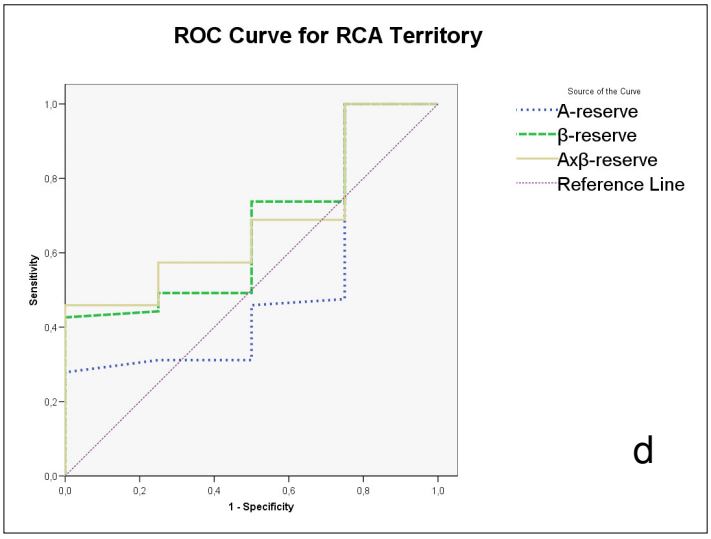


Figure 11 c-d. *Receiver operator characteristics curves of the quantitative perfusion measurements A-, β- and Axβ- reserve in LCx territory (c) and RCA territory (d), as compared with ischemia at SPECT.*
LCx, left circumflex; RCA, right coronary artery; A-r, peak signal intensity reserve; β-r, myocardial blood flow velocity reserve; Axβ-r, myocardial blood flow reserve.

When dividing the patient population into those without and those with ischemia at SPECT, there were significant differences for A-r, β-r and Axβ-r in the LAD territory and for β-r in all territories. All variables and territory differences are given in Table 13.

Table 13*. t-test for quantitative variables (QV) peak signal intensity (A), myocardial blood flow velocity (β) and myocardial blood flow (Axβ) at rest, stress and their respective reserves, if no ischemia or ischemia at SPECT.*

		No Ischemia at SPECT			Ischemia at SPECT		
QV	CA	Rest	Stress	Reserve	Rest	Stress	Reserve
A	All	46.9 ± 14.6	55.7 ± 10.1	1.13 ± 0.60	44.3 ± 15.3	52.0 ± 9.6	1.33 ± 1.26
A	LAD	48.7 ± 9.7	56.7 ± 11.7	1.05 ± 0.32	51.2 ± 7.9	51.3 ± 4.9	0.86 ± 0.20 *
A	LCx	37.1 ± 14.1	53.8 ± 6.5	1.48 ± 0.91	26.0 ± 12.4	46.7 ± 1.3 **	2.78 ± 2.00
A	RCA	54.9 ± 13.3	56.3 ± 10.9	0.92 ± 0.28	51.5 ± 13.5	57.2 ± 17.0	0.82 ± 0.34
β	All	1.12 ± 0.41	4.7 ± 3.3	2.52 ± 1.98	1.26 ± 0.38	3.2 ± 1.6 **	1.55 ± 0.87 *
β	LAD	1.15 ± 0.36	3.8 ± 1.6	1.77 ± 0.75	1.28 ± 0.25	2.7 ± 1.3	1.20 ± 0.59 *
β	LCx	1.16 ± 0.47	6.4 ± 4.3	3.58 ± 2.74	1.04 ± 0.26	3.9 ± 2.3	2.39 ± 1.14
β	RCA	1.04 ± 0.40	4.1 ± 2.8	2.28 ± 1.59	1.51 ± 0.64	3.4 ± 1.8	1.41 ± 0.65 *
Axβ	All	55.7 ± 32.4	261 ± 18	3.54 ± 4.60	59.6 ± 31.5	172 ± 100 **	2.87 ± 4.13
Axβ	LAD	60.2 ± 26.9	222 ± 118	2.21 ± 1.38	68.0 ± 17.2	138 ± 58 **	1.11 ± 0.71 *
Axβ	LCx	47.0 ± 34.5	346 ± 235	6.20 ± 7.24	28.5 ± 16.7	187 ± 111	7.91 ± 6.18
Axβ	RCA	60.2 ± 33.7	232 ± 166	2.53 ± 2.17	79.7 ± 45.8	222 ± 147	1.35 ± 0.85 *

*CT, coronary territory; * = p < 0.05; ** = p < 0.01 significant difference between no ischemia and ischemia at SPECT.*

RTP-ASE Variability

Visual inter- and intra-observer variability is presented in Table 14.

Table 14. *Inter and intra observer agreement of myocardial contrast echocardiography ischemia interpretation for RTP-ASE angio-mode (AM) and high resolution (HR) (n=33).*

	Total	LAD	LCx	RPD
Inter-observer (%) AM	91	88	97	88
Kappa	0.72***	0.73***	0.90***	0.28 ns
Intra-observer (%) AM	94	94	91	97
Kappa	0.76***	0.84***	0.62***	0.78***
Inter-observer (%) HR	95	95	95	95
Kappa	0.70***	0.64***	0.64***	0.78***
Intra-observer (%) HR	97	95	95	100
Kappa	0.78***	0.64**	0.64	1.00***

*LAD, Left anterior descending coronary artery; LCx, Left Circumflex artery; RPD, Right posterior descending coronary artery; **=p<0.01; ***=p<0.001, ns=not significant.*

Intra-observer variability for A and β in study IV was assessed by a second blinded reading of 10 randomly selected patients from the study. Variability was 6.0 % and 18 % for A and β, respectively.

DISCUSSION

The results of the studies in this thesis suggest possible ways to use RTP-ASE - visually, or by parametric or quantitative analysis - to detect myocardial ischemia in a population of patients with known or suspected stable CAD. Visual interpretation by AM or HR RTP-ASE showed excellent agreement with SPECT in studies I, II and IV. The kappa values were significant and consistently high, from around 0.67 in all coronary territories, and the accuracy was over 90 %, which is in line with previous studies (Mor-Avi, Caiani et al. 2001; Mor-Avi, Korcarz et al. 2003; Olszowska, Kostkiewicz et al. 2003; Sieswerda, Yang et al. 2003; Tsutsui, Xie et al. 2005; Winter, Gudmundsson et al. 2005; Korosoglou, Dubart et al. 2006).

Both parametric and quantitative analysis by Qontrast® in studies III and IV, demonstrated generally lower agreement than visual analysis, as compared with SPECT, but showed significant and reasonable accuracy in the LAD territory for the parametric analysis, with the best kappa value and area under the curve using parametric, 8 colour map segmentation. The quantitative Qontrast® analysis showed a somewhat lesser kappa for both perfusion reserves β and Axβ, and also somewhat smaller area under the curve.

When comparing AM and HR in study II, besides good agreement with SPECT, the results suggest that a dual use of AM and HR might complement and improve the accuracy of RTP-ASE in detecting ischemia.

Furthermore, when scrutinizing the ROC curves from studies III and IV (Figures 10 and 11), they show that specificity is better than sensitivity and that specificity remains at 100 % with preserved sensitivity in the majority of the variables tested.

Additionally, a high NPV for the assessment of myocardial ischemia by RTP-ASE was consistently found in all studies of this thesis. The NPV is especially important in patients like those of the study cohorts of this thesis, since the prevalence of reversible ischemia was quite low (26%). Wall motion abnormalities were present in 60% of the patients, indicating a high prevalence of ischemic heart disease. However, the objective was to assess the accurate detection of reversible ischemia using AM and HR, respectively, not to reveal the presence or absence of ischemic heart disease. Hence, results regarding PPVs become somewhat less important. High NPV, on the other hand, is of great importance when myocardial ischemia is to be acquitted from patients of this kind. It is reassuring to know that the method allows safe and accurate preclusion of myocardial ischemia, as compared with SPECT, which is associated with good prognosis (Underwood, Anagnostopoulos et al. 2004).

A very important aspect of the results of this thesis is the matter of non-interpretable territories. The results point to a great advantage of using visual RTP-ASE reading in this regard, where the combination with wall motion analysis in segments with perfusion artefacts upholds the number of interpretable territories without any noticeable loss of accuracy. This is something that quantitative methods alone cannot live up to, as previously shown and as observed in this thesis (Yu, Skyba et al. 2004; Malm, Frigstad et al. 2006). Study I shows (Table 5) that many artefacts were in the basal segments, which supports the finding of low agreement of ischemia detection in LCx and RCA territories, compared to SPECT in studies III and IV. LCx and RCA territories exclusively contain basal and mid segments of the left ventricle.

Interestingly, even when excluding territories considered to contain artefacts, the visual RTP-ASE interpretation is still better than the parametric or quantitative assessments. This reason for this finding might be that the human brain is still better than the computer at compensating for e.g. image quality changes during one RTP loop, or appreciating conformity differences in perfusion depending on different depth, which might falsely indicate differences in perfusion. Such changes are still not built into the algorithms of automatic tissue recognition and myocardial border detection.

Earlier studies have shown promising but variable results for the detection of myocardial ischemia using RTP-ASE, as compared with SPECT, and also varying fractions of interpretable segments in the study population (Mor-Avi, Caiani et al. 2001; Main, Magalski et al. 2003; Olszowska, Kostkiewicz et al. 2003; Sieswerda, Yang et al. 2003). This may be due to the fact that RTP is a quite new technique and that echocardiographers still are learning how to use the technique properly.

Myocardial areas judged ischemic by RTP-ASE but not by SPECT, could be small, perhaps sub-endocardial areas that are truly ischemic. These might not be detected by SPECT due to the lower spatial resolution of this method (Mor-Avi, Caiani et al. 2001; Main, Magalski et al. 2003; Olszowska, Kostkiewicz et al. 2003; Sieswerda, Yang et al. 2003). Thus, although it cannot be concluded from the results of the present study that RTP-ASE is more sensitive in detecting ischemia compared to SPECT, the possibility should be considered. Indeed, there are cases and studies suggesting that this might be true (Tiemann, Ghanem et al. 2001; Hagendorff, Pfeiffer et al. 2003; Senior, Lepper et al. 2004). Nevertheless, prognosis has been proven favourable when myocardial ischemia has been excluded by SPECT (Underwood, Anagnostopoulos et al. 2004), which suggests that ischemia detected with RTP-ASE but not with SPECT probably will not affect prognosis substantially.

Perfusion defects according to RTP-ASE but not as judged by SPECT, may also be due to apical perfusion artefacts. This phenomenon is caused by apical overlapping of ultrasound beams when the focal zone is placed close to the base of the ventricle in the apical projections. This increases the local mechanical index in the apex and, therefore, increases bursting of contrast micro-bubbles compared to the mid- and basal parts (Becher and Burns 2000). This particular problem can be overcome by placing the focal zone near the apex in cases of suspected apical perfusion defects. Another possible solution to this problem is to collect triggered RTP images (Yu, Skyba et al. 2004), thus avoiding continuous ultrasound exposure, which would result in fewer apical perfusion artefacts. However, then the advantage of combining assessment of perfusion and wall motion would of course be lost, which is one of the main advantages of the visual RTP-ASE analysis. Furthermore, triggered imaging would result in reduced statistical power of the quantitative analysis, due to fewer frames of per-

fusion to analyse, which might be needed to improve signal to noise ratio in quantification.

The results from study II indicate both similarities and differences between the two RTP power modulation techniques, AM and HR. As compared with SPECT, there were highly significant agreements for both AM and HR in detecting ischemia, which is in line with previous findings and study I (Mor-Avi, Caiani et al. 2001; Olszowska, Kostkiewicz et al. 2003; Sieswerda, Yang et al. 2003; Winter, Gudmundsson et al. 2005). Although there were no significant differences between AM and HR as regards the agreement with SPECT, there were some differences worth discussing.

Using SPECT as reference, HR showed higher values of kappa and of accuracy, suggesting that HR is better suited than AM in this kind of population, i.e. with low pre-test probability of reversible myocardial ischemia. On the other hand, AM had somewhat higher sensitivity, which means that the risk of a false negative test might actually be higher for HR. Sensitivity and specificity are connected to PPV and NPV, respectively, but are less affected by the prevalence of reversible ischemia in the actual patient sample examined. As compared with PPV and NPV, sensitivity and specificity values may, therefore, provide more reliable information about the precision of the test in any patient sample, rather than about the health of the patients in the actual sample examined. The finding that sensitivity values were higher for AM than for HR indicates that AM RTP-ASE may be better at detecting the true reversibly ischemic patient, while the higher specificity values for HR might represent better identification of non-ischemic patients, as judged by SPECT. These complementary features could indicate different areas of effective use of the two techniques. AM may be useful as a first line reading, with additional reading using HR in cases with suspected ischemia according to AM. Consequently, AM could be the preferred technique to more quickly identify non-ischemic patients in e.g. the emergency ward, while HR primarily might be more effective in decision-making for targeted invasive therapy in high-risk patients not suited for bypass surgery and who cannot be fully revascularised with percutaneous coronary intervention (PCI).

The better sensitivity for AM might be due to the potentially easier visual assessment with this modality, since echoes from contrast bubbles

are displayed as coloured pixels, in distinction to HR, where echoes are displayed in grey scale. The higher kappa and accuracy values could therefore be due to superiority, as compared with HR, in correctly detecting non-ischemic territories, which were predominating in the present study population.

The similarities and differences between AM and HR were approximately similar for the sole perfusion analysis and the combined perfusion and wall motion analysis. This indicates that agreement with SPECT can be maintained at a high level using wall motion analysis in coronary territories with perfusion artefacts as indicated by study I. This should be viewed in relation to the results regarding non-interpretable coronary areas, where the high number of non-interpretable coronary areas in the sole perfusion analysis clearly illustrates lower feasibility, compared to the combined perfusion and wall motion analysis. Therefore, our results suggest that combined analysis is superior for maintaining good feasibility as well as good accuracy.

The results in study III indicate that, evaluation of parametric images generated by Qontrast® during RTP-ASE, may reasonably correctly identify myocardial ischemia, at least in the LAD territory, in patients with known or suspected stable coronary artery disease. As might be expected from previous studies (Moir, Haluska et al. 2005; Malm, Frigstad et al. 2006), good agreement was achieved through interpretation of β or Axβ images, especially from the 8-colour map segmentation. Even the 4-colour map segmentation was better than the full-colour segmentation, which could be due to overestimation of ischemia in the higher resolution images. However, the overestimation could actually be due to true ischemic territories, since SPECT has lower spatial resolution than echocardiography and no possibility to gather replenishment curves, which as is a cornerstone of RTP. However, SPECT is, as mentioned previously, well known for correctly acquitting ischemia with good prognosis (Underwood, Anagnostopoulos et al. 2004).

SUM and SoftSUM evaluations seemed to further improve the agreement with SPECT, which supports the hypothesis that a single parametric parameter can be too sensitive, either to small defects or – more likely, since there were no clear differences between sensitivity and specificity – to artefacts not visually discernible.

LAD was apparently the most suitable coronary territory for evaluation of myocardial ischemia by parametric imaging and by quantification using Qontrast® during RTP-ASE. This is probably due to the fact that the LAD territory is in the near field of the transducer and, therefore, is less sensitive to ultrasound attenuation, as suggested by recent studies (Malm, Frigstad et al. 2006; Toledo, Jacobs et al. 2006). Interpretation of the other territories might be improved in the future, by further development of image quality in myocardial contrast echocardiography and digital contrast and tissue recognition algorithms.

More detailed review of study IV additionally reveals that all three quantitative parameters, A-r, β-r and Axβ-r showed significant agreement with SPECT in the LAD territory. Only β-r and Axβ-r showed significant agreement with SPECT in the all territory analysis, and the agreement was still only significant in the LAD territory when the analysis was performed in the specific coronary territories. Sensitivity and positive predictive value could perhaps have been higher if the segments analysed had been more detailed, i.e. according to a 17-segment model, but then there probably would have been a greater anatomical mismatch between echocardiography and SPECT. These results indicate that the methods for quantification of myocardial ischemia are still in need of substantial improvements to become clinical useful, which is in line with previous studies (Moir, Haluska et al. 2005; Malm, Frigstad et al. 2006).

The remarkable difference between the visual evaluation of myocardial ischemia and the quantitative automated method using Qontrast®, is probably due to the high level of automatic tissue recognition in the software, which might be improved if higher demands on image quality can be satisfied. Quantitative evaluation of myocardial ischemia is expected to be improved in the future, when both echocardiographic image quality improves and the algorithms for tissue or contrast recognition become more advanced and obtain higher signal to noise ratio. Still, in echocardiography, brain and experience seem to beat the computer in perfusion contrast echocardiography. Nevertheless, a combination of visual and software analysis might be of interest, as also suggested by others (Korosoglou, da Silva et al. 2004; Yu, Skyba et al. 2004).

A major disadvantage of the quantitative evaluation of ischemia, as compared with the visual interpretation, is the consistently observed considerably lower feasibility, where studies III and IV show 15 % non-interpretable segments using Qontrast®. Visual interpretation with complementary wall motion analysis could, however, be performed in all of the included patients.

RTP-ASE is a highly available technique with possible bedside accessibility, which makes it appealing for clinical use, even in smaller hospitals with low access to angiographic or scintigraphic techniques.

Limitations

One limitation of this thesis is the relatively few ischemic patients, which diminish the importance of the PPV results. However, the results are valid in this type of population, specially when the aim is to rule out ischemia. The findings cannot automatically be transferred to patient populations with acute coronary syndrome.

Another shortcoming may be that it is difficult to guarantee similarity between echocardiographic and SPECT coronary territory selection, which can cause some territory mismatch and, therefore, falsely incorrect interpretation in some cases.

Coronary angiography might have added some value through comparison with significant coronary stenosis. However, SPECT evaluates myocardial perfusion and coronary angiography merely the morphology of the vessels, which is why SPECT was chosen as reference method in the present study.

One apparent limitation mentioned previously, is the subjectivity when visually analysing ischemia in RTP-ASE, since it is based on visual estimation of both perfusion and wall motion. Although software tools for quantification of perfusion are now available and have shown promising results in animal models (Lafitte, Higashiyama et al. 2002; Agati, Tonti et al. 2004) and a few clinical studies (Bekeredjian, Hilbel et al. 2003; Korosoglou, da Silva et al. 2004; Peltier, Vancraeynest et al. 2004; Vogel, Indermuhle et al. 2005), the results of studies III and IV in this thesis indicate that quantification of perfusion is not yet ready for clinical use. It may however soon be ac-

curate enough to use in clinical practice, but not until the technique has been further developed and clinical studies of appropriate size have evaluated its feasibility, particularly in the LCx and RCA territories.

In line with the previous limitation, is the obvious limitation that the RTP technique demands skilful operators and interpreters with a substantial amount of experience and knowledge of both ultrasound physics, contrast agent characteristics and micro bubble behaviour when exposed to ultrasound. However, this ultrasonic technique is more available than many other modalities and is useful for bedside evaluation, which still makes RTP-ASE one of the most appealing and accessible alternatives.

Overall feasibility using the quantitative methods in studies III and IV, is a major limitation. Up to 15 % of territories were non-interpretable, which also seems to be the case for other presently available quantitative methods (Yu, Skyba et al. 2004; Malm, Frigstad et al. 2006).

Even if the parametric images in study III were quantitatively generated, there is still a visual and, therefore, subjective component involved; the reader of the images has to decide if there is a difference, and to what extent, between images at rest and stress. However, compared to studies with merely visual analysis, the results show less agreement (kappa) but generally higher sensitivity and at least the same negative predictive values (Sieswerda, Yang et al. 2003; Korosoglou, da Silva et al. 2004; Senior, Lepper et al. 2004; Winter, Gudmundsson et al. 2005).

One could argue that higher contrast infusion rate would have increased the amount of detectable contrast in basal parts of the myocardium and, thus, increased accuracy for LCx and RCA territories. Even if not tested within the scope this thesis, it would probably not have been so; larger amounts of contrast would have caused shadowing and, thus, rather diminished the contrast echoes from basal segments instead of increasing them. The only available way of testing for optimal amount of contrast infusion rate, is still a visual, on-line assessment, where a high and uniform level of contrast through the depth of the left ventricular cavity is achieved.

CONCLUSIONS

I

RTP-ASE using power modulation is an accurate and feasible tool for the evaluation of myocardial ischemia in patients with suspected stable coronary artery disease. The combination of perfusion and wall motion evaluation increases the number of interpretable segments and, hence, the feasibility, when using adenosine as stressor.

The results of this study need confirmation in a larger study.

II

There are both similarities and differences between the two RTP power modulation techniques, AM and HR. Both AM and HR showed highly significant agreements for the detection of reversible myocardial ischemia, using SPECT as method of reference. There was a slight, borderline-significant difference between AM and HR in correctly detecting myocardial ischemia as judged by SPECT, where HR seemed to be somewhat better. However, AM and HR complement each other, suggesting the use of both AM and HR for optimum evaluation of ischemia by RTP-ASE in patients with suspected CAD. For both AM and HR, the agreement with SPECT was similar whether or not complementary wall motion evaluation was used. However, when using the combined perfusion and wall motion interpretation, feasibility was higher, with almost no non-interpretable segments. Therefore, our results suggest that combined analysis of perfusion and wall motion, using both AM and HR, provides superior feasibility and accuracy of RTP-ASE.

III

Using SPECT as reference, myocardial ischemia can be adequately evaluated in the LAD coronary territory, using Qontrast® parametric images generated from RTP-ASE, in patients with suspected stable myocardial ischemia. However, caution should be exercised regarding the LCx and, particularly, RCA territories, where the technique needs further improvements. Combined A, β and Axβ image interpretation, especially with summations and averaging, improved the agreement with SPECT, compared to their individual values.

IV

The results from study IV indicate that RTP-ASE Qontrast® quantification of myocardial ischemia, although less accurate than visual evaluation, can be used for quantification of ischemia in the LAD territory, but probably not in the LCx or RCA territories, unless further developed. Visual evaluation of myocardial ischemia shows excellent agreement with SPECT with good feasibility, without non-interpretable territories.

The human brain still seems superior to the computer at detecting myocardial ischemia from RTP-ASE images, in patients with known or suspected CAD, as compared with SPECT, at least in LCx and RCA areas.

Finally.

Echocardiography and, in the context of this thesis, MCE is patient friendly, highly available and inexpensive and, therefore, research of new ways of using echocardiography and MCE is worth the effort. So, my final statement of this thesis is:

RTP-ASE – Worth fighting for!

POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ SVENSKA.

Diagnostisering av syrebrist i hjärtmuskeln med hjälp av kontrastförstärkt ultraljudsundersökning av hjärtmuskelns genomblödning.

Ultraljudsundersökning av hjärtat (ekokardiografi) används mycket inom hjärtsjukvården idag, oftast för att utreda hjärtats och hjärtklaffarnas anatomi och funktion. Ekokardiografi är en ofarlig och ganska enkel undersökning och apparaturen som används är förhållandevis billig och mobil om man jämför med andra bildgivande undersökningar av hjärtat, såsom röntgen, isotopundersökning eller magnetresonans tomografi.

Syrebrist (ischemi) i hjärtmuskeln uppkommer genom att inte tillräckligt med syreförande blod når ett eller flera områden av hjärtmuskeln, vanligen pga. förträngningar i hjärtats kranskärl. Ischemi orsakar ofta, men inte alltid bröstsmärtor, vilket kallas ”kärlkramp” i hjärtat. Utvärdering av ischemi i hjärtmuskeln rekommenderas för optimal behandling av patienter med misstänkt sjukdom i hjärtats kranskärl. Denna utvärdering kan göras med hjälp av olika typer av belastningstest. Metoder som finns att tillgå är arbets-EKG, isotopundersökning (99mTc-sestamibi single-photon emission computed tomography – SPECT) och stressekokardiografi. Liksom arbets-EKG utförs både SPECT och stressekokardiografi med olika typer av belastning. Belastning vid SPECT sker vanligen genom ergometercykling, men kan också utföras genom provokation med läkemedel (dobutamin, adenosin) som ger en belastning på

hjärtmuskeln liknande den vid fysisk ansträngning. Vid stress-ekokardiografi görs belastningen vanligast med dobutamin (dobutamin-atropin stress-ekokardiografi – DSE) men kan också göras med ergometercykling eller adenosin.

SPECT påvisar ischemi i hjärtmuskeln genom att jämföra genomblödningen (perfusionen) i hjärtmuskeln vid maximal belastning och vila. Minskad genomblödning under belastning är tecken på ischemi. Genomblödningen i hjärtmuskeln uppskattas vid SPECT genom att en radioaktiv isotop injiceras i patientens blod. Isotopen fördelas i hjärtmuskeln på samma sätt som blodet och strålarna från isotopen kan detekteras av en speciell detektor (gammakamera). Från områden utan eller med nedsatt genomblödning detekteras ingen eller nedsatt strålning och i dessa områden föreligger således ischemi. Vid DSE jämför man vanligen hjärtmuskelns förmåga att dra ihop sig (kontraktionsförmåga) i arbete och vila. Om kontraktionen minskar i någon del av hjärtmuskeln under belastning så talar det för ischemi i detta område.

Både SPECT och DSE är väldokumenterade och bättre men dyrare än arbets-EKG. Att belasta hjärtat med dobutamin är ofta påtagligt obehagligt för patienten, medan belastning med adenosin ofta uppfattas som mindre obehaglig . Själva belastningen är dessutom oftast kortare än vid både ergometercykling och dobutamin-belastning. Till skillnad från sedvanlig stress-ekokardiografi räcker det dock inte att bedöma hjärtmuskelns kontraktion vid adenosin-belastning, utan det krävs även att genomblödningen bedöms. Genomblödningen i hjärtmuskeln har inte tidigare varit möjlig att värdera vid adenosin-stress-ekokardiografi (ASE). Det blev dock möjligt för några år sedan, då en typ av ny ultraljuds-kontrastmedel introducerades inom ekokardiografi. Kontrastmedlet för ultraljud som användes inom ramen för denna avhandling var Sonovue®. En kombination av ny ultraljudsteknik (realtids perfusion – RTP) och kontrastmedel har gjort det möjligt att samtidigt bedöma genomblödningen i hjärtmuskeln och dess kontraktionsförmåga. Därigenom skulle RTP i kombination med ASE (RTP-ASE) kunna användas för att tillförlitligt detektera ischemi i hjärtmuskeln. Om RTP-ASE visar sig kunna detektera ischemi lika bra som en redan erkänd undersökning, exempelvis SPECT, kan RTP-ASE bli en bra alternativ undersökning. Jämfört med SPECT är RTP-ASE mer tillgänglig och utan radioaktiv strålning, jämfört

med DSE är den snabbare och mindre obehaglig, och den är mera noggrann än arbets-EKG.

Studierna i denna avhandling gjordes för att undersöka om RTP-ASE kan detektera ischemi i hjärtmuskeln med samma noggrannhet som SPECT, hos patienter med känd eller misstänkt stabil kranskärlssjukdom. Jämförelsen med SPECT gjordes både genom visuell tolkning av RTP-bilderna och genom kvantitativ analys av genomblödningen från samma bilder, med hjälp av dataprogrammet Qontrast®.

Resultaten i denna avhandling visade att bedömning av ischemi i hjärtmuskeln genom visuell tolkning av bilder från RTP-ASE överensstämde väl med SPECT. Visuell tolkning är alltså en alternativ metod för att bedöma ischemi hos patienter med känd eller misstänkt stabil kranskärlssjukdom. Bedömning av ischemi genom kvantitativ analys visade dock inte lika god överensstämmelse och kan därför inte rekommenderas för kliniskt bruk i dag. Genom en förväntad teknisk utveckling kan dock dessa problem överkommas inom en snar framtid, varvid denna metod kan komma att visa sig kliniskt användbar.

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