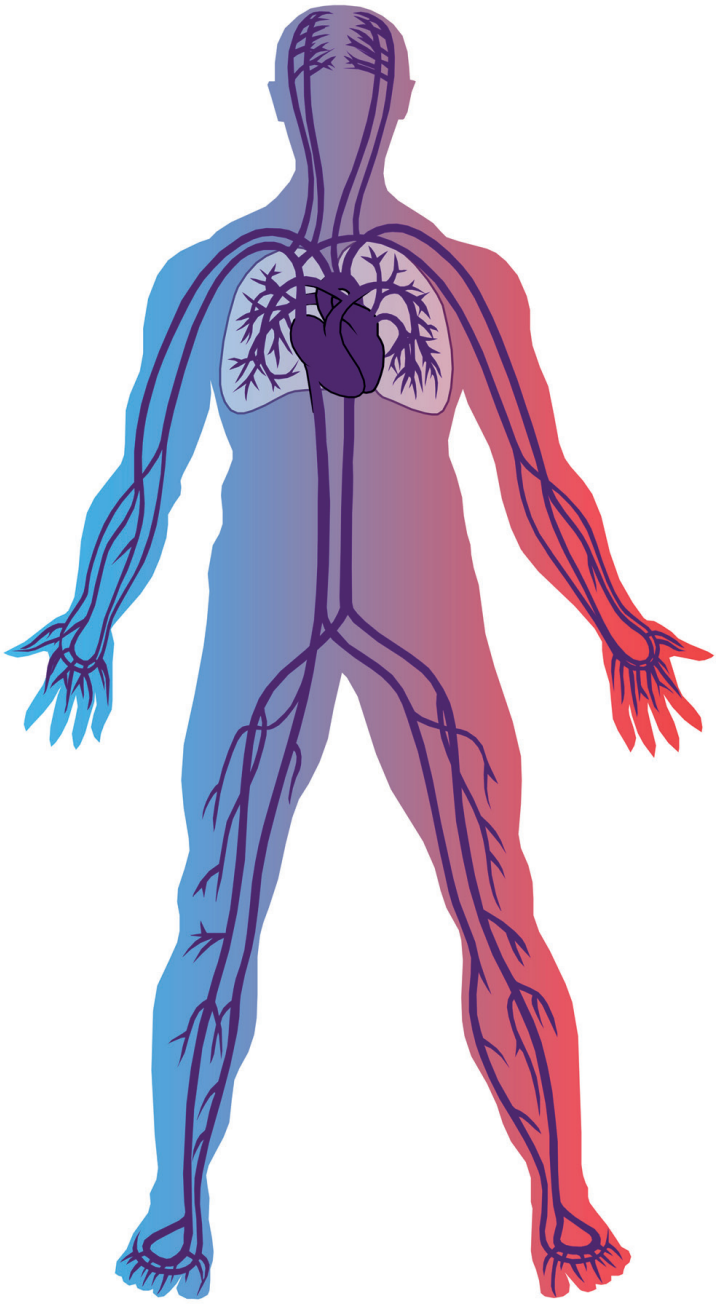
VENOUS AND ARTERIAL THROMBOEMBOLISM

Prevention and prognosis



Sharif M. Pasha

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Prevention and prognosis

Sharif Mohammed Pasha

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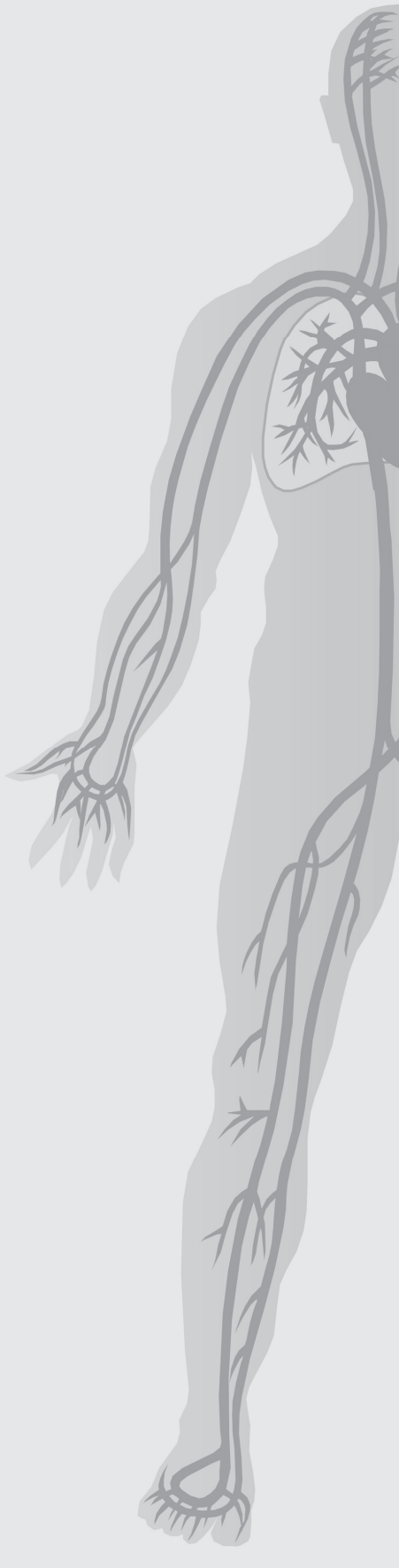

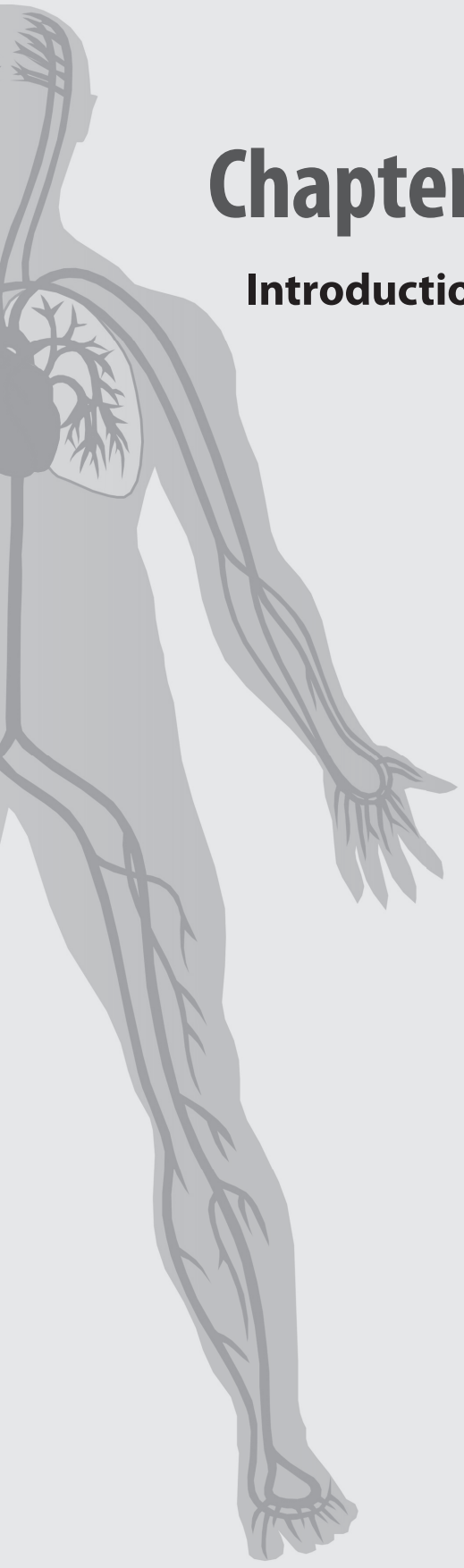

Chapter 1

Introduction

Excessive, non-traumatic bleeding and intravascular coagulation is prevented by a constant equilibrium of Virchow's triad which comprises changes in blood composition, vessel wall properties and blood flow (1). An imbalance in Virchow's triad towards coagulation may result in thrombotic events in venous or arterial systems. The most recognized entities of venous thromboembolic events (VTE) are deep vein thrombosis (DVT) of the leg and acute pulmonary embolism (PE) (2). Deep vein thrombosis is defined as the presence of thrombus in the deep vein system (3). It affects pre-dominantly the deep veins of the lower limbs. It must be distinguished from superficial thrombophlebitis which has a more benign prognosis (4). Acute pulmonary embolism is defined as an acute thrombus in the pulmonary artery (3). VTE can be provoked or unprovoked based on the presence of contributing risk factors effecting Virchow's triad. The most relevant risk factors for VTE include recent surgery or fracture of the lower extremity (odds ratio > 10), active malignancy (odds ratio 2-9), pregnancy or peri-partum period (odds ratio 2-9), hormone replacement therapy and/or use of oral contraception (odds ratio 2-9), immobility for more than three days or a recent long flight (at least 4 hours) (odds ratio < 2) (5). Unprovoked VTE is defined as VTE occurring in the absence of known risk factors.

Patients with DVT typically present with a painful, swollen leg, although non-specific symptoms of the calf muscle and lower extremities are common (6;7). The diagnosis has to be confirmed by compression ultrasonography, which is a highly accurate diagnostic test for detecting DVT (8). The classic presentation of pulmonary embolism is the sudden onset of pleuritic chest pain, shortness of breath, and hypoxia, although PE patients may present with a wide variety of symptoms ranging from non-specific thoracic pain, usually due to irritation of the visceral pleura, or gradually progressive dyspnea to sudden hemodynamic collapse (9;10). Because the signs and symptoms are non-specific, the diagnosis of pulmonary embolism should also be considered in patients with respiratory symptoms unexplained by an alternative diagnosis. The preferred imaging test for patients suspected of having PE is computed tomography pulmonary angiography (CTPA) (3;11). CTPA is readily available in most hospitals and has been shown to have high sensitivity and specificity for PE, which is fully comparable with the traditionally golden standard invasive pulmonary angiography (12;13). Sensitivity is especially high in central PE compared to segmental and especially sub-segmental levels. However, the clinical significance of sub-segmental PE is highly debated and a lower sensitivity for these smaller emboli is therefore accepted (14;15). Notably, important concerns regarding the increased frequency of CTPA use are long-term radiation complications, allergic reactions to iodinated contrast material and contrast-induced nephropathy (15-17). The latter complication is mainly present in patients with chronic kidney disease. The likelihood of this complication could be minimized by prophylactic protocols requiring intravenous fluid infusions like saline or sodium bicarbonate (18). Of note, the clinical risk for lifetime

need of renal replacement therapy due to contrast-induced nephropathy is very low (19).

To reduce the number of required imaging tests, an algorithm has been introduced to enable ruling out suspected PE based on a clinical scoring system and a D-dimer blood test (3;20-22). Examples of such clinical scoring systems are the Wells rules for DVT and PE and the (revised) Geneva scores in PE patients (20;23;24). The Wells' clinical decision rule for suspect PE is a scoring system based on objective signs, symptoms and risk factors of PE, which are assigned 1 till 3 points each, and a final subjective judgment, of treating clinicians regarding the likelihood of PE (3 points) (20). Using the Wells decision rule patients can be categorized in PE 'unlikely' (Wells score ≤ 4) or PE 'likely' (Wells score > 4) groups. In **chapter 2** we systemically review and quantify whether anticoagulant therapy can safely be withheld without the need for any further radiological testing in patients with an "unlikely" Wells' clinical decision rule in combination with a normal D-dimer test (<500 ng/ml). In **chapter 3** we focus on the potential adverse events of iodized contrast media by conducting a meta-analysis to assess the risk of contrast induced nephropathy and the risk of renal replacement therapy after contrast enhanced computed tomography in patients with chronic kidney injury. We also evaluated specific patient subgroups for their risk of developing contrast induced nephropathy.

Pulmonary embolism is a potentially lethal condition (10;25). Mortality is thought to be caused by right ventricular pressure overload secondary to acute pulmonary hypertension caused by PE (26). Radiological findings and cardiac biomarkers can be applied to identify patients with PE at higher risk of adverse clinical outcome, i.e. hemodynamic collapse or death. This is clinically relevant since these patients might benefit from reperfusion therapy on top of standard anticoagulation (3). Radiological signs predicting worse outcome include thrombotic burden and right ventricular function (27). Recent studies have evaluated whether RV dysfunction can be assessed using CTPA. For example, the ratio of RV to LV short-axis diameters was identified to be an accurate sign of RV dysfunction (27). Absence of RV dysfunction, described as a RV/LV ratio of 1.0 or less and a pulmonary artery obstruction index of 40% or less, is predictive for an event free outcome (28). It can be plausibly assumed that a delay in diagnosis and treatment initiation may be a strong predictor of adverse outcome as well. Notably, recent studies have not been able to confirm this association and reported that a patients' delay, i.e. time between symptom onset and presentation at the hospital, did not result in worse clinical outcome (29-32). **Chapter 4** reports on a study in patients with CTPA-proven PE in which we aimed to further investigate the impact of patient delay on PE prognosis by analyzing whether delay between symptom onset and presentation at the hospital influenced thrombotic burden and/or aggravated right ventricular function.

In addition to radiological signs of poor cardiac function, several biomarkers may predict adverse outcome after PE (33-35). A well-known cardiac biomarker that predicts

clinical outcome in PE is Brain-type Natriuretic Peptide (BNP). BNP is an amino-peptide predominantly excreted on hemodynamic changes by the myocytes in the left ventricle as pro-BNP (36). Pro-BNP is subsequently cleaved in the circulation into BNP and the inactive N-terminal-pro-BNP. The BNP release is directly proportional to the ventricular expansion and volume overload (37). The effects of BNP are vasodilatation, natriuresis and diuresis. These actions are thought to be primarily beneficial in pathological states of the left ventricle such as hypertension and compensated heart failure (38). Nonetheless, BNP levels are also raised in patients with acute PE, which predominantly affects the right ventricle and not the muscular left ventricle (39). To further understand the source of BNP release in acute PE, we analyzed associations between NT-pro-BNP levels and CT assessed right and left ventricular volumes and function in patients with and without PE (**chapter 5**).

The major pathophysiological mechanism of arterial thromboembolic events (ATE) is atherosclerosis. In short, atherosclerosis starts as fatty streaks caused by influx of lipids into the tunica intima, the innermost layer of artery walls (40). This initiates an inflammatory response with influx of monocytes, macrophages, T-cells and also platelets. Eventually, a protective fibrous cap is generated over fatty deposits (41). These lesions may fill the vascular lumen, lowering the post-lesion blood flow which ultimately results in transient ischemic disease such as angina pectoris in the coronary arteries, transient ischemic attack in the cerebral arteries or intermittent claudication in peripheral arteries (42). When this atherosclerotic plaque ruptures, a local thrombus is immediately formed that may cause a total obstruction of the arterial lumen causing acute ischemic disease as myocardial infarction and stroke (42). Most relevant non-genetic risk factors for ATE are smoking, hypertension, diabetes and hypercholesterolemia which are predominately diseases of the developed world but become also more and more prevalent in third world countries (43;44). Nonetheless, 80% of the worlds' cardiovascular burden occurs in low-income and middle-income countries (44). The individual contribution of these risk factors for cardiovascular disease is not easy to assess, since patients often have combination of multiple risk factors. For instance, only a small fraction of the hypertensive population has an elevation of blood pressure alone, with the majority exhibiting additional cardiovascular risk factors such as obesity or smoking (45). Notably, when concomitantly present, different risk factors may potentiate each other, leading to a total cardiovascular risk that is greater than the sum of its individual components (45). In **chapter 6** we focus on the effect of blood pressure in the occurrence of atherosclerosis in a unique study population living on the island of Flores, Indonesia. This is a treatment naïve population with a traditional "non-western" life style at the secondary epidemiological transition, which means a high burden of infectious disease and low amount of cardiovascular diseases. In this population we studied carotid intima media thickness — as a marker of atherosclerosis — in relation to blood pressure class.

Untill the beginning of the 21st century VTE and ATE were considered vascular diseases with different entities. However in 2003 a strong correlation between VTE and atherosclerosis was reported (48). In the latter study, ultrasonography of the carotid arteries was performed as a surrogate measurement of atherosclerosis in patients with DVT and healthy control patients. Carotid plaque was detected in 47.1% of the patients with unprovoked DVT compared to 27.4% (odds ratio 2.3; 1.4-3.7) of the patients with provoked DVT, and 32.0% (odds ratio 1.8; 1.1-2.9) in healthy control patients. These results indicated an association between ATE and VTE. In the years following his publication, several different studies have reported a higher incidence of ATE in patients with unprovoked VTE when compared to patients with provoked VTE or in whom VTE was suspected but ruled out by radiological examination (49-52). A meta-analysis showed that these findings could partly be explained by individual cardiovascular risk factors including obesity and hypertension, although the exact mechanisms remains unknown (53). Most of the studies on this subject included patients with VTE in general or with acute PE only, and did not focus solely on DVT patients. We assessed the incidence of ATE in patients with a first episode of compression ultrasonography proven provoked and unprovoked proximal DVT and compared them with the incidence of ATE in patients in whom DVT was clinically suspected but ruled out (**chapter 7**). In **chapter 8** we summarize and discuss the results of the studies presented in this thesis.

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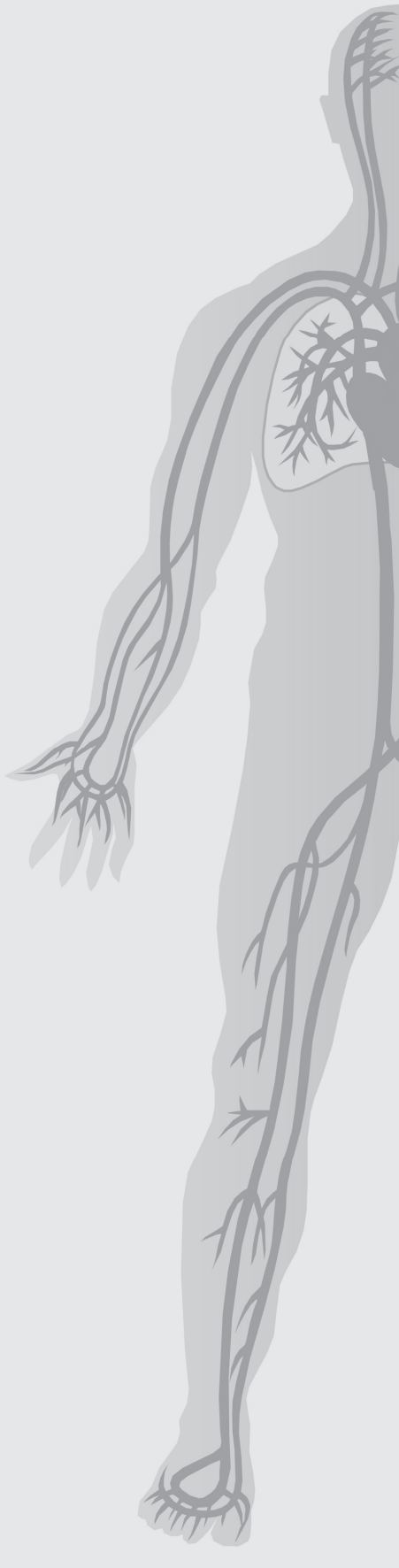

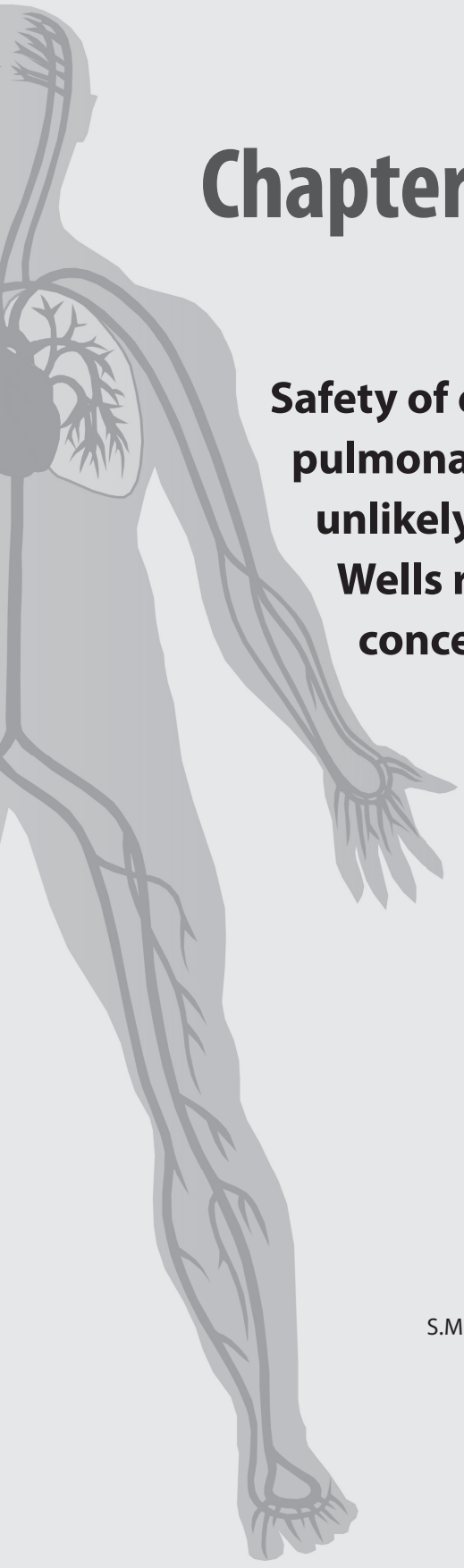

Chapter 2

Safety of excluding acute pulmonary embolism based on an unlikely clinical probability by the Wells rule and normal D-dimer concentration: a meta-analysis

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Thromb Res 2010; 125: e123-7

ABSTRACT

Introduction

The Wells clinical decision rule (CDR) and D-dimer tests can be used to exclude pulmonary embolism (PE). We performed a meta-analysis to determine the negative predictive value (NPV) of an "unlikely" CDR (≤ 4 points) combined with a normal D-dimer test and the safety of withholding anti-coagulants based on these criteria.

Methods

Prospective studies that withheld anti-coagulant treatment from patients with clinically suspected PE and an "unlikely" CDR in combination with a normal D-dimer concentration without performing further tests were searched for in Medline, Cochrane and Embase. Primary endpoints were the recurrence rate of venous thromboembolism (VTE) and PE-related mortality during 3-months follow-up.

Results

Four studies including 1660 consecutive patients were identified. The pooled incidence of VTE after initial exclusion of acute PE based on an "unlikely" CDR and normal D-dimer was 0.34% (95%CI 0.036-0.96%), resulting in a NPV of 99.7% (95%CI: 99.0-99.9%, random effects-model). The risk for PE related mortality was very low: 1/1660 patients had fatal PE (0.06%, 95%CI 0.0017-0.46%).

Conclusion

Acute PE can be safely excluded in patients with clinically suspected acute PE who have an "unlikely" probability and a negative D-dimer test and anticoagulant treatment can be withheld. There is no need for additional radiological tests in these patients to rule out PE.

INTRODUCTION

The possibility of excluding the diagnosis of acute pulmonary embolism (PE) without the need for radiological imaging is a great step forward in the complex management of patients suspected of this disease. In this way diagnostic time, costs and potential complications from performing computed tomographic pulmonary angiography (CTPA) or ventilation-perfusion (V-Q) lung scintigraphy, including radiation exposure and allergic reaction to contrast dye, are kept limited.

The Wells rule (Table 1) is widely used as a clinical decision rule for the assessment of clinical probability for PE (1-5). Originally using this rule, patients could be divided into three categories of increasing risk for having PE. These categories were low (<2.0 points, 2.0% PE), moderate (2.0-6.0 points, 18.8% PE) and high (>6.0 points, 50% PE) clinical probability.(5) It has previously been shown that anticoagulant therapy could be safely withheld from patients classified as low risk with a normal D-dimer concentration (28.4% of the population) (5). In a post hoc analysis, it was suggested that using dichotomization of the Wells rule – dividing patients in to PE "unlikely" (≤4 points) and PE "likely" (>4 points) – PE might be safely excluded based on a low as well as an "unlikely" clinical probability and a normal D-dimer test, with very low three months venous thromboembolism (VTE) recurrence (5). Importantly, in that study 50% of patients had a Wells score

Table 1. The Wells clinical decision rule.[5]

Variable	Points
Clinical signs and symptoms of DVT (minimum of leg swelling and pain with palpation of the deep veins)	3
An alternative diagnosis is less likely than PE	3
Heart rate greater than 100	1.5
Immobilisation or surgery in the previous four weeks	1.5
Previous DVT/PE	1.5
Hemoptysis	1
Malignancy (on treatment, treated in the last 6 months or palliative)	1
Clinical probability	
Low	< 2 total
Intermediate	2-6 total
High	>6 total
Simplified assessment	
Likely	≤ 4 total
Less likely	> 4 total

of 4 points or less (5.1% PE) compared to only 31% of patients who had a score of 2 points or less (2.0% PE). Thus, the 2-level approach increases the number of patients in whom radiological tests can be avoided although it puts more weight on the sensitivity of the D-dimer test. An additional advantage of the dichotomization of the Wells rule is that it involves a simpler triage in patients.

In spite of the important advantages of a diagnostic strategy including the dichotomized Wells rule and D-dimer testing to exclude acute PE, widely implementation of such a diagnostic algorithm is lacking (6). In one recent study, only 58% of the patients with a positive D-dimer underwent, as should be, CT-scanning, and in 7% of the patients with negative D-dimer results, superfluous CT scans were performed (7). One explanation for this is that the safety of this algorithm is understudied and untreated PE is a major concern for every physician since that has been shown to have a high mortality rate ranging from 9.2-51% (8,9).

For this reason, we have performed a systematic review and meta-analysis of studies in patients that excluded acute PE on the basis of an "unlikely" clinical probability (Wells rule ≤4 points) and a normal high sensitive D-dimer test to evaluate the safety of such a diagnostic strategy.

METHODS

Data sources

A literature search was performed to locate all prospective studies using a diagnostic strategy including a dichotomized clinical decision rule and a D-dimer test to rule out PE. Predefined search terms were used as Mesh terms as well as free text words. All full articles published after the introduction of the Wells score (2000) till the December 1st 2008 (date of our final search) were eligible for inclusion in the analysis. The articles were limited to the English, German, French or Dutch language. In addition to Medline, Embase and Cochrane databases were searched, but did not enclose any additional studies useful for inclusion in our meta-analysis. All patients with an "unlikely" clinical probability and normal D-dimer test result who did not undergo any further radiological imaging aimed at evaluating the presence of PE or DVT were included. Finally, a clinical follow-up of at least three months was demanded.

Study outcome

The outcome of this meta-analysis was the safety of not treating patients with suspected acute PE, an "unlikely" clinical probability according to the Wells rule (≤4 points) and a normal D-dimer test result. The primary study endpoint was defined as all objectively confirmed fatal and non-fatal VTE defined as PE or deep vein thrombosis (DVT). As a

secondary endpoint, we studied total VTE attributable mortality. Mandatory for inclusion was the consecutive enrollment of patients and the prospective assessment of the Wells rule and D-dimer concentration.

Data abstraction

All identified articles were individually reviewed by two reviewers (S.P and F.K). In case of disagreement, a third reviewer (M.H.) was consulted. Data on the study design, patient characteristics, the incidence of PE or DVT in the follow-up period and the mortality rate attributable to PE were abstracted following the Guidelines proposed by the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group (10). Individual study quality was assessed by the following items: patient enrollment, outcome assessment, duration of follow-up, loss-to-follow-up and funding source.

When studies were identified that excluded PE in patients based on an "unlikely" clinical probability and a normal D-dimer test but the paper itself did not contain all necessary information for our analysis, the authors of these papers were asked to provide the missing numbers.

Statistical analysis

The proportions of total and fatal recurrent VTE in patients in whom acute PE was ruled out based were pooled to assess the safety of not treating these patients. Patients who were lost to follow-up were excluded from the analysis. For our primary endpoint analysis, patients receiving anticoagulants and patients in whom additional diagnostic tests were performed despite both an "unlikely" clinical probability and a negative D-dimer were excluded. Since it is not unlikely that patients in whom additional tests were performed despite formal exclusion of pulmonary embolism (which are protocol violations) are more prone to be diagnosed with a venous thrombotic event during follow-up, a second calculation of the incidence of the occurrence of thrombosis during follow-up was performed without excluding these patients, which leads to a more conservative estimation of the safety of this strategy. The upper limit of the 95% confidence interval of the fatal and non-fatal three months thromboembolic rate after a negative invasive pulmonary angiography was defined as the cut-off point for the safe exclusion of PE (11). To pool the proportions of venous thrombosis during follow-up, both random and fixed effect models were employed. Since the incidences of events were very low and even zero in some studies, individual studies by inverse variance weighting could not be pooled (using this strategy, studies with a proportion and a variance of zero would not be included in the model). Therefore inverse arcsine variance weights for the fixed effects model and DerSimonian-Laird weights for the random effects were used, (12) as embodied in the statistical package StatsDirect (StatsDirect Ltd, Cheshire, UK). To assess heterogeneity among included studies the I2 statistic with its 95% confidence

interval was calculated, which is the percentage of variation across studies that is due to heterogeneity rather than chance. All calculations were performed using StatsDirect (StatsDirect Ltd, Cheshire, UK).

RESULTS

Study selection

The literature search revealed 157 articles. Of these, 139 were excluded after review of the title and abstract. After full review another 14 articles were excluded because of use of other clinical decision rules, retrospective assessment of the Wells rule or performance of additional diagnostic tests to rule out PE. Eventually, 4 studies were approved for inclusion in this meta-analysis (figure 1)(1-4).

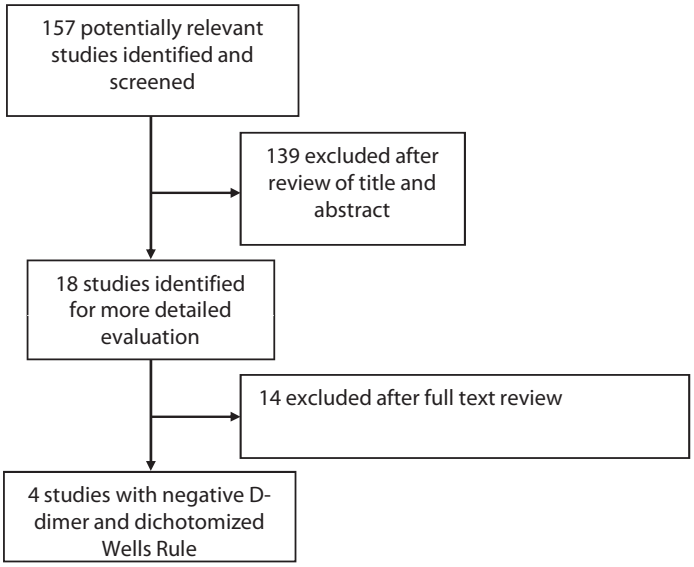

Figure 1. Flowchart of study inclusion

Quali and characteristics of included studies

All four identified studies were prospective studies with a follow-up period of three months. The loss to follow-up was very low (0.0-0.7%, table 2). The mean age of the patients was in the range of 51 to 54 years (table 2) and 34.2 to 42.6% of the patients were of male gender. The vast majority of the patients were outpatients (70.9-100%

Table 2. Study characteristics

Study	Rodger et al[1,*]	van Belle et al[2]	Goekoop et al[3]	Anderson et al[4,#]
Characteristic				
Study population	199	3306	879	NA
Age, mean (SD), y	53.5 (18)	53.0 (18.4)	51 (18)	NA
Male	68 (34.2)	1409 (42.6)	329 (37.4)	NA
History of VTE	22 (11.1)	480 (14.5)	83 (9.4)	NA
Malignancy	56 (28.1)	476 (14.4)	17 (1.9)	NA
Recent surgery, immobilization or trauma	29 (14.6)	610 (18.5)	50 (5.7)	NA
Outpatients	141 (70.9)	2701 (81.7)	879 (100)	NA
Duration of follow-up	3 months	3 months	3 months	3 months
Lost to follow-up	0 (0%)	4 (0.12%)	6 (0.7%)	0 (0%)
Study design	Prospective	Prospective	Prospective	Prospective
D-dimer test	Simplired[$] and Accuclot latex agglutination[†]	VIDAS[‡] and Tinaquant[§]	VIDAS[‡]	NA[Δ]
D-dimer cut-off value	NA	500 ng/mL	500 ng/mL	NA
Funding source	The Heart and Stroke Foundation of Canada, Ottawa	Participating hospitals	Participating hospitals	The Canadian Institutes of Health research

* Only the Bioped arm is included since this arm contained the needed group of patients.

[#] Patient group was already excluded before characterisation

[$] SimpliRED (Agen Biomedical Limited, Acaccia Ridge, Australia)

[†] Accuclot latex agglutination D-dimer test (Sigma Diagnostics, St Louis, Mo)

[‡] VIDAS D-dimer assay (BioMérieux, Marcy- l'Etoile, France)

[§] Tinaquant assay (Roche Diagnostica, Mannheim, Germany)[Δ]Patients underwent automated D-dimer testing from plasma samples according to local practice in the participating 5 academic health centers

between the studies, table 2). Different D-dimer tests were used; VIDAS D-dimer assay (BioMérieux, Marcy- l'Etoile, France) SimpliRED (Agen Biomedical Limited, Acaccia Ridge, Australia), Tinaquant assay (Roche Diagnostica, Mannheim, Germany) and the Accuclot latex agglutination D-dimer test (Sigma Diagnostics, St Louis, Mo). A total of 88 protocol violations (0-22% between the studies) were observed: in 45 patients, CT- or V-Q scans were performed to rule out PE although the assessment of clinical probability indicated "unlikely" and the D-dimer concentration was normal. Two of these 45 patients (3.3%) were diagnosed with PE and one (1.7%) received OAC therapy because of a medical history of acute PE and a clinical presentation of hemoptysis even though a subsequently performed CT-scan ruled out PE (3). In 14 additional patients undefined protocol violations were reported (1). Finally, 29 patients receiving anticoagulant therapy for other

reasons than PE were left out of the analysis in one study (2). In contrast to the former 59 patients, follow-up in these 29 patients could not be retrieved.

Meta-analysis

The 4 included studies comprised 5801 patients of who 1660 (29%) were left untreated because of unlikely clinical decision rule and normal D-dimer test result. Of these 1660 patients 6 patients (6/1660, weighted pooled incidence 0.34%; 95%CI 0.036- 0.96%) were eventually diagnosed with symptomatic VTE during three months follow-up (Table 3, figure 2). One of these patients (1/1660, weighted pooled incidence 0.10%; 95%CI 0.0017-0.46%) died possibly as a direct consequence of fatal PE (Table 3, figure 3). The pooled negative predictive value (NPV) of having VTE during three months follow up after an "unlikely" clinical probability in combination with a normal D-dimer test was 99.7% (95%CI 99.0-99.9%).

After including patients in whom additional diagnostic tests were performed despite both an "unlikely" clinical probability and a negative D-dimer, the pooled analysis included 1719 patients of whom 8 were diagnosed with VTE during follow-up (8/1719, weighted pooled incidence 0.53%; 95%CI 0.24-0.92%). Of these, one patient (1/1719, weighted pooled incidence 0.096%; 95%CI 0.0008-0.42) was diagnosed with fatal PE. This results in a NPV of having VTE on follow-up of 99.5% (95%CI 99.1-99.8).The assessment of heterogeneity in our primary analysis showed an I2 statistic of 46.2% (95%CI 0-80.8%) in the VTE in follow-up analysis and an I2 statistic of 37.2% (95%CI 0-78.5%) in the mortality analysis. The use of a fixed effects model did not materially change the study outcome.

The possibility of publication bias in our meta-analysis was assessed with the use of funnel plots. No indication of publication bias was detected.

Table 3. Outcome of PE related morbidity and deaths of the included studies (3 months follow up)

Study	Number of patients with "unlikely" clinical probability and negative d-dimer test (n)	Incidence of VTE in follow-up (n)	Number of mortality due to PE in follow up (n)
Rodger et al[1]	49	1 (%)	1 (%)
van Belle et al[2]	1028	5 (%)	0 (%)
Goekoop et al[3]	405	0 (%)	0 (%)
Anderson et al[4]	178	0 (%)	0 (%)

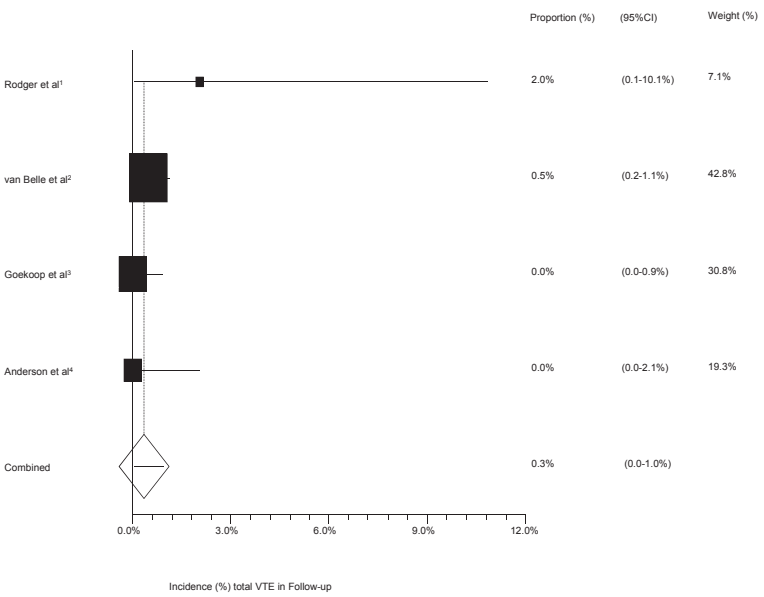

Figure 2. Incidence of total VTE during follow up (random effects model)

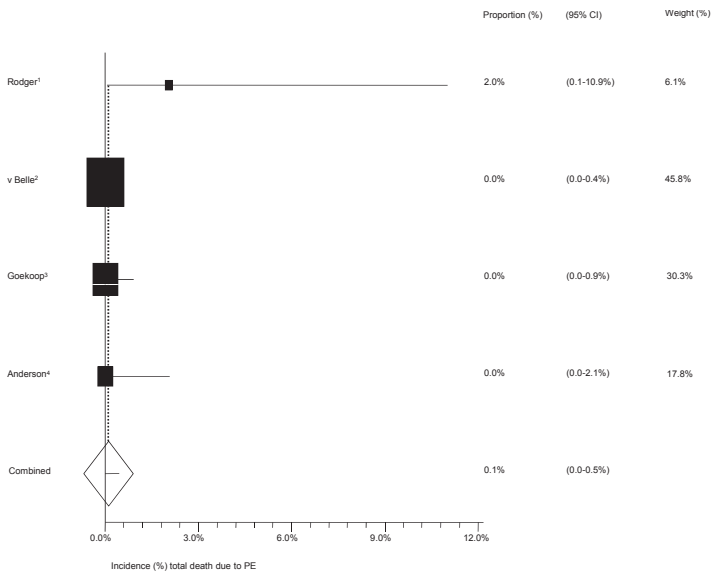

Figure 3. Incidence of death due to VTE on follow up (random effects model)

DISCUSSION

The main finding of our study is that the pooled incidence of morbidity due to PE after an "unlikely" clinical decision rule (Wells Rule ≤ 4 points) in combination with a normal D-dimer test is 0.34% (95%CI 0.036- 0.96%), resulting in a NPV of 99.7% (95%CI 99.0-99.9%). The upper limit of the 95% confidence interval of the three months VTE-rate after negative pulmonary angiography is 2.7% (11). Using this 2.7% as the threshold for the safe exclusion of PE, our analysis shows that ruling out PE on basis of a negative D-dimer test and an "unlikely" clinical decision rule is a very safe criterion even after inclusion of the patients in whom additional radiological tests were performed (weighted pooled incidence of VTE 0.53%).

Additionally, the three months mortality risk of PE in these patients was very low (0.10%; 95%CI 0.002-0.46%). This pooled three months mortality rate compares favourable to the mortality rate after a negative pulmonary angiography (0.3%) (11).

The patients with "unlikely" clinical probability for PE and a normal D-dimer concentration represent almost one third of the total patient population with suspected acute PE. Importantly, CT scans can be withheld in all these patients, saving diagnostic time, costs and preventing CT related complications as contrast induced nephropathy or cancer caused by radiation exposure.

We consider our results representative because our results were based on a pooled analysis of a large cohort of 1660 patients. Given the extremely narrow confidence intervals we found, it is highly unlikely that future studies would materially change our results and influence the conclusions based on the present analysis. The data was abstracted from high quality studies with a prospective design, consistent follow-up time and comparable demographic patient characteristics. Furthermore, all endpoints were well-defined and confirmed by predefined criteria. In addition, there was no indication for important inter-study heterogeneity since the I2 statistics for all analysis were rather low and the use of random or fixed effect models did not substantially alter our study outcome. We decided to present the results of the random effect models, since random effects models lead to more conservative estimates than fixed effect models. Therefore, underestimation of the incidence of VTE or death due to fatal PE is highly improbable. In addition, even after including patients in whom the study protocols were violated (i.e. additional diagnostic testing despite "unlikely" clinical probability and negative D-dimer) the incidences of morbidity and mortality due to recurrent disease remained very low. Furthermore, funnel plots provided no indication for the existence of publication bias. Of note, one potential limitation of our study is the use of different D-dimer assays among the included studies. Nonetheless, since all used assays are reported to have high sensitivity for detection of venous thromboembolism, we are convinced that this has not greatly influenced our study outcome.

Our results have important clinical implications. The results of our meta-analysis show that anticoagulant therapy can safely be withheld without the need for any further radiological tests in patients with an "unlikely" CDR in combination with a normal D-dimer test. These patients represent almost one third of the total patient population with suspected PE. It is possible to take fast management decisions by this combination of bedside tests in these patients as the results of these tests are ready in less than 1-2 hours after presentation.

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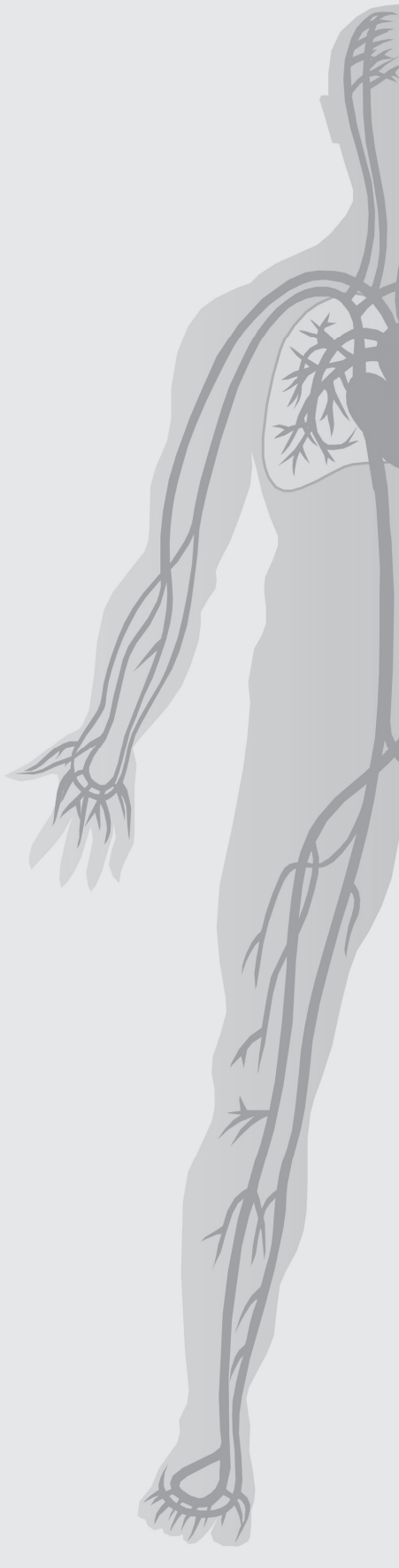

Chapter 3

Meta-analysis: Serum creatinine changes following contrast enhanced CT imaging

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ABSTRACT

Introduction

Contrast induced nephropathy (CIN) is a decrease in renal function following administration of contrast media (CM) and is defined as an increase in serum creatinine over 25% or 44 µmol/ L from baseline in the absence of other causes. The aim of this meta-analysis was to asses the overall risk of CIN and the need for renal replacement therapy (RRT) after a contrast enhanced CT-scan. Finally, we aimed to identify subgroups at increased risk for CIN.

Methods

A literature search was performed to identify studies evaluating the incidence of CIN after contrast enhanced CT in Pubmed, Medline, Embase and Cochrane databases. Our primary endpoint was the pooled incidence of CIN. Secondary endpoint was the pooled incidence of CM induced need for RRT or chronic loss of kidney function after contrast enhanced CT. Furthermore, prespecified subgroup analyses were performed for different patient categories. Meta-analysis was performed using an exact likelihood approach.

Results

In total, 40 studies evaluating the incidence of CIN after contrast enhanced CT were included in this meta-analysis. The pooled incidence of CIN after contrast enhanced CT was 6.3% (95% CI 4.9-8.1). The risk of RRT as a result of CIN was low, 0.06% (95% CI 0.008-0.4) and the decline in renal function persisted in 1.1% of patients (95% CI 0.6-2.1%). The incidence of CIN was not influenced by study design or quality. Patients with chronic kidney disease (estimated filtration rate < 60 ml/min/1.73m2, incidence 7.7%, 95% CI 5.3-11.1) or diabetes mellitus (incidence 10.5%, 95% CI 6.4-16.7) might be at slightly increased risk for the development of CIN after contrast enhanced CT.

Conclusion

CIN occurs in about 6% of patients after contrast enhanced CT. In 1 % of all patients (95% CI 0.6-2.1%) the decline in renal function persisted. The clinical relevance of these findings should be elucidated in further studies on clinically relevant endpoints.

INTRODUCTION

Contrast media are often administered intravenously to improve imaging in patients undergoing CT-scanning. Besides allergic reactions, contrast induced nephropathy (CIN) is one of the most feared complications of iodinated contrast media (1). Contrast induced nephropathy is usually defined as an increase in serum creatinine over 25% or 44 µmol/L from baseline value in 48-72 hours after contrast media administration in the absence of other aetiologies (2,3). Although CIN is mostly mild and reversible, it can occasionally lead to a need for renal replacement therapy (RRT). Furthermore, after intra arterial contrast administration CIN, even in milder forms, is associated with an increased morbidity and mortality during hospitalization (3-5).

Risk factors for the development of CIN have particularly been studied after intra arterial contrast administration used for procedures like percutaneous transluminal (coronary) angiography. Those risk factors such as diabetes mellitus, cardiovascular disease, use of nephrotoxic medication and chronic kidney disease are common in patients with an indication for contrast media enhanced CT-scanning. However, contrast enhanced CT might sometimes be withheld in patients at increased risk for developing CIN in order to prevent nephrotoxicity in patients with already multiple co-morbidities (6,7). This could result in a diagnostic delay or a reduced accuracy of the diagnostic process in a major group of patients (6,8). Therefore, detailed knowledge about the risk and the prevention of CIN is necessary (9).

Various studies on the incidence and prevention of CIN have been performed over the last years and show an incidence of CIN after contrast enhanced CT varying between 0-25 % (1,10). This wide variability could possibly be explained by the difference in co-morbidities and the amount of contrast administered to perform contrast enhanced CT between the published studies. The aim of this meta-analysis was to assess the overall risk of CIN, persistent loss of renal function and the need for RRT in patients undergoing contrast enhanced CT. Finally, we aimed to identify subgroups at increased risk for CIN.

METHODS

Data sources and study selection

A literature search was performed to identify studies evaluating the incidence of CIN after contrast enhanced CT. Predefined search terms were used as Mesh terms and free text words, including "CIN", "contrast media" and "venous". Since the use of high osmolar contrast media decreased substantially after the year 2000, articles published thereafter till April 1st 2010 (date of final search) were eligible for inclusion in the analysis. The articles were limited to the English, Dutch, German and French language. In addition

to the Pubmed search, Medline, Embase and Cochrane databases were scrutinized, but did not enclose any additional studies eligible for inclusion. There were no restrictions to the included patient population. Finally, a follow-up period for the occurrence of CIN of at least 24 hours after contrast enhanced CT was demanded for studies to be eligible.

Study outcome

The main outcome of this meta-analysis was the pooled incidence of objectively confirmed occurrences of CIN after contrast enhanced CT. As a secondary endpoint, we assessed the incidence of contrast media induced need for RRT. Furthermore, predefined subgroup analyses were performed for different patient categories at increased risk for CIN due to co-morbidities such as chronic kidney disease (estimated filtration rate < 60 ml/min/1.73m2), diabetes mellitus and cardiovascular disease or the use of nephrotoxic medication. In addition a meta-regression was performed to estimate the effect of the administered CM dose and study quality on the occurrence of CIN.

Data abstraction

All identified articles were reviewed independently by two reviewers (S.P. and J.K.). In case of disagreement a third reviewer (W.Z.) was consulted. Data on the study design, patient's characteristics, the incidence of CIN in the follow-up period and the known risk factors for CIN were abstracted following the Guidelines proposed by the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group (11). When studies did assess the occurrence of CIN after contrast enhanced CT but the paper did not contain the necessary data for the analysis, the corresponding authors of the papers were contacted to provide the missing data. Individual study quality was assessed by an adaption of the Newcastle-Ottawa Scale (12). This scale contains among others data on the assessment of exposure and outcome, timeframe, adequacy of follow up and demonstration of a stable renal function at start of the study, see table 2. First of all, the timeframe of follow up could be of major influence on the reported incidence of CIN. We considered a time frame of 24-96 hours after CT adequate. Second, when a large group of patients is lost to follow up, the observed incidence of CIN might not be accurate. Studies with at least 90% of patients with a complete follow up were considered ample. Third, if renal function is already declining prior to contrast enhanced CT, it might be difficult to establish whether a patient has developed CIN. For that reason, studies demonstrating renal function to be stable at baseline were regarded as sufficient. Fourth, studies were considered representative if the studied population comprised all patients undergoing CT in daily practice.

Statistical analysis

The main outcome of the present meta-analysis was the pooled proportion of patients with CIN after contrast enhanced CT. For all studies, the proportion of patients with CIN was calculated as the number of patients with CIN divided by the total number of patients undergoing contrast enhanced CT. Exact 95% confidence intervals were calculated for all proportions.

Meta-analysis was performed using an exact likelihood approach. The method used was a logistic regression with a random effect at the study level (13). Given the heterogeneity of clinical conditions, a random effects model was performed by default and no fixed effects analyses were performed. I2 statistics cannot be calculated from meta-analyses based on exact likelihood approaches, and I2 estimates were therefore not reported. For meta-analysis of proportions the exact likelihood approach based on a binomial distribution has advantages compared to a standard random effects model that are based on normal distributions (14). Firstly, estimates from a binomial model are less biased than estimates from models based on a normal approximation (13,15). This is especially the case for proportions that are close to 0 or 1. Secondly, no assumptions are needed for the exact approximation when dealing with zero-cells, whereas the standard approach needs to add an arbitrary value (often 0.5) which contributes to the biased estimate of the model (16,17). The meta-regression was based on a binomial distribution.

All analyses were performed with STATA 10.0 (Stata Corp, Texas, USA).

RESULTS

Study selection

The literature search identified 963 unique studies (figure 1). After evaluation of title and abstract 903 studies were excluded. Another 20 studies were excluded after more thorough examination: eight of these 20 studies did not contain original data (5,18-24) and in five studies contrast media were administered intra arterially as well (23-27). Patients were included before the year 2000 in four studies and therefore were not eligible for inclusion (28-31). The endpoint of CIN was lacking in two studies (32,33). One study had a case control design so the incidence of CIN could not be determined (4). Finally, a total of 40 studies were eligible for inclusion in this meta-analysis.

Study Characteristics

Included studies were published between 2000 and 2010 and contained a total of 19,585 patients, with the largest study comprising 11,516 patients (34). Serum creatinine was measured 48-96 hours after contrast enhanced CT in the majority of studies and in 7

cohorts after 48-168 hours. All included studies defined CIN as an increase in serum creatinine over 25% or over 44 µmol/ L (0.5 mg/dL) after contrast enhanced CT.

Study characteristics are summarized in table 1. Mean age ranged from 44-74 years old, the percentage of female gender varies from 3-58% and patients were administered between 10-230 ml of contrast media in concentrations of 200-400 mg/mL Iodine. In 25 out of 33 studies (75%) including patients with chronic kidney disease, hydration prophylaxis regimes were advised.

Table 1. Summary characteristics of 40 included studies

Study Characteristics	
Year of publication	2000 – 2010
Study design	
Prospective follow-up study	21 (53%)
Retrospective follow-up study	19 (48%)
Studies including only high risk patients (i.e. eGFR < 60 ml/min/1.73m²)	16 (40%)[·]
Total number of patients	19 585
Clinical Characteristics	
Mean age	58
Gender, m/f[£]	10 992 / 7 963
Mean serum creatinine at baseline (µmol/L)	102[¥]
Patients with chronic kidney disease (%)*[§]	35[‡]
Patients with diabetes mellitus (%)	28[†]
Patients with hypertension (%)	19[§]
Range of means NaCl or NaHCO3[ƒ] *administered (mL)*	500-3000
Concentration Iodine in contrast media (mg/mL)	200-400
Range of means of amount contrast administered (mL)	10-300[#]
Risk of bias assessment	
Representativeness of the exposed cohort (% studies)	72.5
Demonstration outcome of interest not present at start of study (% studies)	22.5
Adequate follow-up timeframe for outcomes to occur (% studies)	72.5
Adequacy of follow up of cohorts (% studies)	48

[£] Data on 4 studies missing, [¥] Data on 8 studies missing, [·]data on 2 studies missing
*** eGFR: estimated glomerular filtration rate, [ƒ] NaCl: sodium chloride, NaHCO3: Sodium bicarbonate**
[§] Chronic Kidney Disease, estimated glomerular filtration rate < 60 ml/min/1.73m²
[‡] Data of 5 studies missing, [†] data of 17 studies missing, [§] data of 30 studies missing, [#] data on 11 studies missing

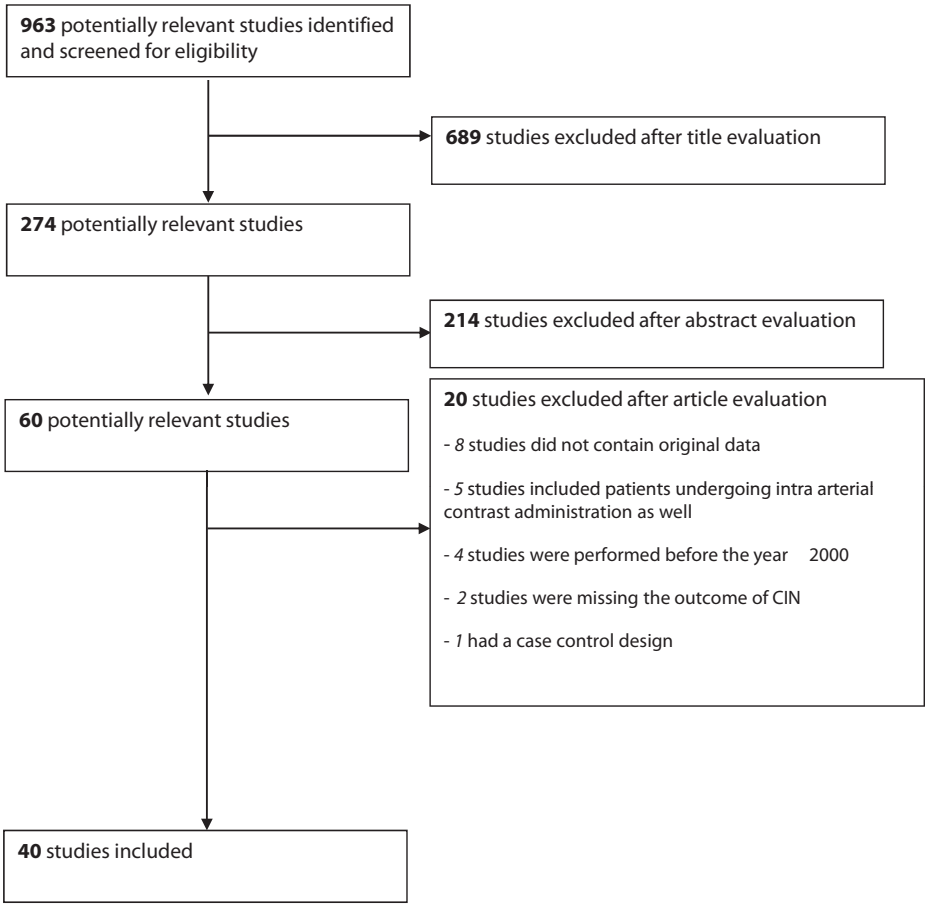

Figure 1. Flowchart of study inclusion

Study quality

In 29 studies (73%) the cohort was representative for the population undergoing contrast enhanced CT in daily practice. In 29 (73%) studies the timeframe of follow-up was adequate for diagnosing CIN, between 24 and 96 hours after contrast enhanced CT. In other studies, serum creatinine was measured until one week after contrast enhanced CT. However, in only 18 studies (45%) follow up was completed in at least 90% of the included patients. Importantly, only nine studies (23 %) assured renal function to be stable at baseline, therefore a laboratory confirmed CIN would be unlikely to be due to an instable renal function.

Meta-analysis

Reported incidences of CIN after contrast enhanced CT ranged from 0 to 25% (35,36). The weighted pooled incidence of CIN was 6.3% (95% CI 4.9-8.1) in a random-effects model (see figure 2). The need for RRT as a result of CIN was rare with a weighted pooled incidence of 0.06% (95% CI 0.01-0.4). Stratified analyses showed that only patients with chronic kidney disease or diabetes mellitus might be at slightly increased risk for developing CIN after contrast enhanced CT, with an incidence of 7.7% (95% CI 5.3-11.1)

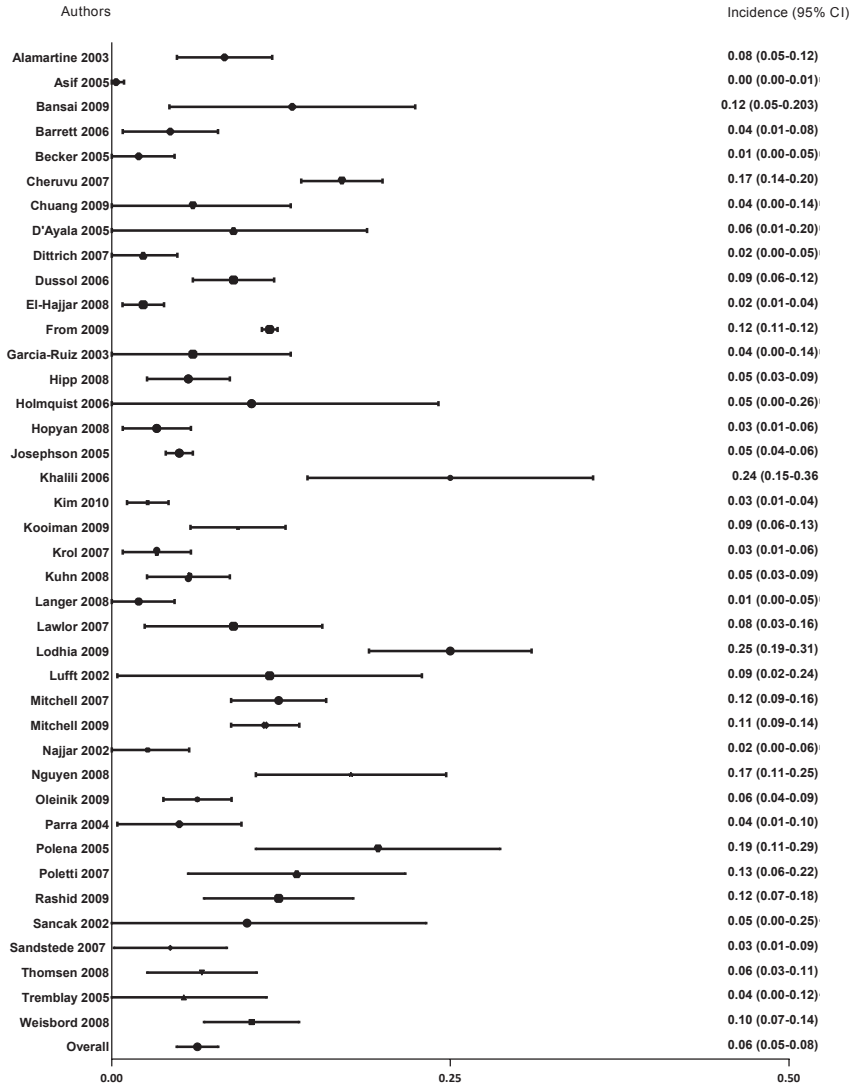

Figure 2. Funnel plot of 40 included studies on the incidence of contrast induced nephropathy

in patients with chronic kidney disease versus 5.5% (95% CI 3.2-9.2) in patients without chronic kidney disease and 10.5% (95% CI 6.4-16.7) in patients with diabetes mellitus versus 4.5% (95% exact CI 2.1-9.3) in patients without diabetes mellitus (see figure 3). Twenty studies reported the course of renal function in CIN positive patients at least one week after contrast enhanced CT, varying from one week till two months after contrast enhanced CT. In 1.1% of all patients undergoing CT (95% CI 0.6-2.1%) the decline in renal function persisted.

In a meta-regression the administered Iodine dose was not evidently associated with the occurrence of CIN (p=0.58). Restricting the analysis to studies with an adequate follow-up had no significant effect on the reported incidence of CIN. Neither had a random-effects meta-regression on the difference between prospective and retrospective studies (p=0.90) or the consecutiveness of included patients (p=0.73).

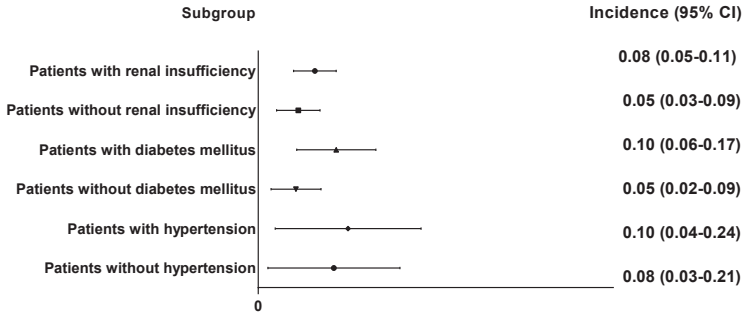

Figure 3. Meta-analyses according to patient category

DISCUSSION

This is the first meta-analysis to assess the incidence of CIN after contrast enhanced CT. The main finding of this meta-analysis is a pooled incidence of CIN after contrast enhanced CT of 6.3%. In addition, the risk of RRT as a result of CIN after contrast enhanced CT was very low,(0.06%) and in 1% of all patients undergoing CT the decline in renal function persisted. Finally, patients with chronic kidney disease or diabetes mellitus seem to be at slightly increased risk for developing CIN after contrast enhanced CT.

There are some limitations as a result of the variable design of the studies included in our meta-analysis. First, it should be noticed that the incidence found in our study is probably an overestimation due to the following reasons; the definition of CIN requires absence of other aetiologies for the sudden rise in serum creatinine (2,3). If follow-up is short and the diagnostic work up is limited other causes of acute kidney injury might be

underappreciated (6). Moreover, ten studies in this meta-analysis included patients who were admitted at an emergency department (7,10,18,37-43). Those patients might not be hemodynamically stable, which might lead to a, often clinically irrelevant, fluctuation in serum creatinine values in the first days after admission which is independent of contrast enhanced CT.

Second, the clinical course of CIN positive patients could not be studied by this meta-analysis since only four out of the 40 included studies addressed hospitalization and morbidity after contrast enhanced CT and nine studies only addressed mortality as a secondary endpoint. In our opinion, further studies should all mention these items as an endpoint in their analysis. Furthermore, the 20 studies observing the course of renal function after patients were diagnosed with CIN had different timeframes for this endpoint, varying from one week to two months. It would be of great value when all studies could measure creatinine at various points in time.

Third, not all studies reported risk factors for CIN detailed enough for proper subgroup analyses of our meta-analysis. As a result not all co-morbidities generally considered to be relevant risk for developing CIN, including heart failure and peripheral artery disease were shown to be risk factors according to this meta-analysis (9). Hence, only diabetes mellitus and chronic kidney disease were demonstrated to yield a slightly higher incidence of CIN.

Fourth, we were unable to incorporate a control group. This control group might be able to indicate whether the risk of acute kidney injury is higher in patients receiving contrast media vs. patients undergoing CT without it. This might rule out the effect of other causes of acute kidney injury on the risk assessment of CIN. In studies including patients in the emergency department or intensive care unit such a study design could make sense. However for studies reporting the incidence of CIN in an elective outpatient cohort, this control group would be of low relevance. We included four elective outpatient cohorts in our meta-analyses, reporting incidences of 1.8, 2.5, 4.1 and 7.7% respectively (44-47). The profound evidence for CIN studied by a trial randomizing between the administration of contrast media or placebo will always be lacking due too the clinical indication for contrast administration.

Additionally, our meta-analysis has some intrinsic limitations as well. First, our pooled incidence risk of CIN is influenced by preventive hydration regimes in patients at high risk for CIN. In 75% of studies including patients at high risk for CIN some form of hydration regime was applied. This might lead to an underestimation of the risk for CIN in high risk patients without preventive measures. Due to our design the magnitude of this effect on the incidence of CIN could not be assessed. No randomized controlled trials in patients undergoing contrast enhanced CT within our inclusion criteria for eligible studies were found.

Second, our meta-analyses might be under the influence of bias. Whether publication bias is present and what its influence might be on the effect estimate is difficult to establish. Observational studies on the incidence of CIN are less likely to lead to publication bias since studies reporting a relatively high or low incidence are both of interest for publication. Therefore if publication bias has occurred, it is not likely to be of strong influence on the incidence in our meta-analyses.

Third, if the majority of trials in our meta-analyses only included patients at high risk for CIN, an overestimation of the incidence of CIN might be the result. In this analyses, 16 out of 40 trials (40%) restricted the inclusion to patients at high risk for CIN, i.e. eGFR < 60 ml/min/1.73m2, including 2,477 out of the total of 11,516 patients (22%). In daily practice, this proportion ranges from 10-39% (40,48). Furthermore, neither the consecutiveness of included patients nor study design had influence on the incidence of CIN. Therefore the effect of selection bias is most likely to be insignificant.

However, we do regard our results as representative since they are based on pooled proportions in a total of 19,563 patients. Furthermore, the incidence of CIN had a small 95% confidence interval, implying that the point estimate we showed is reliable. Moreover, the quality of individual studies did not clearly influence the incidence of CIN. Therefore, it is unlikely that bias as a result of low study quality influenced our results considerably. In addition, follow-up time for the assessment of CIN was consistent for the great majority of studies. Also, although the incidence of CIN in our meta-analysis might be under the influence of preventive hydration regimes, the incidence of CIN and the exceptional rare persistent decline in renal function indicate that contrast media can be safely administered to high risk patients when preventive measures are taken.

Chronic kidney disease and diabetes mellitus are often describes as risk factors for CIN in literature (9,27,49,50). The higher incidence of CIN in patients with chronic kidney disease could very well be due to the definition of CIN which is defined by a rise in serum creatinine (6). In patients with a low glomerular filtration rate (GFR) serum creatinine rises more steeply when hemodynamic changes occur or contrast is administered (6). In order to prove a higher nephrotoxicity in patients with chronic kidney disease further studies should analyse the expression of markers for acute renal injury after contrast enhanced CT, such as neutrophil gelatinase associated lipocalin (NGAL) or kidney injury marker 1 (KIM-1) as shown by recent work (51,52). An explanation for this increased risk in patients with diabetes mellitus might be the result of a disturbed auto regulation in those patients (40,53,54). On the other hand, it is argued as well that patients with diabetes have a wider variability in serum creatinine which might lead to a spuriously higher incidence of CIN (6,36,55).

Contrast induced nephropathy and the prevention of it in high risk patients is a difficulty in the diagnostic work up. Contrast enhanced CT is a frequently used diagnostic tool and the proportion of patients at high risk for CIN is increasing, due too the aging

of the population which is accompanied by chronic kidney disease and other co morbidities. Contrast induced nephropathy preventing guidelines recommend hydration regiments in patients at high risk for CIN or even the use of a different, less accurate, diagnostic tool without contrast administration (56). This meta-analysis shows an incidence of CIN of 6% with an incidence of RRT due to CIN of 0.06%. Moreover, in only 1% of all patients undergoing CT the decline in renal function persisted. Whether it is safe to perform contrast enhanced CT in high risk patients cannot be directly concluded from our results since most of the included studies involved some sort of protection measure against contrast nephropathy.

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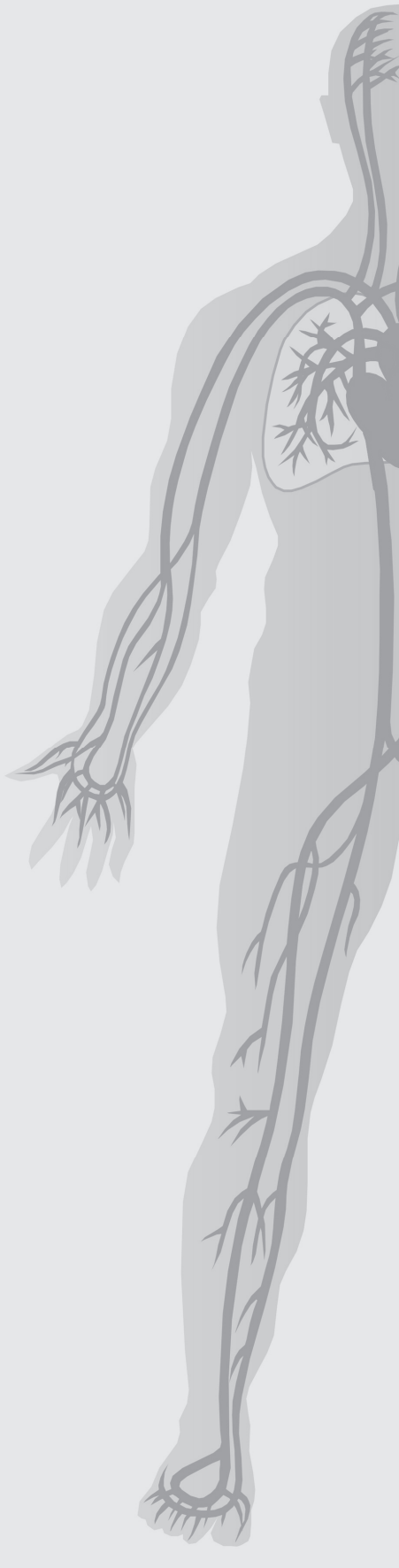

Chapter 4

Right ventricular function and thrombus load in patients with pulmonary embolism and diagnostic delay

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ABSTRACT

Introduction

It has earlier been demonstrated that the time between symptom onset and objective diagnosis of pulmonary embolism (PE) does not influence outcome on re-thrombosis and mortality. This time frame consists of patient's delay and doctor's delay. It is unknown whether this patient's delay is of influence on the thromboembolic burden and right ventricular function. We sought to evaluate this by measuring Qanadli-score and RV/LV-ratio in PE patients with and without patient's delay.

Methods

Post-hoc analyses of an observational prospective outcome study in 113 consecutive CT proven PE patients. In all patients Qanadli-score and RV/LV ratio were scored and duration from symptom onset until the clinical presentation was requested. Also mortality and hospital readmission in a 6-weeks follow-up period were collected.

Results

Twenty patients with and 93 patients without delay, with identical baseline characteristics and comorbidities, were included. In a linear analysis, Qanadli-scores were not correlated to delay with a $R2$ of 0.021 ($p=0.130$). RV/LV-ratio had a $R2$ <0.001 ($p=0.991$). Likewise, longer delay was not associated to 6-week mortality (Odds ratio: 0.65; 95%CI 0.08-5.57) or hospital readmission (Odds ratio: 0.75; 95%CI 0.15-3.65).

Conclusion

In our patient cohort, patient's delay was not associated with higher thrombus load or right ventricular dysfunction. This could provide a possible explanation for the lack of association between delay in diagnosing PE and clinical outcome as found in earlier studies.

INTRODUCTION

Acute pulmonary embolism (PE) is a frequently diagnosed disease with an incidence of 1-2 per 1000 persons (1). Mortality rates range between 2-30%, depending on the size of the embolism and the cardiopulmonary condition of the patient (2). Especially in the first hours of the acute PE event, patients may die from PE (3). Nonetheless, in 17% of the patients PE is diagnosed more than a week after symptom onset (4). In recent studies no correlation was found between patient's delay, defined as time between symptom onset and presentation at the hospital, and clinical outcome (5-7). In these studies it is hypothesized that, from symptom onset until presentation, thrombus growth and aggravation of right ventricular function could be negligible in some patients (5-7). However, these studies were unable to confirm or reject this hypothesis.

The aim of present study was to investigate whether elevation of thrombotic burden and aggravated right ventricular function was irrespective of patient's delay. This was done by measuring Qanadli-scores and RV/LV-ratio in 113 consecutive PE patients presenting with and without delay.

METHODS

Patients

This was a post-hoc analysis of an observational prospective outcome study conducted in an academic and a peripheral teaching-hospital, as described earlier (8-10). In short, during the period September 1st 2005 and December 1st 2008 all consecutive hemodynamically stable in- and outpatients with a clinical suspicion for PE were eligible for inclusion. Patients with a likely clinical decision rule (Wells > 4 points total) and/or an elevated D-dimer blood test (> 500 ng/mL) underwent a computed tomography pulmonary angiography (CTPA; Aquillion 64; Toshiba Medical Systems, Otaware, Japan). In patients with an unlikely clinical decision rule (Wells ≤ 4 points total) and a normal D-dimer blood test (≤ 500 ng/mL) PE was ruled out without further imaging (10). CTPA was considered positive for PE when at least one filling defect in the pulmonary artery tree was present. All patients with a clinical suspicion of PE, regardless of the outcome of the CTPA, were followed for a period of 6 weeks. Patients aged below 18 years, with impossibility to follow-up, hemodynamically unstable at presentation, pregnant, with known allergy to contrast agents, with renal impairment function and patients unable to give informed consent were excluded from this study. The study was approved by the Institutional Review Board of both participating hospitals and all patients provided written informed consent.

Radiological examinations

To quantify the vascular obstruction of the pulmonary arteries caused by PE, the scoring system as proposed by Qanadli et al. was used (12). In this score the left and right lung were regarded having 10 segmental arteries each (3 for the upper lobes, 2 for the middle lobe and to the lingula, and 5 for the lower lobes). Each individual segmental artery is scored 0 points when there is no thrombus present, 1 point for partial occlusion of the artery and 2 points for total occlusion. So, in total the maximal Qanadli-score was 40 per patient. Thrombi in the most proximal artery were scored a value equal to the number of segmental arteries present distally. Subsegmental PE was considered as a partial occlusion and was assigned 1 point.

RV/LV-ratio was calculated by measuring the right and left ventricular dimensions on a post-processing workstation (Vitrea, version 2, Vital Images, Minnetonka, USA). This was done, as described earlier (13), in reconstructed CT-4 chamber view, by identifying the maximal distance between the ventricular endocardium and the interventricular septum, perpendicular to the ventricular long-axis. A RV/LV-ratio greater than 1.0 was considered as right ventricular enlargement.

Patient's delay

Patient's delay was defined as the time, expressed as number of days, between onset of symptoms and clinical presentation at the hospital. In case patients presented within 24 hours of symptom onset, delay was scored in hours. Patient's delay was scored at the day of presentation. In case patients had slowly progressing symptoms, the day the complaints started was scored as the onset of symptoms. If there were complains for a longer period with an acute change in symptoms, the acute moment was scored as the start of onset of symptoms.

Variables and study endpoints

The primary outcome of the study was the Qanadli-score and RV/LV-ratio relative to the duration of symptoms before presentation to the hospital. Secondary endpoints were mortality and hospital readmission 6 weeks after diagnosis of PE compared to patient's delay for less and more than 7 days, as proposed in previous studies (5-7). In addition, subsegmental PE, a prior history of VTE and active malignancy were scored.

Statistical analysis

Baseline patient characteristics are presented as mean ± standard deviation (SD). Qanadli-score, RV/LV-ratio and patient's delays are presented as median with an interquartile range (IQR). Nominal data are presented as N, %. Linear analysis was used for the analysis of correlations between delay and Qanadli-score and between delay and RV/LV-ratio. For the correlation between delay and the secondary endpoints mortality and

hospital readmission, odds ratios with 95% confidence intervals (CI) were calculated. Because patients with massive PE are possibly more prone to present at the first or second day after symptom onset, our analyses could be biased. Therefore, all analyses were performed for a second time after excluding patients with a delay less than 2 days. Statistical analysis was performed using SPSS statistics 17.0.2 (SPSS Inc., Chicago, Illinois, The USA). P-values <0.05 were considered statistically significant.

RESULTS

PE was confirmed after CTPA in 113 of the 439 patients eligible for inclusion (25.7%). The baseline characteristics are depicted in table 1. Mean age was 56 ± 17 years. There were more male patients (60, 53.1%) than female patients (53, 46.9%; p<0.001), and more outpatients (93, 82.3%) than inpatients (20, 17.7%; p<0.001). The amount of subsegmental PE did not significantly differ in patients with and without PE (p=0.706).

The median time from onset of symptoms to presentation was 2 days, IQR 1-6 days. In patients without PE (data not shown) median time from onset of symptoms to clinical presentation was identical (2 days, IQR 1-7 days). Patient's delay of more than 7 days was present in 20 patients (17.7%) and 4 patients (3.5%) had a delay of more than a month. Forty-one patients (36.3%) presented within 24 hours of onset of complains of whom 23

Table 1. Baseline patient characteristics

Variable	All patients (n=113)	Patients without delay (n=93)	Delayed patients (n=20)	Significance (p-value)
Age (years ± SD)	56 ± 17	55 ± 17	59 ± 17	0.322
Male sex (n,%)	60 (53.1)	53 (57.0)	7 (35.0)	0.075
Outpatients (n,%)	93 (82.3)	76 (81.7)	17 (85.0)	0.730
Previous VTE (n,%)	25 (22.1)	21 (22.6)	4 (20.0)	0.803
Malignancy (n,%)	24 (21.2)	20 (21.5)	4 (20.0)	0.883
COPD	7 (6.2)	4 (4.3)	3 (15.0)	0.073
Heart failure	5 (4.4)	4 (4.3)	1 (5.0)	0.892
D-dimer (ng/mL ± SD)	2770 ± 1678	2701 ± 1673	3090 ± 1706	0.350
Delay (days ± SD)	5.7 ± 9.2	2.4 ± 1.8	21.0 ± 13.4	<0.001
Qanadli-score (score ± SD)	12.3 ± 9.3	11.7 ± 9.5	14.6 ± 7.8	0.212
RV/LV-ratio (ratio ± SD)	1.09 ± 0.33	1.09 ± 0.35	1.09 ± 0.21	0.962
Subsegmental PE (n,%)	14 (12.4)	12 (12.9)	2 (10.0)	0.706
All-cause mortality (n,%)	8 (7.1)	7 (7.5)	1 (5.0)	0.693
Hospital re-admission (n,%)	14 (12.4)	12 (12.9)	2 (10.0)	0.706

SD, Standard Deviation; VTE, Venous Thromboembolic Event; COPD, Chronic Obstructive Pulmonary Disease; PE, Pulmonary Embolism

(20.4%) within the first 12 hours. Twenty-one patients (18.6%) presented on the second day (Figure 1).

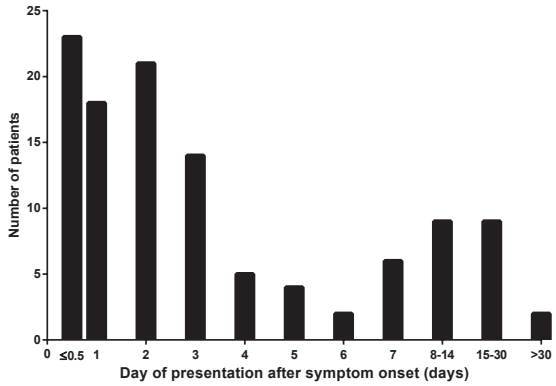

Figure 1. Number of patients presenting per day delayed after symptom onset

The median Qanadli-score was 10 points with an IQR of 4-21 points. The mean Qanadli-score was 11.7 ± 9.5 in patients without delay and 14.6 ± 7.8 in patients with delay (p=0.212). When analyzed in a linear analysis, the Qanadli-score was not correlated to delay after symptom onset (R2 0.021 p=0.130 Figure 2a.). Likewise, in our second analysis after excluding patients presenting at the first 2 days after symptom onset, the Qanadli-score was not associated with delay (R2 0.003, p=0.726 Figure 2b.).

The median RV/LV-ratio was 1.02 with an IQR of 0.92-1.16. Mean RV/LV-ratio was 1.09 ± 0.35 in patients without delay and 1.09 ± 0.21 in patients with delay (p=0.962). In a linear analysis, RV/LV-ratio did not correlate to patient's delay (R2 <0.001 p=0.991 Figure 3a.). After excluding patients presenting at day 1 and 2 similar results were found (R2 0.002 p=0.998 Figure 3b.)

Death after 6 weeks of follow-up occurred in 8 patients (7.1%) in which 7 out of 93 without delay (7.5%) and 1 of 20 patients with delay (5.0%) died (Odds ratio: 0.65; 95% CI 0.08 – 5.6). A total of 14 out of 113 patients (12%) were readmitted to the hospital. In the group of patients with delay, 12 of 93 (13%) were readmitted and 2 of 20 patients (10%) with delay were readmitted to the hospital (Odds ratio: 0.75; 95% CI 0.15 – 3.6). Excluding the patients who presented within 48 hours of onset of symptoms or those with subsegmental PE did not change these findings.

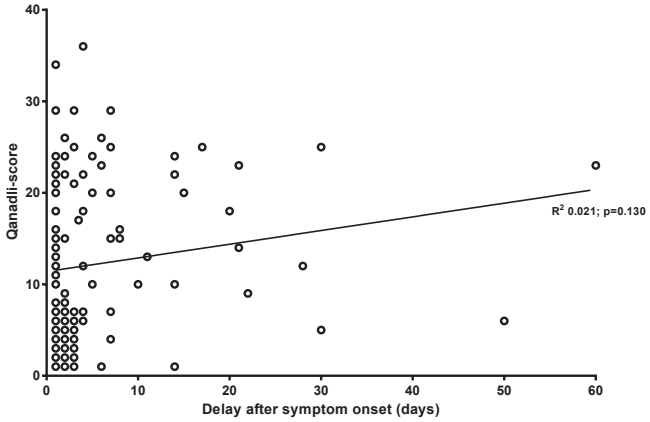

Figure 2a. Scatter plot of the Qanadli-score and patients delay after symptom onset

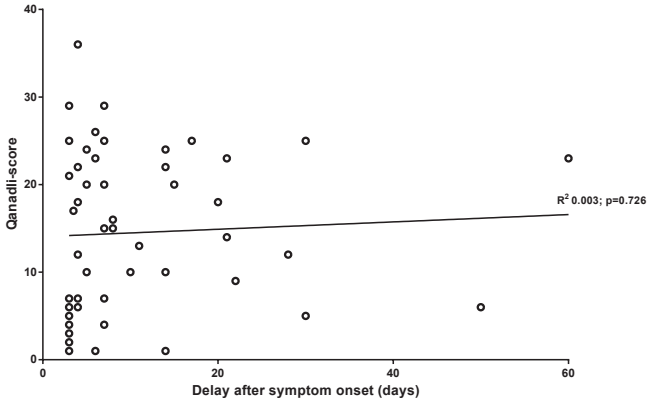

Figure 2b. Scatter plot of the Qanadli-score and patients delay in patients presenting after 2 days of symptom onset

DISCUSSION

Our results show that the thromboembolic burden (assessed by the Qanadli-score) and right ventricular function (measured by the RV/LV-ratio on CT-scan) in patients diagnosed with acute symptomatic PE were not adversely affected in patients with a delay. Also, the 6-weeks survival and hospital readmission rates are identical in patients with and without patient's delay. The latter findings correlate with earlier studies. One study showed that 3-month survival and recurrence VTE rates were not adversely affected by patient's delay in 397 symptomatic PE patients presenting at the emergency ward. Seventy-two of these patients had patient's delay of more than 7 days (18%). Deaths were identical in both groups (OR 0.9; 95% CI 0.4-2.0) (5). These results were confirmed by a second study,

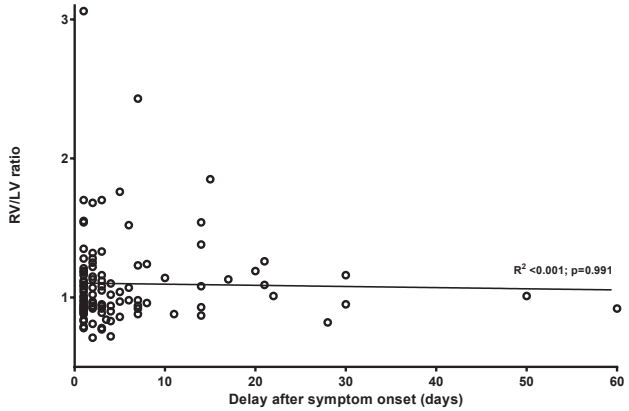

Figure 3a. Scatter plot of the RV/LV ratio and patients delay after symptom onset

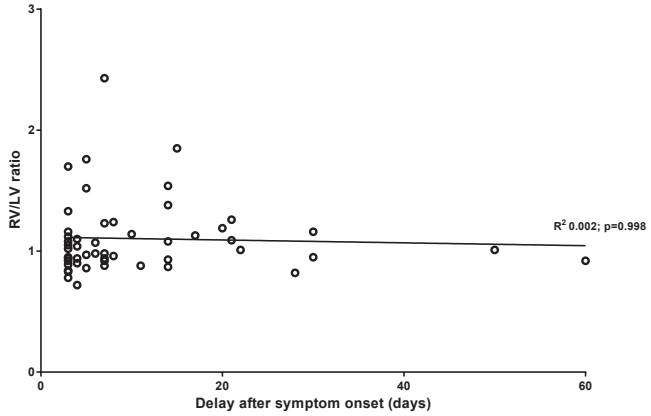

Figure 3b. Scatter plot of the RV/LV ratio and patients delay in patients presenting after 2 days of symptom onset

in which 375 patients, admitted with the diagnosis of PE, were included. Patient's delay of more than 7 days was present in 186 patients (50%). The primary outcome, mortality, was identical in patients with and without delay (6). A third retrospective study in 454 consecutive PE patients, in which 113 patients (28%) were delayed more than a week, found that mortality was identical in patients with and without delay (7). However, none of these studies provided a pathophysiological explanation for these findings and all suggested further studying this observation. Our study strengthens the hypothesis by showing that, besides outcome on mortality and hospital readmission, also thrombo-embolic burden was identical in patients with and without patient's delay.

In an earlier study of our group, the predictive value of RVD and Qanadli-score on outcome during 3-months follow-up of patients with PE was evaluated (12). In that study, the RV/LV ratio and fatal PE related with a regression coefficient of 1.55 (p=0.04). PE

patients with an obstruction index of 40%, 16 points or more which is the cut-off value as proposed by Qanadli (11), had an 11-fold risk of PE-related mortality than patients with an index smaller than 40% (13). Since our present study showed identical RV/LV ratios and Qanadli-scores in patients with and without patient's delay, we assume that our data strengthens the hypothesis that patient's delay does not influence outcome in patients presenting with PE. A previous study suggested that the lack of effect of delay on outcome might affect the guidelines that advise initial anticoagulant treatment before radiological examinations are performed (5). Although our data showed identical results regarding outcome on mortality and morbidity, only the effect of patients delay, time between symptom onset and presentation at the emergency ward, was studied. Withholding anticoagulant treatment before radiological examinations is doctors delay and has not been studied. Therefore we do not believe the results should affect current guidelines.

Nonetheless, our thrombus loads are identical to those found in earlier studies using the Qanadli and/or Miller-index (11;14). An explanation could be the actual duration of the delay in our study, which was defined by reported time between the moment of symptom onset up to the moment of diagnosis. Notably, it is unknown whether the subjectively reported initial symptoms were actually caused by a PE. Another limitation to our study is that we only included hemodynamically stable patients who survived the first hours to undergo evaluation at the emergency ward. All these patients underwent a CT-scan based on an elevated Wells clinical decision rule and/or elevated D-dimer levels causing a selection bias in which only patients with multiple complains or patients with a certain degree of pulmonary artery obstruction were included. Therefore, the complaints and thromboembolic burden in patients with and without delay could be identical at the day of presentation in the hospital. However, there was a big interval in D-dimer levels indicating that our study population included both larger and smaller PE. It could also be debated that patients with patient's delay had smaller PE at onset of symptoms and presented at the emergency ward after exacerbated symptoms due to thrombus growth. Still, the amount of subsegmental PE did not significantly differ between patients with and without patient's delay. Finally, differences in patient characteristics or VTE risk factors between patients with and without delay could also act as a confounder. However, the presence of active malignancy, COPD, congestive heart failure or a prior history of VTE was not different between the 2 study groups.

We conclude that both RV/LV ratio, and the arterial pulmonary obstruction assessed by the Qanadli-score, was identical in hemodynamic stable patients with and without patient's delay. This strengthens the findings that PE-related mortality after a 6-week follow-up period was not related with patient's delay.

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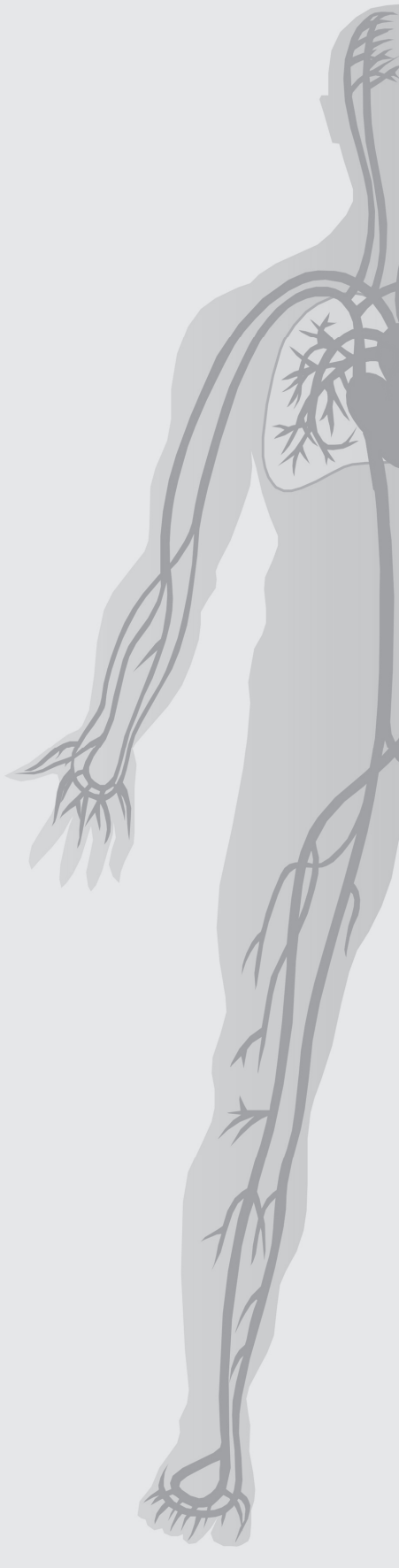

Chapter 5

NT-PRO-BNP levels in patients with acute pulmonary embolism are correlated to right but not left ventricular volume and function

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ABSTRACT

Introduction

NT-pro-BNP is primarily secreted by left ventricular (LV) stretch and wall tension. Notably, NT-pro-BNP is a prognostic marker in acute pulmonary embolism (PE), which primarily stresses the right ventricle (RV). We sought to evaluate the relative contribution of the RV to NT-pro-BNP levels during PE.

Methods

A post-hoc analysis of an observational prospective outcome study in 113 consecutive patients with CT proven PE and 226 patients in whom PE was clinically suspected but ruled out by CT. In all patients RV and LV function was established by assessing ECG-triggered-CT measured ventricular end-diastolic-volumes and ejection fraction (EF). NT-pro-BNP was assessed in all patients. The correlation between RV and LV end-diastolic-volumes and systolic function was evaluated by multiple linear regression corrected for known confounders.

Results

In the PE cohort increased RVEF (β-coefficient (95%CI) -0.044(±-0.011); p<0.001) and higher RV end-diastolic-volume (β-coefficient 0.005(±0.001); p<0.001) were significantly correlated to NT-pro-BNP, while no correlation was found with LVEF (β-coefficient 0.005(±0.010); p=0.587) and LV end-diastolic-volume (-0.003(±0.002); p=0.074). In control patients without PE we found a strong correlation between NT-pro-BNP levels and LVEF (β-coefficient -0.027(±-0.006); p<0.001) although not LV end-diastolic-volume (β-coefficient 0.001 (±0.001); p=0.418). RVEF (β-coefficient -0.002(±-0.006); p=0.802) and RV end-diastolic-volume (β <0.001(±0.001); p=0.730) were not correlated in patients without PE.

Conclusion

In PE patients, lower RVEF and higher RV end-diastolic-volume were significantly correlated to NT-pro-BNP levels as compared to control patients without PE. These observations provide pathophysiological ground for the well known prognostic value of NT-pro-BNP in acute PE.

INTRODUCTION

There are several methods to triage and assess the severity of acute pulmonary embolism (PE). Probably the easiest method is by using a clinical decision rule, as the PESI score (1). Other means include the assessment of right ventricle function, by ultrasound or CT (2), or the measurement of biomarkers including troponin (3), D-dimer (4) and B-type Natriuretic Peptide (BNP) (5).

BNP is an amino-peptide secreted as pre-pro-BNP by the myocytes in the left ventricle (LV) and right ventricle (RV). Elevated levels of BNP can cause a variety of physiological effects, including lowering of blood pressure, reducing peripheral vascular resistance and regulating sodium balance by influencing the renin-angiotensin-aldosteron system (6). These actions are thought to be beneficial in pathological states such as hypertension and compensated heart failure (7).

Since the RV has a relative thin wall compared to the LV, changes in BNP levels are mostly studied in conditions caused by hemodynamic changes in the LV (7). BNP levels are therefore well established markers for the prognosis and diagnosis of LV disease (8). Nonetheless, Tulevski et al. showed that BNP levels were also elevated in patients with acute PE when compared to healthy controls (9). This result was hypothesized by the authors to be caused by RV distress. It has additionally been shown that a low BNP level is a predictor for benign clinical outcome in patients with acute PE (10) and elevated BNP level a predictor for adverse clinical outcome (11). Another study showed that PE patients with RV enlargement had higher NT-pro-BNP and BNP levels (12). And it was shown that elevated NT-pro-BNP levels are a predictor of RV dysfunction in patients diagnosed with PE (13). In a recent meta-analysis it has been shown that elevated BNP predicts worse outcome of PE with an OR of 7.6 for 30-day mortality (5). These data reason that the increase of (NT-pro-)BNP levels in PE patients are caused by the relatively thin RV. However, to what extent elevation of BNP levels in PE patients are actually caused by the RV or LV distress is still unknown. Therefore we sought to evaluate the relative contribution of the RV and LV to NT-pro-BNP levels in patients with acute PE and compared it to the contribution of the RV and LV to NT-pro-BNP levels in PE negative patients.

METHODS

Patients

This was a post-hoc analysis of an observational prospective outcome study (14,15). Between September 1st 2005 and December 1st 2008 hemodynamically stable, consecutive in- and outpatients with clinically suspected acute PE and a strict indication for computed tomography pulmonary angiography (CTPA) were included. This indication

comprised patients with a likely clinical decision rule (Wells > 4 points total) and/or an elevated D-dimer blood test (>500 ng/mL). PE was ruled out in patients with an unlikely clinical decision rule and a normal D-dimer test without further imaging (16). All other patients underwent multi-detector row CTPA (Aquillion 64; Toshiba Medical Systems, Otawara, Japan) of the chest during breath-hold at inspiration (inter-observer agreement 83% κ 0.85; intra-observer agreement 93% κ 0.87) (17). PE was considered present in case of at least one filling defect in the pulmonary artery tree. After the diagnostic CTPA, an ECG-synchronized dynamic cardiac CT was performed. Patients in whom PE was demonstrated were treated according to international guidelines with therapeutic doses of low molecular weight heparin for at least 5 days, and vitamin K antagonists for a minimum period of 3 months aiming at an INR of 2.0-3.0. In case of severe PE or clinical deterioration, admission to the intensive care unit and/or administration of thrombolytic drugs was considered by the attending clinician. All patients who underwent CTPA, regardless of the outcome, were followed for a period of 6 weeks. Exclusion criteria were age below 18 years, impossibility of follow-up, hemodynamically instability at presentation, pregnancy, patients with a known allergy to intravenous contrast media, renal function impairment and patients unable to give written informed consent. This study was approved by the Institutional Review Board of the two participating hospital and all patients provided written informed consent. PE was confirmed after CTPA in 113 of the 439 patients eligible for inclusion (prevalence of PE 25,7%). To double match the number of PE patients, the first 226 consecutive patients in whom PE was ruled out were included for further analysis. Four patients with PE were excluded from the final analysis due to missing data (NT-pro-BNP in 3 patients and cardiac CT in 1 patient).

Image acquisition

Image acquisition was validated and described previously (18). In short, all patients received 35-40 mL of contrast agent in order to perform the low-dose ECG-synchronized cardiac CT scanning. The scan parameters were: tube voltage of 120 kV and tube current from 100 to 200 mA. Rotation time ranged between 0.4 and 0.5 seconds and pitch factor between 0.25-0.50. Both parameters were automatically determined based on the heart rate of each individual patient in order to obtain the best resolution. Radiation dose was determined with ImPACT CT Patient Dosimetry Calculator, version 1.0.2; ImpactSCAN; London, England [www.impactscan.org] and was an estimated 3.3 mSv. Image acquisition was done with a slice-thickness of 2 mm.

Cardiac function

Ventricular volumes and function were assessed using dedicated cardiac function analysis software (CT-MASS; Medical Imaging Systems, Leiden, The Netherlands) as previously described (15). First, the phases with the largest and the smallest RV volumes

were selected using running cine movies on the midventricular level. These selections represented the end-diastolic and end-systolic phases. The endocardial borders for the RV and LV were drawn in every other transverse section, from apex to the level of the pulmonary outflow tract, in the end-diastolic and end-systolic phase. Out of these drawings, RV and LV end-diastolic volume (RV inter-observer variability 1.0%, intra-observer variability 0.8%; LV inter-observer variability 2.3%, intra-observer variability 1.0%), end-systolic volume (RV inter-observer variability 1.8%, intra-observer variability 0.9%; LV inter-observer variability 3.8%, intra-observer variability 1.3%), stroke volume (RV inter-observer variability 3.1%, intra-observer variability 1.8%; LV inter-observer variability 4.4%, intra-observer variability 2.1%) and ejection fraction (RV inter-observer variability 3.1%, intra-observer variability 1.8%; LV inter-observer variability 4.4%, intra-observer variability 1.3%) were calculated (15;19). Threshold values were RVEF ≤47%; LVEF ≤57%; RVEDV ≥227mL for males and ≥154mL for females; LVEDV ≥195mL for males and ≥141mL for females (20).

Blood sampling

Venous plasma and serum samples were obtained at admission and immediately stored at -80 °C. After inclusion of all patients, samples were analyzed after a single thaw. NT-pro-BNP levels were measured with a quantitative immunoassay (Elecsys 2010 analyzer, Roche Diagnostics, Mannheim, Germany). The claimed CV's by the manufacturer at concentrations above 70 pg/mL is below 4% for intra-assay precision and below 5% for inter-assay precision. Functional sensitivity (lowest concentration measured with inter-assay precision of 20% CV) was measured at 10 pg/mL. Both the cardiac CT-measurements and the NT-pro-BNP level assessment were performed post-hoc by different researchers blinded for clinical information.

Statistical analysis

For all baseline characteristics, data are presented as mean ± standard deviation (SD). NT-pro-BNP is presented as median with an interquartile range (IQR). Since NT-pro-BNP levels were not normally distributed, they were Log-transformed. Receiver operating characteristic (ROC)-curve was used to analyze at which NT-pro-BNP level RV/LV ratio starts to be abnormal. Two multiple linear regressions were used to assess the relation between log NT-pro-BNP measurements and cardiac function. Data is given as β-coefficient ± SE which reflects the difference in log NT-pro-BNP associated with a difference of 1 unit of SD. P values <0.05 were considered significant. Non significant p-values are referred as 'ns'. In model 1, the association of the RVEF and LVEF and RV and LV end-diastolic volumes and log NT-pro-BNP was evaluated. This model was corrected for known risk factors causing elevation of NT-pro-BNP, which are age, sex and creatinine clearance. In addition, RV and LV end-diastolic volume were included as covariates in

this model. Since the EF is calculated by dividing the stroke volume by the end-diastolic volume, a second model for the association of the RV and LV end diastolic volume and log NT-pro-BNP was sought. Model 2 was corrected for age, gender and creatinine clearance. Statistical analysis was done using SPSS statistics 17.0.2 (SPSS Inc., Chicago, Illinois, The USA)

RESULTS

The baseline characteristics of the study participants are depicted in table 1. The mean age was 56 years in the patients with PE and 55 years in patients without PE (p=0.707). In the patients with acute PE, 54% were male compared to 44% in the patients without PE (p=0.071). A history of previous venous thromboembolic events (VTE) was present in 23% of the patients with PE and in 13% of the patients without PE (OR 1.95, 95% CI 1.08-3.52). COPD was more frequent in the patients without PE (6% vs. 16%, OR 0.35, 95% CI 0.14-0.78). Active malignancy (23% vs. 25%, p=0.710) and left sided heart failure (4.6% vs. 7.5%, p=0.176) were equally distributed between both groups. Mean systolic

Table 1. Baseline patient characteristics

Variable	Patients with PE (N=109)	Patients without PE (N=226)	Significance (p-value)
Age (years ± SD)	56 ± 16	55 ± 17	0.707
Male sex (n, %)	59 (54)	99 (44)	0.071
Previous VTE (n, %)	25 (23)	30 (13)	0.024
Active malignancy (n, %)	23 (21)	52 (23)	0.710
COPD (n, %)	7 (6)	37 (16)	0.012
Left sided heart failure (n, %)	4 (3,7)	17 (7,5)	0.176
Inpatient (n, %)	19 (17)	58 (26)	0.097
NT-pro-BNP > 600 pg/mL (n,%)	31 (28)	56 (25)	0.460
Creatinine clearance (mL/min/1.73m^2 ± SD)	87 (29)	93 (53)	0.234
Systolic blood pressure (mmHg ± SD)	139.1 (17.2)	137.5 (21.5)	0.610
Diastolic blood pressure (mmHg ± SD)	78.7 (12.2)	76.8 (12.5)	0.356
Heart rate (BPM ± SD)	89.9 (18.6)	84.4 (16.7)	0.066
LV ejection fraction (%± SD)	52.7 (9.3)	54.0 (12)	0.312
RV ejection fraction (%± SD)	45.2 (12)	50.5 (10)	<0.001
LV end diastolic volume (mL ± SD)	154 (49)	155 (49)	0.789
RV end diastolic volume (mL ± SD)	190 (61)	167 (61)	0.002

PE indicates pulmonary embolism; SD, standard deviation; VTE, venous thromboembolic event; COPD, chronic obstructive pulmonary disease; BPM, beats per minute; LV indicates left ventricular; RV, right ventricular

blood pressure was 139.1 mmHg in PE patients and 137.5 mmHg in patients without PE (p=0.610), diastolic blood pressure was 78.7 in PE patients and 76.8 in patients without PE (p=0.356) and heart rate was 89.9 beats per minutes (BPM) in patient with PE and 84.4 BPM in patients without PE (p=0.066).

NT-pro-BNP level >600 pg/mL, which has a high discriminative power as predictor for adverse clinical events after PE (14), was present in 28% of the patients with PE and in 25% of the patients without PE (p=0.460). In patients with PE the median NT-pro-BNP level was 223.0 pg/ml with an IQR of 54.9-1021.5 pg/ml. In patients without PE the median NT-pro-BNP level was 146.4 with an IQR of 53.2-598.6 (p=0.230).The RVEF was significantly lower in patients with PE than in those without PE (45.2% ± 12 vs. 50.5% ± 10; p<0.001) and 50 PE patients (46%) had abnormal RVEF while in the patients without PE only 62 (27%) had abnormal RVEF (p<0.001). In addition, RV end-diastolic volume was significantly larger in the PE patients (190 mL ± 61 vs. 167 mL ± 61 p=0.002) and in 49 PE patients (45%) RV end-diastolic volume was abnormal versus 66 patients (29%) without PE (p=0.005). LVEF (p=0.312) and LV end-diastolic volume (p=0.789) did not significantly differ between the 2 patient cohorts. The 77 (out of 336) patients with pathological cardiac function had significantly higher NT-pro-BNP values compared to patients with normal ventricular function (210 vs. 156 pg/mL, p<0.001). ROC-curve showed that RV/LV ratio started to be abnormal at 180 pg/mL (sensitivity 67.5%; specificity 57.0%). At the cut-off point of 600 pg/mL sensitivity was 42.9% and specificity 79.0%.

With regard to the first multiple linear regression (table 2), the RV and LVEF were related with log NT-pro-BNP. In an age-, sex-, creatinine clearance- and end-diastolic volume-adjusted model, a decreased RVEF was significantly associated to increased NT-pro-BNP levels in patients with PE. Every percent increase of the RVEF caused a rise of log NT-pro-BNP with β-coefficient -0.044 ± -0.011 (p<0.001). A change in LVEF was

Table 2. Association of right- and left ventricular ejection fractions with log NT-pro-BNP

	Patients with PE			Patients without PE		
Parameter	**β-coefficient†**	**Standard error**	**P-value**	**β-coefficient†**	**Standard error**	**P-value**
RV ejection fraction (%)	-0.044	-0.011	<0.001	-0.002	-0.006	0.802
LV ejection fraction (%)	0.005	-0.010	0.587	-0.027	-0.006	<0.001
Age (years)	0.021	0.004	<0.001	0.016	0.003	<0.001
Sex (being female)	0.551	0.138	<0.001	0.097	0.096	0.310
eGFR (mL/min/1.73m^2)	0.002	0.002	0.488	-0.001	0.001	0.146
RV end diastolic volume (mL)	-0.001	0.002	0.522	0.001	0.001	0.274
LV end diastolic volume (mL)	0.003	0.002	0.145	-0.001	0.001	0.451

RV indicates right ventricular; LV, left ventricular; eGFR, estimated glomerular filtration rate

† β-coefficient reflects the difference in log NT-pro-BNP associated with a difference of 1 unit of SD, for example, for each SD unit higher RV ejection fraction, log NT-pro-BNP is higher by β.

not significantly correlated to NT-pro-BNP levels (β-coefficient 0.005 ± 0.010; p=0.587). In contrast, NT-pro-BNP levels in patients without acute PE were not dependent on RVEF (β-coefficient -0.002 ± -0.006; p=0.802) but strongly correlated to LVEF (β-coefficient -0.027 ±0.006; p<0.001). Also increasing age (β-coefficient 0.021 ± 0.004; p<0.001) and being female (β-coefficient 0.551 ± 0.138; p<0.001) significantly influenced the log NT-pro-BNP levels in patients with PE. In patients without PE only age had a significant contribution (β-coefficient 0.016 ± 0.003; <0.001). Being female did not contribute significantly. This could be explained by the significantly higher age and both RV and LV end-diastolic volumes in male patients in the PE negative patients (data not shown).

In the second multiple linear regression (table 3), EF was taken out of the model. In latter model, RV and LV end diastolic volumes were related to log NT-pro-BNP levels. In an age-, sex- and creatinine clearance-adjusted model, a decrease in RV end diastolic volume significantly increased NT-pro-BNP levels (β-coefficient 0.005 ± 0.001; p<0.001) in patients with PE while LV end diastolic volume did not significantly influence log NT-pro-BNP levels (β-coefficient -0.003 ± -0.002; p=0.074). Also an increase in age significantly raised log NT-pro-BNP (β-coefficient 0.024 ± 0.005; <0.001). In patients without PE on the other hand, LV (β-coefficient 0.001 ± -0.001; p=0.418) and RV (β <0.001 ± -0.001; p=0.730) end diastolic volumes were not associated to NT-pro-BNP levels. Increase in age, like in all analysis before, contributed significantly to a change in log NT-pro-BNP (β-coefficient 0.018 ± 0.003; <0.001).

Table 3. Association of right- and left ventricular end-diastolic volumes with log NT-pro-BNP

	Patients with PE			Patients without PE		
Parameter	**β-coefficient†**	**Standard error**	**P-value**	**β-coefficient†**	**Standard error**	**P-value**
RV end diastolic volume (mL)	0.005	0.001	<0.001	<0.001	0.001	0.730
LV end diastolic volume (mL)	-0.003	0.002	0.074	0.001	0.001	0.418
Age (years)	0.024	0.005	<0.001	0.018	0.003	<0.001
Sex (being female)	0.455	0.157	0.004	0.010	0.105	0.922
eGFR (mL/min/1.73m^2)	0.001	0.003	0.734	-0.001	0.001	0.113

RV indicates right ventricular; LV, left ventricular; eGFR, estimated glomerular filtration rate

† β-coefficient reflects the difference in log NT-pro-BNP associated with a difference of 1 unit of SD, for example, for each SD unit higher RV end diastolic volume, log NT-pro-BNP is higher by β.

DISCUSSION

This study assessed the relative contribution of the right and left ventricle to NT-pro-BNP levels in the setting of patients with acute PE. Our results indicate that NT-pro-BNP levels in PE patients were strongly correlated with RVEF but not with LVEF and volume in PE patients. In patients in whom PE was suspected but ruled out by CTPA, the opposite was

shown: in these patients a decrease in LVEF was significantly associated with elevated NT-pro-BNP levels, while RVEF and RV and LV end-diastolic volumes were not.

In a previous study in 50 patients with PE presented at the emergency ward of a University hospital, it was already suggested that a rise in NT-pro-BNP already occurs before the rise of cardiac damage-related biomarkers (12). NT-pro-BNP is therefore, possibly, a biomarker released in an early stage of pulmonary embolism-associated myocardial stretch. This was confirmed in a second study in 77 PE patients, which found a similar correlation between NT-pro-BNP and right ventricular enlargement (13). Our study supports the hypothesis that elevated NT-pro-BNP levels are caused in an early stage by mechanical stress of the RV in acute PE patients, and not or in a far lesser extent to LV hemodynamics. In comparison with the 2 studies mentioned above, we included all in- and out-hospital patients with suspicion of PE and were therefore able to compare patients with PE to patients in whom PE was expected but ruled out on CTPA. Our observations are also in line with similar findings in other pathological conditions leading to RV overload, such as in pulmonary hypertension, although this represents chronic and not acute RV overload with pathophysiological thickening of the RV wall. In this condition BNP levels have been shown to correlate with RV remodeling, severity of pulmonary hypertension and prognosis (21-24).

The clinical use of NT-pro-BNP level assessment in acute PE has been studied in recent years. Several studies have shown that because of the benign prognosis of PE associated with low NT-pro-BNP levels, patients can be safely treated in an out-of-hospital setting (25). A prospective outcome study has indicated that NT-pro-BNP levels, as compared to several biomarkers including troponin and D-dimer, as well as ventricular function measurement, had superior accuracy and clinical utility for identification of PE patients with low risk of adverse events (14). This was also shown in a recent study that measured the negative predictive value of the Pulmonary Severity Index (PESI) (26) and compared it to NT-pro-BNP levels. A PESI score with a cut-off of 86 (NPV 88%) points was inferior to a NT-pro-BNP below 300 pg/mL (NPV 100%) (27).

Our study has some limitations. First, we only included 113 patients with PE in our study. Due to this relatively limited amount of PE patients our study and observations are prone to unmeasured confounders. However, previous studies correlating NT-pro-BNP with right ventricular enlargement and dysfunction were done in 50 and 77 PE patients. Therefore we assumed we had a reasonable amount of patients. Furthermore, we did not include all patients in whom PE was ruled out on CTPA. Nonetheless, by using the first 226 consecutive patients without PE we consider our selected group representative for the entire population. Third, mean NT-pro-BNP levels are identical in patients with and without PE. We believe this is caused by other cardiopulmonary morbidities, like heart failure and COPD, which were more commonly present in patients without PE. Fourth, ECG-synchronized cardiac CT scanning was performed to assess RV

and LV function while MRI is considered as the golden standard. However, recent studies have shown that ECG-synchronized cardiac CT scanning and MRI give comparable accuracy and reproducibility in assessing the cardiac ventricles (28;29). Unfortunately, these studies were all performed in healthy patients without the acute condition of PE. In these patients heart rate was normal and regular. To prevail this fact, we excluded all unstable patients. Furthermore, none of the patients had irregular heartbeats. Therefore we assumed cardiac CT to be sufficiently accurate and reproducible and comparable to cardiac MRI. Fifth, we did not measure pulmonary artery pressure, which is the pathophysiological link between PE and RV enlargement and dysfunction. However, we do not assume that the pulmonary artery pressure will influence the correlation of NT-pro-BNP and RV function. At last, we used a cut-off point for NT-pro-BNP of 600 pg/mL. This cut-off point was, in a previous study in this population (14), found to have a high sensitivity (90%, 95% CI 56-99.8) and specificity (78%, 95% CI 70-84) for adverse clinical outcome. However, the cut-off point of NT-pro-BNP is not well assessed and varies between 300 to 1000 pg/mL in current literature (30-32)

In summary, our data show that in patients with hemodynamically stable PE, lower RVEF and higher RV end-diastolic-volume were significantly correlated to NT-pro-BNP levels as compared to control patients without PE. These observations provide pathophysiological ground for the well known prognostic value of NT-pro-BNP in acute PE.

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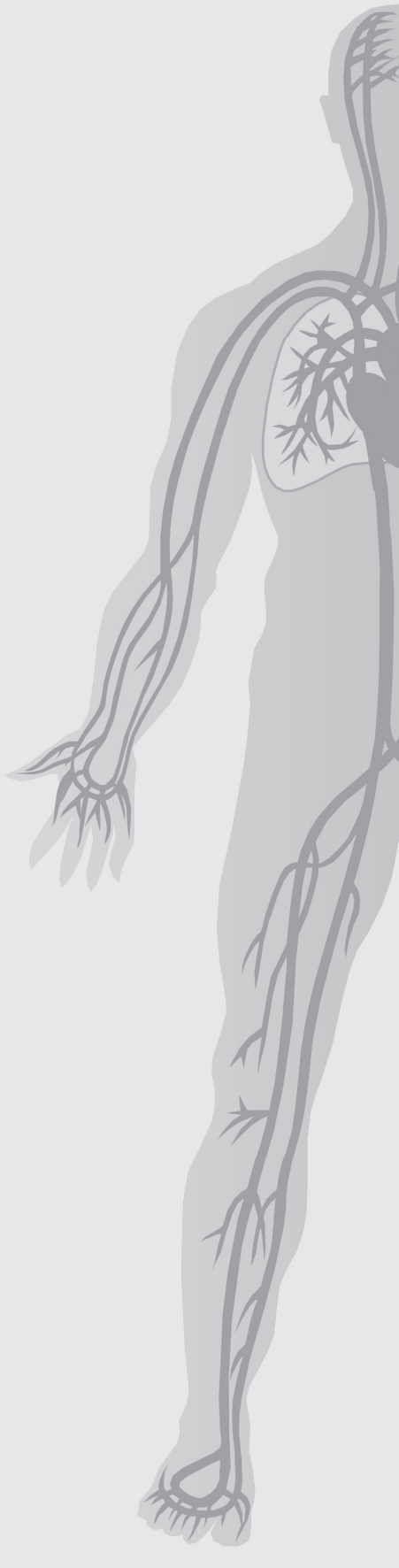

Chapter 6

Blood pressure class and carotid artery intima-media thickness in a population at the secondary epidemiological transition

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ABSTRACT

Introduction

Data relating blood pressure class to subclinical organ damage are infrequently reported in populations with a traditional 'non western' lifestyle. As the relevance of blood pressure stratification to cardiovascular prognosis has not been elucidated in these low-income countries at the second epidemiological transition, we aimed to study the effect of blood pressure class on carotid artery intima-media-thickness (IMT) on Flores Island, Indonesia.

Methods

A cross-sectional study was performed in 476 inhabitants (male/female) of Flores. Blood pressure was classified using the ESH/ESC classification. The primary endpoint was mean carotid-IMT measured by ultrasonography in classes of blood pressure. Covariate analysis was performed adjusting for conventional cardiovascular risk factors.

Results

Blood pressure ranged from 94-250 mmHg systolic and 50-125 mmHg diastolic, 35% of the population had "Grade-I-hypertension" or higher, 1,7% of the population was short-term treated with antihypertensive therapy. IMT significantly differed for blood pressure classes ($p<0.001$). Mean(±SEM) IMT was 587.8(±9.3)µm, 621.5(±7.6)µm, 653.6(±10.5)µm, 717.9(±14.0)µm, and 750.1(±21.8)µm for "optimal", "(high) normal", "grade-I, grade-II and grade-III-hypertension" classes, respectively. After adjustment for cardiovascular risk factors, similar results were obtained.

Conclusions

A strong association was found between blood pressure class and carotid artery intima media thickness in treatment naïve subjects of a population with a traditional lifestyle, at the second epidemiological transition. Intriguingly, the increase of intima media thickness was already observed at the "high normal" BP class. This study may help to prioritize preventive and therapeutic measures to lower blood pressure in countries at the second epidemiological transition.

INTRODUCTION

Eighty percent of global cardiovascular mortality occurs in low and middle income countries (1). Bibliographic studies looking at global burden of diseases, have reported that hypertension is the leading cardiovascular risk factor (2) with a modeled estimate of 639 million hypertensive adults in the year 2000 in economically developing countries(3).

The main cause of mortality defines the stage of epidemiological transition. Before the first epidemiological transition death was mostly due to war and accidents/injuries. During the second epidemiological transition mortality is mainly characterized by a high burden of infectious disease, degenerative and man-inflicted diseases are slowly emerging. During the third epidemiological transition, cardiovascular mortality replaces infectious diseases as the primary cause of death. Many low income countries are at the second stage of epidemiological transition (4). The island of Flores, Indonesia, is regarded to be at this stage, nevertheless besides infectious diseases, cardiovascular disease and elevated blood pressure are becoming important causes of visits to health centers (5). Subjects living at Flores Island have little exposure to "Western type risk factors". In addition, access to antihypertensive medication and statins is very limited.

Blood pressure is an established and important risk factor for cardiovascular disease. Blood pressure can be classified in stages. These stages or classes are related to cardiovascular prognosis (6). In high income countries, Stamler et al showed that "Grade II hypertension" is associated with an adjusted relative risk of 4.57 for coronary heart mortality if compared to subjects in the "optimal" blood pressure class (7). A Finnish study described the relation between blood pressure classes and echographically measured carotid artery intima media thickness, an established intermediate marker of atherosclerosis (8-10). The relevance of blood pressure classes with respect to subclinical organ damage has not been assessed in low income countries at the second epidemiological transition. Carotid artery intima media thickness is listed as a measure of subclinical organ damage in the guidelines of hypertension (6). If blood pressure is a universal risk factor it is to be expected that a higher blood pressure class results in higher carotid artery intima media measures also in low income countries at secondary transition.

It is conceivable that currently available data may underestimate the effect of blood pressure class on carotid artery intima media thickness due to the high level of care given to patients either in the primary or secondary prevention setting in high and middle income countries. In this respect, studies in treatment naïve populations may add valuable information. We hypothesize that blood pressure class defines carotid artery intima media thickness in a population with a traditional "non-western" life style

at the secondary epidemiological transition. Therefore, we studied carotid artery intima media thickness in relation to blood pressure class in a nearly treatment naïve population on Flores Island, Indonesia.

METHODS

Study area on Flores Island, Indonesia

Flores Island, East Indonesia, is one of the Lesser Sunda Islands with a surface area of 17,164 sq km. In Nangapanda, Ende District, a rural area in the mid-South of Flores, which is the study area, the inhabitants follow a traditional lifestyle, the main sources of income being labor-intensive agriculture, unassisted by mechanical tools, decorative stone collecting and sarong weaving. The diet mainly consists of the freshly grown vegetables and fresh fish. The fish is brined to preserve and stored for times of scarcity hereby becoming the most important source of salt intake. Coconut oil and fresh coconut milk are used for frying and cooking. (Information derived of local sources, questionnaires administered during the surveys and from "The official site of Ende District, East Nusa Tenggara. www.endekab.go.id 20-04-2010").

Study population and design

All included patients were participants of the ImmunoSPIN project. The ImmunoSPIN project (www.immunospin.org) was established in Nangapanda area, which has been described in detail before (11). The ImmunoSPIN project was approved by the Research Ethical Committee of the University of Indonesia, Jakarta, Indonesia. Because of the high rate of illiteracy amongst patients, verbal informed consent was obtained from each subject. A total of 500 randomly selected inhabitants of Nangapanda, between the age of 18 and 80 years, were invited for carotid artery IMT measurements on ten in advance specified days in July 2009. Patients were selected based on their address. By choosing 10 research days all parts of the village were covered and therefore the group is representative for the inhabitants of Nangapanda. Of the 500 invited patients, 476 (95.2%) accepted the invitation. Here, we describe the results of a cross sectional observational study. The primary aim of the study was to study the effect of blood pressure class on carotid artery intima media thickness in an environment at the secondary epidemiological transition, unaffected by conventional "Western-type cardiovascular risk factors" and unbiased by cardiovascular treatment on Flores Island, Indonesia.

Clinical data

Interviews were performed for medical history, family history, medication and cigarette smoking. Medical past and family history were categorized in cardiac events, hyperten-

sion, cerebrovascular events and diabetes mellitus type II. Age estimates were derived from interviews and missionary records. In case age was unknown, age estimates were done using a collection of information gathered from birth related events, neighbours, relatives, or from calculations derived out of their children's age. Waist hip ratio (WHR) and body-mass index (BMI) were measured in all patients. In all patients the Systematic Coronary Risk Evaluation (SCORE)-score was calculated. Since Flores is considered a low risk population, the low risk SCORE-chart of Southern Europe was used. In order to calculate a SCORE-score in all individual patients, even below the age of 40 and above the age of 65, all patients were extrapolated to the age of 60, as proposed in the original ESH/ESC guidelines (12).

Blood pressure measurement

Blood pressure measurements were taken of all patients in supine position after a 5 minute rest. Measurements were done 3 consecutive times on the left arm in all patients using a digital sphygmomanometer from Omron 705IT (HEM-759P-E2) (OMRON Healthcare Europe BV, The Netherlands). The average of three systolic and diastolic blood pressure measurements was used in the analysis. The mean blood pressures were grouped using the criteria published by The European Society of Hypertension (ESH) and The European Society of Cardiology (ESC) 2007 classification for adults above 18 years of age. (6) "Optimal" blood pressure was a blood pressure <120/80 mmHg, "(high) normal" is defined as a blood pressure of ≥120/80 mmHg and lower than 140/90 mmHg, "grade I hypertension" as ≥140/90 mmHg and lower than 160/100 mmHg, "grade II hypertension" as ≥160/100 mmHg and lower than 180/110 mmHg and grade III hypertension as a blood pressure ≥180/110 mmHg.

Carotid artery intima media thickness measurement

IMT was measured while the patient was lying in a supine position. Measurements were conducted at 3 different angles of both the right and left common carotid artery at 10 mm proximal of the carotid artery bulb after shipping a mobile ultrasound device (Mylab®25 ultrasound system with a LA523 13-4MHz transducer, ESAOTE, S.p.A, The Netherlands) from the Netherlands to Flores Island. The mean of these 6 measurements was used in the analysis. First, a pilot study on 46 randomly chosen patients was performed in order to evaluate the quality and reproducibility of the measurements and to evaluate the infrastructure. These IMT measurements were done twice by 2 trained ultrasonographists (LJW and AEW) after training under supervision (of JTT) at the vascular and internal medicine outpatients-ward at Leiden University Medical Centrum (LUMC), The Netherlands. A highly significant correlation was observed between both echographists with a Pearson's coefficient (r) of 0.933. A Bland-Altman plot (not shown) showed no significant difference between both echographists. An acceptable variance around the

mean (within 1.96 standard deviations) was observed without downward or upward bias. The IMT measurements of both echographists showed high ICC for absolute agreement (0.963, $p<0.01$). After this pilot study, one of the physicians (AEW) performed all intima-media thicknesses measurements in the 476 patients included in this study. Carotid artery plaque was scored by the absence of presence of plaque. The definition of carotid artery plaque was the presence of any vascular irregularities or widening of the carotid artery, with or without protrusion into the lumen (13).

Laboratory measurements

All patients were instructed to be fasting before venous sampling. Blood glucose was analyzed using Breeze®2 glucose meter (Bayer Health Care LLC, Basel, Switzerland). Lipid profiles (Total cholesterol, Triglycerides, HDL-cholesterol) were measured using commercial enzymatic kits Cat:11489232 for TC and HDL and Cat:11488872 for TG (Roche Molecular Biochemicals) and determined using ELISA reader (LabSystem Multiscan, MHC347, Finland). LDL-cholesterol was calculated by using the Friedwald calculation.

Sample size calculation

No studies have been performed in high, middle or low income countries that analyzed IMT stratified by blood pressure levels. A formal power calculation was therefore not possible. The aimed number of included patients (n=500) was based on the capacity of the research team that performed IMT measurements to conduct the work within a 2 week period.

Statistical analysis

For all baseline characteristics data are presented as mean ± SD. The mean IMT is presented as mean ± SEM. Analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were used to assess the relation between mean IMT measurements and classes of systolic blood pressure. Bonferroni correction was applied for comparison between all groups. P values <0.05 were considered to be significant. Model 1 is not corrected for any additional 'classical' cardiovascular risk factors (ANOVA). Model 2 is corrected for age and gender (ANCOVA 1). Model 3 is corrected like model 2 with addition of smoking, blood glucose, waist-hip ratio, total cholesterol/HDL ratio, medical history and family history (ANCOVA 2). Statistical analysis was done using SPSS statistics 17.0.2 (SPSS Inc., Chicago, Illinois, The USA).

RESULTS

Demographics

Out of the 500 patients invited, 476 (95.2%) responded and presented at our research centre. Of these 476 patients, 471 (99%) had both IMT and blood pressure measured and were included in the analysis. Patients' characteristics are given in table 1. The average age was 51 ± 10 years, ranging from 20 to 80 years. There was a slight predominance of female patients (58%) and a third of all patients were smokers (33.2%). Pharmacological treatment was virtually absent: Fourteen patients (2.9%) used any form of medication of whom eight patients (1.7%) were taking anti-hypertensive medication mostly on irregular basis (3 patients used nifedipine, 6 captopril and 3 hydrochlorothiazide). Twenty patients (4.2%) had suffered a stroke and/or a myocardial infarction, 30 patients (6.3%) had diabetes mellitus type II. A positive familial history of cardiovascular events was present in 30 patients (6.3%). The mean waist-hip ratio was 0.89 ± 0.07 (0.92 ± 0.06 in male and 0.87 ± 0.06 in female). The mean blood glucose levels measured was 6.1 ± 1.8 mmol/L. Mean blood pressure was 135 mmHg systolic and 78 mmHg diastolic. Hypertension (grade I and II) was present in 35.6% of the subjects. The mean total cholesterol level was 5.05 ± 1.12 mmol/L, HDL cholesterol was 1.54 ± 0.39 mmol/L, triglycerides 1.30 ± 0.68 mmol/L and the mean calculated LDL-cholesterol level was 2.92 ± 1.01 mmol/L. The mean total cholesterol / HDL-cholesterol ratio was 3.44 ± 1.07. Mean carotid artery IMT was 635.4 ± 5.1 µm. Mean SCORE-score was 2.4 ± 2.1 %. Carotid plaque was present in 16.6% of the patients. (Table 1)

Carotid artery IMT and blood pressure class

Blood pressure was measured in 471 patients. Mean blood pressure (±SD) was 111.7/66.8 (±6.0/6.1) mmHg in the "optimal" blood pressure class group (n=121), 128.4/75.6 (±5.8/7.3) mmHg in the "(high) normal" blood pressure class group (n=180), 147.1/85.3 (±5.5/6.0) mmHg in the "grade I hypertension" class group (n=95), 166.2/92.2 (±8.3/10.4) mmHg in the "grade II hypertension" class group (n=53) and 194.4/101.6 (±18.7/12.1) mmHg in the "grade III hypertension" class group (n=22).

Mean carotid artery IMT (±SEM) was 587.8 (±9.3) µm, 621.5 (±7.6) µm, 653.6 (±10.5) µm, 717.9 (±14.0) µm and 750.1 (±21.8) µm in the "optimal" blood pressure, "(high) normal" blood pressure, "grade I, II and III hypertension" classes, respectively. If corrected for multiple testing the carotid artery IMT measurements of all blood pressure classes remained significantly different compared to the grade II hypertension class ($P<0.01$).

To address the potential influence of age and gender, a subsequent analysis was performed adjusting for the variables 'age' and 'sex' (model 2). This resulted in a mean IMT (±SEM) of 605.1 (±8.2) µm, 623.7 (±6.5) µm, 647.4 (±9.0) µm, 696.2 (±12.2) µm and 716.4 (±18.9) µm in the "optimal" blood pressure to the "grade III hypertension" class,

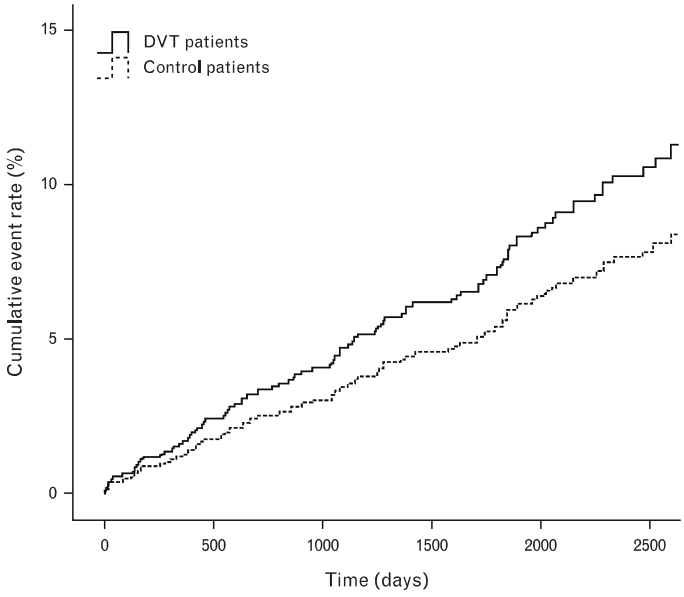
Table 1. Baseline patient characteristics: demographic and metabolic variables.

Variable	Total	Male	Female	Optimal	(High)Normal	Grade I	Grade II	Grade III
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Age (year)	51±10	52±11	50±11	47±10	50±9	52±10	56±11	58±9.3
Systole (mmHg)	135±23	135±23	136±22	112±6	128±6	147±6	166±8	194±19
Diastole (mmHg)	78±12	78±13	79±12	67±6	76±7	85±6	92±10	102±12
BMI (kg/m^2)	22.9±3.8	22.4±3.7	23.2±3.8	21.5±3.5	23.2±3.7	23.3±3.6	23.8±4.2	24.2±3.2
WHR	0.89±0.07	0.92±0.06	0.87±0.06	0.88±0.07	0.88±0.07	0.90±0.07	0.90±0.07	0.92±0.06
Glucose (mmol/L)	6.0±1.8	6.0±1.5	6.1±2.0	5.7±1.4	5.8±0.8	6.2±2.3	7.1±3.4	6.1±1.4
Total cholesterol (mmol/L)	5.05±1.12	4.91±1.07	5.14±1.14	4.93±1.14	4.98±1.13	5.14±1.14	5.28±0.98	5.41±0.99
HDL-cholesterol (mmol/L)	1.54±0.39	1.45±0.4	1.61±0.38	1.60±0.37	1.55±0.40	1.52±0.39	1.49±0.42	1.43±0.29
Triglycerides (mmol/L)	1.30±0.68	1.45±0.84	1.23±0.61	1.19±0.63 166±8 92±10 23.8±4.2 0.90±0.07 7.1±3.4 5.28±0.98 1.49±0.42	1.32±0.76	1.35±0.75	1.50±0.73	1.42±0.68
LDL-cholesterol (mmol/L)	2.92±1.01	2.82±0.97	2.98±1.03	2.75±1.00	2.78±1.02	3.00±1.07	3.07±0.89	3.29±1.02
TC/HDL ratio	3.44±1.07	3.56±1.06	3.35±1.07	3.21±0.89	3.37±1.07	3.60±1.24	3.77±1.05	3.88±0.83
Diabetes (yes/no)	7.4%	7.3%	7.4%	3.3%	2.8%	9.5%	17%	9.1%
Smoking (ever/never)	33.2%	78.8%	0.7%	36.4%	29.4%	34.7%	34.0%	36.4%
RR medication (yes/no)	1.7%	1.0%	2.1%	0.0%	0.0%	2.1%	5.7%	4.5%
Cardiovascular Events (yes/no)	4.2%	5.1%	3.6%	2.5%	3.3%	5.3%	5.7%	13.6%
- Myocardial (yes/no)	0.7%	1.0%	0.4%	0%	0.6%	0%	0%	3.8%
- Stroke (yes/no)	3.8%	4.5%	3.2%	2.5%	2.8%	5.3%	3.8%	13.6%
Family history of CVD (yes/no)	6.3%	6.1%	6.5%	7.4%	7.8%	5.3%	3.8%	0.0%
Carotid plaque (%)	16.6%	27.3%	9.4%	9.9%	14.5%	18.9%	26.9%	36.4%
SCORE-score	2.4±2.1%	4.6±2.2%	1.2±0.6%	2.1±1.3%	1.7±1.1%	2.4±2.0%	3.9±2.7%	6.1±4.4%

SD: standard deviation, BMI: body mass index, WHR: waist-hip ratio, TC/HDL: Total cholesterol / HDL cholesterol, RR: blood pressure

respectively (P<0.01). Adjusting for 'age', 'sex', 'smoking', 'blood glucose', 'waist-hip-ratio', 'total cholesterol / HDL-cholesterol ratio', 'medical history' and 'familial history' (model 3) resulted in a mean IMT (±SEM) of 609.3 (±10.1) µm, 622.7 (±8.1) µm, 653.4 (±11.2) µm, 681.7 (±16.5) µm and 709.5 (±24.2) µm in the respective blood pressure classes (figure 1).

Table 2 shows the contribution of every individual 'classical' cardiovascular risk factor on the progression of IMT (model 3). Blood pressure classes significantly contributed to carotid artery IMT (P<0.01) regardless of age (p<0.01), gender (p=0.11), blood glucose (p=0.73), lipid levels (p=0.49), waist-hip ratio (p=0.68), smoking (p=0.74), medical history (p=0.48) and familial history (p=0.58). Using LDL-cholesterol instead of the total cholesterol / HDL-cholesterol ratio yielded identical results (data not shown).



References	991	853	776	718	584	309
DVT patients	244	148	105	82	51	21

Figure 1. Cumulative event rate for atherothrombotic events in patients with and without deep vein thrombosis (DVT)

DISCUSSION

This is the first study that relates blood pressure class to carotid artery intima media thickness in a population with a traditional "non western" lifestyle at the secondary epidemiological transition. The results show a clear increase of carotid artery intima

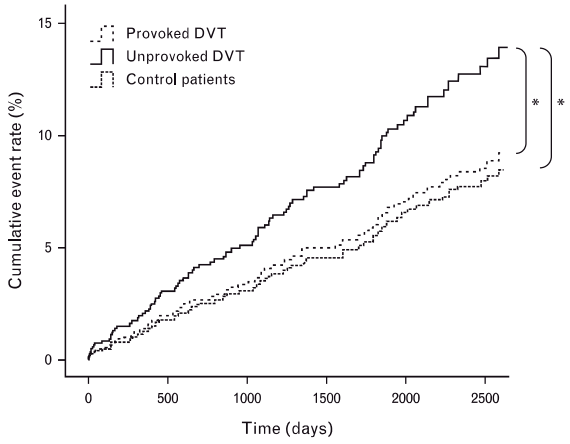
Table 2. Parameters estimates: prediction of IMT.

	Model 1			Model 2			Model 3		
	Increase in IMT (±95% CI) (µm/unit)	**Partial eta^2 (r)**	**Sig. (p=)**	**Increase in IMT (±95% CI) (µm/unit)**	**Partial eta^2 (r)**	**Sig. (p=)**	**Increase in IMT (±95% CI) (µm/unit)**	**Partial eta^2 (r)**	**Sig. (p=)**
Hypertension class	.	.	<0.01[1]	.	.	<0.01[1]	.	.	<0.01[1]
Optimal	Reference	.	.	reference	.	.	reference	.	.
(High) Normal	33.8 (±23.5)	0.017	<0.01[2]	18.6 (±20.5)	0.007	0.08[2]	13.4 (±25.4)	0.004	0.30[2]
Grade I	65.8 (±27.5)	0.045	0.01[3]	42.3 (±24.0)	0.025	<0.01[3]	44.1 (±30.0)	0.028	0.04[3]
Grade II	130.1 (±33.0)	0.114	<0.01[4]	91.1 (±29.5)	0.074	<0.01[4]	72.4 (±39.1)	0.045	<0.01[4]
Grade III	162.4 (±46.5)	0.092	<0.01[5]	111.3 (±41.1)	0.058	<0.01[5]	100.2 (±52.4)	0.047	<0.01
Parameter									
Age (years)	.	.	.	4.7 (±0.8)	0.220	<0.01	5.5 (±1.1)	0.266	<0.01
Sex (being male)	.	.	.	38.0 (±16.3)	0.043	<0.01	28.7 (±36.1)	0.009	0.12
Smoking (no)	.	.	.	.	.	.	6.8 (±37.5)	<0.001	0.72
Blood glucose (mmol/L)	.	.	.	.	.	.	1.2 (±5.4)	0.001	0.67
Waist-hip ratio	.	.	.	.	.	.	-37.5 (±169.6)	0.001	0.66
Total cholesterol / HDL ratio	.	.	.	.	.	.	3.5 (±10.3)	0.002	0.51
Medical history	.	.	.	.	.	.	16.6 (±48.3)	0.002	0.50
Familial history	.	.	.	.	.	.	12.5 (±42.6)	0.001	0.57

[1] Significance of the grade II hypertension class compared to the optimal class
[2] Compared to the optimal class
[3] Compared to the (High) Normal class
[4] Compared to the Grade I hypertension class
[5] Compared to the Grade II hypertension class

media thickness with higher blood pressure classes. An effect that remained statistically significant after correction for potential confounders. Interestingly, this observation has been done in an environment where medication is not yet continuously present. Thus, the results probably reflect the real and remarkable strong impact of blood pressure class in carotid artery intima media thickness. These results show that the relevance of blood pressure stratification is not restricted to subjects in high income countries at the third epidemiological transition (7,9,14).

Intriguingly, the effect of blood pressure carotid artery intima media thickness may start at an early level of blood pressure elevation. Although, the effect of optimal versus (high) normal blood pressure on the intima media thickness did not remain statistically significant in ANCOVA model 1 and 2, the statistically significant uncorrected data (ANOVA) suggest the increase of carotid artery intima media thickness may already occur in subjects in the high normal blood pressure group. This is in line with the dose-effect relation between BP classes and intima media thickness as observed. Importantly, the population studied may well reflect the situation in millions of subjects living "non-Western" societies at the secondary epidemiological transition. Given this strong effect of blood pressure class on IMT, in these subjects, and given the fact that increased IMT predicts cardiovascular events later in life, the study results underline the importance to establish preventive and therapeutic measures for these patients. The knowledge and resulting therapeutic means developed in high income countries should be studied and



* $P < 0.05$

References	991	853	776	718	584	309
Provoked DVT patients	170	102	70	55	37	15
Unprovoked DVT patients	74	46	35	27	14	6

Figure 2. Cumulative event rate for atherothrombotic events in patients with a first provoked deep vein thrombosis (DVT), unprovoked DVT, and in control without DVT

especially become available in low income countries even if they are at the secondary epidemiological transition.

Our study population had an incidence of grade I and II hypertension of over 35%, which is relatively high compared to multiple studies in rural areas in Asia and Africa. (15,16) In addition, 17-37% of the 180 patients with "(high) normal" blood pressure may progress to hypertension if the data in literature apply to the subjects now studied on Flores Island (17). We were however surprised to find this high prevalence of hypertension in this population. A consideration regarding this high prevalence is that salt intake on Flores Island may be relatively high due to the use of brined seafood (18). Unfortunately, we did not collect urine samples to confirm this hypothesis by measuring urine sodium secretion. Also local data on salt ingestion have never been gathered and are therefore not available. Given this, it is currently not possible to correlate salt intake to blood pressure class, nor to study the relation of sodium intake on the carotid IMT in this population(19,20).

It could be argued that the results of our study are confounded by other cardiovascular risk factors for instance smoking. Therefore, an analysis for covariance was performed to assess the influence of age, gender, smoking, blood glucose level, waist-hip-ratio, total cholesterol / HDL-cholesterol ratio, familial history and medical history. The results of these analyses (figure 1) show that blood pressure class significantly relate to carotid artery intima media thickness. In the ANCOVA models, age significantly contributed to IMT. This is in line with the effect of age on IMT previously described in western populations (21,22). Another weakness is that IMT measurements can not differentiate between the intima and media of the vessel wall. The elevation of carotid artery IMT does therefore not differentiate between atherosclerotic intima vessel wall thickening and media vessel wall hypertrophy due to hemodynamic stimulation by the elevated systolic blood pressure (23,24).

We are aware the study has limitations. Patients with known hypertension and patients with a medical or familial history of cardiovascular disease may have been more eager to participate in the study. However, the inclusion of patients at ten randomly chosen days and the high response rate argue against a sample bias. Second, a cross-sectional study design without follow-up period, is not a design to register cardiovascular events. Thus, analysis relating high IMT values to future cardiovascular events could not be performed in the context of the current study. Thus, the extrapolation of the results of multiple studies in Western populations that established carotid artery IMT as marker for the presence of atherosclerosis and as approved intermediate endpoint for randomized intervention studies may be unjustified and should be confirmed in follow-up studies

(8-10) Third, there is a relatively high amount of carotid artery plaque. (25) This could be elucidated by defining plaque as any vascular irregularities or widening of the carotid artery, with or without protrusion into the lumen. However, the presence of plaque increases according to the blood pressure class which is as expected.

The results of the present study are to our knowledge the first to show the direct relation of blood pressure classes and carotid artery IMT in an untreated population at the stage of second epidemiological transition. This study underlines the importance of blood pressure as risk factor for cardiovascular disease and the potential enormous benefit of blood pressure lowering strategies in subjects living in low income countries at the second epidemiological transition.

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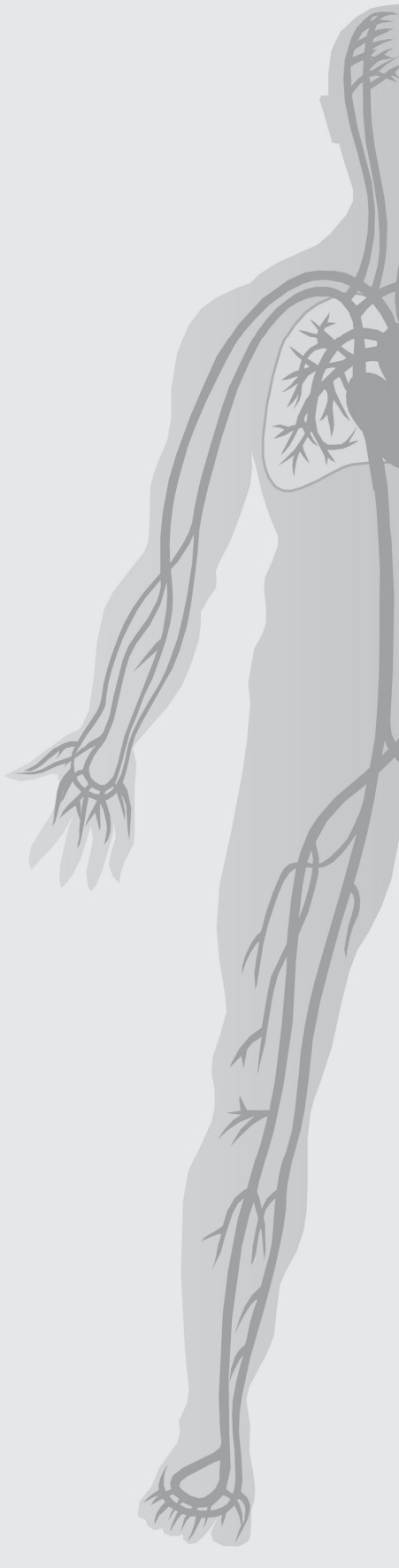

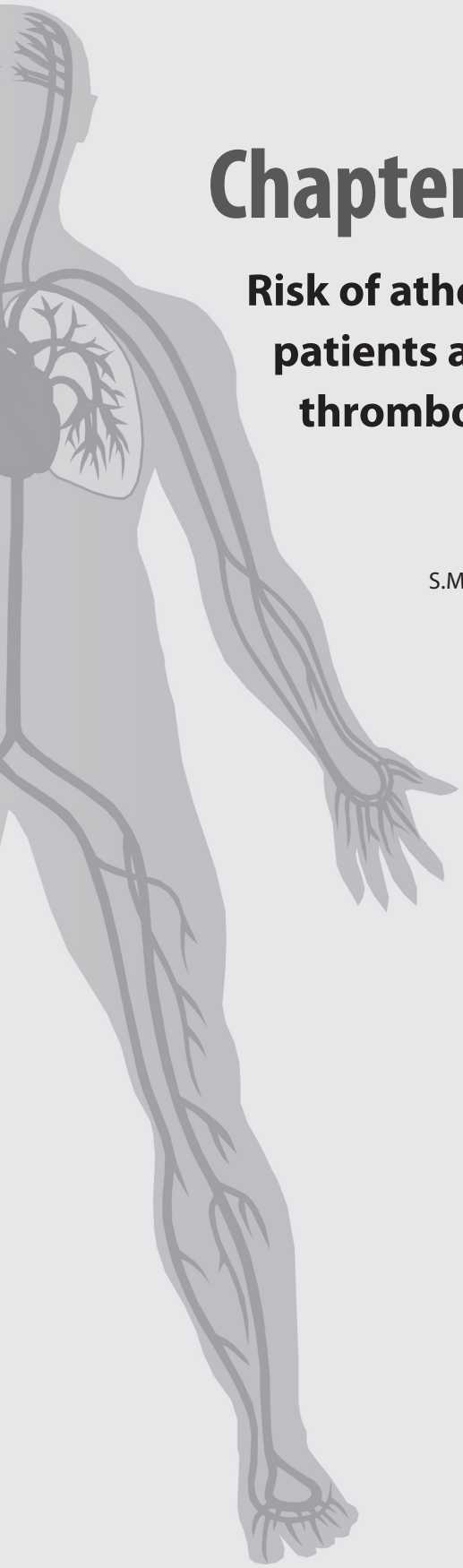

Chapter 7

Risk of atherothrombotic events in patients after proximal deep-vein thrombosis

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ABSTRACT

Introduction

Several studies showed elevated incidences of atherothrombotic events (ATE) in patients with unprovoked venous thromboembolic events (VTE). This association remains understudied in patients presenting with deep vein thrombosis (DVT). We evaluated the incidence of ATE in patients with DVT and compared it to patients with provoked DVT and controls without DVT.

Methods

Patients with compression ultrasonography (CUS) proven unprovoked DVT, provoked DVT, and symptomatic patients, in whom DVT was excluded by CUS, were followed and scored for the occurrence of ATE.

Results

170 patients with provoked, 74 patients with unprovoked DVT and 991 patients without DVT were included. During follow-up 128 ATE occurred (incidence 6.5/100 patient-years). Adjusted hazard ratio (HR) was not different between patients with DVT and without DVT (1.4; 95%CI 0.76-2.4). In contrast, patients with unprovoked DVT suffered ATE more frequent than provoked DVT patients (3.16; 95%CI 1.1-9.1) and control patients (HR 2.8; 95%CI 1.3-5.7). Notably, when fully adjusted for known ATE risk factors the risk differences between controls, provoked and unprovoked DVT patients diminished: HR 1.1 (95%CI 0.47-2.5) and 1.7 (95%CI 0.80-3.6) respectively.

Conclusion

Our study showed that risk of ATE in patients with unprovoked DVT was higher than in patients with provoked DVT or control patients. Interestingly, after full adjustment for multiple known risk factors, the significant difference between unprovoked DVT patients and provoked DVT patients or control patients diminished. This implicates that the correlation between ATE and DVT is non-causal and the measured cardiovascular risk factors are confounders in this correlation.

INTRODUCTION

Several studies have observed an increased risk of atherothrombotic events (ATE) after a diagnosis of venous thromboembolism (VTE) of unknown origin (unprovoked) (1-4). A meta-analysis by Becattini et al. (5), showed an incidence rate ratio (IRR) of 1.87 for the risk of ATE in patients with unprovoked VTE when compared to healthy controls and an IRR of 1.86 for the risk of ATE in unprovoked VTE when compared to patients with VTE of known origin (provoked). Three of the studies included in this meta-analysis concerned patients with pulmonary embolism (PE) (3, 6, 7), while only one study examined the incidence of ATE after deep vein thrombosis (DVT) (3). In the latter population-based cohort study, the relative risk for overall ATE in the first year after VTE was higher in unprovoked PE patients than in unprovoked DVT patients (2.84 vs 1.87) when compared to population controls and when population controls were compared to provoked PE and provoked DVT, the relative risk was 2.28 and 1.82 respectively (8). Importantly, in that study, the incidence of ATE in patients with DVT had been compared with the incidence in population controls without VTE, who may have a lower a priori risk for arterial disease than patients with established VTE. Moreover, due to the study design, Sorensen et al. were unable to adjust for patients' characteristics and known individual cardiovascular risk factors. Therefore, our goal was to further explore the association between DVT and ATE by including a control population with more similar baseline characteristics than population controls and to collect their individual cardiovascular risk factors. Therefore we selected a group of consecutive patients presenting to our hospital who were clinically suspected of having DVT, but in whom DVT was ruled out by compression ultrasonography (CUS) as a control group. In this prospective cohort study, we assessed and compared the incidence of ATE in patients with a first episode of CUS confirmed proximal DVT with the ATE incidence of patients in whom DVT was suspected but ruled out. Further, we repeated this analysis for patients with provoked and unprovoked DVT separately.

METHODS

Study design

A prospective cohort study was conducted to define the risk of ATE and the event-free survival in patients with a first unprovoked and/or provoked proximal DVT compared to control patients without a medical history of VTE in whom DVT was suspected but ruled out. Primary study endpoints were new episodes of confirmed VTE and ATE, the latter defined as myocardial infarction, ischemic stroke, transient ischemic attack, intermittent claudication, carotid endarterectomy, unstable angina, coronary artery bypass grafting,

peripheral arterial angioplasty, bypass or death of unknown cause in the period from diagnosis of DVT until 31-12-20109.

Patients

All in- and out-patients in our hospital with clinically suspected DVT between July 2002 and December 2005 were eligible for inclusion. Their medical charts were searched for the occurrence of study endpoints at the end of the follow-up period. Whenever a patient had died before December 2010, the pathology report, whenever available, was examined for date and cause of death. This was double checked with data from the Office of National Statistics of the Netherlands. The surviving patients were contacted by mail and requested to complete a questionnaire with questions regarding medical history, smoking status, medication use and current clinical condition. In case the resubmitted questionnaires contained missing data, we contacted the patients by telephone. Patients who did not respond to our first request were sent a reminder. If patients could not be reached, the last medical report of their treating general practitioner was used to complete our database. In case of a reported episode of VTE or ATE during the follow-up period, information regarding diagnosis, treatment regimen and treatment time was collected. The study protocol was reviewed and approved by the Institutional Review Board of the Leiden University Medical Centre (LUMC).

Patients with a first acute DVT

The diagnosis of proximal DVT was based on non-compressibility in the popliteal, femoral and/or iliac veins on compression ultrasonography (CUS). Data on risk factors for DVT were derived from the original medical charts. Unprovoked DVT was defined as DVT occurring in the absence of risk factors: active malignancy, immobility for more than three days or a recent long flight (at least 4 hours), recent surgery or fracture of lower extremity, pregnancy or peri-partum period, hormone replacement therapy and use of oral contraception. Patients diagnosed with acute DVT were initially treated according to hospital policy with low-molecular-weight heparin (LMWH) followed by oral vitamin K antagonists (VKA) for at least 3 months for patients with provoked DVT and at least 6 months for patients with unprovoked DVT. Because therapy with VKA reduces the risk for myocardial infarction10 patients with an alternative indication for VKA therapy, in whom these anticoagulants could not be withdrawn after a 6-month treatment period, were excluded from this analysis.

Patients in whom DVT was suspected but ruled out

The control population consisted of patients in whom a first DVT was clinically suspected but ruled out by serial CUS or CUS with a normal D-dimer test11. They were not treated

with anticoagulant drugs. As with the proven DVT patients, those with an alternative indication for oral anticoagulation were excluded from this analysis.

Statistical analysis

Baseline characteristics are given as mean ± standard deviation (SD). Nominal data are presented as the number of patients (N) and the percentage in the study cohort (%). The incidence of ATE in patients with provoked and unprovoked DVT compared to patients in whom a DVT was clinically suspected but ruled-out by CUS was assessed. Cumulative event rates were estimated using a Kaplan-Meier life-table. To rule out the effect of VKA on the incidence of ATE, the start date was set at the day of VKA cessation. Hazard ratios (HR) were calculated using a Cox proportional hazard model. These were adjusted for known confounders including sex, age, malignancy, positive smoking status, hypertension, diabetes and hypercholesterolemia and a previous history of ATE. Patients with (recurrent) thrombosis or non-vascular death were censored in the analysis. Analysis was performed using SPSS version 14.0 (SPSS Inc, Chicago, IL).

RESULTS

Patients

In a total of 1425 patients, the presence of acute DVT was suspected during the inclusion period. Because of a prior history of acute VTE, 190 (13%) patients were excluded from further analysis. From the remaining 1235 patients, 244 (20%) were diagnosed with and treated for DVT, leaving 991 patients (80%) without DVT as control population. Alternative diagnosis in these latter patients without DVT included Baker's cyst, thrombophlebitis, cellulitis, erysipelas, intramuscular bleeding and varicose veins.

Baseline characteristics of the study population are depicted in Table 1. There were significantly more females in the provoked DVT and control groups than in the unprovoked DVT group. Provoked DVT patients were significantly younger than the other study patients and a history of ATE was significantly less prevalent in unprovoked DVT patients. There was no difference within the study groups for the presence of diabetes, hypercholesterolemia and smoking. The total number of patient-years (py) was 5882 in the entire population, 5169 py in the control patients, 482 py in the patients with provoked DVT and 231 py in the patients with unprovoked DVT. The median follow-up was 6 years.

Atherothrombotic Events

A total of 128 fatal and non-fatal ATE occurred during the study period in a total of 384 person years (incidence 6.5/100 py). No significant difference was found in event free

Table 1. Baseline patient characteristics

	All patients (n=1235)	Control patients (n=991)	Patients with provoked DVT (n=170)	Patients with unprovoked DVT (n=74)
Sex (Female, %)[a]	725 (59)	598 (60)	102 (60)	25 (34)
Age (years ± SD)[a]	56 ± 17	57 ± 18	53 ± 17	59 ± 16
History of ATE (n,%)[a]	142 (12)	124 (13)	15 (9)	3 (4)
Active malignancy (n,%)[a]	221 (19)	157 (16)	64 (38)	-
Smoking (n,%)	187 (15)	148 (15)	27 (16)	12 (16)
Diabetes (n,%)	110 (9)	96 (10)	7 (4)	7 (10)
Hypertension (n,%)[a]	375 (30)	309 (31)	39 (23)	27 (37)
Hypercholesterolemia (n,%)	195 (16)	163 (16)	18 (11)	14 (19)
Number of patient years in follow-up	5882	5169	482	231

[a] Significantly different; DVT=Deep Vein Thrombosis; SD=Standard Deviation; ATE=Atherothrombotic Event

survival between all DVT patients compared to control patients without DVT (figure 1) (HR 1.4; 95%CI 0.80-2.5). When adjusted for age, sex, smoking, active malignancy, hypertension, diabetes, hypercholesterolemia and a history of a prior ATE, the HR remained non-significant (1.4; 95% CI 0.76-2.4).

After categorizing the DVT group in patients with unprovoked and patients with provoked DVT, the incidence of ATE in patients with unprovoked DVT was 18/100 py (10 events in 41.6 py), 3.4/100 py in patients with provoked DVT (12 events in 16.4 py) and 6.3/100 py in control patients (106 events in 325.8 py) without DVT. A significant difference was found in the unadjusted event free survival between control patients and patients with unprovoked DVT (HR 2.8; 95% CI 1.3-5.7, figure 2) and the event free survival between unprovoked and provoked DVT patients (H.R.3.16; 95% CI 1.1-9.1, figure 2). The event free survival between patients with provoked DVT patients and control patients was not different (H.R. 0.87; 95% CI 0.38-2.0).

When fully adjusted for known risk factors (age, sex, smoking, active malignancy, hypertension, diabetes, hypercholesterolemia and a history of a prior ATE), the difference in event free survival between controls, provoked and unprovoked DVT patients almost disappeared (provoked vs. control 1.1; 95% CI 0.47-2.5; unprovoked vs. control 1.7; 95%CI 0.80-3.6; unprovoked vs. provoked 1.6; 95% CI 0.52-4.6).

Venous thromboembolic events

A total of 46 fatal and non-fatal VTE occurred during the follow-up period. Fifteen control patients without DVT endured a new VTE in a total of 58.9 py (incidence 1.1/100 py). Recurrent VTE occurred in 14 unprovoked DVT patients (total of 29.4 py, incidence 12.7/100 py) and in 17 provoked DVT patients (total of 31.8 py, incidence 6.6/100 py). The

risk for VTE in follow-up was significantly higher in patients with unprovoked DVT than in control patients (Relative Risk (RR) 12.5; 95% confidence interval 6.3-25) and in patients with provoked DVT than in patients without DVT (RR 6.61; 95% CI 3.4-13). The risk for recurrent thrombosis in unprovoked DVT patients was nearly significant higher than in patients with provoked DVT (RR 1.89; 95% CI 0.99-3.6).

Deaths

A total of 355 study patients died during the follow-up period. The majority (154 patients, 43%) died of cancer, 30 patients (8.5%) died of a myocardial infarction, 24 patients (6.8%) suffered a fatal stroke, 8 patients (2.3%) had fatal PE. The remaining 139 patients (39.2%) died of other reasons, including infections and traumatic events.

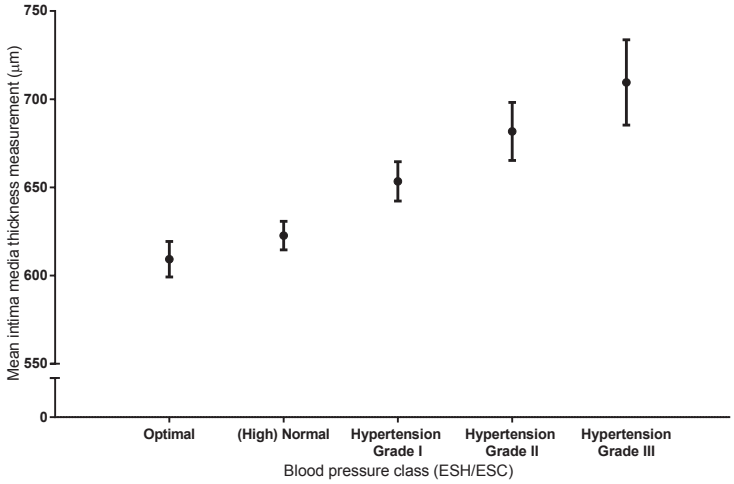

Figure 1. Carotid artery intima–media thickness and blood pressure class Korte titels

DISCUSSION

The results of our study indicate that the risk of ATE in patients with unprovoked DVT was higher than in patients with provoked DVT and in control patients in whom DVT was clinically suspected but ruled-out by CUS. Interestingly, after full adjustment for multiple known risk factors, the significant difference between unprovoked DVT patients and provoked DVT patients or control patients diminished considerably. By demonstrating this, our study extends earlier observations and implicates that the correlation between ATE and DVT is non-causal and that the measured cardiovascular risk factors are confounders in this correlation.

Our study design and study population was different from previous reports analyzing the incidence of ATE after VTE. Only one previous study examined solely the incidence of ATE in DVT patients and compared it to control patients without VTE (3). They found that patients with a DVT had an increased risk for the development of ATE, which was most pronounced during the first year but persisted after 20 years of follow-up. However, this study was performed in a population-based cohort from a nationwide database and nationwide patients were used as controls, which may have a lower a-priori risk for ATE than patients with proven VTE. The inclusion of patients in whom DVT was clinically expected but ruled out by CUS resulted in a control group with more comparable baseline characteristics. In addition, our study is the first study in patients, presenting with proximal DVT of the leg, in which it was possible to adjust for known individual cardiovascular risk factors and to exclude patients using vitamin K antagonists from the analysis.

Our study group has earlier evaluated the risk for ATE after PE, and identical control patients –i.e. in whom PE was suspected but ruled out- were selected to adjust for confounding factors (7). Though our unadjusted results are very comparable to this earlier study, there is an interesting contrast to the PE cohort with regard to the influence of classical ATE risk factors on the adjusted hazard ratio's for ATE. While the unadjusted as well as adjusted hazard ratios for ATE in unprovoked PE patients were significantly higher compared to provoked PE and control patients, correction of the hazard ratios for traditional ATE risk factors in the DVT cohort resulted in loss of all significant differences. Two possible explanations for this difference between DVT and PE patients seem obvious. First, since the number of patients in the unprovoked DVT cohort is relatively limited, our study could be underpowered for an analysis that includes almost 10 different correction factors. A second possible explanation could be that risk factors -and therefore the pathophysiological mechanism- for PE and DVT are not uniform. For instance, a previous study has shown that, when compared to non-carriers, the relative risk for the occurrence of DVT was 7.0 in factor V Leiden carriers while the relative risk for the occurrence for PE was 2.812. This paradox has also been found in use of oral contraceptives13 and pulmonary diseases, including asthma, COPD and pneumonia (14-16). In addition, ATE after PE may occur following another more direct mechanism. One could hypothesize that the presence of a thrombus in the pulmonary artery and direct cardiac stress by the pulmonary embolus could lead to cytokine release and local inflammatory reaction which enhances the progression of already present atherosclerotic plaques (17). However, there are no currently published studies that reject or prove this hypothesis. A direct causal effect of DVT on future occurrence of ATE is difficult to explain. Considering this, the large influence of traditional cardiovascular risk factors on the hazard ratios for ATE in patients with unprovoked versus provoked DVT patients and controls might in-

dicate that these risk factors contribute to both ATE as well as VTE, especially in patients with unprovoked DVT.

Strengths of this study include the fact that we used data from the general practitioner and the Office of National Statistics of The Netherlands in addition to the medical record of our hospital and the questionnaire to score our study endpoints. Therefore no patients were lost to follow-up. Second, we used well defined and serious medical events as study endpoints, which are likely to be recorded and therefore we believe we did not miss out any ATE in follow-up. Third, we had an adequately long follow-up period, with a total number of 5882 patient years. Limitations of our study were, despite the large consecutive sample size, a relatively small number of patients with unprovoked DVT. Second, possible confounding factor including the classical ATE risk factors were only assessed at baseline. Therefore our analyses were not corrected for patients receiving preventive medication for ATE after study inclusion.

In conclusion, our data indicates that patients after unprovoked DVT are at increased risk of ATE compared to patients after provoked DVT and control patients without DVT, as was previously shown in patients with unprovoked PE. However, full adjustment for several well established ATE risk factors diminished these findings. These results raise the question whether the known risk factors for ATE and VTE attribute equally in PE and DVT patients and contradict a causal relation between ATE and VTE. Large prospective trials are needed to identify the underlying mechanism in both PE and DVT patients for developing ATE complications and to identify an adequate treatment regimen for future arterial and venous complications in patients presenting with a first VTE. This treatment could include vitamin-K antagonists, direct factor IIa or Xa inhibitors, aspirin or statins.

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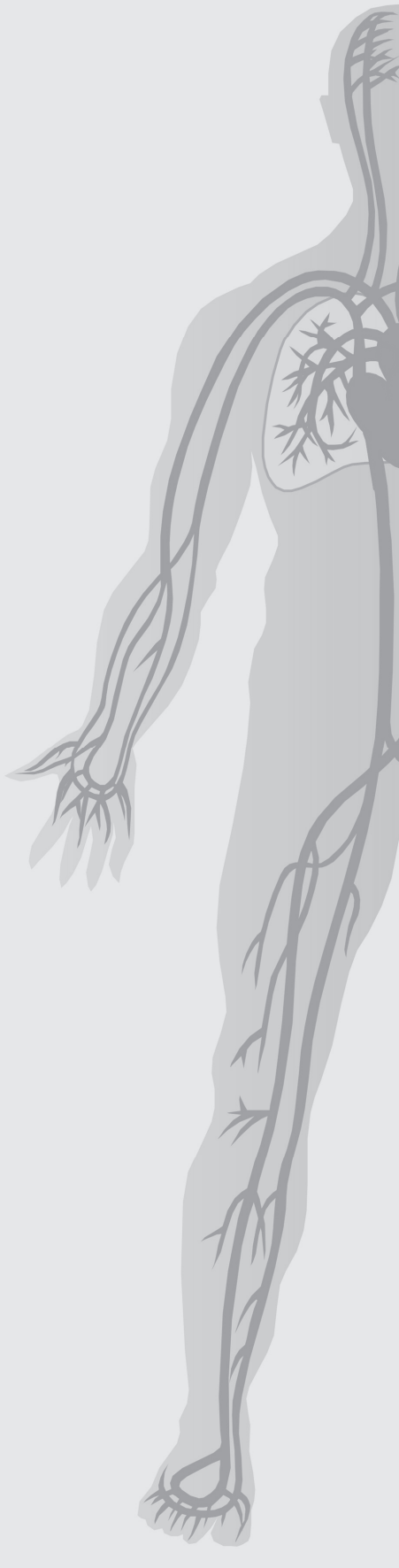

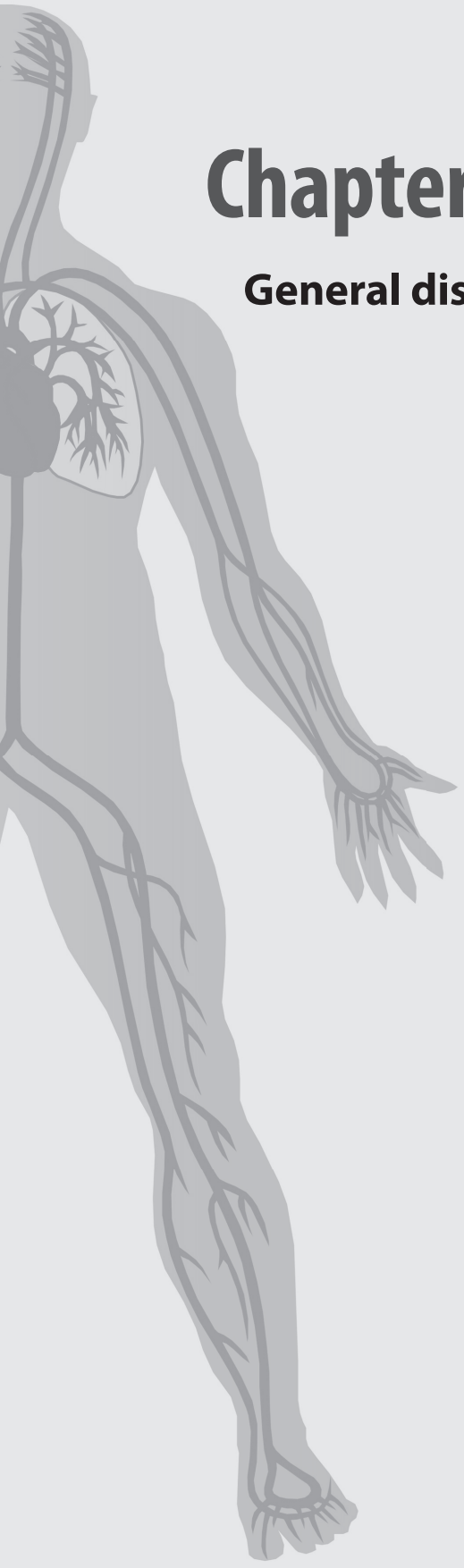

Chapter 8

General discussion and summary

The aims of this thesis were to i) improve the diagnostic management of acute pulmonary embolism (PE), ii) study predictors of adverse clinical outcome of PE, iii) study the association between venous thromboembolism (VTE) and arterial thromboembolic events (ATE), and iv) highlight the relevance of hypertension in atherosclerosis and the effect of aspirin on blood pressure. For this purpose, we conducted a randomized controlled trial, several cohort studies and meta-analyses. **Chapter 1** provides a general introduction to this thesis.

Chapter 2 is a meta-analysis of 4 prospective management studies (1660 consecutive patients) in which the diagnosis of acute PE was excluded on the basis of a "unlikely" clinical decision rule (Wells rule ≤4 points) and a normal high sensitive D-dimer test to evaluate the safety of such a diagnostic strategy. We demonstrated that this dichotomized threshold of the Wells rule is safe with a negative predicative value (NPV) of 99.7% (95%CI 99.0-99.9%). Also, the 3-month mortality risk of PE was very low (0.10%; 95%CI 0.002-0.46%). In **chapter 3**, we performed a meta-analysis of 40 studies focusing on the risk for contrast induced nephropathy (CIN) and the need for renal replacement therapy due to persistent loss of renal function after intravenous contrast enhanced CTPA. In 19563 patients the weighted incidence of CIN was 6.4% (95% CI 5.0–8.1) and the need for renal replacement therapy was 0.06% (95% CI 0.01–0.4). From those numbers, we conclude that intravenous contrast media could be safely administered in the overall majority of patients with suspected PE.

Although PE is a potentially lethal disease, it is diagnosed more than a week after symptom onset in 17% of the patients. In **chapter 4**, we describe an observational prospective outcome study in 113 consecutive patients with CT proven PE to evaluate whether patient's delay is of influence on the thromboembolic burden and right ventricular function by measuring Qanadli-score and right ventricular (RV)/left ventricular (LV)-ratio. In a linear analysis, neither the Qanadli score (R^2 0.003, p=0.726) nor the RV/LV-ratio (R^2 <0.001 p=0.991) were associated with this patient's delay. Also, the mortality rates after 6 weeks of follow-up did not significantly differ in patients with and without delay (Odds ratio: 0.65; 95% CI 0.08 — 5.6). Hence, we concluded that we could not demonstrate an association between patient's delay and either heavier thrombus load, more severe right ventricular dysfunction or mortality.

BNP or NT-pro-BNP, the inactive N-terminal of BNP, is a known biomarker for unfavourable clinical outcome in patients with acute PE. Whether the elevation in BNP level is caused by RV distress due to pulmonary artery obstruction or whether the rise is caused by LV distress due to hemodynamic changes was debated up till recently. In **chapter 5** we measured RV and LV ejection fraction (EF) and end-diastolic volumes (EDV) in 109 patients with and 226 patients without PE, and related those to NT-pro-BNP levels. In patients with PE, an increased RVEF (β-coefficient (95% confidence interval [CI)) −0.044 (± −0.011); p<0.001) and a higher RV end-diastolic volume (β-coefficient 0.005 (±

0.001); p<0.001) were significantly correlated to NT-pro-BNP, while no correlation was found with LVEF (β-coefficient 0.005 (± 0.010); p=0.587) and LV end-diastolic-volume (β-coefficient –0.003 (± 0.002); p=0.074). In patients in whom PE was suspected but ruled out by CTPA, we found a strong correlation NT-pro-BNP levels and LV function. With this study we have provided evidence that elevated NT-pro-BNP levels in PE patients are indeed caused by RV distress.

In **chapter 6**, we examined the effect of blood pressure on intima media thickness, as a measure of atherosclerosis, in a population at the second epidemiological transition, in which mortality is mostly characterized by infectious diseases. We found that IMT significantly differed between BP classes (P<0.001). Mean (±SEM) IMT was 587.8 (±9.3) mm, 621.5 (±7.6)mm, 653.6 (±10.5)mm, 717.9 (±14.0)mm, and 750.1 (±21.8)mm for 'optimal', '(high) normal', 'grade-I, grade-II, and grade-III hypertension' classes, respectively. After adjustment for cardiovascular risk factors, similar results were obtained. Therefore we conclude that there is indeed a strong correlation between blood pressure class and intima media thickness, which already occurs in the "high normal" blood pressure class.

In **Chapter 7**, we evaluated the association between VTE and ATE by comparing the incidences of cardiovascular events (defined as myocardial infarction, ischemic stroke, transient ischemic attack, intermittent claudication, carotid endarterectomy, unstable angina, coronary artery bypass grafting, peripheral arterial angioplasty, bypass or death of unknown cause) in patients with provoked and unprovoked deep vein thrombosis (DVT) and in patients in whom DVT was suspected but ruled out by compression ultrasonography. After a median follow-up of 6 years, no significant difference was found in the incidence of ATE in patients with and without DVT (HR 1.4; 95%CI 0.80-2.5). If the DVT group was separated in provoked and unprovoked DVT, we could demonstrate a significant difference in the unadjusted event free survival between control patients and patients with unprovoked DVT (HR 2.8; 95% CI 1.3-5.7) and the event free survival between unprovoked and provoked DVT patients (H.R.3.16; 95% CI 1.1-9.1). The event free survival between patients with provoked DVT patients and control patients was not different (H.R. 0.87; 95% CI 0.38-2.0). When fully adjusted for known cardiovascular risk factors (age, sex, smoking, active malignancy, hypertension, diabetes, hypercholesterolemia and a history of a prior ATE), the difference in event free survival between controls, provoked and unprovoked DVT patients almost disappeared (provoked vs. control 1.1; 95% CI 0.47-2.5; unprovoked vs. control 1.7; 95%CI 0.80-3.6; unprovoked vs. provoked 1.6; 95% CI 0.52-4.6). We concluded that patients with an unprovoked DVT are at increased risk for ATE, which could largely be explained by overlapping cardiovascular risk factors..

FUTURE PERSPECTIVES

The diagnostic management of acute pulmonary embolism (PE) has evolved during the last 20 years. Nowadays, emergency doctors can rely on safely excluding this condition by a clinical decision rule in combination with D-dimer blood test levels. However, incorrect use of tests like D-dimer and the fear for consequences of missed PE has resulted in a dramatic increase of patient undergoing further imaging. This results in unnecessary radiation exposure and over use of computed tomography pulmonary angiogram (CTPA). Therefore an even more simplified diagnostic algorithm that can easily be implemented and more closely fits the busy clinical practice should be searched for. A promising new diagnostic algorithm is that of 'Years' (1). This algorithm consists of 3 items (clinical signs of DVT, hemoptysis and the subjective clinical assessment whether PE is more likely than another diagnosis or not) in combination a D-dimer blood test. In patients scoring none of the items, PE can safely be discarded without CTPA, in case of a D-dimer blood test <1000 ng/ml. In patient in whom at least 1 of the items is positive, a D-dimer cut-off of <500 ng/ml is used to exclude PE without CTPA. All other patients undergo CTPA. The YEARS algorithm has been proven to reduce the number of necessary CT-scans even further and to be safe in post-hoc analyses of the Christopher and Prometheus studies. The safety and efficiency of this algorithm is currently being studied in a Dutch prospective outcome study.

Another possibility to lower the risk of radiation and contrast mediated nephropathy is the use of magnetic resonance-pulmonary angiography (MRPA).MRI has been reported to have high specificity (97-100%) and good inter-observer agreement (kappa value 0.93; 0.88-0.99)for the diagnosis of acute PE, although only in analyses that excluded technically inadequate scans (2). However MRPA frequently resulted in technically inadequate and non-interpretable images (30%) especially in centres not routinely performing MRPA. Overall sensitivity (78.7-89.6%) (2) of MRPA was shown to be comparable to that of single-slice helical CT (62-78%) (3). Hence, with the current available technique MRPA is not suitable for use in the day to day clinical practice. Hypothetically, inclusion of compression ultrasonography after normal MRPA could possibly complement this lack of sensitivity. In addition, further technical improvement could overcome current limitations. Especially young and pregnant patients, in whom exposure to radiation is associated with the greatest risk, could benefit from the availability of MRPA.

Second, venous and arterial thromboembolic events should no longer be regarded as two worlds apart. For instance, identification of VTE patients at especially high risk for arterial cardiovascular events is relevant since these patients may benefit from modified treatment regimens including preventive use of antiplatelet and cholesterol synthesis inhibiting treatment. Prospective studies are needed to examine the association of the presence and extend of cardiovascular risk factors at the moment of VTE diagnosis and

the future risk of suffering from ATE. Also, the cost-efficacy of implementation of strict cardiovascular disease prevention strategies in patients with acute VTE should be the focus of future research.

Finally, management of hypertension remains a challenge. In this thesis, we have confirmed the well-known role of hypertension in the occurrence of arterial thromboembolic events. Despite current antihypertensive regimens, therapy resistant hypertension, is estimated to occur in ~13 % of patients (4;5). This resistant hypertension is caused by several factors, including incompliance, irreversible arterial stiffness due to late diagnosis of hypertension and lack of recognition of secondary causes such as sleep apnea. Therefore new treatment regimens to overcome these factors are needed. These treatment regimens should concentrate on improving therapeutic adherence by increasing patient awareness but also optimize therapeutic effect of current and future medication. Promising new options are neprilysin inhibitors, which have been shown to be effective in heart failure patients (6). Also optimizing the time of intake of medication, based on circadian rhythm, could reduce the amount of medication needed and thereby improve patient adherence. A previous study (7) showed that aspirin taken at awakening significantly lowered plasma renin and urine dopamine and norepinephrine excretion when compared to taken at bedtime. This is a pathophysiologically plausible mechanism in the reduction of blood pressure. Another study showed that aspirin at bedtime significantly reduced COX1-dependent platelet activity when compared to aspirin taken on awaking. This could potentially prevent an additional amount of arterial thromboembolic events (8). Therefore, future clinical trials should investigate whether switching aspirin intake to bedtime lowers the blood pressure and ultimately lowers the risk of arterial thromboembolic events. A promising new invasive therapeutic option is carotid sinus baro-receptor stimulating therapy. This is an implantable device that electrically stimulates the carotid baro-receptors. This ultimately leads to decreased sympathetic nerve activity, but also decreased heart rate, cardiac contractility and activation of the renin-angiotensin-aldosteron system and ADH release (9). A phase-III study showed a significant reduction of systolic blood pressure of up to 35 mmHg in patients with therapy resistant hypertension (10). However, this trial did not meet a pre-specified safety condition: the procedural safety had an event-free rate ~75% while 82% was pre-specified. Future clinical trials should further address the safety and efficacy of carotid sinus baro-receptor stimulating therapy in the treatment of hypertension.

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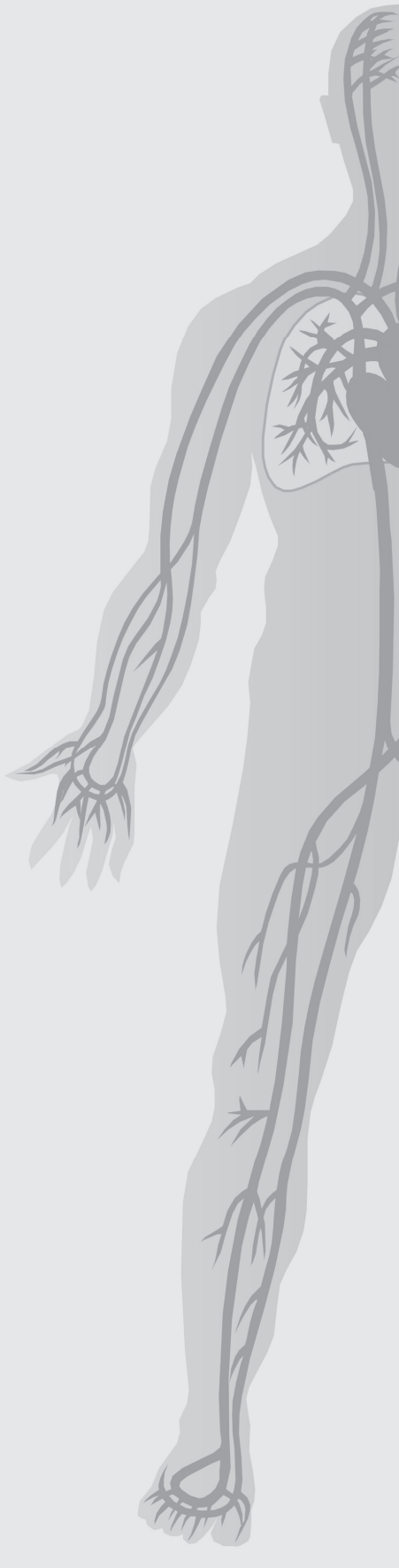

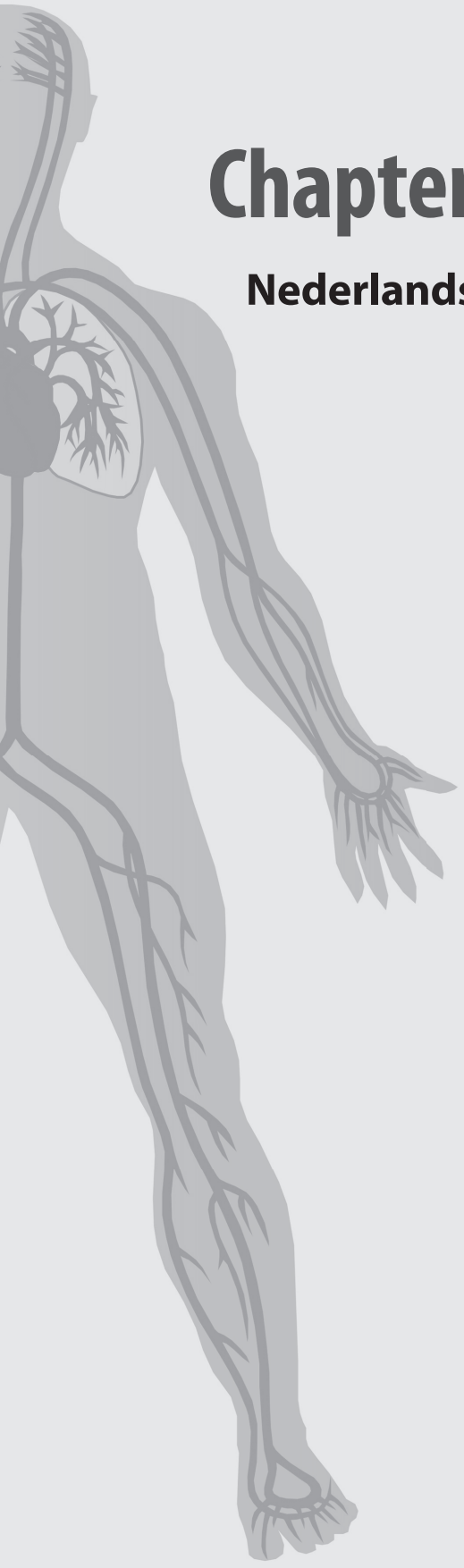

Chapter 9

Nederlandse samenvatting

Dit proefschrift bevat studies die als doel hebben om 1) de diagnostiek van longembolieën te verbeteren, 2) bij patiënten met een longembolie de factoren te ontdekken die een slechte uitkomst veroorzaken, 3) een associatie aan te tonen tussen veneuze en arteriële trombose en 4) het belang van hoge bloeddruk in het ontwikkelen van aderverkalking te benadrukken.

Hoofdstuk 1 is een algemene inleiding over het ontstaan en de presentatie van veneuze en arteriële trombose.

Hoofdstuk 2 is een meta-analyse van vier prospectieve studies die 1660 opeenvolgende patiënten behelst. In al deze studies werd onderzocht of een longembolie veilig kan worden uitgesloten op basis van een klinische beslisregel met als uitkomst "onwaarschijnlijk" (Wells ≤ 4 punten) en een normale D-dimeertest. Wij demonstreerden dat dit algoritme veilig is met een negatief voorspellende waarde van 99.7% met een 95% betrouwbaarheidsinterval (95% CI) van 99.0%-99.9%. De kans op overlijden aan een longembolie in de daaropvolgende 3 maanden was 0.1.% (95% CI 0.002%-0.46%).

In hoofdstuk 3 wordt een meta-analyse besproken, waarin werd gekeken hoeveel patiënten er niervervangende therapie nodig hebben wegens blijvend nierfunctieverlies na het toedienen van intraveneus contrast bij de diagnostiek naar longembolieën. In de 19.563 geïncludeerde patiënten was er een gewogen incidentie van contrastnefropatie in 6.4% (95% CI 5.0%-8.1%). Niervervangende therapie was in 0.06% (95% CI 0.01%-0.4%) van de patiënten nodig. Op basis van deze gegevens concluderen wij dat intraveneus contrast veilig toegediend kan worden in de grote meerderheid van de patiënten verdacht voor een longembolie.

Ondanks dat longembolie een potentieel dodelijke ziekte is, wordt de diagnose in 17% van de gevallen pas na meer dan een week na het starten van symptomen gesteld. In hoofdstuk 4 beschrijven we een observationele prospectieve uitkomststudie in 113 opeenvolgende patiënten met een op CT bewezen longembolie. In deze groep patiënten werd gekeken of de duur tussen het starten van de klachten en het stellen van de diagnose van invloed was op de grootte van de trombose en de rechter-ventrikelfunctie. Dit werd gedaan door de Qanadli-score en de rechter-ventrikel/linker-ventrikel-ratio te meten. Uit een lineaire analyse werd aangetoond dat een latere presentatie geen invloed had op de Qanadli-score (R^2 0.003, p=0.726), noch op de rechter-ventrikel/linker-ventrikel-ratio (R^2 <0.001, p=0.991). De mortaliteit na 6 weken was ook niet verschillend in de patiënten met ten minste 1 week bestaande klachten, vergeleken met patiënten met minder dan een week klachten (Odds ratio: 0.65; 95% CI 0.08 — 5.6). Hieruit maakten wij op, dat de duur tot presentatie niet van invloed is op de ernst van de longembolie in de zin van grotere trombose, verminderde rechter-ventrikelfunctie of mortaliteit.

Er zijn meerdere biomarkers die een ongunstige uitkomst voorspellen in patiënten met een longembolie. BNP of NT-pro-BNP, het inactieve N-terminaal van BNP, is een van die biomarkers. Het is echter niet duidelijk of dit gemaakt wordt door rek op het

rechter-ventrikel, veroorzaakt door obstructie van de longembolie, of dat het komt door hemodynamische veranderingen in het linkerventrikel. In hoofdstuk 5 werd de rechter- en linker-ventrikel-ejectiefractie en het einddiastolisch volume gemeten in 109 patiënten met een longembolie en in 226 patiënten bij wie een longembolie op CT was uitgesloten. De NT-pro-BNP-waardes werden tussen deze twee groepen vergeleken. In patiënten met een longembolie was een betere rechter-ventrikel-ejectiefractie (β-coëfficiënt -0.044 (95% CI ±-0.011); p < 0.001) en een hogere rechter-ventrikel-einddiastolisch volume (β-coëfficiënt 0.005 (95% CI ±0.001); p=0.587), significant gecorreleerd met NT-pro-BNP-waardes. Er is daarentegen geen relatie tussen linker-ventrikel-ejectiefractie (β-coëfficiënt 0.005 (± 0.010); p=0.587) en linker-ventrikel-einddiastolisch volume (β-coëfficiënt -0.003 (± 0.002); p=0.074) met NT-pro-BNP-waardes. In de patiënten waarin een longembolie is uitgesloten op CT was er juist een sterke relatie met linker-ventrikelfunctie en NT-pro-BNP-waardes. Middels deze studie hebben we bewijs geleverd dat verhoogde NT-pro-BNP-waardes in patiënten met een longembolie veroorzaakt worden door stress op het rechter-ventrikel.

In hoofdstuk 6 hebben we naar de associatie gekeken tussen veneuze en arteriële trombose. Dit hebben we gedaan door de incidentie van cardiovasculaire events (omschreven als myocardinfarct, beroerte, transient ischemische attack (TIA), claudicatio intermittens, carotisendarterectomie, instabiele angina pectoris, bypassoperatie, operatie aan de beenvaten wegens atherosclerose en dood met onbekende oorzaak) te vergelijken in patiënten met uitgelokte en niet-uitgelokte diep-veneuze trombose en patiënten waarin een diep-veneuze trombose werd verdacht, maar uitgesloten middels echografie. Na een gemiddelde follow-up van 6 jaar was er geen verschil in incidentie van cardiovasculaire events tussen patiënten met en zonder een diep-veneuze trombose (HR 1.4; 95% CI 0.80-2.5). Als de groep met patiënten met een diep-veneuze trombose werd opgesplitst in uitgelokt en niet-uitgelokt, dan waren er wel significante verschillen. In de onaangepaste event free survival-analyse tussen de gezonde controlegroep en patiënten met een niet-uitgelokte diep-veneuze trombose, behelsde dit een hazard ratio van 2.8 (95% CI 1.3-5.7) en tussen de uitgelokte en niet-uitgelokte trombose was de hazard ratio 3.16 (95% CI 1.1-9.1). De hazard ratio tussen patiënten met een uitgelokte trombose en de controlegroep was niet verschillend (HR 0.87; 95% CI 0.38-2.0). Na volledige correctie met de bekende cardiovasculaire risicofactoren (namelijk: leeftijd, geslacht, roken, maligniteit, hypertensie, diabetes mellitus, hypercholesterolemie en een belaste voorgeschiedenis) verdwenen zo goed als alle verschillen tussen de drie groepen (uitgelokt vs controle HR 1.1; 95% CI 0.47-2.5; niet-uitgelokt vs controle HR 1.7; 95% CI 0.80-3.6; niet-uitgelokt vs uitgelokt HR 1.6; 95% CI 0.52-4.6). Hieruit concludeerden wij dat patiënten met een niet-uitgelokte diep-veneuze trombose een verhoogd

risico op arteriële complicaties hebben, die grotendeels verklaard kunnen worden door reeds bekende cardiovasculaire risicofactoren.

Hypertensie is een van deze risicofactoren. In het jaar 2000 waren er wereldwijd 972 miljoen mensen met hypertensie. De verwachting is dat dit aantal alleen maar zal stijgen tot 1.56 miljard in 2025. In hoofdstuk 7 bekeken we het effect van hypertensie op de vaatwand, middels het meten van de intima media-dikte (IMT), in een populatie die de tweede epidemiologische transitie ondergaat (de oorzaak van overlijden verschuift van infectieziekten naar hart- en vaatziekten). Wij vonden dat de IMT significant verschilde tussen de hypertensie-klasses ($p<0.001$). De gemiddelde (±SEM) IMT was 587.8 (± 9.3) mm, 621.5 (±7.6)mm, 653.6 (±10.5)mm, 717.9 (±14.0)mm, en 750.1 (±21.8)mm in respectievelijk de klasses "optimaal", "hoog normaal", "graad I hypertensie", "graad II hypertensie" en "graad III hypertensie". Na correctie voor bekende risicofactoren vonden we vergelijkbare resultaten. Aan de hand hiervan concludeerden wij, dat er inderdaad een sterke correlatie is tussen de mate van hypertensie en de IMT en dit effect is al aantoonbaar in patiënten met "hoog-normale" bloeddruk.

Toekomstperspectieven

De diagnostiek van acute longembolieën is de afgelopen 20 jaar snel veranderd. Hedendaagse artsen kunnen vertrouwen op een klinische beslisregel in combinatie met een D-dimeer om een longembolie onwaarschijnlijk te maken. Echter, het verkeerd implementeren van deze beslisregel en de angst om een longembolie te missen, heeft geresulteerd in een forse toename van het aantal patiënten dat alsnog een beeldvorming ondergaat. Hierdoor volgt een onnodige stralingsbelasting en meer CT-scans die verricht moeten worden. Daarvoor is het belangrijk om de beslisregels te blijven verbeteren. Deze moeten makkelijker en sneller te hanteren zijn om bruikbaar te blijven op een drukke eerste hulp. Een veelbelovende nieuwe beslisregel is de "YEARS-score". Deze beslisregel bestaat uit slechts drie punten (klinische tekenen van een diep-veneuze trombose, haemoptoë en het subjectieve punt of een longembolie de meest waarschijnlijke diagnose is) in combinatie met een D-dimeer. In patiënten die geen punten scoren, kan een longembolie zonder beeldvorming veilig worden uitgesloten bij een D-dimeer van <1000 ng/ml. In patiënten die ten minste 1 punt scoren is de grenswaarde een D-dimeer <500 ng/ml. Alle andere patiënten ondergaan beeldvorming. De "YEARS-score" heeft in de Christopher en Prometheus-studie post hoc reeds bewezen dat hij veilig is te gebruiken en het aantal CT-scans reduceert. Momenteel is er een prospectieve uitkomststudie bezig om de veiligheid en effectiviteit van deze score verder te bestuderen.

Een andere mogelijkheid om radiatie en de kans op contrastnefropatie te verlagen is het gebruik van een Magnetic Resonance Pulmonary Angiography (MRPA). MRI heeft reeds bewezen een hoge specificiteit (97-100%) en goede inter-observer agreement (kappa-waarde 0.93; 0.88-0.99) te zijn in de diagnostiek naar longembolie. Echter, al

deze analyses excludeerden de technisch inadequate beelden. Juist deze technisch inadequate en niet te interpreteren beelden komen frequent voor bij MRPA (30%), met name in centra die dit onderzoek niet frequent verrichten. De sensitiviteit van MRPA (78.7-89.6%) is vergelijkbaar met single-slice CT (62-78%). Deze sensitiviteit zou hypothetisch verbeterd kunnen worden door een echo van de benen te verrichten bij een negatief resultaat. Echter, dit is met de hedendaagse beschikbaarheid van de MRI nog geen adequaat alternatief en verbeteringen van de techniek zouden huidige limitaties moeten doen vergeten. Vooral jonge en zwangere patiënten, in wie straling het meeste risico geeft, kan de MRPA uiteindelijk veelbelovend zijn.

Ten tweede, veneuze en arteriële trombose moeten niet langer gezien worden als twee verschillende identiteiten. Het identificeren van patiënten met veneuze trombose die een verhoogd risico hebben op arteriële trombose is zeer belangrijk, omdat zij potentieel kunnen profiteren van plaatjesremming en cholesterolverlagers. Prospectieve studies zijn nodig om het verband tussen cardiovasculaire risicofactoren bij patiënten met een veneuze trombose en het risico op een arteriële trombose uit te kristalliseren. Deze studies moeten zich ook richten op de kosteneffectiviteit van strikte cardiovasculaire preventie in patiënten met veneuze trombose.

Als laatste blijft het management van hypertensie een uitdaging. In dit proefschrift hebben we nogmaals het verband aangetoond tussen hypertensie en het ontwikkelen van arteriële trombose. Ondanks onze huidige behandelingsmogelijkheden, blijft therapieresistente hypertensie (13% van de patiënten) een groot probleem. Therapieresistente hypertensie is het gevolg van meerdere factoren waaronder therapie-ontrouw, onomkeerbare vaatstijfheid door het te laat stellen van de diagnose en de verminderde alertheid op secundaire oorzaken, zoals het obstructieve slaapapneusyndroom. Nieuwe behandelmethode zijn nodig om deze factoren te overkomen. Deze behandelmethode moet zich aan de ene kant richten op het verbeteren van de therapietrouw door patiënten bewuster te maken van de risico's, en aan de andere kant moet het therapeutische effect van de huidige en toekomstige medicatie worden geoptimaliseerd. Veelbelovende nieuwe middelen zijn neprilysine-remmers, welke reeds hebben aangetoond zeer effectief te zijn bij patiënten met hartfalen. Ook het tijdstip van inname van de medicatie aan de hand van het cicardiaanse ritme is van groot belang. Dit kan de hoeveelheid medicatie verminderen en daarmee de therapietrouw verbeteren. Zo heeft een eerdere studie aangetoond dat aspirine ingenomen voor het slapen gaan, het serum renine en urine dopamine en norepinefrine significant verlaagt ten opzichte van inname in de ochtend. Op basis van dit pathofysiologische mechanisme kan aspirine dus aanvullend gebruikt worden om de bloeddruk te verlagen. Prospectieve studies zijn nodig om dit effect te bevestigen. Een andere veelbelovende, nieuwe invasieve therapeutische optie is carotis baroreceptor-stimulerende therapie. Dit is een implementeerbaar apparaat dat de carotis baroreceptor stimuleert met behulp van elektriciteit. Dit leidt uiteindelijk

tot verminderde sympatische activiteit en daarmee tot een verlaging van de hartslag, verlaging van de rek op het hart, verminderde activatie van het renine-angiotensine-aldosteron-systeem en verminderde ADH-afgifte. Een recente fase-III-studie presenteerde een significante reductie van de bloeddruk tot wel 35mmHg in patiënten met therapieresistente hypertensie. Echter, dit onderzoek haalde niet de vooraf gestelde veiligheidsmarges; de procedure werd in 75% van de gevallen zonder complicaties uitgevoerd, waar een minimum van 82% was vereist. Toekomstige klinische onderzoeken moeten zich verder richten op de veiligheid en effectiviteit van carotis baroreceptorstimulatie in de behandeling van therapieresistente hypertensie.

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CURRICULUM VITAE

Sharif Mohammed Pasha werd geboren op 3 maart 1985 te Leiden. In juni 2003 behaalde hij zijn vwo-diploma aan het Rijnlands Lyceum te Oegstgeest. Na de zomervakantie startte hij met de studie Geneeskunde aan de Universiteit Leiden. In 2009 behaalde hij zijn doctoraal examen met een scriptie getiteld "Safety of excluding acute pulmonary embolism based on an unlikely clinical probability by the Wells rule and normal D-dimer concentration", onder supervisie van prof. dr. M.V. Huisman. In 2007 was hij reeds met zijn coschappen gestart, waarna hij in augustus 2009 cum laude zijn artsexamen behaalde. Aansluitend werkte hij als arts-onderzoeker op de afdeling Algemene Interne Geneeskunde van het Leids Universitair Medisch Centrum te Leiden onder begeleiding van prof. dr. M.V. Huisman, dr. J.T. Tamsma en dr. F.A. Klok. De resultaten van zijn werkzaamheden zijn beschreven in dit proefschrift. In januari 2013 is hij begonnen met zijn opleiding tot internist in het HAGA ziekenhuis te 's-Gravenhage (opleider dr. M.O. van Aken).

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