NEUROENDOCRINE ACTIVATION AND DIAGNOSTICS IN PULMONARY EMBOLISM:

TRANSLATIONAL STUDIES

Henrik Gutte

This review has been accepted as a thesis together with three previously published papers by University of Copenhagen 1st of July 2010 and defended on 26th of October 2010.

Tutors: Andreas Kjær & Jann Mortensen

Official opponents: Per Hildrebrand, Marika Bajc & Inge Lise Kanstrup

Correspondence: Department, Department of Clinical Physology, Nuclear Medicine & PET, Rigshospitalet, Copenhagen University Hospital. Blegdamsvej , 210 0 Copenhagen, Denmark

E-mail: henrikgb@gmail.com

Dan Med Bull 2011;58(3):B4258

LIST OF PAPERS

The following papers constitute the basis of this thesis, and will be referred to in the text by their Roman numerals.

I. Henrik Gutte, Jann Mortensen, Claus Verner Jensen, Camilla Bardram Johnbeck, Peter von der Recke, Claus Leth Petersen, Jesper Kjærgaard, Ulrik Sloth Kristoffersen, Andreas Kjær. Detection of pulmonary embolism with combined ventilation/perfusion SPECT and low-dose CT: Head-to-head comparison with multidetector CT-angiography. J Nucl Med. 2009. (50) 12: 1987-50.

II. Henrik Gutte, Jann Mortensen, Claus Verner Jensen, Peter von der Recke, Claus Leth Petersen, Ulrik Sloth Kristoffersen, Andreas Kjær. ANP, BNP and D-dimer predict Right Ventricular Dysfunction in Patients with Acute Pulmonary Embolism. Clin Physiol Funct Imaging. 2010. 30(6):466-72.

III. Henrik Gutte, Jytte Oxbøl, Ulrik Sloth Kristoffersen, Jann Mortensen, Andreas Kjær. Gene expression of ANP, BNP and ET-1 in cardiac chambers of rats during experimental pulmonary embolism. PLoS One. 2010. 14;5(6):e11111.

SUMMARY

Acute pulmonary embolism (PE) is a severe and potentially fatal disease which acutely augments the right ventricle (RV) strain. Development of RV dysfunction (RVD) in the disease process is synonymous with an overall poor prognosis.

The diagnosis of PE is usually established by a combination of clinical assessment, D-dimer test and medical imaging with either lung scintigraphy or pulmonary multidetector computer tomography (MDCT) angiography. Which of the two methods to use in PE diagnostic has not been determined and very limited data comparing these modalities are available.

Assessment of RV function is cumbersome due to complex geometry. RVD is usually established by echocardiography which is observer dependent, has low reproducibility, and requires expertise. Therefore, a simple and reproducible biochemical method to assess RVD in patients with PE would be desirable.

Brain natriuretic peptide (BNP), pro-atrial natriuretic peptide (pro-ANP), cardiac troponin I (TnI), and endothelin-1 (ET-1) have been the most studied plasma biomarkers in the context of risk stratification in PE. BNP is mainly produced in the ventricles of the heart. It is released from the left ventricle in response to increased filling pressure and is increased in chronic left heart failure. Pro-ANP is primarily produced in the atria, is released by atrial distention and is elevated in chronic pulmonary hypertension and could be an early marker for RVD. Plasma level of ET-1 has been shown to correlate with pulmonary pressure and is released from endothelial cells in the pulmonary vessels. Additionally, increases in circulating levels of ET-1 have been reported in an experimental animal model of PE.

TnI is part of a complex of regulatory proteins in the cardiac myofilaments and is released upon myocyte injury. It is related to short term clinical outcome, prolonged hypotension, and cardiogenic shock after myocardial infarction and is a predictor of 30-day mortality and RVD using echocardiography in patients with PE.

Our hypothesis was therefore that the neuroendocrine activation of BNP, pro-ANP, ET-1, and TnI alone or in combination could serve as markers of RVD in patients with PE. The use of plasma biomarkers would be much simpler than reproducible medical imaging methods such as magnetic resonance imaging (MRI), radionuclide based methods etc.

This ph.d. thesis is based on three manuscripts and assesses the diagnostics of PE and the usability of biomarkers in diagnosing RVD in patients with PE.
In manuscript I we performed V/Q-SPECT, pulmonary MDCT angiography, low-dose CT, and cardiac CT on 100 patients suspected of PE. We found that both V/Q-SPECT alone and V/Q-SPECT combined with a low-dose CT scan had a higher sensitivity than pulmonary MDCT-angiography in the diagnosis of PE. In addition, both pulmonary MDCT-angiography and V/Q-SPECT in combination with low-dose CT had high specificities, whereas V/Q-SPECT alone had a lower specificity. With the use of hybrid scanners, V/Q-SPECT in combination with low-dose CT without contrast enhancement has "revitalized" lung scintigraphy and should probably be considered first-line imaging test in diagnosing PE.
The results from manuscript I are used in manuscript II. By using exact measurement with cine CT of cardiac function and geometry as reference we found that the plasma levels of BNP and pro-ANP can be used in diagnosing RVD in PE patients.
In manuscript III, gene expression of ANP, BNP, and ET-1 in the cardiac atria and ventricles in response to graded acute PE in an animal model was measured. By using an experimental animal model it was possible to gradually induce PE in different severities and at the same time measure the neuroendocrine secretion and gene expression of BNP, pro-ANP, and ET-1 in order to establish the relation between PE and neuroendocrine activation. By obtaining tissue from different chambers in the heart the origin of the peptides could be demonstrated.
We found a close correlation between PE degree and gene expression of ANP and BNP in the cardiac chambers with a selective increase in the right chambers of the heart. Furthermore, plasma BNP, TnI, and ET-1 levels dose-dependently increased with the degree of PE.
Since measurements of cardiac biomarkers are inexpensive and easily obtained they may prove useful in the clinical diagnosis of RVD in PE. The results of this thesis support this idea.

BACKGROUND

Pulmonary embolism

Acute pulmonary embolism (PE) is a severe and potentially fatal disease with a mortality rate of approximately 30% if untreated[1]. The incidence is 2 per 1,000 person years in the Western countries[2-5]. PE is a blockage of the main pulmonary artery or one of its branches by a dislodged thrombus (typically a blood clot from the deep veins of the lower extremities). PE reduces the cross-sectional area of the pulmonary blood vessels, resulting in an increase in total pulmonary vascular resistance and pulmonary hypertension. The right ventricle (RV) is then subjected to increased work, increased wall tension, shear force injury to myocytes, and compression of the coronary vessels which leads to ischemia. The obstruction of the pulmonary flow and the resulting pressure on the RV of the heart leads to the symptoms and signs of PE[6].
The risk of PE is increased in congenital hypercoagulable states (antithrombin III deficiency, factor V Leiden mutation, antiphospholipid antibodies etc.) and in acquired hypercoagulable states (cancer, increasing age, immobilization, surgery, venous stasis, injury etc). PE presents with an extensive clinical continuum, from asymptomatic over systemic hypotension and cardiogenic shock to sudden death. Symptoms of PE include dyspnea, pleuritic pain, cough, and palpitations. Clinical signs include tachypnea, tachycardia, unspecific stethoscope findings, and oxygen desaturation[7].

Establishing the diagnosis of pulmonary embolism

The clinical presentation of PE is highly variable, and many of its associated symptoms are non-specific, which make diagnosis difficult. The diagnosis of PE is usually established by a combination of clinical assessment, D-dimer test and imaging with either lung-scintigraphy or multidetector computer tomography (MDCT) angiography. Both imaging methods have their pros and cons, and none can diagnose all cases (sensitivity <100%). Among the weaknesses of traditional two-dimensional (2-D) planar ventilation-perfusion (V/Q)-scintigraphy when using the Prospective Investigation of Pulmonary Embolism Diagnosis (PIOPED) interpretation criteria are high proportions of equivocal studies[8;9] as well as only moderate interobserver agreement[10]. Accordingly, in recent years V/Q-scintigraphy has had a diminished role in the diagnosis of PE.
Pulmonary MDCT angiography has a higher diagnostic accuracy and specificity than conventional planar V/Q scintigraphy[11;12]. Thus, in many institutions MDCT is first-line imaging test in daily clinical routine in patients suspected of PE[13-15]. In addition, MDCT has the ability to yield an alternative diagnosis and has a high degree of interobserver agreement[16-19]. Several studies have demonstrated that MDCT angiography is sensitive with a high specificity[20-23]. However the positive predictive value for pulmonary MDCT angiography declines when thrombi are located in smaller pulmonary vessels. Positive predictive values were 97 % (116 of 120 patients) for PE in a main or lobar artery, 68 % (32 of 47 patients) for a segmental vessel, and 25 % (2 of 8 patients) for a subsegmental branch[24;25]. However, data are sparse in the subsegmental group.
This study also demonstrated that predictive values varied substantially when clinical probability of PE was taken into account. In patients with high or intermediate clinical probability, the positive predictive value of MDCT was high (96% and 92%, respectively) but decreased to 58% in the case of low clinical probability. Negative predictive value of MDCT was high in patients with low or intermediate clinical probability (96% and 89%, respectively) but was low in patients with high clinical probability (60%)[26;27].
At present, many centers use only pulmonary MDCT. This might not be beneficial because of a possible lower sensitivity and higher radiation dose compared with lung-scintigraphy. Reasons for extensive use of MDCT may include its round-the-clock availability, lower cost, and high frequency of conclusive results, as well as the departments' inexperience with V/Q-SPECT. Recently proposed algorithms for evaluation of patients suspected of having PE have totally omitted the use of lung-scintigraphy in the diagnostic work-up[28]. Some guidelines only include lung-scintigraphy as an alternative imaging technique, when patients can not have a MDCT performed due to severe renal insufficiency or allergy to intravenous contrast agents or when a CT-based strategy is inconclusive[29;30].
Yet, the introduction of three-dimensional (3-D) V/Q-single-photon-emission-computer-tomography (SPECT) technology instead of 2-D planar V/Q-scintigraphy suggests an improvement in the diagnostic performance of scintigraphy[31-34].

Recently, hybrid gamma camera/MDCT systems have been introduced, which allows for simultaneous lung V/Q-SPECT and MDCT angiography and may be used for diagnosing PE[32;35]. However, very limited data directly comparing these two 3-D modalities are available[36;37] and a head to head comparison of simultaneous V/Q-SPECT and pulmonary MDCT angiography for the detection of PE has to our knowledge never previously been undertaken.

Right ventricular dysfunction

The extent of pulmonary vascular obstruction, the preexisting status of the cardiopulmonary system, and the physiologic consequences of both hypoxically and neurohumorally mediated vasoconstriction are probably the most important prognostic factors in PE patients[38-40]. Acute obstruction of the pulmonary circulation by a thrombus acutely augments the RV strain. In patients without preexisting cardiopulmonary disease, obstruction of less than 20% of the pulmonary circulation results in several compensatory actions with adverse hemodynamic consequences. Recruitment and distension of pulmonary vessels occur as obstruction increases. Increases in pulmonary artery and right atrial pressure occur when the extent of pulmonary vascular obstruction reaches 40%. The Frank-Starling mechanism sustains the RV stroke work and cardiac output. When the degree of pulmonary artery obstruction reaches ~50%, compensatory mechanisms are overcome, cardiac output declines, and right atrial pressure increases severely. With further acute obstruction, the RV dilates, RV wall tension increases, RV ischemia develops, cardiac output falls, arterial systemic hypotension occurs, and eventually cardiogenic shock and death occurs[41].

RVD may correlate with the degree of obstruction of the pulmonary circulation[41;42] and assessment of RV function may therefore offer additional insight into the pathophysiological consequences of PE[43]. RV ejection fraction (RVEF) is a sensitive and robust measurement of RV function. Right heart strain or RVD defined as RVEF <0.45, (RVEF normal value: 0.45-0.57[44;45]) holds prognostic information and can be used as management guide in treatment[46-48].

Many patients with PE remain in a stable condition with normal blood pressure and RV function and have an excellent prognosis with anticoagulants alone and can be referred to a non-intensive care unit. However a large group of patients which may appear to be in a stable condition with normal arterial systemic blood pressure actually have compromised RV function and increased afterload due to obstruction of the pulmonary artery by embolism. PE patients can be categorized into three groups based on systemic arterial blood pressure and RV function. The groups are; normal systemic arterial blood pressure and no RVD (minor PE), normal systemic blood pressure and RVD (submassive PE), and systemic hypotension and RVD (massive PE), where the two latter are considered as hemodynamically unstable high-risk patients[49]. The prognosis of patients with normal systemic arterial blood pressure and RVD is grave compared with patients who have normal systemic arterial blood pressure and normal RV function and size. These patients appear stable but have impending RV failure and a high mortality. Identification of these high-risk patients is difficult and may permit use of more aggressive interventions[50-52]. In about 5%, acute PE is represented by massive PE with hypotension and cardiogenic shock. About 50% of normotensive patients have transthoracic echocardiographic pattern of RVD, and approximately 10% of these will die[53]. According to current guidelines, hypotensive patients with PE should receive thrombolytic therapy and require intensive observation[54;55]. However there is no consensus in the management of normotensive PE patients with RVD[56].

Assessment of RV function is cumbersome due to complex geometry and limited sharpness of the endocardial surface caused by the profoundly trabeculated myocardium. Two-dimensional echocardiography can be used in evaluating right-side heart function and is the method of choice in daily clinical routine. But the technique is only semiquantitative, less accurate and reproducible and requires cardiology expertise. Thus, it is only useful for bedside assessment of severe dilation of the right chambers[32]. If more accurate measurements are needed, radionuclide based methods, CT or cardiac magnetic resonance imaging (MRI) should be preferred[57;58].

Enhanced CT technology has allowed for improved delineation of the cardiac chambers. The dilation of the RV compared with the LV size (RV/LV-ratio) on non-ECG-gated CT of the thorax has been proposed as a prognostic factor for short-term adverse outcomes in patients with acute PE[59]. But RV/LV-ratio obtained on non-ECG-gated CT cannot give an exact estimation of RV function[60]. As a research tool we used an ECG-gated cardiac CT angiography which created volumetric cardiac cine images and assessed quantitative cardiac function which is comparable with the current criterion standard; MRI[61;62].

However, these medical imaging modalities cannot be used in a daily clinical routine. It is therefore warranted to have simpler and more reproducible methods, which can define potential candidates to therapy, especially in the group of PE patients with normal arterial systemic blood pressure and RVD. This thesis will therefore focus on normotensive patients with PE induced RVD.

Cardiac biomarkers

Biomarkers that are associated with increased pressure in the right heart and in the pulmonary circulation may have clinically relevance in risk stratification in PE patients. Several biomarkers have been studied in patients suspected of acute PE. The natriuretic peptide system has emerged as one of the most important hormonal systems in the control of cardiovascular homeostasis and function. Brain natriuretic peptide (BNP), atrial natriuretic peptide (ANP) and endothelin-1 (ET-1) seem to be the most promising biomarkers in the context of risk stratification in PE and assessment of RVD.

Cardiac electron microscope studies have demonstrated that atrial cardiocytes, contains features associated with polypeptide hormone-production. Based on these findings ANP were identified in the human heart in 1984 and BNP in 1989. This demonstrated a new functional endocrine role of the heart in the modulation of blood volume and vascular tone[63]. ET-1 was identified in 1988 and is released with increased pressure from the endothelium of the pulmonary vascular bed[64]. ANP, BNP, and ET-1 are synthesized as amino acid precursor proteins and undergo intracellular modification to prohormones.

ANP is primarily produced in the atria and is released by atrial distention[65]. ANP mRNA encodes a precursor protein of 151 amino acids (prepro-ANP). Removal of a 25-amino acids signal peptide results in a 126 amino acids pro-ANP. Upon secretion, pro-ANP is further processed into a circulating active form (ANP-28) and its N-terminal fragment[66;67]. Secretion of ANP has a very rapid response and release into circulation from a pool of previously stored hormones in secretory granules of atrial cardiomyocytes. The active form of ANP is rapidly cleared from the circulation with a half life of 3-4 min. Pro-ANP has a much longer half-life (60-120 min) which leads to higher concentrations in blood compared to ANP-28. As circulating levels of pro-ANP are less sensitive to rapid fluctuations of ANP-28 levels, they better reflect the total amount of secreted ANP. Regulation of ANP release occurs mainly at the level of hormone secretion and a critical decrease in ANP stores must be reached before transcriptional activation occurs[68].

The plasma level of ANP is elevated in patients with chronic pulmonary hypertension[69] and seems to correlate with mean pulmonary arterial pressure, right atrial pressure, RV end-diastolic

pressure, and total pulmonary resistance[70;71]. Thus, it could be an early marker for RVD. However only limited data are available for ANP in relation to acute PE[72].

BNP mRNA is translated into prepro-BNP containing 134 amino acids. A 26-amino acid signal peptide is cleaved of, resulting in the 108-amino acids long prohormone termed pro-BNP. Pro-BNP is sequestered in storage granules and cleaved into a 76-amino acids N-terminal fragment (NT-pro-BNP) and the active hormone (BNP-32) on release into circulation[73]. BNP is constitutively released and is secreted in burst from physiological and patophysiological stimuli[74;75]. The regulation of BNP release occurs mainly at the level of gene transcription with only minor stores of pro-BNP and processed BNP within cardiomyocytes. The cells are thus dependent on activation of the BNP gene resulting in increased gene expression. In contrast to ANP, BNP gene expression can increase very rapidly in response to an appropriate stimulus[52]. ANP and BNP appear to form a dual, integrated system, with ANP as a very rapidly responding hormone, and BNP may be a backup hormone activated after extended cardiac strain. The natriuretic peptides are biologically active substances capable of counteracting rising cardiac preload by increasing glomerular filtration rate and inhibiting of sodium reabsorption in the collecting duct causing natriuresis and diuresis. Natriuretic peptides relax vascular smooth musculature, causing arterial and venous dilation and leading to reduction in systemic arterial blood pressure (decrease in afterload). Additionally, the hormones inhibit the renin-angiotensin-aldosterone axis[76]. The natriuretic peptides exert their effects on the target cells through interactions with specific surface located receptors linked to the cGMP-dependent signalling cascade[77;78].
In the normal functioning heart the atria is the main source of both BNP and ANP. However with chronic myocyte stretch there is an upregulation of ventricular natriuretic peptide secretion[79]. Hence, both ANP and BNP are released from the left ventricle (LV) in response to increased filling pressure as seen in chronic left heart failure[80-82]. Moreover, an acute increase in both atrial and ventricular BNP mRNA levels by pressure overload in vivo occurs within one hour and corresponds well with observed increase in plasma BNP levels[83].
So while the role of BNP in relation to LV failure is known for many patient groups, the potential role of elevated BNP in the differentiation of patients with PE and RVD has not been fully established. Indeed, two recent meta-analysis demonstrated an association between elevated BNP plasma concentration and the presence of RVD in patients with PE[84;85]. However, echocardiography[86-92] or non-gated pulmonary CT[93] which are not very precise methods was used in most studies to assess the association between biomarkers and the degree of RVD. Moreover, most of the patient groups in the present studies investigating BNP in relation to PE and RVD have been mixtures of hemodynamically stable and unstable patients yielding higher prevalence of RVD and PE than is normally the case. Likewise, RVD definition differs between the studies.
ET-1, which is found abundantly in the lungs, is an extremely potent vasoconstrictor and bronchoconstrictor. ET-1 is released by stimuli such as endothelial stretch in the pulmonary vascular bed, endothelial injury, hypoxia, or exposure to thrombin, a key product in the coagulation cascade[94]. ET-1 is produced from a precursor (prepro-endothelin). After the removal of a signal peptide, it is selectively processed to yield big-ET-1, a biologically inactive intermediate. Big-ET-1 is further converted into active ET-1 (21–amino acids peptide) by endothelin-converting-enzyme. ET-1 elicits vasoconstriction through selective endothelin receptors. The receptors are located in myocytes, fibroblasts, and smooth muscle cells especially in the endothelium of the pulmonary circulation[95].
Plasma ET-1 is correlated strongly with pulmonary pressure and numerous studies has shown increased levels of ET-1 both in patients and in experimental animal models with chronic thromboembolic pulmonary hypertension[96;97]. Furthermore an increased level of ET-1 both in the arterial and venous plasma has been reported in an experimental canine PE model[98] and similar results have been reported in sheeps[99] and rats[100]. In keeping with these animal experimental findings, one human study has shown an increase in the ET-1 plasma level of patients in the early phase of a PE[101].
Previous studies have shown that the gene expression of BNP, ANP and ET-1 is upregulated both in the RV and LV in an animal model of chronic pulmonary hypertension[102-105]. However, the exact nature and source of BNP, ANP and ET-1 expression and secretion following PE has not previously been studied.
The troponin complex consists of proteins of the thin filaments that regulate the contraction of cardiac and skeletal muscles. When cardiac myocytes are injured, the cytosolic components of troponins are released first. Following an irreversible injury, myocyte cell membrane degrades and troponin stored in the myofilaments are released gradually[106]. Serial measurement of plasma cardiac Troponin I (TnI) has become an important tool for diagnosis and risk stratification of patients suspected of or presenting with acute coronary syndrome. However, cardiac troponins are also raised in many patients presenting with conditions other than acute coronary syndromes[107]. In patients with PE, RVD can induce troponin release from injured cells of the right ventricle[108]. A number of studies involving patients suspected of PE have evaluated the correlation between elevated TnI plasma level and short term clinical outcome, prolonged hypotension, cardiogenic shock, predictor of 30-day mortality and RVD at echocardiography[109-111]. However no studies have so far established the precise correlation between TnI and RVD using an exact measurement of RV function.

Hypothesis

Our hypothesis was therefore that the gene expression of ANP, ET-1 and BNP was upregulated in the RV during PE and that the neuroendocrine activation measured as the plasma level of ANP, BNP, ET-1, and TnI could serve to diagnose RVD and was correlated with extent of RVD. Moreover, our hypothesis was that combined SPECT-CT scanners could have a role in modern diagnostics of PE.

Aims

Hence, the overall aim of the present study was to evaluate different methods for the diagnosing of PE and to investigate the role of biomarkers during RVD and their potential role in detection of RVD in patients suspected of PE.

The specific objectives, corresponding to papers I-III were

I. In a prospective design to study the diagnostic ability of V/Q-SPECT compared with that of pulmonary MDCT-angiography obtained simultaneously in patients suspected of having PE.

II. To investigate the diagnostic value of plasma levels of pro-ANP, BNP, ET-1 and TnI for the detection of RVD as observed on an ECG-gated cardiac MDCT scan in patients suspected of PE.

III. To quantitatively study the gene expression of BNP, ANP and ET-1 in all 4 cardiac chambers in rats during PE and to investigate the association between the plasma concentrations of the biomarkers and their local gene expression in the heart.

MATERIALS & METHODS

Animal studies

Animal model of pulmonary embolism

In a rat model polystyrene microspheres were used to create PE. Injection of polystyrene microparticles is an established model for experimental PE. Microparticles occlude the pre- capillaries in the lungs with physiological alterations similar to PE as observed in humans and mimicking RVD[112-117]. The rats were randomly assigned into 4 groups. Microspheres were injected via a tail vein to produce mild (0.87 million beads /100 g body weight, referred to as PE 0.87), moderate (1.30 million beads /100 g body weight, referred to as PE 1.30) and severe PE (1.95 million beads /100 g body weight, referred to as PE 1.95). The control group consisting of sham animals received vehicle alone. After 16 hours of treatment, the animals were euthanized and blood was collected and the RV, LV, right atrium (RA) and left atrium (LA) was excised.
The method allows accurate grading of the pulmonary vascular occlusion. Pulmonary vascular resistance ensues and afterload increases with an increasing load of microparticles.
It has previously been demonstrated that the induction of PE 1.95 initially produces a peak reduction in mean arterial systemic blood pressure of approximately 25% from basal measurements followed by partial recovery of arterial blood pressure to ~10% below basal level. This dose also causes the *in vivo* RV systolic blood pressure to increase from 30 mmHg at baseline to 55 mmHg measured 30 min after embolization, suggesting a ~75% pulmonary vascular occlusion. The group is also characterized by a high mortality and systemic hypoxemia. PE 1.30 produces a mean RV systolic pressure of ~40 mmHg two hours after injection with normal systemic arterial blood pressure[118;119].

Quantitative real-time PCR

To study regulation of ANP, BNP, and ET-1 gene expression in our rat model of PE we used quantitative real-time polymerase chain reaction (QPCR).
The QPCR assay was developed by Heid et al in 1996[120] and is a quantitative method for assessing expression of specific genes of interest (GOI) in tissue. It is a system which couples product amplification by PCR to fluorescence intensity carried by a reporter molecule. The method is based on the following steps 1) extraction of total RNA (ribonucleic acid) from the tissue samples 2) reverse transcription of the RNA into cDNA (complementary deoxyribonucleic acid) - using a viral enzyme, a transcriptase and 3) amplification of the target cDNA using polymerase chain reaction (PCR). The amplified cDNA is detected and quantified as it accumulates in the reaction after each amplification cycle. The first PCR cycle at which the fluorescence of the amplified target product can be detected significantly above the background signal is termed the threshold cycle (C_t). The C_t value associates with the start amount of target which means that a low C_t correlates a higher initial target level (because the fluorescence increase above background level at an earlier cycle).
The detection of the amplified cDNA was based on sequence-specific fluorescent probes (TaqMan® probes). The probes consists of a reporter flourophore at the 5`-end and a quencher fluorophore at the 3`-end. By adding DNA Taq Polymerase the 5`-3`-exonuclease activity of the enzyme will cause cleaving of the probe and releasing of both flourophores resulting in the reporter flourophore to fluoresce. The advantage of using the TaqMan® chemistry (apart from a higher specificity for the investigated genes) is the ability to measure several genes on the same template (multiplex assays).
In the study all gene expressions were calculated using the comparative method, $2^{-\Delta\Delta Ct}$[121], whereby the GOI is initially normalized to a "housekeeping" gene. The housekeeping gene is per definition a gene that is constantly expressed, unaffected by the experimental conditions[122]. The optimal housekeeping gene for this study was selected from a panel of 8 common rat endogenous genes: ACTB (NM_031144), B2M (NM_012512), GAPDH (NM_017008), HPRT1 (NM_012583), RPL13 (NM_031101), TBP (NM_001004198), UBC (NM_017314) and 18S (NC_001665). The gene expression stabilities of the candidate reference genes were compared using the software program NormFinder[123]. The "NormFinder" algorithm calculates a stability value for every gene tested using a statistical approach to estimate overall gene expression variation whereby the most stable reference gene can be established. All genes were tested in tissues from rat hearts representing the 4 groups: 3 treated and 1 untreated (n=10 in each group). The housekeeping gene that was found to be the overall most stable was TBP (TATAA-box Binding Protein).
In the present study relative quantification expressed as fold changes was achieved. First all GOIs from each animal samples (RV, LV, RA, and LA) were quantified, then normalized to the housekeeping gene, TBP and finally related to the untreated sample group (calibrator group). In addition, RV was used as a calibrator group in order to calculate the relative contribution of the chambers.

Blood sample analysis

After euthanizing the rats, blood was collected in EDTA containers with 100 µL Trasylol, 10.000 KIE/mL, centrifuged, and plasma was transferred to a fresh tube and stored at −80 °C until analyzed.
The active form of BNP differs between rat and human. In the rat the active form is BNP-45. The kit was based on enzyme immunoassay and had a cross reactivity of 100% between BNP-45 and BNP-32. The sensitivity of the assay was 0.3 ng/mL and the intra- and interassay coefficients of variation (CV) were <5% and <14%, respectively.
For ET-1, pro-ANP, TnI and D-dimer plasma analyzing, see blood sample analysis in the Clinical studies section.

Clinical studies

Patients and study design

The patients were recruited consecutively and prospectively at Rigshospitalet and Frederiksberg Hospital, Copenhagen, from June 2006 to February 2008. All patients were referred to the Departments of Nuclear Medicine at Rigshospitalet or Frederiksberg Hospital, to a V/Q-SPECT as first line imaging procedure. Patients were eligible if there was suspicion of PE, defined as an acute onset of new or worsening shortness of breath or chest pain without any obvious cause combined with a positive D-dimer (>0.5 mg/mL) or a clinical assessment with a Wells-score>2[124] (table 1). Most of the patients referred to our department to a

V/Q-SPECT either have low or moderate clinical probability according to the Wells score. We used Wells score[125] and a positive D-dimer test as inclusion criteria in order to increase the pre-test probability of PE and RVD and thereby increase the power of the study.

All patients consented to diagnostic testing, including a pulmonary MDCT angiography, a low-dose CT scan without contrast enhancement, a V/Q-SPECT, blood pressure measurement and blood sampling. All scans were performed on a dedicated hybrid SPECT/MDCT-16 slice scanner in the same session.
All patients had at least six months of follow-up after the scans with telephone interview and review of hospital files.
During the 18-months recruiting period, 196 patients were screened and 100 were eligible for the study. Of the 100 patients included in the study, six patients were excluded due to poor contrast enhancement in the pulmonary vessels and eight were excluded due to V/Q-SPECT being non-interpretable in the sense of poor technical quality.

Clinical Feature	Score
Clinical signs and symptoms of DVT (objectively measured leg swelling and pain with palpation in the deep-vein system)	3.0
Heart rate >100 beats/min	1.5
Immobilization for ≥3 consecutive days (bed rest except to go to bathroom) or surgery in previous 4 weeks	1.5
Previous objectively diagnosed pulmonary embolism or DVT	1.5
Hemoptysis	1.0
Cancer (with treatment within past 6 mo or palliative treatment)	1.0
Pulmonary embolism likely or more likely than alternative diagnoses (on the basis of history, physical examination, chest radiography, ECG, and blood tests)	3.0

Table 1. Model for determining the clinical probability of pulmonary embolism, according to the Wells score. The condition of patients is scored according to the following criteria: less than 2.0 low probability: 2.0 to 6.0 moderate probability: and more than 6.0 high probability. DVT: deep venous thrombosis: ECG: electrocardiography[126].

V/Q-SPECT
Nuclear medicine imaging uses radioactive isotopes (radionuclides) and relies on the process of radioactive decay and emitting photons. The photons can be detected using a gamma camera. This technique is known as scintigraphy and produces scintigrams. Radionuclides can be combined with other chemical compounds or used alone and once administered to the patient, can localize specific organs or cellular receptors. This unique ability of radionuclides allows a noninvasive quantification and spatial localization of physiological, metabolic, or pathological processes rather than relying on the anatomy as opposed to the CT modality. A V/Q lung scan involves simultaneous imaging and evaluation of the distribution of pulmonary blood flow and alveolar ventilation. The ventilation scan (with the use of ^{81m}Kr) assesses the ability of air to reach all parts of the lungs. ^{81m}Kr is an ultra-short lived (T½: 13 seconds) isotope which is eluted of the Rb-Kr generator by oxygen and flows directly to the lungs. By continuous inhalation of ^{81m}Kr the scintigram illustrates the distribution of airflow/ventilation[127].
The perfusion scan shows how blood circulates within the lungs and is most commonly performed in order to check for the presence of a blood clot or abnormal blood flow inside the lungs. Perfusion lung scanning is performed after intravenous (i.v.) injection of radiolabelled microparticles (^{99m}Tc-MAA; macroaggregated albumin) which are trapped in the pulmonary precapillaries on a first pass transit. The principle underlying the diagnosis of PE is that whereas pulmonary perfusion is abnormal, the pulmonary alveolar ventilation usually remains intact as a result of its bronchial ventilation supply (mismatch defect).

SPECT is a tomographic (3-D) imaging technique which acquires multiple 2-D scintigrams from multiple angles. A computer is then used to apply a tomographic reconstruction algorithm to the projections, yielding a 3-D dataset[128;129]. The main advantage of using the SPECT technique compared to planar imaging in relation to V/Q scanning is a higher image "contrast" because superimposing of surrounding normal activity onto lesions are eliminated[130;131] and the images can then be viewed in sagital, axial and coronal views.
In the present study a V/Q-SPECT study was obtained simultaneously in 72 steps a 20 seconds through a 180° projection on a dual headed gamma-camera and was performed immediately after the MDCT acquisition with the patient in a supine position. Accordingly, the total V/Q-SPECT acquisition time was 13 minutes. The perfusion study was performed after i.v. injection of 150 MBq of ^{99m}Tc-MAA. The ventilation study was performed when inhaling of ^{81m}Kr. Both studies were performed with low-energy general purpose collimators and acquired in a 128 x 128 matrix.

In the present study all scintigraphic mismatch defects (> 0.5 segment) were classified as having PE. Using PIOPED criteria is inappropriate since these criteria were derived from a single view ^{133}Xe ventilation and planar perfusion imaging which is very different from V/Q-SPECT[132]. Reinartz et al used a simplified reporting scheme that regarded all mismatches defects as PE resulting in high sensitivity (97%) and specificity (91%) on V/Q-SPECT[133;134].
The best way to report V/Q-SPECT has not been clarified. However, there seems to be consensus about a more simplified reporting scheme in V/Q-SPECT reading[135-138].
The reader was blinded to the clinical history of the patients. V/Q-SPECT datasets were reviewed alone and in combination and fused with the pulmonary low-dose CT without contrast enhancement. Finally Q-SPECT in combination with low-dose CT was reviewed.

Low-dose CT
The use of CT technology has increased rapidly. A CT scanner is an X-ray source where the detector system is mounted opposite on a rotating ring. A motorized table moves the patient through the CT imaging system. At the same time, the X-ray source rotates within the circular opening, and a set of X-ray detectors rotates in synchrony. This results in the generation of a large series of two-dimensional X-ray images obtained around a single axis of rotation. These datasets are finally reconstructed as tomographic images and provides information on the density of tissues derived from the attenuation of X-rays and is derived into anatomical information.
The first CT acquisition in our study consisted of a low dose CT scan without contrast enhancement (140kV, 20mAs/slice, collimator 16 x 1.5 mm, rotation time 0.5 seconds and pitch 0.813, 512 x 512 matrix) and was obtained during tidal breathing. The low-dose CT was used for attenuation correction of the V/Q-SPECT data and for fusion with the V/Q-SPECT images.

Pulmonary MDCT angiography

Pulmonary MDCT angiography was carried out after a deep inspiration breath hold at 120 kV, 230 mAs/slice, in cranial-caudal direction, with 16 x 0.75 mm collimation and pitch 0.94 and rotation time 0.5 seconds and acquired in a 512 x 512 matrix. Biphasic administration of 350 mg iodine/mL contrast media was performed with an automatic injection pump. The first phase consisted of administration of 80 mL contrast at 4.3 mL/s into a cubital vein followed by a saline chaser bolus of 45 mL injected with a flow rate of 4.3 mL/s.
To ensure optimal opacification of the lung arteries the scan was initiated using bolus tracking, entering a circular region of interest in the trunk of the pulmonary artery. The threshold for triggering was preset at 100 Hounsfield units (HU). Images were acquired with the maximum intensity of radio-opaque contrast in the pulmonary arteries. Because of the contrast enhancement, any mass filling defects, such as an embolus, will appear dark in place of the contrast, filling the space where blood should be flowing into the lungs.
All angiographic scans were evaluated blinded to clinical history by two experienced readers in evaluating pulmonary MDCT angiography.

ECG gated cardiac MDCT angiography
The third MDCT acquisition was focused on the cardiac cavities and function and consisted of an electrocardiogram (ECG)-gated MDCT angiographic examination of the entire heart (140 kV, 500 mAs/slice, 16 x 0.75 mm collimation, pitch of 0.298, and rotation time of 0.4 seconds) in a cranial caudal direction after a deep inspiration. Cardiac images were obtained after a 10 second standardized delay with administration of 45 ml of contrast at 4 mL/s followed by saline chaser bolus of 50 mL injected with a flow rate of 4 mL/s.

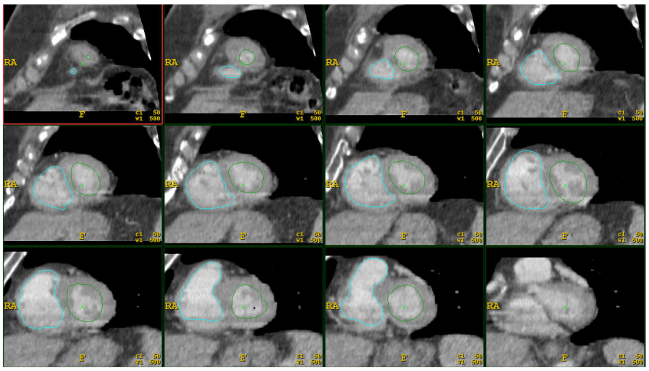

Figure 1. Short axis images of right ventricle and left ventricle with endocardial contours are drawn in both chambers. The blue line delineates the right ventricle endocardium and the green line delineates the left ventricle endocardium.

Each cardiac scan was retrospectively reconstructed in 5% steps, ranging from 5% to 95%, through the entire ECG R-R interval. In this technique, each portion of the heart is imaged more than once while an ECG trace is recorded. The ECG is then used to correlate the CT data with the corresponding phases of cardiac contraction. Multiplanar reconstructions cine images can be created at increments throughout the R-R interval in any scan plan desired.
Image reconstruction was performed with 1 mm slices and contiguous. These images were than reoriented and reconstructed in short axis datasets. Analyses of the reconstructed short axis 5% data sets were performed with dedicated semiautomatic cardiac software analysis tool, which provided semi-automatically segmentation of the endo- and epi-luminal borders of RV and LV (figure 1). The RV and LV basal slice was defined as earlier described[139]. RV and LV EF, RV and LV stroke volume and cardiac output were automatically calculated by the dedicated software.

Gold standard: final pulmonary embolism diagnosis
In our study the PE diagnosis was made at a consensus reading by side-by-side reading of all lesions detected on the pulmonary MDCT angiography, low-dose CT without contrast enhancement, and the V/Q-SPECT using all the available information from ECG, transthoracic echocardiography, Doppler ultrasound examinations of the lower extremities veins, D-dimer levels, clinical data, and follow-up from hospital files or telephone interviews. A negative final PE diagnosis was established in patients if they had no evidence of PE in the clinical data and follow-up including clinical time course, response to treatment within 6 months, or if they died and PE was an unlikely cause of death.

Blood sample analysis
Blood samples were obtained in the supine resting position after 10 minutes of rest. Venous blood was drawn into tubes containing EDTA and 500 µL aprotinin and immediately centrifuged at 2,000 x g for 15 minutes; plasma was then transferred to glass tubes and immediately frozen and kept at –80 °C until analyzed. BNP and TnI were measured by an automated two-site sandwich immunoassay technique using chemiluminescence (Centauer). ANP was measured using an enzyme-linked immunosorbent assay (ELISA) kit and the lower detection limit for NT-pro-ANP (1–98) was 0.05 nmol/L and the intra- and interobserver CV were 2% and 4%, respectively.
The physiologically active C-terminal peptide of BNP-32 was measured. The sensitivity of the assay was 2 pg/mL and the intra- and interassay CVs were 1.2% and 2.3%, respectively.
The sensitivity of the TnI assay was 0.015 ng/mL and the interassay CV was 10%.
ET-1 was measured using ELISA. The assay measures the physiologically active ET peptide (ET-1 1-21). The lower detection limit was 0.02 fmol/mL and the intra- and interassay CV were 4% and 6%, respectively.
D-dimer was analyzed at the time of the scan. The intraassay CV was below 5%, and the interassay CV was below 6%. The detection threshold was 0.20 µg/mL. The dynamic range was from 0.20 to 20 µg/mL.
All blood samples were analyzed at Cluster for Molecular Imaging, University of Copenhagen, Denmark, except for the D-dimer, which was analyzed at The Department of Clinical Biochemistry at Frederiksberg Hospital or Rigshospitalet (Tina quant, Roche Diagnostics, Hvidovre, Denmark). The intraassay CV was below 5% and the interassay CV was below 6%. The detection threshold was 0.20 mg/mL. The dynamic range was from 0.20 to 20 mg/mL.

Statistical analysis
Diagnostic performance was calculated as sensitivity, specificity, positive predictive value, negative predictive value and accuracy. 95% confidence intervals (95% CI)[140] were calculated on the parameters.
Descriptive data and biomarker levels were presented as mean ± SD or median (interquartile range). A one-sample Kolmogorov-Smirnov test was performed on all variables to test for the assumption of normal distribution. The hormone values demonstrated a right skewed distribution and accordingly $\log_{10}$ transformation was performed leading to normal distribution. Means were compared using a two-sample t-test. We examined the

diagnostic performance of each biomarker using receiver operating characteristic (ROC) curve analysis with area under the curve (AUC). Fisher's exact test and Chi-square statistics was used when comparing the number of categorical variables between two independent groups. Correlation between hormones and cardiac parameters was tested for by linear regression. R (Pearson's correlation coefficient) analyzed correlations between the tested parameters. To assess the dependence between the tested variables, multivariate linear regression was performed with backward stepwise elimination of least and non-significant parameters. We subsequently performed multinomial logistic analyses to determine the independent predictors for the occurrence of RVD for the cardiac biomarkers. The odds ratios (ORs) and 95% CI were calculated to compare the prognostic value of each variable. Gene expression data were presented as mean ± SEM. Comparisons between gene expression levels in the four cardiac chambers were made by t-tests followed by Bonferroni correction for multiple comparisons. P<0.05 was considered significant.

Ethics

The regional ethical committee approved the clinical study and the study protocol was compliant with guidelines in the Helsinki Declaration on Human Experimentation. Written, informed consent was obtained from all participating patients. Included patients received an additional ~20 millisievert (mSv) in radiation dose from the CT scans compared to the standard evaluating procedure, 11 mSv from the pulmonary MDCT angiography[141], <1 mSv from the low-dose CT[142], and approximately 8 mSv from the cardiac MDCT angiography[143].

The natural risk of fatal cancer in the Danish population is about 20%, meaning that the risk of getting a fatal cancer[144] increased from 20% to about 20.1% for the included patients.

The animal studies were conducted under the approval of the Danish Animal Welfare Council.

RESULTS

Study I

Data from a total of 81 patients were available for analysis of the diagnostic performance of the different imaging modalities. Of these 81 patients, 31 (38%) had PE. There were no difference in patient characteristics between the group with PE and the group without PE, except for D-dimer plasma level which was increased in patients with PE. Patient characteristics are presented in table 2.

	No PE (n=50)	PE (n=31)	P-value
Age (years)	68 (±12)	63 (±14)	0.14
Height (cm)	171 (±11)	175 (±9)	0.33
Weight (kg)	74 (±18.2)	81 (±23.5)	0.18
BMI (kg/m^2)	25.1 (±5.6)	26.5 (±6.6)	0.30
Systolic BP (mmHg)	138 (±20)	133 (±17)	0.31
Diastolic BP (mmHg)	78 (±15)	82 (±13)	0.57
Pulse (bpm)	82 (±18)	87 (±14)	0.88
Wells score	2.2(±1.8)	3.1(±2.4)	0.05
D-dimer (mg/mL)	2.0(±2.0)	5.0(±4.8)	>0.0001#

Table 2. Patient characteristics. Values are mean (± SD). BP: blood pressure. BMI: body mass index. Bpm: beats/minute. # Statistics are based on log 10 transformed values.

V/Q-SPECT scan alone had a sensitivity of 97% (95% CI: 82-100%) and a specificity of 88% (95% CI: 75-95%). When a low-dose CT scan was added to the V/Q-SPECT investigation, the sensitivity was still 97% (95% CI: 83-99%) but the specificity increased to 100% (95% CI: 93-100%). Pulmonary MDCT angiography alone had a sensitivity of 68% (95% CI: 49-83) and a specificity of 100% (95% CI: 93-100%). Q-SPECT in combination with a low-dose CT scan had a sensitivity of 93% (95% CI: 81-98%) and a specificity of 51% (95% CI: 43-55%).

V/Q-SPECT	
Sensitivity	97 (82-100)
Specificity	88 (75-95)
PPV	82 (65-93)
NPV	98 (88-100)
Accuracy	91 (83-93)
Non-diagnostic rate	5 (1-12)

Q-SPECT + low-dose CT	
Sensitivity	93 (81-98)
Specificity	51 (43-55)
PPV	57 (49-60)
NPV	91 (76-98)
Accuracy	68 (58-72)
Non-diagnostic rate	17 (10-28)

V/Q-SPECT + low-dose CT	
Sensitivity	97 (83-99)
Specificity	100 (93-100)
PPV	100 (88-100)
NPV	98 (90-100)
Accuracy	99 (93-100)
Non-diagnostic rate	0 (0-4)

Pulmonary MDCT angiography	
Sensitivity	68 (49-83)
Specificity	100 (93-100)
PPV	100 (84-100)
NPV	83 (71-92)
Accuracy	88 (78-94)
Non-diagnostic rate	0 (0-4)

Table 3 summarizes the diagnostic performance of the imaging modalities.

V/Q-SPECT	
Sensitivity	97 (82-100)
Specificity	88 (75-95)
PPV	82 (65-93)
NPV	98 (88-100)
Accuracy	91 (83-93)
Non-diagnostic rate	5 (1-12)

Q-SPECT + low-dose CT	
Sensitivity	93 (81-98)
Specificity	51 (43-55)
PPV	57 (49-60)
NPV	91 (76-98)
Accuracy	68 (58-72)
Non-diagnostic rate	17 (10-28)

V/Q-SPECT + low-dose CT	
Sensitivity	97 (83-99)
Specificity	100 (93-100)
PPV	100 (88-100)
NPV	98 (90-100)
Accuracy	99 (93-100)
Non-diagnostic rate	0 (0-4)

Pulmonary MDCT angiography	
Sensitivity	68 (49-83)
Specificity	100 (93-100)
PPV	100 (84-100)
NPV	83 (71-92)
Accuracy	88 (78-94)
Non-diagnostic rate	0 (0-4)

Table 3 Diagnostic performances of the imaging modalities. All values are presented in percentages (95% CI). PPV: positive predictive value, NPV: negative predictive value.

Study II

A total of 71 patients were available for analysis of the potential role of biomarkers in the diagnosis of RVD in patients suspected of PE. Of these 71 patients, 29 (41%) had PE and 7 (24%) of the patients with PE had RVD. There was no difference in biomarker levels, except for D-dimer which was increased in patients with PE (p=0.0001). **Table** 4 displays the biomarker levels for patients according to PE classification.

	No PE (n=42)	PE (n=29)	P-value#
D-dimer (mg/L)	1.3 (0.7-2.9)	3.1 (2.0-7.1)	0.0001
Pro-ANP (nmol/L)	3.78 (2.59-5.42)	3.63 (2.25-9.12)	0.86
BNP (pg/mL)	36.9 (15.7-141.4)	29.5 (9.0-228.9)	0.82
ET-1 (fmol/mL)	1.32 (0.68-2.13)	1.71 (0.60-2.60)	0.88
TnI (mikrog/L)	0.006 (0.002-0.020)	0.007 (0.003-0.025)	0.38

Table 4 Biomarker levels for patients according to PE classification. Values are median (interquartile range), #: t-test on log10 transformed data.

Patients with RVD had significantly higher plasma levels of BNP (p=0.02), pro-ANP (p=0.005), and D-dimer (p=0.003) than patients without RVD (table 5).

	No RVD (n=22)	RVD (n=7)	P-value[#]
D-dimer (mg/L)	3.0 (1.8-5.3)	8.6 (3.0-15.8)	0.003
Pro-ANP (nmol/L)	3.57 (1.87-4.66)	9.12 (4.51-10.00)	0.005
BNP (pg/ml)	19.6 (8.2-47.9)	251.0 (103.8-672.4)	0.02
ET-1 (fmoll/ml)	1.53 (0.55-2.32)	2.60 (0.63-4.10)	0.29
TNI (µg/l)	0.007 (0.003-0.168)	0.006 (0.001-0.071)	0.97

Table 5. Biomarker levels for patients with PE according to RVD classification. Values are median (interquartile range), #: t-test on log10 transformed data.

In figure 2, AUCs and ROC curves are presented for each biomarker based upon RVD classification. Pro-ANP, BNP, and D-dimer all had significant AUC (compared to the random ROC curve) in the ROC analysis. Arrows indicate cut-off values yielding the highest accuracy.

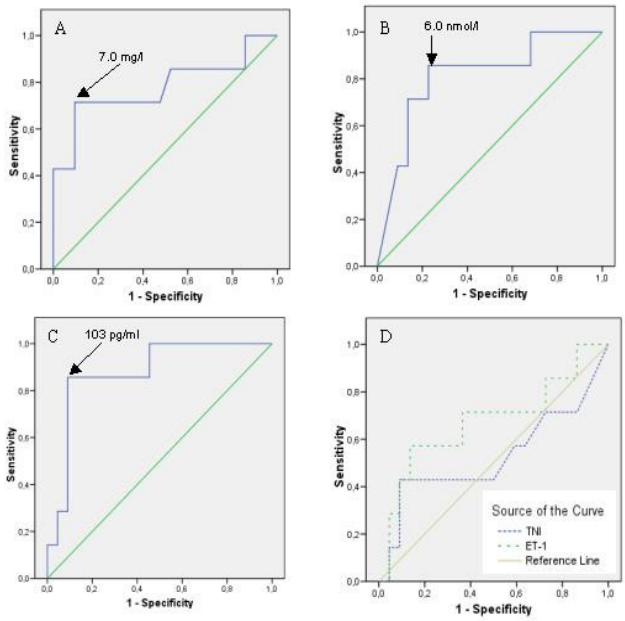

Figure 2. ROC curve analysis in classifying RVD for D-dimer, AUC= 0.78 (95% CI: 0.54-1.00;, p=0.03; panel A), Pro-ANP, AUC= 0.82 (95% CI: 0.63-1.00; p=0.01; panel B), BNP, AUC= 0.88 (95% CI: 0.74-1.00; p=0.003; panel C) and TnI (AUC= 0.51 (95% CI: 0.21-.081; p=0.94; panel D) and ET-1 (AUC= 0.67 (95% CI: 0.42-0.93; p=0.18; panel D) in all patients with PE. Arrows indicate cut-off values yielding highest the accuracy.

Sensitivity, specificity, negative predictive value and positive predictive value for diagnosing RVD in patients with PE were calculated for different cut-off values of D-dimer, pro-ANP, and BNP.

At the most accurate cut-off value of D-dimer (7.0 mg/L), the sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 71% (95% CI, 41-88), 91% (95% CI, 82-97), 71% (95% CI, 41-89), 91% (95% CI, 82-97) and 86% (95% CI, 73-95), respectively (figure 2, panel A). At the most accurate cut-off value of pro-ANP (6.0 nmol/L), the sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 71% (95% CI, 41-90), 86% (95% CI, 77-92), 63% (95% CI, 36-79), 91% (95% CI, 80-97) and 83 (95% CI, 68-91), respectively (figure 2, panel B). At the most accurate cut-off value of BNP (103 pg/mL), the sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 86% (95% CI, 73-95), 91% (95% CI, 82-97), 75% (95% CI, 50-90), 95% (95% CI, 80-97) and 90% (95% CI, 73-96), respectively (figure 2, panel C). ET-1 and TnI could not discriminate PE patients with RVD (figure 2, panel D).

In univariate linear regression analyses, RVEF was associated with plasma levels of BNP (R=0.41, RVEF = 58.7 – 5.4 x log BNP, p=0.03) and D-dimer (R=0.45, RVEF = 57.4 – 12.9 x log D-dimer, p=0.02). In multiple linear regression analysis including all cardiac biomarkers in the model, only log D-dimer remained in the final model as an independent factor associated with RVEF.

Univariate logistic regression analyses revealed that pro-ANP (OR=1.46 (CI 1.08-1.99), p=0.015) and D-dimer (OR=1.3 (CI 1.04-1.70), p=0.023) were predictive cardiac biomarkers in all patients with PE for the occurrence of RVD. Multinomial logistic regression analysis using D-dimer and pro-ANP revealed that D-dimer was the only predictive independent cardiac biomarker in patients with PE for the occurrence of RVD.

Study III

Pulmonary embolism induced gene expression

BNP

PE dose-dependently increased BNP gene expression in the RV (2-6 fold; p<0.05-0.001) relative to the control group (untreated animal group). In contrast PE dose-dependently decreased BNP gene expression in both the LV (0.6-0.5 fold, p<0.001) and the LA (0.8-0.3, p<0.01-0.001) (figure 3).

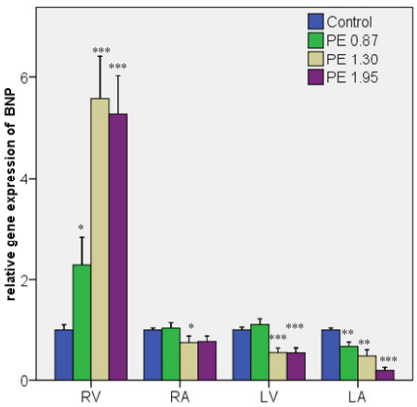

Figure 3. BNP gene expression in the cardiac chambers relative to the control group. Error bars indicates SEM. RV: right ventricle, RA: right atrium, LV: left ventricle, LA: left atrium. *P<0.05; **p<0.01; ***p<0.001 vs. control group.

ANP

PE dose-dependently increased gene expression of ANP in the RV 8-9 fold (p<0.05) and in the RA 1.5 fold (p<0.001) compared to the control group. There was an increase of ANP gene expression in LV in the PE 1.30 group (p<0.05) (figure 4).

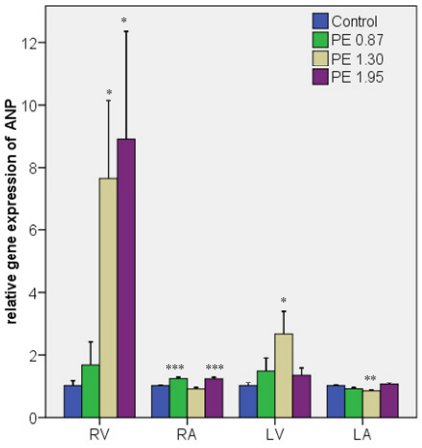

Figure 4. ANP gene expression in the cardiac chambers relative to the control group. Error bars indicates SEM. RV: right ventricle, RA: right atrium, LV: left ventricle, left atrium: LA. *P<0.05; **p<0.01; ***p<0.001 vs. control group.

ET-1

No systematic changes were seen in ET-1 gene expression in the RA, LA, LV, or RV during PE.

Relative gene expression level in the cardiac chambers

BNP

In the untreated animals (the control group), BNP gene expression was 18 fold higher in the RA and 6-7 fold higher in LV and LA relative to BNP gene expression in the RV (p<0.001). In the PE 0.87 group, BNP gene expression in RA decreased to 9 fold higher relative to the RV (p<0.001) and also 9 fold higher compared to LV and LA (p<0.001). In the PE 1.30 group, BNP gene expression in RA was 3.5 fold higher relative to RV (p<0.01) and 3.5 fold higher compared to LA. In the PE 1.95 group, the relative BNP gene expression in the RA was only 1.5 fold higher relative to RV (p<0.01) (figure 5).

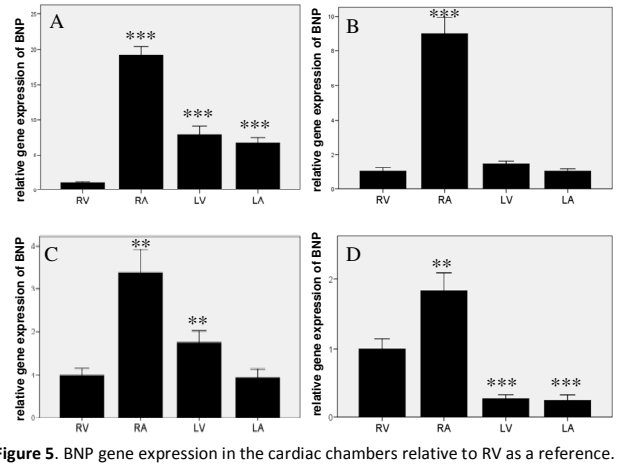

Figure 5. BNP gene expression in the cardiac chambers relative to RV as a reference. Error bars indicates SEM. Panel A shows the control group. Panel B shows the PE 0.87 group. Panel C shows the PE 1.30 group. Panel D shows the PE 1.95 group. RV: right ventricle, RA: right atrium, LV: left ventricle, LA: left atrium. *P<0.05; **p<0.01; ***p<0.001 vs. RV group.

ANP

In figure 6 it is illustrated that ANP was predominately expressed in LA and RA in both the control and the PE treated groups relative to the RV (300-1200 fold; p<0.001). The ANP gene expression in the atria compared to the RV decreased as the degree of PE increased.

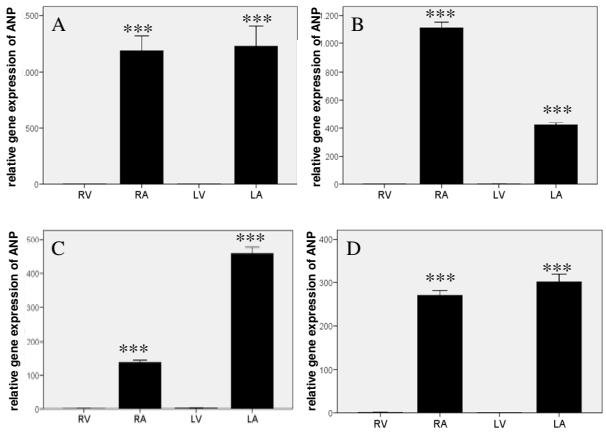

Figure 6. ANP gene expression in the cardiac chambers relative to RV as reference. Error bars indicate SEM. Panel A shows the control group. Panel B shows the PE 0.87 group. Panel C shows the PE 1.30 group. Panel D shows the PE 1.95 group. RV: right ventricle, RA: right atrium, LV: left ventricle, LA: left atrium; ***p<0.001 vs. RV group.

ET-1

Gene levels of ET-1 were primarily expressed in LA (4 fold), LV (2 fold) and RA (4.5 fold) in the control group (p<0.001) compared with RV. This did not change during PE (p<0.001-0.01).

Plasma level of pro-ANP, TnI, ET-1 and BNP

Plasma levels of pro-ANP, TnI, ET-1, and BNP are shown in figure 7.

Plasma BNP level (figure 7, panel A) showed a significant increase from the control group to PE 1.30 and a decrease from PE 1.30 to 1.95.
There was no significant change in the pro-ANP plasma level (figure 7, panel B).
Plasma ET-1 level (figure 7, panel C) and TnI (figure 7, panel D) significantly and dose-dependently increased after 16 hours in the groups with PE.

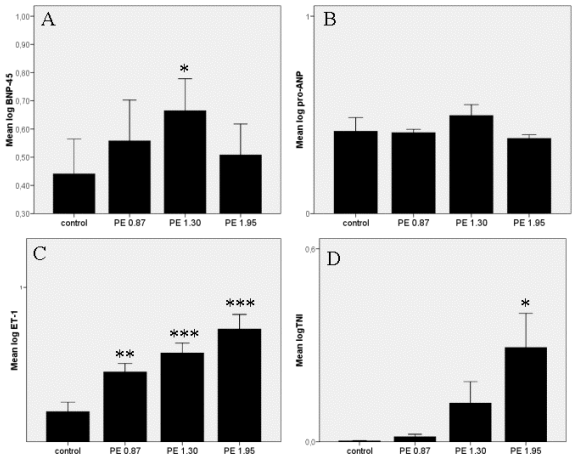

Figure 7. Plasma hormone levels of BNP, pro-ANP, ET-1 and TnI. Error bar represents SEM. Panel A shows mean log BNP. Panel B shows mean log pro-ANP. Panel C shows mean log ET-1. Panel D shows the mean log TnI. *P<0.05; **p<0.01; ***p<0.001 compared to the control group.

DISCUSSION

Diagnostic performance of V/Q-SPECT and CT in pulmonary embolism

This is to our knowledge the first prospective study (I) on simultaneously obtained V/Q-SPECT and pulmonary MDCT angiography. We found that a V/Q-SPECT had a sensitivity of 97% and a specificity of 88%. When adding the information of a low-dose CT scan, the sensitivity was still 97% but the specificity increased to 100%. A MDCT angiography alone had in our study a sensitivity of 68% and a specificity of 100%.
We found good agreement with regard to sensitivity, specificity and accuracy to a previous retrospective study that had values of 97%, 91%, and 94%, respectively for V/Q-SPECT alone and values of 86%, 98%, and 93%, respectively for MDCT[145].
The use of SPECT technique in our study showed to have a much lower frequency of equivocal tests than is known from traditional planar lung scans which in previous studies have been reported to have up to 73% non-conclusive exmaniations[146]. This is in accordance with previous studies which demonstrated that the use of SPECT in V/Q scintigraphy reduced the frequency of equivocal tests markedly[135;147;148].
The addition of low-dose CT without a contrast agent to the V/Q-SPECT resulted in an even higher confidence of the reading with a reduction of inconclusive studies from 5% with SPECT alone to 0% with SPECT + low-dose CT. In addition, the specificity was improved with fewer false positive interpretations (from 18% to 0%). This was mainly due to findings on the low-dose CT scan that gave alternative explanations for subtle perfusion defects that else would have been interpreted as PE on SPECT alone. Although a low dose CT scan without a contrast agent is inherently inferior to those acquired by a diagnostic CT scan with a contrast agent, the low dose CT scan provided satisfactorily relevant diagnostic information to determine the origin of the V/Q-SPECT lesions. When assessing the V/Q-SPECT datasets alone, six patients obtained a false positive PE diagnose, three patients had mismatched defects on the V/Q-SPECT scans due to interlobar fissures, and three patients with COPD had mismatched defects because of paraseptal emphysema, pneumonic infiltration, atelectasis, and pleural fluid. These lesions were all demonstrated on the low-dose CT.
We reported all mismatched defects (> 0.5 segment) on the V/Q-SPECT as PE. Alternative reasons for mismatched V/Q defects include non-embolic diseases such as pulmonary vasculitis and extrinsic vascular compression, such as vessel stenosis, lung cancer, nodal enlargement, and therapeutic interventions such as radiation therapy[127;149]. However, such differential diagnoses could be excluded herein by follow-up, history, blood samples, and, most importantly, findings on the head-to-head comparison with pulmonary MDCT angiography, so that the risk of an incorrect conclusion was further reduced.
The fact that we did not have an independent gold standard for establishing the PE diagnosis produced difficulties for the evaluation and comparison of the diagnostic accuracy of different modalities in PE. In order to compare the diagnostic performance of the tested modalities, we used a combination of composite and head-to-head consensus reading as criterion standard. The use of this combined method which includes all tested modalities to classify PE patients raises methodological and conceptual problems and is controversial. It is important to keep in mind the possibility that some patients may be incorrectly assigned to a disease category by the examination that are being studied giving exaggerated or underestimated accuracies. This concern was also commented on in an invited perspective with reference to our study[150] published in the Journal of Nuclear Medicine[151]. However we believe that our approach can be justified and as long as the results are viewed in that respect we believe it was the best available criterion standard.

Plasma levels of biomarkers in relation to right ventricular dysfunction

We demonstrated that the plasma levels of BNP, pro-ANP, and D-dimer can be used in discriminating between RVD and no RVD in PE patients (II).
To the best of our knowledge this is the first study in which significant differences are shown in pro-ANP plasma levels in PE patients with and without RVD. At a cut-off value of pro-ANP of 6.0 nmol/L, the sensitivity, specificity, positive predictive value, and negative predictive value were 71%, 86%, 63%, and 91%, respectively for discrimination of RVD. The high negative predictive value indicates that pro-ANP might be a valuable tool to exclude patients from having RVD.
In animals with chronic RV dysfunction ANP plasma level is correlated to RVEF[152]. In patients with RV overload due to atrial septal defect and primary or thromboembolic pulmonary hypertension, ANP plasma level is correlated with mean pulmonary arterial pressure, RA pressure, RVEF, RV end-diastolic pressure, cardiac output, and total pulmonary resistance[153;154]. However, in the present study we did not find a correlation between RVEF as a continuous variable and pro-ANP. However we found that pro-ANP was predictive of RVD in patients with PE in the logistic regression analysis.

We also demonstrated that patients with acute PE and RVD had significantly higher plasma BNP concentration than patients with PE and no RVD. We determined that plasma BNP at a cut-off

value of 103 pg/mL had a sensitivity, specificity, positive predictive value, and negative predictive value for detecting RVD which was comparable to previous PE studies. Previous studies have shown that a BNP cut-off value of 90 pg/mL had a sensitivity and specificity of 94% and 92%, respectively[155] and of 65% and 94%, respectively[156] in patients with PE and RVD. These results support the potential use of plasma BNP as a rule-out-test of RVD in PE patients.
High concentrations of BNP can distinguish PE patients at high risk of complicated in-hospital course and death from those with lower risk. BNP has been shown to have a high negative predictive value (97-99%) for adverse outcomes in normotensive patients with PE. However, the positive predictive value of adverse outcomes of an elevated BNP level is low[157;158] and augmented BNP alone does not seem to justify more invasive treatment regimes[159]. Furthermore, the rationale behind thrombolysis for submassive PE has been challenged[160]. However, it has been suggested that thrombolysis should be considered in patients with high natriuretic peptide level to prevent clinical deterioration in submassive PE[161], This approach is inadvisable because of the low positive predictive values. Hence, the role of natriuretic peptides in the assessment of RVD in PE seems to associate to the excellent negative predictive value.
Plasma BNP is known to correlate with LV dysfunction in LV heart failure[162]. In isolated chronic RVD, BNP level is elevated and correlates positively with mean pulmonary pressure and RV end-diastolic pressure and negatively with cardiac output and RVEF[163;164]. This is in accordance with our study where we demonstrated that RVEF and not LVEF was associated in the regression analysis with BNP plasma level.
An association has been demonstrated between BNP plasma level, PE severity, and degree of RVD on echocardiography[165;166]. BNP plasma concentration has been shown to have prognostic information in PE patients[167]. In our study, difference in BNP plasma levels was seen between RVD and no RVD in PE patients. However, no difference was seen in BNP levels between patients with PE and no PE. This may indicate that the prognostic information of BNP in PE patients may relate to the involvement of the RV.

Additionally we did not find an association between venous plasma levels of ET-1 and PE and RVD. This is in accordance with an earlier study which demonstrated that plasma ET-1 concentration assessed on venous blood was not elevated in patients with acute PE as compared to control subjects, concluding that plasma ET-1 does not have an important role in the acute phase of PE[168]. However it has been reported that a net increase in ET-1 assessed on arterial plasma in PE patients was significantly elevated in PE patients as compared to control subjects[169-171]. In experimental PE models both the expression of the ET-1 gene and the expression of ET-related genes were augmented in the lung tissue after the introduction of PE, demonstrating an activation of the ET-1 system in acute PE[172;173]. However, the clinical significance of ET-1 abnormalities in PE needs to be established.

We showed that at the cut-off value of D-dimer of 7.0 mg/L, the sensitivity was 71% and the specificity was 91% for detection of RVD in patients with PE. D-dimer is currently used to rule out PE due to its high negative predictive value. As D-dimer is a degradation product of fibrin, the degree of elevation of D-dimer could be associated with the extent of thrombus burden. It is hypothesized that a larger thrombus will augment the RV afterload and the RVD due to the increased pulmonary vascular occlusion[174]. A previous study demonstrated that high D-dimer levels at admission was associated with increased risk of mortality in patients with PE[175]. Therefore D-dimer seems to be a promising tool to classify PE patients in RVD, however further studies need to be done.

We did not find an association between TnI and RVD and PE. Elevation of cardiac TnI levels in plasma is a reliable indicator of myocardial injury[176] and correlates significantly with ECG and echocardiography assessment of RV pressure overload and myocardial dysfunction in PE patients[93;177;178]. However detection of a subtle troponin elevation in PE is only possible during a short diagnostic window with a maximum plasma level TnI within 4 hours[179]. Others have stated that the dynamics of cardiac TnI release in acute PE in patients who present with symptoms of less than 72 hours duration could be different from those who present with longer duration of symptoms. Therefore, the use of cardiac TnI in risk stratification of PE might be limited to the patients presenting within 72 hours from the onset of symptoms[180]. This probably resembles the kinetics of TnI in patients with unstable angina pectoris, however with a release of shorter duration[181]. The time interval between symptom onset and diagnostic imaging and biomarker testing in our study may possibly have been too long. We obtained blood sampling at scan time, not at the hospital admission and therefore may have underestimated the significance of TnI elevation in the present study.
Limitations of the study include the small sample size (29 patients with PE and 9 with RVD) which caused the statistical power to decrease and therefore the findings in this study should be confirmed in a large multi-centred study design.

Methodological difficulties are introduced when using ECG-gated cardiac CT in assessing RV function. The technique provides an excellent spatial resolution with an advantageous signal-to-noise ratio; however the temporal resolution of current MDCT technology is still inadequate. In the present study (II) the patients scanned had a relatively high heart rate (mean 85 beats per minute). Because of the relatively limited temporal resolution in CT technology, images obtained in patients with higher heart rate are of lower quality and impair delineation of endocardial contour which can underestimate EF[182;183]. In coronary CT-angiography, beta-blockade is given to reduce the heart rate in order to reduce artifacts in the coronary arteries. Artificially reduced heart rate or contractility of the myocardium with a beta-blocker may underestimate the cardiac function; especially the stroke volume and cardiac output[184]. We did not want to alter the cardiac function of the patients; therefore beta-blockade was not an option in our study.

Gene expression in the cardiac chambers of biomarkers in pulmonary embolism
In this study, we found that gene expression of ANP and BNP were strongly and dose dependently up-regulated in the RV during PE.
We demonstrated that in the control group, the highest level of BNP expression was in the RA followed by the LV, LA, and RV and is in accordance with previous studies[185;186]. In PE, the BNP gene expression is increased in the RV and decreased in the LV thereby making the contribution of BNP secretion from the RV relatively more important. Taking the weight of the different chambers into account, 70% of all circulating plasma BNP derives from the ventricles under normal conditions and up to 88% under pathophysiological conditions[187;188].

Plasma hormone levels of BNP were dose-dependently increased in the PE model. However plasma BNP decreased significantly at PE 1.95 which corresponded to the decrease of BNP gene expression in the RV. This also supports the idea that RV is the dominant chamber in the secretion of BNP in PE.

This study is to our knowledge the first that investigates regulation of gene expression using QPCR in both atria in an animal model of acute PE. It has previous been shown that ANP gene expression in the RV is increased in an animal model of chronic pulmonary hypertension and RV hypertrophy[189;190]. Our results showed that ANP was dose-dependently up-regulated on the right side of the heart in rats with PE.
In the control group and during PE we found that the ANP gene expression was higher in the atria than in the ventricles. Previous studies on tissue specific expression of the ANP gene have shown high-level expression in the RA and virtually undetectable amounts of ANP mRNA and peptides in the LV of healthy adult mammals[191]. Over 90% of ANP released from the normal adult heart originates from the atria[192]. Despite the increase of ANP expression, pro-ANP hormone plasma level was unaffected in our study of experimental PE. The acute response to atrial stretch results in enhanced temporary secretion of the ANP in the atria[193]. The depletable nature of the ANP pool and the relatively low weight of the atrias in rats might explain the lack of a significant increase of ANP secretion during PE[194]. Due to the dynamic nature of the release of these hormones into the plasma it seems essential to study different time points during pulmonary embolism. However, the aim of our study was not to study the fluctuations of the hormone plasma concentration.
This supports the idea that ANP is a very rapidly responding hormone and BNP may be a backup hormone only activated after extended cardiac strain.

PE did not systematically affect the gene expression of ET-1 in the heart probably because ET-1 is mainly synthesized and secreted from the pulmonary endothelial cells[195]. However, ET-1 plasma hormone levels were dose-dependently increased in our PE model. We collected blood from the large vessels after decapitation of the rats, which means that the blood sampled was a mixture of venous and arterial blood. This could explain why ET-1 plasma level was significantly increased in rats and not in the clinical study (II).

Contrary to the clinical study (I) plasma hormone levels of TnI were dose-dependently increased in the PE model (study III). One explanation could be that we collected blood early (16 hours) after PE induction in contrary to the clinical study. Another explanation could be that the rat hearts were under more hypoxic stress and therefore had a higher TnI plasma concentration. Unfortunately, we did not measure oxygen saturation and analyze myocardial damage in the rat heart in order to speculate about the origin of the plasma TnI.

The animal model used to induce PE in rats is an established experimental PE model[196-201]. However, RV function (EF, cardiac output, etc.) has never been studied in this model. With precise measurement of pressure and function of RA, LA, RV, and LV, we could have established the degree of RVD and correlated the degree of pulmonary obstruction with gene expression in the different heart chambers. Regrettably, this was not done. In the moderate and mild PE model, RV generated pressures were increased without altering the LV pressure. But in our rats we actually do not know the precise function of the heart - neither in the PE group, nor in the controls.

CONCLUSIONS

Based on the results of this thesis, it can be concluded that V/Q-SPECT in combination with a low dose CT scan has a high sensitivity and specificity and outperforms MDCT angiography in the diagnosis of PE. Hence, the superiority of MDCT over planar lung scintigraphy does not apply when 3-D scintigraphy is used and low dose CT scan information is added. With use of combined scanners, V/Q-SPECT in combination with low-dose CT without contrast enhancement can “revitalize” lung scintigraphy and should probably be considered first-line imaging test in diagnosing PE.
The present thesis also supports the idea of natriuretic peptides as biomarkers of RVD in PE and it can be concluded that the plasma levels of BNP, pro-ANP, and D-dimer can be used in discriminating between RVD and no RVD in PE patients. Since measurements of cardiac biomarkers are inexpensive and easily obtained they may prove useful in the clinical diagnosis of RVD.
At last, it can be concluded that there is a close correlation between PE degree and gene expression of ANP and BNP in the cardiac chambers with a selective increase in the right chambers of the heart in an experimental PE model. This supports our hypothesis that the origin of ANP and BNP in PE patients is the right side of the heart.

Perspectives and future directions

The present thesis also raises several important questions. Is low-dose CT in fact necessary in combination with V/Q-SPECT or is it enough to perform an x-ray of the chest? Does the higher diagnostic accuracy for V/Q-SPECT also account for decrease in short term and long term morbidity and mortality? The findings of small emboli located in segmental and subsegmental vessels needs also to be discussed; the clinical or prognostic significance of these small mismatched defects is far from clear.
Does a biomarker approach have an influence on short and long term mortality and morbidity? Further studies with greater power are necessary before definite conclusions can be drawn concerning the added value of natriuretic peptides with regard to risk stratifying patients suspected of PE? Randomized studies with extended follow-up time should be carried out in order to establish whether aggressive therapeutic intervention such as thrombolysis or admittance to a cardiology emergency unit can decrease the mortality rate and be beneficial for patients with RVD and PE?
There is no consensus regarding the cut-off values for the investigated plasma markers and an important question is which cut-off value facilitates precise discrimination between patients with PE and without PE or RVD or no RVD? It seems that the biomarkers described are rule out tests, but what about rule-in tests? Measurement of biomarkers in patients with PE might in the future be standard when risk stratifying patients suspected of RVD and useful when choosing the method of intervention. However, the right algorithm still needs to be established.

References

(1) Barritt DW, Jordan SC. Anticoagulent Drugs in the Treatment of Pulmonary Embolism: A Controlled Trial. Lancet 1960; 275(7138):1309-1312.

(2) Anderson FA, Jr., Wheeler HB, Goldberg RJ, Hosmer DW, Patwardhan NA, Jovanovic B et al. A population-based perspective of the hospital incidence and case-fatality rates of deep vein thrombosis and pulmonary embolism. The Worcester DVT Study. Arch Intern Med 1991; 151(5):933-938.

(3) Hansson PO, Welin L, Tibblin G, Eriksson H. Deep vein thrombosis and pulmonary embolism in the general population. 'The Study of Men Born in 1913'. Arch Intern Med 1997; 157(15):1665-1670.

(4) Silverstein MD, Heit JA, Mohr DN, Petterson TM, O'Fallon WM, Melton III LJ. Trends in the Incidence of Deep Vein Thrombosis and Pulmonary Embolism: A 25-Year Population-Based Study. Arch Intern Med 1998; 158(6):585-593.

(5) Oger E. Incidence of venous thromboembolism: a community-based study in Western France. EPI-GETBP Study Group. Groupe d'Etude de la Thrombose de Bretagne Occidentale. Thromb Haemost 2000; 83(5):657-660.

(6) Wood KE. Major Pulmonary Embolism: Review of a Pathophysiologic Approach to the Golden Hour of Hemodynamically Significant Pulmonary Embolism. Chest 2002; 121(3):877-905.

(7) Goldhaber SZ. Pulmonary Embolism. In: Braunwald E, editor. Braunwalds: Heart Disease, A Textbook of Cardiovascular Medicine. 5 ed. Philadelphia: Saunders; 1997.

(8) Strashun AM. A Reduced Role of V/Q Scintigraphy in the Diagnosis of Acute Pulmonary Embolism. J Nucl Med 2007; 48(9):1405-1407.

(9) PIOPED Investigators. Value of the ventilation/perfusion scan in acute pulmonary embolism. Results of the prospective investigation of pulmonary embolism diagnosis (PIOPED). The PIOPED Investigators. JAMA 1990; 263(20):2753-2759.

(10) Blachere H, Latrabe V, Montaudon M, Valli N, Couffinhal T, Raherisson C et al. Pulmonary Embolism Revealed on Helical CT Angiography: Comparison with Ventilation--Perfusion Radionuclide Lung Scanning. Am J Roentgenol 2000; 174(4):1041-1047.

(11) Grenier PA, Beigelman C. Spiral computed tomographic scanning and magnetic resonance angiography for the diagnosis of pulmonary embolism. Thorax 1998; 53(2):25S-31.

(12) Blachere H, Latrabe V, Montaudon M, Valli N, Couffinhal T, Raherisson C et al. Pulmonary Embolism Revealed on Helical CT Angiography: Comparison with Ventilation--Perfusion Radionuclide Lung Scanning. Am J Roentgenol 2000; 174(4):1041-1047.

(13) Stein PD, Fowler SE, Goodman LR, Gottschalk A, Hales CA, Hull RD et al. Multidetector Computed Tomography for Acute Pulmonary Embolism. N Engl J Med 2006; 354(22):2317-2327.

(14) Stein PD, Woodard PK, Weg JG, Wakefield TW, Tapson VF, Sostman HD et al. Diagnostic Pathways in Acute Pulmonary Embolism: Recommendations of the PIOPED II Investigators. Radiology 2007; 242(1):15-21.

(15) Strashun AM. A Reduced Role of V/Q Scintigraphy in the Diagnosis of Acute Pulmonary Embolism. J Nucl Med 2007; 48(9):1405-1407.

(16) Blachere H, Latrabe V, Montaudon M, Valli N, Couffinhal T, Raherisson C et al. Pulmonary Embolism Revealed on Helical CT Angiography: Comparison with Ventilation--Perfusion Radionuclide Lung Scanning. Am J Roentgenol 2000; 174(4):1041-1047.

(17) Mayo JR, Remy-Jardin M, Muller NL, Remy J, Worsley DF, Hossein-Foucher C et al. Pulmonary embolism: prospective comparison of spiral CT with ventilation-perfusion scintigraphy. Radiology 1997; 205(2):447-452.

(18) Stein PD, Fowler SE, Goodman LR, Gottschalk A, Hales CA, Hull RD et al. Multidetector Computed Tomography for Acute Pulmonary Embolism. N Engl J Med 2006; 354(22):2317-2327.

(19) Coche E, Verschuren F, Keyeux A, Goffette P, Goncette L, Hainaut P et al. Diagnosis of Acute Pulmonary Embolism in Outpatients: Comparison of Thin-Collimation Multi-Detector Row Spiral CT and Planar Ventilation-Perfusion Scintigraphy. Radiology 2003; 229(3):757-765.

(20) Blachere H, Latrabe V, Montaudon M, Valli N, Couffinhal T, Raherisson C et al. Pulmonary Embolism Revealed on Helical CT Angiography: Comparison with Ventilation--Perfusion Radionuclide Lung Scanning. Am J Roentgenol 2000; 174(4):1041-1047.

(21) Grenier PA, Beigelman C. Spiral computed tomographic scanning and magnetic resonance angiography for the diagnosis of pulmonary embolism. Thorax 1998; 53(2):25S-31.

(22) Mayo JR, Remy-Jardin M, Muller NL, Remy J, Worsley DF, Hossein-Foucher C et al. Pulmonary embolism: prospective comparison of spiral CT with ventilation-perfusion scintigraphy. Radiology 1997; 205(2):447-452.

(23) van Rossum AB, Pattynama PM, Ton ER, Treurniet FE, Arndt JW, van Eck B et al. Pulmonary embolism: validation of spiral CT angiography in 149 patients. Radiology 1996; 201(2):467-470.

(24) Stein PD, Fowler SE, Goodman LR, Gottschalk A, Hales CA, Hull RD et al. Multidetector Computed Tomography for Acute Pulmonary Embolism. N Engl J Med 2006; 354(22):2317-2327.

(25) Stein PD, Woodard PK, Weg JG, Wakefield TW, Tapson VF, Sostman HD et al. Diagnostic Pathways in Acute Pulmonary Embolism: Recommendations of the PIOPED II Investigators. Radiology 2007; 242(1):15-21.

(26) Stein PD, Fowler SE, Goodman LR, Gottschalk A, Hales CA, Hull RD et al. Multidetector Computed Tomography for Acute Pulmonary Embolism. N Engl J Med 2006; 354(22):2317-2327.

(27) Stein PD, Woodard PK, Weg JG, Wakefield TW, Tapson VF, Sostman HD et al. Diagnostic Pathways in Acute Pulmonary Embolism: Recommendations of the PIOPED II Investigators. Radiology 2007; 242(1):15-21.

(28) British Thoracic Society guidelines for the management of suspected acute pulmonary embolism. Thorax 2003; 58(6):470-483.

(29) Kyrle PA, Eichinger S. New diagnostic strategies for pulmonary embolism. Lancet 2008; 371(9621):1312-1315.

(30) Remy-Jardin M, Pistolesi M, Goodman LR, Gefter WB, Gottschalk A, Mayo JR et al. Management of Suspected Acute Pulmonary Embolism in the Era of CT Angiography: A Statement from the Fleischner Society. Radiology 2007; 245(2):315-329.

(31) Gutte H, Mortensen J, Jensen C, Recke PVd, Petersen CL, Kristoffersen US et al. Comparison of V/Q-SPECT and planar V/Q-lung scintigraphy in diagnosing acute pulmonary embolism. Nucl Med Comm 2010; 31(1):82-86.

(32) Gutte H, Mortensen J, Jensen C, Recke PVd, Kristoffersen US, Kjær A. Added value of combined simultaneous lung ventilation–perfusion single-photon emission computed tomography/multi-slice-computed tomography angiography in two patients suspected of having acute pulmonary embolism. Clin Resp J 2008; 1(1):52-55.

(33) Reinartz P, Wildberger JE, Schaefer W, Nowak B, Mahnken AH, Buell U. Tomographic Imaging in the Diagnosis of Pulmonary Embolism: A Comparison Between V/Q Lung Scintigraphy in SPECT Technique and Multislice Spiral CT. J Nucl Med 2004; 45(9):1501-1508.

(34) Bajc M, Bitzen U, Olsson B, Perez de Sa V, Palmer J, Jonson B. Lung Ventilation/Perfusion SPECT in the Artificially Embolized Pig. J Nucl Med 2002; 43(5):640-647.

(35) Bailey D, Roach P, Bailey E, Hewlett J, Keijzers R. Development of a cost-effective modular SPECT/CT scanner. Eur J Nucl Med Mol Imaging 2007; 34(9):1415-1426.

(36) Miles S, Rogers KM, Thomas P, Soans B, Attia J, Abel C et al. A Comparison of SPECT Lung Scintigraphy and CTPA For the Diagnosis of Pulmonary Embolism. Chest 2009; 136(6):1546-1543.

(37) Reinartz P, Wildberger JE, Schaefer W, Nowak B, Mahnken AH, Buell U. Tomographic Imaging in the Diagnosis of Pulmonary Embolism: A Comparison Between V/Q Lung Scintigraphy in SPECT Technique and Multislice Spiral CT. J Nucl Med 2004; 45(9):1501-1508.

(38) Wood KE. Major Pulmonary Embolism: Review of a Pathophysiologic Approach to the Golden Hour of Hemodynamically Significant Pulmonary Embolism. Chest 2002; 121(3):877-905.

(39) Lualdi JC, Goldhaber SZ. Right ventricular dysfunction after acute pulmonary embolism: pathophysiologic factors, detection, and therapeutic implications. Am Heart J 1995; 130(6):1276-1282.

(40) Elliott CG. Pulmonary Physiology during Pulmonary Embolism. Chest 1992; 101(4):163S-171S.

(41) Fedullo P. Pulmonary Embolism. In: Fuster V, Walsh R, O'Rourke R, Poole-Wilson P, editors. Hurst's The Heart. 12 ed. New York: McGraw-Hill Medical Publishing Division; 2008.

(42) van der Meer RW, Pattynama PMT, van Strijen MJL, van den Berg-Huijsmans A, Hartmann IJC, Putter H et al. Right Ventricular Dysfunction and Pulmonary Obstruction Index at Helical CT: Prediction of Clinical Outcome during 3-month Follow-up in Patients with Acute Pulmonary Embolism. Radiology 2005; 235(3):798-803.

(43) Ghuysen A, Ghaye B, Willems V, Lambermont B, Gerard P, Dondelinger RF et al. Computed tomographic pulmonary angiography and prognostic significance in patients with acute pulmonary embolism. Thorax 2005; 60(11):956-961.

(44) Kjaer A, Lebech AM, Hesse B, Petersen CL. Right-sided cardiac function in healthy volunteers measured by first-pass radionuclide ventriculography and gated blood-pool SPECT: comparison with cine MRI. Clin Phys Funct Imag 2005; 25(6):344-349.

(45) Haddad F, Hunt SA, Rosenthal DN, Murphy DJ. Right ventricular function in cardiovascular disease, part I: Anatomy, physiology, aging, and functional assessment of the right ventricle. Circulation 2008; 117(11):1436-1448.

(46) Goldhaber SZ, Haire WD, Feldstein ML, Miller M, Toltzis R, Smith JL et al. Alteplase versus heparin in acute pulmonary embolism: randomised trial assessing right-ventricular function and pulmonary perfusion. Lancet 1993; 341(8844):507-511.

(47) Kreit JW. The Impact of Right Ventricular Dysfunction on the Prognosis and Therapy of Normotensive Patients With Pulmonary Embolism. Chest 2004; 125(4):1539-1545.

(48) Quiroz R, Kucher N, Schoepf UJ, Kipfmueller F, Solomon SD, Costello P et al. Right Ventricular Enlargement on Chest Computed Tomography: Prognostic Role in Acute Pulmonary Embolism. Circulation 2004; 109(20):2401-2404.

(49) Woods J, Monteiro P, Rhodes A. Right ventricular dysfunction. Curr Opin Crit Care 2007; 13(5):532-540.

(50) Goldhaber SZ, Haire WD, Feldstein ML, Miller M, Toltzis R, Smith JL et al. Alteplase versus heparin in acute pulmonary embolism: randomised trial assessing right-ventricular function and pulmonary perfusion. Lancet 1993; 341(8844):507-511.

(51) Kreit JW. The Impact of Right Ventricular Dysfunction on the Prognosis and Therapy of Normotensive Patients With Pulmonary Embolism. Chest 2004; 125(4):1539-1545.

(52) Quiroz R, Kucher N, Schoepf UJ, Kipfmueller F, Solomon SD, Costello P et al. Right Ventricular Enlargement on Chest Computed Tomography: Prognostic Role in Acute Pulmonary Embolism. Circulation 2004; 109(20):2401-2404.

(53) Goldhaber SZ, Visani L, De Rosa M. Acute pulmonary embolism: clinical outcomes in the International Cooperative Pulmonary Embolism Registry (ICOPER). Lancet 1999; 353(9162):1386-1389.

(54) British Thoracic Society guidelines for the management of suspected acute pulmonary embolism. Thorax 2003; 58(6):470-483.

(55) Task FM, Torbicki A, Perrier A, Konstantinides S, Agnelli G, Galie N et al. Guidelines on the diagnosis and management of acute pulmonary embolism: The Task Force for the Diagnosis and Management of Acute Pulmonary Embolism of the European Society of Cardiology (ESC). Eur Heart J 2008; 29(18):2276-2315.

(56) Konstantinides S. Should thrombolytic therapy be used in patients with pulmonary embolism? Am J Cardiovasc Drugs 2004; 4(2):69-74.

(57) Koch K, Oellig F, Oberholzer K, Bender P, Kunz P, Mildenberger P et al. Assessment of right ventricular function by 16-detector-row CT: comparison with magnetic resonance imaging. Eur Radiol 2005; 15(2):312-318.

(58) Coche E, Vlassenbroek A, Roelants V, D´Hoore W, Verschuren F, Goncette L et al. Evaluation of biventricular ejection fraction with ECG-gated 16-slice CT: preliminary findings in acute pulmonary embolism in comparison with radionuclide ventriculography. Eur Radiol 2005; 15(7):1432-1440.

(59) van der Meer RW, Pattynama PMT, van Strijen MJL, van den Berg-Huijsmans A, Hartmann IJC, Putter H et al. Right Ventricular Dysfunction and Pulmonary Obstruction Index at Helical CT: Prediction of Clinical Outcome during 3-month Follow-up in Patients with Acute Pulmonary Embolism. Radiology 2005; 235(3):798-803.

(60) Vorobiof G, Kadiev S. Evaluation of Right Ventricular Size on Computed Tomography: Unreliable at Best. Am J Med 2008; 121(8):e15.

(61) Müller M, Teige F, Schnapauff D, Hamm B, Dewey M. Evaluation of right ventricular function with multidetector computed tomography: comparison with magnetic resonance imaging and analysis of inter- and intraobserver variability. Eur Radiol 2009; 19(2):278-289.

(62) Koch K, Oellig F, Oberholzer K, Bender P, Kunz P, Mildenberger P et al. Assessment of right ventricular function by 16-detector-row CT: comparison with magnetic resonance imaging. Eur Radiol 2005; 15(2):312-318.

(63) de Bold AJ, Ma KK, Zhang Y, de Bold ML, Bensimon M, Khoshbaten A. The physiological and pathophysiological modulation of the endocrine function of the heart. Can J Physiol Pharmacol 2001; 79(8):705-714.

(64) Galie N, Manes A, Branzi A. The endothelin system in pulmonary arterial hypertension. Cardiovasc Res 2004; 61(2):227-237.

(65) Kjaer A, Hesse B. Heart failure and neuroendocrine activation: diagnostic, prognostic and therapeutic perspectives. Clin Phys Funct Imag 2001; 21(6):661-672.

(66) Levin ER, Gardner DG, Samson WK. Natriuretic Peptides. N Engl J Med 1998; 339(5):321-328.

(67) McGrath MF, de Bold AJ. Determinants of natriuretic peptide gene expression. Peptides 2005; 26(6):933-943.

(68) Mair J. Biochemistry of B-type natriuretic peptide - where are we now? Clin Chem Lab Med 2008; 46(11):1507-1514.

(69) Hirata Y, Suzuki E, Hayakawa H, Matsuoka H, Sugimoto T, Kojima M et al. Role of endogenous ANP in sodium excretion in rats with experimental pulmonary hypertension. Am J Physiol Heart Circ Physiol 1992; 262(6):H1684-H1689.

(70) Nagaya N, Nishikimi T, Okano Y, Uematsu M, Satoh T, Kyotani S et al. Plasma Brain Natriuretic Peptide Levels Increase in Proportion to the Extent of Right Ventricular Dysfunction in Pulmonary Hypertension. J Am Coll Cardiol 1998; 31(1):202-208.

(71) Nagaya N, Nishikimi T, Uematsu M, Satoh T, Kyotani S, Sakamaki F et al. Plasma Brain Natriuretic Peptide as a Prognostic Indicator in Patients With Primary Pulmonary Hypertension. Circulation 2000; 102(8):865-870.

(72) Kiely DG, Kennedy NS, Pirzada O, Batchelor SA, Struthers AD, Lipworth BJ. Elevated levels of natriuretic peptides in patients with pulmonary thromboembolism. Respir Med 2005; 99(10):1286-1291.

(73) Mair J. Biochemistry of B-type natriuretic peptide - where are we now? Clin Chem Lab Med 2008; 46(11):1507-1514.

(74) Hama N, Itoh H, Shirakami G, Nakagawa O, Suga Si, Ogawa Y et al. Rapid Ventricular Induction of Brain Natriuretic Peptide Gene Expression in Experimental Acute Myocardial Infarction. Circulation 1995; 92(6):1558-1564.

(75) Mair J. Biochemistry of B-type natriuretic peptide - where are we now? Clin Chem Lab Med 2008; 46(11):1507-1514.

(76) de Lemos JA, McGuire DK, Drazner MH. B-type natriuretic peptide in cardiovascular disease. Lancet 2003; 362(9380):316-322.

(77) Levin ER, Gardner DG, Samson WK. Natriuretic Peptides. N Engl J Med 1998; 339(5):321-328.

(78) McGrath MF, de Bold AJ. Determinants of natriuretic peptide gene expression. Peptides 2005; 26(6):933-943.

(79) Hall C. Essential biochemistry and physiology of (NT-pro)BNP. Eur J Heart Fail 2004; 6(3):257-260.

(80) Mukoyama M, Nakao K, Hosoda K, Suga S, Saito Y, Ogawa Y et al. Brain natriuretic peptide as a novel cardiac hormone in humans. Evidence for an exquisite dual natriuretic peptide system, atrial natriuretic peptide and brain natriuretic peptide. J Clin Invest 1991; 87(4):1402-1412.

(81) Goetze JP, Friis-Hansen L, Rehfeld JF, Nilsson B, Svendsen JH. Atrial secretion of B-type natriuretic peptide. Eur Heart J 2006; 27(14):1648-1650.

(82) Klok FA, Mos IC, Huisman MV. BNP Levels in the Prediction of Adverse Outcome in Patients with Pulmonary Embolism: A Meta-analysis. Am J Respir Crit Care Med 2008; 178(4):425-430.

(83) Magga J, Marttila M, Mantymaa P, Vuolteenaho O, Ruskoaho H. Brain natriuretic peptide in plasma, atria, and ventricles of vasopressin- and phenylephrine-infused conscious rats. Endocrinology 1994; 134(6):2505-2515.

(84) Klok FA, Mos IC, Huisman MV. BNP Levels in the Prediction of Adverse Outcome in Patients with Pulmonary Embolism: A Meta-analysis. Am J Respir Crit Care Med 2008; 178(4):425-430.

(85) Cavallazzi R, Nair A, Vasu T, Marik P. Natriuretic peptides in acute pulmonary embolism: a systematic review. Intensive Care Med 2008; 34(12):2147-2156.

(86) Pruszczyk P, Kostrubiec M, Bochowicz A, Styczynski G, Szulc M, Kurzyna M et al. N-terminal pro-brain natriuretic peptide in patients with acute pulmonary embolism. Eur Respir J 2003; 22(4):649-653.

(87) Yardan T, Altintop L, Baydin A, Yilmaz O, Guven H. B-type natriuretic peptide as an indicator of right ventricular dysfunction in acute pulmonary embolism. Int J Clin Pract 2008; 62(8):1177-1182.

(88) Binder L, Pieske B, Olschewski M, Geibel A, Klostermann B, Reiner C et al. N-Terminal Pro-Brain Natriuretic Peptide or Troponin Testing Followed by Echocardiography for Risk Stratification of Acute Pulmonary Embolism. Circulation 2005; 112(11):1573-1579.

(89) Pieralli F, Olivotto I, Vanni S, Conti A, Camaiti A, Targioni G et al. Usefulness of Bedside Testing for Brain Natriuretic Peptide to Identify Right Ventricular Dysfunction and Outcome in Normotensive Patients With Acute Pulmonary Embolism. Am J Cardiol 2006; 97(9):1386-1390.

(90) Logeart D, Lecuyer L, Thabut G, Tabet JY, Tartiére JM, Chavelas C et al. Biomarker-based strategy for screening right ventricular dysfunction in patients with non-massive pulmonary embolism. Intensive Care Med 2007; 33(2):286-292.

(91) Kucher N, Printzen G, Doernhoefer T, Windecker S, Meier B, Hess OM. Low Pro-Brain Natriuretic Peptide Levels Predict Benign Clinical Outcome in Acute Pulmonary Embolism. Circulation 2003; 107(12):1576-1578.

(92) Kruger S, Graf J, Merx M, Koch K, Kunz D, Hanrath P et al. Brain natriuretic peptide predicts right heart failure in patients with acute pulmonary embolism. Am Heart J 2004; 147(1):60-65.

(93) Vuilleumier N, Righini M, Perrier A, Rosset A, Turck N, Sanchez JC et al. Correlation between cardiac biomarkers and right ventricular enlargement on chest CT in non massive pulmonary embolism. Thromb Res 2008; 121(5):617-624.

(94) Battistini B. Modulation and roles of the endothelins in the pathophysiology of pulmonary embolism. Can J Physiol Pharmacol 2003; 81(6):555-569.

(95) Masaki T. Historical review: Endothelin. Trends Pharmacol Sci 2004; 25(4):219-224.

(96) Stewart DJ, Levy RD, Cernacek P, Langleben D. Increased Plasma Endothelin--1 Pulmonary Hypertension: Marker or Mediator of Disease? Ann Intern Med 1991; 114(6):464-469.

(97) Miyauchi T, Yorikane R, Sakai S, Sakurai T, Okada M, Nishikibe M et al. Contribution of endogenous endothelin-1 to the progression of

cardiopulmonary alterations in rats with monocrotaline-induced pulmonary hypertension. Circ Res 1993; 73(5):887-897.

(98) Lee J, Chun Y, Lee I, Tuder RM, Hong S, Shim T et al. Pathogenic Role of Endothelin 1 in Hemodynamic Dysfunction in Experimental Acute Pulmonary Thromboembolism. Am J Respir Crit Care Med 2001; 164(7):1282-1287.

(99) Miyahara T, Koizumi T, Kubo K, Hanaoka M, Kaneki T, Yamamoto H et al. Endothelin receptor blockade attenuates air embolization-induced pulmonary hypertension in sheep. Eur J Pharmacol 1999; 385(2-3):163-169.

(100) Ayach B, Tsang J, Jeng AY, Blouin A, Gosselin M, Wang FH et al. Effects of a selective endothelin A receptor antagonist, ABT-627, in healthy normotensive anaesthetized rats developing acute pulmonary air embolism. Clin Sci 2002; 103(48):371S-375S.

(101) Sofia M, Faraone S, Alifano M, Micco A, Albisinni R, Maniscalco M et al. Endothelin abnormalities in patients with pulmonary embolism. Chest 1997; 111(3):544-549.

(102) Lourenco AP, Roncon-Albuquerque R, Jr., Bras-Silva C, Faria B, Wieland J, Henriques-Coelho T et al. Myocardial dysfunction and neurohumoral activation without remodeling in left ventricle of monocrotaline-induced pulmonary hypertensive rats. Am J Physiol Heart Circ Physiol 2006; 291(4):H1587-H1594.

(103) Adachi S, Ito H, Ohta Y, Tanaka M, Ishiyama S, Nagata M et al. Distribution of mRNAs for natriuretic peptides in RV hypertrophy after pulmonary arterial banding. Am J Physiol Heart Circ Physiol 1995; 268(1):H162-H169.

(104) Roncon-Albuquerque J, Vasconcelos M, Lourenco AP, Brandao-Nogueira A, Teles A, Henriques-Coelho T et al. Acute changes of biventricular gene expression in volume and right ventricular pressure overload. Life Sci 2005; 78(22):2633-2642.

(105) Doyama K, Fukumoto M, Takemura G, Tanaka M, Oda T, Hasegawa K et al. Expression and distribution of brain natriuretic peptide in human right atria. J Am Coll Cardiol 1998; 32(7):1832-1838.

(106) Jimenez Castro D, Diaz G, Molina J, Marti D, Del Rey J, Garcia-Rull S et al. Troponin I and risk stratification of patients with acute non massive pulmonary embolism. Eur Respir J 2007; 31(4):847-53.

(107) Jaffe AS, Babuin L, Apple FS. Biomarkers in acute cardiac disease: the present and the future. J Am Coll Cardiol 2006; 48(1):1-11.

(108) Meyer T, Binder L, Hruska N, Luthe H, Buchwald AB. Cardiac troponin I elevation in acute pulmonary embolism is associated with right ventricular dysfunction. J Am Coll Cardiol 2000; 36(5):1632-1636.

(109) Konstantinides S. Pulmonary embolism: impact of right ventricular dysfunction. Curr Opin Cardiol 2005; 20(6):496-501.

(110) Jimenez Castro D, Diaz G, Molina J, Marti D, Del Rey J, Garcia-Rull S et al. Troponin I and risk stratification of patients with acute non massive pulmonary embolism. Eur Respir J 2007; 31(4):847-53.

(111) Pruszczyk P, Bochowicz A, Torbicki A, Szulc M, Kurzyna M, Fija+ékowska A et al. Cardiac Troponin T Monitoring Identifies High-Risk Group of Normotensive Patients With Acute Pulmonary Embolism*. Chest 2003; 123(6):1947-1952.

(112) Courtney DM, Watts JA, Kline JA. End tidal CO2 is reduced during hypotension and cardiac arrest in a rat model of massive pulmonary embolism. Resuscitation 2002; 53(1):83-91.

(113) Zagorski J, Sanapareddy N, Gellar MA, Kline JA, Watts JA. Transcriptional profile of right ventricular tissue during acute pulmonary embolism in rats. Physiol Genomics 2008; 34(1):101-111.

(114) Zagorski J, Debelak J, Gellar M, Watts JA, Kline JA. Chemokines Accumulate in the Lungs of Rats with Severe Pulmonary Embolism Induced by Polystyrene Microspheres. J Immunol 2003; 171(10):5529-5536.

(115) Jones AE, Watts JA, Debelak JP, Thornton LR, Younger JG, Kline JA. Inhibition of prostaglandin synthesis during polystyrene microsphere-induced pulmonary embolism in the rat. Am J Physiol Lung Cell Mol Physiol 2003; 284(6):L1072-L1081.

(116) Cuenoud HF, Joris I, Majno G. Ultrastructure of the myocardium after pulmonary embolism. A study in the rat. Am J Pathol 1978; 92(2):421-458.

(117) Watts JA, Zagorski J, Gellar MA, Stevinson BG, Kline JA. Cardiac inflammation contributes to right ventricular dysfunction following experimental pulmonary embolism in rats. J Mol Cell Cardiol 2006; 41(2):296-307.

(118) Jones AE, Watts JA, Debelak JP, Thornton LR, Younger JG, Kline JA. Inhibition of prostaglandin synthesis during polystyrene microsphere-induced pulmonary embolism in the rat. Am J Physiol Lung Cell Mol Physiol 2003; 284(6):L1072-L1081.

(119) Watts JA, Zagorski J, Gellar MA, Stevinson BG, Kline JA. Cardiac inflammation contributes to right ventricular dysfunction following experimental pulmonary embolism in rats. J Mol Cell Cardiol 2006; 41(2):296-307.

(120) Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. Genome Res 1996; 6(10):986-994.

(121) Livak KJ, Schmittgen TD. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-DDCT Method. Methods 2001; 25(4):402-408.

(122) Bustin SA, Mueller R. Real-time reverse transcription PCR (qRT-PCR) and its potential use in clinical diagnosis. Clin Sci 2005; 109(4):365-379.

(123) Andersen CL, Jensen JL, Orntoft TF. Normalization of Real-Time Quantitative Reverse Transcription-PCR Data: A Model-Based Variance Estimation Approach to Identify Genes Suited for Normalization, Applied to Bladder and Colon Cancer Data Sets. Cancer Res 2004; 64(15):5245-5250.

(124) Wells PS, Ginsberg JS, Anderson DR, Kearon C, Gent M, Turpie AG et al. Use of a Clinical Model for Safe Management of Patients with Suspected Pulmonary Embolism. Ann Intern Med 1998; 129(12):997-1005.

(125) Wells PS, Ginsberg JS, Anderson DR, Kearon C, Gent M, Turpie AG et al. Use of a Clinical Model for Safe Management of Patients with Suspected Pulmonary Embolism. Ann Intern Med 1998; 129(12):997-1005.

(126) Wells PS, Ginsberg JS, Anderson DR, Kearon C, Gent M, Turpie AG et al. Use of a Clinical Model for Safe Management of Patients with Suspected Pulmonary Embolism. Ann Intern Med 1998; 129(12):997-1005.

(127) Beharry N, Coulden R, Peters M. Pulmonary embolism. In: Ell P, Gambhir S, editors. Nuclear Medicine in Clinical Diagnosis and Treatment. 3 ed. Edinburgh: Churchill Livingstone; 2004. 883-897.

(128) Petersson J, Sanchez-Crespo A, Larsson SA, Mure M. Physiological imaging of the lung: single-photon-emission computed tomography (SPECT). J Appl Physiol 2007; 102(1):468-476.

(129) O'Connor MK, Kemp BJ. Single-Photon Emission Computed Tomography/Computed Tomography: Basic Instrumentation and Innovations. Semin Nucl Med 2006; 36(4):258-266.

(130) Bajc M, Bitzen U, Olsson B, Perez de Sa V, Palmer J, Jonson B. Lung Ventilation/Perfusion SPECT in the Artificially Embolized Pig. J Nucl Med 2002; 43(5):640-647.

(131) Petersson J, Sanchez-Crespo A, Larsson SA, Mure M. Physiological imaging of the lung: single-photon-emission computed tomography (SPECT). J Appl Physiol 2007; 102(1):468-476.

(132) PIOPED Investigators. Value of the ventilation/perfusion scan in acute pulmonary embolism. Results of the prospective investigation of pulmonary embolism diagnosis (PIOPED). The PIOPED Investigators. JAMA 1990; 263(20):2753-2759.

(133) Reinartz P, Wildberger JE, Schaefer W, Nowak B, Mahnken AH, Buell U. Tomographic Imaging in the Diagnosis of Pulmonary Embolism: A Comparison Between V/Q Lung Scintigraphy in SPECT Technique and Multislice Spiral CT. J Nucl Med 2004; 45(9):1501-1508.

(134) Bajc M, Neilly J, Miniati M, Schuemichen C, Meignan M, Jonson B. EANM guidelines for ventilation/perfusion scintigraphy. Eur J Nucl Med Mol Imaging 2009; 36(8):1356-1370.

(135) Bajc M, Olsson CG, Olsson B, Palmer J, Jonson B. Diagnostic evaluation of planar and tomographic ventilation/perfusion lung images in patients with suspected pulmonary emboli. Clin Phys Funct Imag 2004; 24(5):249-256.

(136) Roach PJ, Bailey DL, Harris BE. Enhancing Lung Scintigraphy With Single-Photon Emission Computed Tomography. Semin Nucl Med 2008; 38(6):441-449.

(137) Schumichen C. V/Q-Scanning/SPECT for the Diagnosis of Pulmonary Embolism. Respiration 2003; 70(4):329-342.

(138) Bajc M, Neilly J, Miniati M, Schuemichen C, Meignan M, Jonson B. EANM guidelines for ventilation/perfusion scintigraphy. Eur J Nucl Med Mol Imaging 2009; 36(8):1356-1370.

(139) Alfakih K, Reid S, Jones T, Sivananthan M. Assessment of ventricular function and mass by cardiac magnetic resonance imaging. Eur Radiol 2004; 14(10):1813-1822.

(140) Clopper CJ, Pearson ES. The Use of Confidence or Fiducial Limits Illustrated in the Case of the Binomial. Biometrika 1934; 26(4):404-413.

(141) ImPACT CT Patient Dosimetry Calculator (0.99x) [2008.

(142) Swensen SJ, Jett JR, Sloan JA, Midthun DE, Hartman TE, Sykes AM et al. Screening for lung cancer with low-dose spiral computed tomography. Am J Respir Crit Care Med 2002; 165(4):508-513.

(143) Hunold P, Vogt FM, Schmermund A, Debatin JF, Kerkhoff G, Budde T et al. Radiation Exposure during Cardiac CT: Effective Doses at Multi-Detector Row CT and Electron-Beam CT. Radiology 2003; 226(1):145-152.

(144) Brenner DJ, Hall EJ. Computed Tomography - An Increasing Source of Radiation Exposure. N Engl J Med 2007; 357(22):2277-2284.

(145) Reinartz P, Wildberger JE, Schaefer W, Nowak B, Mahnken AH, Buell U. Tomographic Imaging in the Diagnosis of Pulmonary Embolism: A Comparison Between V/Q Lung Scintigraphy in SPECT Technique and Multislice Spiral CT. J Nucl Med 2004; 45(9):1501-1508.

(146) PIOPED Investigators. Value of the ventilation/perfusion scan in acute pulmonary embolism. Results of the prospective investigation of pulmonary embolism diagnosis (PIOPED). The PIOPED Investigators. JAMA 1990; 263(20):2753-2759.

(147) Leblanc M, Leveillee F, Turcotte E. Prospective evaluation of the negative predictive value of V/Q SPECT using 99mTc-Technegas. Nucl Med Comm 2007; 28(8):667-672.

(148) Bajc M, Olsson B, Palmer J, Jonson B. Ventilation/Perfusion SPECT for diagnostics of pulmonary embolism in clinical practice. J Intern Med 2008; 264(4):379-387.

(149) De Geeter FW, Reinartz P, Buell U. Tomographic Imaging in the Diagnosis of Pulmonary Embolism: Still, We Do Not Know. J Nucl Med 2005; 46(12):2119-2121.

(150) Gutte H, Mortensen J, Jensen C, johnbeck Camilla Bardram, Recke PVd, Petersen CL et al. Detection of pulmonary embolism with combined ventilation/perfusion SPECT and low-dose CT: Head-to-head comparison with CT-angiography. J Nucl Med 2009; 50(12):1987-1992.

(151) Parker JA. Improving Lung Scintigraphy. J Nucl Med 2009; 50(12):1919-1920.

(152) Hirata Y, Suzuki E, Hayakawa H, Matsuoka H, Sugimoto T, Kojima M et al. Role of endogenous ANP in sodium excretion in rats with experimental pulmonary hypertension. Am J Physiol Heart Circ Physiol 1992; 262(6):H1684-H1689.

(153) Tulevski II, Groenink M, van der Wall EE, van Veldhuisen DJ, Boomsma F, Stoker J et al. Increased brain and atrial natriuretic peptides in patients with chronic right ventricular pressure overload: correlation between plasma neurohormones and right ventricular dysfunction. Heart 2001; 86(1):27-30.

(154) Nagaya N, Nishikimi T, Okano Y, Uematsu M, Satoh T, Kyotani S et al. Plasma Brain Natriuretic Peptide Levels Increase in Proportion to the Extent of Right Ventricular Dysfunction in Pulmonary Hypertension. J Am Coll Cardiol 1998; 31(1):202-208.

(155) Yardan T, Altintop L, Baydin A, Yilmaz O, Guven H. B-type natriuretic peptide as an indicator of right ventricular dysfunction in acute pulmonary embolism. Int J Clin Pract 2008; 62(8):1177-1182.

(156) Tulevski II, Hirsch A, Sanson BJ, Romkes H, van der Wall EE, van Veldhuisen DJ et al. Increased brain natriuretic peptide as a marker for right ventricular dysfunction in acute pulmonary embolism. Thromb Haemost 2001; 86(5):1193-1196.

(157) Sohne M, ten Wolde M, Boomsma F, REITSMA JB, Douketis JD, Buller HR. Brain natriuretic peptide in hemodynamically stable acute pulmonary embolism. J Thromb Haemost 2006; 4(3):552-556.

(158) Kucher N, Printzen G, Doernhoefer T, Windecker S, Meier B, Hess OM. Low Pro-Brain Natriuretic Peptide Levels Predict Benign Clinical Outcome in Acute Pulmonary Embolism. Circulation 2003; 107(12):1576-1578.

(159) Klok FA, Mos IC, Huisman MV. BNP Levels in the Prediction of Adverse Outcome in Patients with Pulmonary Embolism: A Meta-analysis. Am J Respir Crit Care Med 2008; 178(4):425-430.

(160) Thabut G, Logeart D. Thrombolysis for Pulmonary Embolism in Patients With Right Ventricular Dysfunction: Con. Arch Intern Med 2005; 165(19):2200-2203.

(161) ten Wolde M, Tulevski II, Mulder JWM, Sohne M, Boomsma F, Mulder BJM et al. Brain Natriuretic Peptide as a Predictor of Adverse Outcome in Patients With Pulmonary Embolism. Circulation 2003; 107(16):2082-2084.

(162) Vogelsang TW, Jensen RJ, Monrad AL, Russ K, Olesen UH, Hesse B et al. Independent effects of both right and left ventricular function on plasma brain natriuretic peptide. Eur J Heart Fail 2007; 9(9):892-896.

(163) Nagaya N, Nishikimi T, Okano Y, Uematsu M, Satoh T, Kyotani S et al. Plasma Brain Natriuretic Peptide Levels Increase in Proportion to the Extent of Right Ventricular Dysfunction in Pulmonary Hypertension. J Am Coll Cardiol 1998; 31(1):202-208.

(164) Tulevski II, Groenink M, van der Wall EE, van Veldhuisen DJ, Boomsma F, Stoker J et al. Increased brain and atrial natriuretic peptides in patients with chronic right ventricular pressure overload: correlation between plasma neurohormones and right ventricular dysfunction. Heart 2001; 86(1):27-30.

(165) Klok FA, Mos IC, Huisman MV. BNP Levels in the Prediction of Adverse Outcome in Patients with Pulmonary Embolism: A Meta-analysis. Am J Respir Crit Care Med 2008; 178(4):425-430.

(166) Pruszczyk P, Kostrubiec M, Bochowicz A, Styczynski G, Szulc M, Kurzyna M et al. N-terminal pro-brain natriuretic peptide in patients with acute pulmonary embolism. Eur Respir J 2003; 22(4):649-653.

(167) Kline JA, Zeitouni R, Marchick MR, Hernandez-Nino J, Rose GA. Comparison of 8 biomarkers for prediction of right ventricular hypokinesis 6 months after submassive pulmonary embolism. Am Heart J 2008; 156(2):308-314.

(168) Kostrubiec M, Pedowska-Wloszek J, Ciurzynski M, Bienias P, Pacho S, Piaskowska M et al. Endothelin is not elevated in acute pulmonary embolism. Thromb Res 2009; 124(2):157-160.

(169) Sofia M, Faraone S, Alifano M, Micco A, Albisinni R, Maniscalco M et al. Endothelin abnormalities in patients with pulmonary embolism. Chest 1997; 111(3):544-549.

(170) Langleben D, Dupuis J, Langleben I, Hirsch AM, Baron M, Senécal JL et al. Etiology-Specific Endothelin-1 Clearance in Human Precapillary Pulmonary Hypertension*. Chest 2006; 129(3):689-695.

(171) Battistini B. Modulation and roles of the endothelins in the pathophysiology of pulmonary embolism. Can J Physiol Pharmacol 2003; 81(6):555-569.

(172) Ayach B, Tsang J, Jeng AY, Blouin A, Gosselin M, Wang FH et al. Effects of a selective endothelin A receptor antagonist, ABT-627, in healthy normotensive anaesthetized rats developing acute pulmonary air embolism. Clin Sci 2002; 103(48):371S-375S.

(173) Lee J, Chun Y, Lee I, Tuder RM, Hong S, Shim T et al. Pathogenic Role of Endothelin 1 in Hemodynamic Dysfunction in Experimental Acute Pulmonary Thromboembolism. Am J Respir Crit Care Med 2001; 164(7):1282-1287.

(174) Ghanima W, Abdelnoor M, Holmen LO, Nielssen BE, Ross S, Sandset PM. D-dimer level is associated with the extent of pulmonary embolism. Thromb Res 2007; 120(2):281-288.

(175) Aujesky D, Roy PM, Guy M, Cornuz J, Sanchez O, Perrier A. Prognostic value of D-dimer in patients with pulmonary embolism. Thromb Haemost 2006; 96(4):478-482.

(176) Katus HA, Remppis A, Neumann FJ, Scheffold T, Diederich KW, Vinar G et al. Diagnostic efficiency of troponin T measurements in acute myocardial infarction. Circulation 1991; 83(3):902-912.

(177) Kostrubiec M, Pruszczyk P, Bochowicz A, Pacho R, Szulc M, Kaczynska A et al. Biomarker-based risk assessment model in acute pulmonary embolism. Eur Heart J 2005; 26(20):2166-2172.

(178) Pruszczyk P, Bochowicz A, Torbicki A, Szulc M, Kurzyna M, Fija+ékowska A et al. Cardiac Troponin T Monitoring Identifies High-Risk Group of Normotensive Patients With Acute Pulmonary Embolism*. Chest 2003; 123(6):1947-1952.

(179) Konstantinides S, Geibel A, Olschewski M, Kasper W, Hruska N, Jackle S et al. Importance of Cardiac Troponins I and T in Risk Stratification of Patients With Acute Pulmonary Embolism. Circulation 2002; 106(10):1263-1268.

(180) Punukollu G, Khan IA, Gowda RM, Lakhanpal G, Vasavada BC, Sacchi TJ. Cardiac troponin I release in acute pulmonary embolism in relation to the duration of symptoms. Int J Cardiol 2005; 99(2):207-211.

(181) Hamm CW, Goldmann BU, Heeschen C, Kreymann G, Berger J, Meinertz T. Emergency Room Triage of Patients with Acute Chest Pain by Means of Rapid Testing for Cardiac Troponin T or Troponin I. N Engl J Med 1997; 337(23):1648-1653.

(182) Dewey M, Muller M, Teige F, Hamm B. Evaluation of a semiautomatic software tool for left ventricular function analysis with 16-slice computed tomography. Eur Radiol 2006; 16(1):25-31.

(183) Miller S, Simonetti OP, Carr J, Kramer U, Finn JP. MR Imaging of the Heart with Cine True Fast Imaging with Steady-State Precession: Influence of Spatial and Temporal Resolutions on Left Ventricular Functional Parameters1. Radiology 2002; 223(1):263-269.

(184) Jensen CJ, Jochims M, Hunold P, Forsting M, Barkhausen Jr, Sabin GV et al. Assessment of left ventricular function and mass in dual-source computed tomography coronary angiography: Influence of beta-blockers on left ventricular function: Comparison to magnetic resonance imaging. Eur J Radiol 2009; 74(3):484-91.

(185) Ogawa Y, Nakao K, Mukoyama M, Hosoda K, Shirakami G, Arai H et al. Natriuretic peptides as cardiac hormones in normotensive and spontaneously hypertensive rats. The ventricle is a major site of synthesis and secretion of brain natriuretic peptide. Circ Res 1991; 69(2):491-500.

(186) Dagnino L, Drouin J, Nemer M. Differential expression of natriuretic peptide genes in cardiac and extracardiac tissues. Mol Endocrinol 1991; 5(9):1292-1300.

(187) LaPointe MC. Molecular regulation of the brain natriuretic peptide gene. Peptides 2005; 26(6):944-956.

(188) Mukoyama M, Nakao K, Saito Y, Ogawa Y, Hosoda K, Suga Si et al. Human brain natriuretic peptide, a novel cardiac hormone. Lancet 1990; 335(8692):801-802.

(189) Akimoto K, Miyata A, Hayakawa K, Kangawa K, Matsuo H. Plasma and atrial levels of Atrial Natriuretic Peptide (ANP) in pulmonary hypertensive rats. Life Sci 1988; 43(14):1125-1132.

(190) Comini L, Agnoletti G, Panzali A, Mantero G, Pasini E, Gaia G et al. Activation of ANP synthesis during congestive heart failure in rats treated with monocrotaline. Am J Physiol Heart Circ Physiol 1995; 268(1):H391-H398.

(191) LaPointe MC. Molecular regulation of the brain natriuretic peptide gene. Peptides 2005; 26(6):944-956.

(192) Ogawa Y, Nakao K, Mukoyama M, Shirakami G, Itoh H, Hosoda K et al. Rat brain natriuretic peptide--tissue distribution and molecular form. Endocrinology 1990; 126(4):2225-2227.

(193) Mangat H, de Bold AJ. Stretch-induced atrial natriuretic factor release utilizes a rapidly depleting pool of newly synthesized hormone. Endocrinology 1993; 133(3):1398-1403.

(194) de Bold AJ, Ma KK, Zhang Y, de Bold ML, Bensimon M, Khoshbaten A. The physiological and pathophysiological modulation of the endocrine function of the heart. Can J Physiol Pharmacol 2001; 79(8):705-714.

(195) Stelzner TJ, O'Brien RF, Yanagisawa M, Sakurai T, Sato K, Webb S et al. Increased lung endothelin-1 production in rats with idiopathic pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 1992; 262(5):L614-L620.

(196) Courtney DM, Watts JA, Kline JA. End tidal CO2 is reduced during hypotension and cardiac arrest in a rat model of massive pulmonary embolism. Resuscitation 2002; 53(1):83-91.

(197) Zagorski J, Sanapareddy N, Gellar MA, Kline JA, Watts JA. Transcriptional profile of right ventricular tissue during acute pulmonary embolism in rats. Physiol Genomics 2008; 34(1):101-111.

(198) Zagorski J, Debelak J, Gellar M, Watts JA, Kline JA. Chemokines Accumulate in the Lungs of Rats with Severe Pulmonary Embolism Induced by Polystyrene Microspheres. J Immunol 2003; 171(10):5529-5536.

(199) Jones AE, Watts JA, Debelak JP, Thornton LR, Younger JG, Kline JA. Inhibition of prostaglandin synthesis during polystyrene microsphere-induced pulmonary embolism in the rat. Am J Physiol Lung Cell Mol Physiol 2003; 284(6):L1072-L1081.

(200) Cuenoud HF, Joris I, Majno G. Ultrastructure of the myocardium after pulmonary embolism. A study in the rat. Am J Pathol 1978; 92(2):421-458.

(201) Watts JA, Zagorski J, Gellar MA, Stevinson BG, Kline JA. Cardiac inflammation contributes to right ventricular dysfunction following experimental pulmonary embolism in rats. J Mol Cell Cardiol 2006; 41(2):296-307.