N-terminal pro-brain natriuretic peptide as a predictor of cardiovascular events and mortality in patients with differentiated thyroid carcinoma

M3 stage wetenschap

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Abstract

Introduction
Differentiated thyroid carcinoma (DTC) has a favorable survival rate. Long-term treatment with thyroid hormone is however associated with an increased risk of cardiovascular disease. N-terminal pro-brain natriuretic peptide (NT-proBNP) has been shown to be an excellent predictor for future cardiovascular events and mortality in several populations. Primary aim was to study NT-proBNP as an independent predictor for cardiovascular events, secondary aim was to study the predictive value of NT-proBNP for all-cause mortality in DTC patients in follow-up. Tertiary aim was to determine the optimal NT-proBNP cutoff value to discriminate between DTC patients at high or low risk for cardiovascular events and mortality.

Patients and Methods
In this retrospective cohort study, a single center cohort of 288 patients in follow-up for DTC was included. NT-proBNP levels were determined in serum samples dating from 2004-2008. At the end of follow-up in 2012, cardiovascular events and death causes that occurred during follow-up were scored. Using Cox regression analyses, NT-proBNP was studied as an independent predictor for outcome. ROC curves were plotted and sensitivity and specificity were calculated to search for the optimal NT-proBNP cutoff values.

Results
Mean baseline age (±SD) was 54.8±14.5 years, median [IQR] NT-proBNP at baseline was 69 [40-119] pg/ml. Median [IQR] follow-up was 6.2 [4.9 – 6.5] years. During follow-up 25 patients (8.7%) had a cardiovascular event, 29 patients (10.1%) died. In crude Cox regression analyses, each SD increase in logarithmically transformed NT-proBNP was associated with a hazard ratio (HR) of 3.0 (95% CI 2.2 – 4.0) for cardiovascular events and HR 3.1 (95% CI 2.2 – 4.3) for mortality. After a backward Cox regression analysis, initially adjustment for age, sex, cardiovascular risk factors and DTC characteristics, HR was 3.3 (95% CI 2.3 – 4.5) for cardiovascular events and 1.9 (95% CI 1.3 – 2.9) for mortality. When NT-proBNP was added to conventional risk factors in the Cox regression model, the predictive model significantly improved for both cardiovascular events (p=0.0003) and mortality (p=0.018). The optimal NT-proBNP cutoff value was found to be 138 pg/ml for cardiovascular events and 85 pg/ml for mortality.

Conclusion
NT-proBNP is an independent predictor of cardiovascular events and mortality for patients in follow-up for DTC. NT-proBNP improves risk stratification in DTC patients. Adding NT-proBNP to traditional cardiovascular risk factors in a new model may help to identify patients eligible for a more stringent treatment of cardiovascular risk factors. Further study is needed to establish whether treatment according to risk assessment by the new model can improve event-free survival in these patients.
**Samenvatting**

**Introductie**
Gedifferentieerd schildklier carcinoom (DTC) heeft een gunstige overleving. Lange termijn behandeling met schildklierhormoon is echter geassocieerd met een verhoogd risico op cardiovasculaire ziekte. Het is aangetoond dat N-terminal pro-brain natriuretic peptide (NT-proBNP) een goede voorspeller is voor toekomstige cardiovasculaire events en mortaliteit in verschillende populaties. Primair doel van de studie was om NT-proBNP als onafhankelijke voorspeller voor cardiovasculaire events en mortaliteit te onderzoeken in DTC patiënten in follow-up. Secundair doel was om de voorspellende waarde van NT-proBNP voor mortaliteit te onderzoeken. Het bepalen van de optimale NT-proBNP afkappwaarde om onderscheid te maken tussen DTC patiënten met een hoog of laag risico voor cardiovasculaire events en mortaliteit was een tertiair doel.

**Patiënten en methode**

**Resultaten**
De gemiddelde baseline leeftijd (±SD) was 54.8±14.5 jaar, mediane [IQR] baseline NT-proBNP was 69 [40-119] pg/ml. Mediane [IQR] follow-up duur was 6.2 [4.9 – 6.5] jaar. Tijdens follow-up kregen 25 patiënten (8.7%) een cardiovasculair event, 29 patiënten (10.1%) kwamen te overlijden. In de ongecorrigeerde Cox regressie analyse was elke SD toename in de logaritmisch getransformeerde NT-proBNP geassocieerd met een hazard ratio (HR) van 3.0 (95% BI 2.2 – 4.0) voor cardiovasculaire events en HR 3.1 (95% BI 2.2 – 4.3) voor mortaliteit. Na een backward Cox regressie analyse, aanvankelijk gecorrigeerd voor leeftijd, geslacht, cardiovasculaire risico factoren and DTC karakteristieken, was de HR 3.3 (95% BI 2.3 – 4.5) voor cardiovasculaire events en 1.9 (95% BI 1.3 – 2.9) voor mortaliteit. Na toevoeging van NT-proBNP aan conventionele risicofactoren in het Cox regressie model nam de voorspellende waarde van het predictiemodel toe voor zowel cardiovasculaire events (p=0.0003) als mortaliteit (p=0.018). De optimale NT-proBNP afkapwaarde was 138 pg/ml voor cardiovasculaire events en 85 pg/ml voor mortaliteit.

**Conclusie**
NT-proBNP is een onafhankelijke voorspeller voor cardiovasculaire events en mortaliteit voor patiënten die in follow-up zijn voor DTC. NT-proBNP verbetert risicostratificatie in DTC patiënten. Toevoeging van NT-proBNP aan conventionele risicofactoren in een nieuw model kan behulpzaam zijn in het identificeren van patiënten die in aanmerking komen voor een stringente behandeling van cardiovasculaire risicofactoren. Verder onderzoek is geïndiceerd om te bepalen of behandeling op basis van risicostratificatie volgens het nieuwe model de event-vrije overleving kan verbeteren in deze patiëntengroep.
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1. Introduction

In recent years the early detection and treatment of many malignancies has improved. Consequently the number of cancer survivors is steadily growing and concerns about late iatrogenic side effects of cancer treatment have been raised.\(^{(1)}\) Differentiated thyroid carcinoma (DTC), the most common endocrine malignancy, is one of these cancers. Each year more than 450 patients in the Netherlands are diagnosed with DTC and the incidence has steadily increased over the past decades.\(^{(2)}\) Despite the relatively low incidence of DTC there is a high prevalence; currently about 4000 patients in the Netherlands are in follow-up for DTC. This number is likely to increase due to the favorable survival of DTC patients and the general increasing life expectancy.\(^{(3)}\)

**Differentiated Thyroid Carcinoma**

DTC consists of both papillary and follicular (including Hürthle cell type) carcinoma, and originates from the follicular epithelial cells.\(^{(4)}\) Papillary thyroid carcinoma is the most common type and accounts for 75% of DTC. Women are affected in a 3:1 ratio compared to men.\(^{(2)}\) DTC survival rates are high with 10-year disease free survival of 92-98%.\(^{(4)}\) DTC represents approximately 90% of all thyroid carcinomas and is therefore the most frequent thyroid carcinoma type. The remaining part consists of medullary carcinoma (a neuroendocrine tumor), and the aggressive dedifferentiated anaplastic carcinoma.

Initial DTC treatment consists of a total thyroidectomy and a central and/or lateral modified radical neck dissection if lymph node metastases are present. Thereafter patients are treated with radiiodine (I-131) ablation therapy to destroy residual thyroid tissue, benign or malignant. As a result of the ablation of thyroid remnants, thyroglobulin (Tg), a protein normally synthesized by the thyroid, can be used as a tumor marker during follow-up. Also, radiiodine scintigraphy can be used as a sensitive method to search for local thyroid remnants, recurrences or distant metastases.\(^{(5)}\) Thyroid Hormone Suppression Therapy (THST) is subsequently given to lower or suppress the thyrotropin (TSH) level by administration of supraphysiological doses of levothyroxine (T4). The rationale of THST is that TSH is considered a growth factor for DTC cells. THST has been shown to improve survival, especially in intermediate and high risk patients.\(^{(6)}\) Furthermore T4 administration is necessary to correct the hypothyroid state caused by the total thyroidectomy and thyroid remnant ablation with radiiodine.

**Cardiovascular effects of thyroid hormone**

Since the survival rate of DTC is high, there is an increasing interest in possible harmful long-term effects of treatment. Particularly the use and extent of THST have become controversial,\(^{(7)}\) since an excess of thyroid hormone is associated with adverse effects on the heart and vascular system.\(^{(8)}\) The goal of THST is to suppress the TSH level by oversubstituting patients with thyroid hormone (T4, or triiodothyronine (T3) during initial treatment). In the peripheral tissues, T4 is largely converted to T3, which is the main biologically active thyroid hormone. Thyroid hormone, especially T3, has several cardiovascular effects, by both genomic and nongenomic pathways.\(^{(8)}\) In cardiac myocytes T3 can bind to the thyroid hormone nuclear receptor, inducing transcription of important structural and regulatory genes. Several genes regulating intracellular calcium levels, affecting contractile cardiac function and diastolic relaxation, are regulated by the presence or absence of T3. T3 also has several rapid, nongenomic effects on cardiac myocytes and vascular smooth muscle cells. It interacts with ion channels in myocytes, rapidly affecting intracellular levels of sodium, calcium and potassium. Intracellular signaling pathways in the
heart and vascular smooth muscle cells are influenced by T3 as well.\(^\text{8}\) Thyroid hormone therefore has a substantial influence on the cardiovascular system.

In case of increased thyroid hormone concentrations, depolarization is accelerated in myocytes (including the pacemaker cells). This results in an increased heart rate (positive chronotropy). Also cardiac contraction is increased as a result of intracellular changes in sodium, potassium and calcium levels (positive inotropy). These effects are combined with a T3 induced increased sympathetic tone and a decreased parasympathetic tone, resulting in an even more enhanced positive chronotropic and inotropic effect. Furthermore, T3 increases tissue oxygen consumption and tissue thermogenesis. Systemic vascular resistance is decreased due to interactions between thyroid hormone and vascular smooth muscle cells, resulting in a decreased effective arterial filling volume, an increased renal sodium absorption and an increased blood volume. Taken together, these effects lead to an increased cardiac output (figure 1) and therefore increased workload for the heart, which can be harmful on a long-term basis.\(^\text{8,9}\)

Adverse cardiovascular effects have been described in DTC patients. Abdulrahman et al. found an impaired myocardial systolic function in DTC patients on THST.\(^\text{10}\) In another study performed in DTC patients at least 10 years on THST, diastolic dysfunction and an increased left ventricular mass were found.\(^\text{11}\) Furthermore a decreased arterial elasticity, adverse metabolic and prothrombotic effects and recently an increased prevalence of Atrial Fibrillation (AF) were found in this patient group.\(^\text{12-14}\) Moreover, our own data show an increased risk of cardiovascular mortality in DTC patients as compared to a matched control group, independent of cardiovascular risk factors. A lower TSH level was associated with an increased cardiovascular mortality, suggesting a role of THST in the pathogenesis of cardiovascular adverse effects once more (see attachment 1 for the abstract).

The adverse effects of excess thyroid hormone on the heart have been studied more extensively in non-DTC patients with overt and subclinical hyperthyroidism (a state in which the TSH level is suppressed and T3 and T4 are in the normal range).\(^\text{15}\) Meta-analyses and large population based studies suggest an increased incidence of AF, heart failure, cardiovascular events, all-cause mortality and cardiovascular mortality in patients with hyperthyroidism or subclinical hyperthyroidism.\(^\text{16-20}\)

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**Figure 1. Effects of Thyroid Hormone on Cardiovascular hemodynamics.**
Reproduced from: I. Klein, N Engl J Med. 2001\(^\text{19}\)
Predictors for cardiovascular morbidity and mortality

In the past decade the indication and extent of THST have been reconsidered, to seek for an optimal balance between decreasing the risk of a recurrence and preventing iatrogenic adverse effects. Despite concerns on the long-term effects of THST, this treatment is still recommended by the European and American Thyroid Associations (ETA and ATA) during the initial follow-up period in all DTC patients. THST remains indicated for intermediate and high risk patients after initial treatment, while for low risk patients THST can be tempered after the initial follow-up period. Nevertheless, many patients remain exposed to long-term THST and potential late iatrogenic cardiovascular side effects, while predictors for late cardiovascular morbidity and mortality have never been studied in this patient group.

In recent years the interest in the literature for a wide range of biomarkers as a predictor of cardiovascular morbidity and mortality has grown. Various biomarkers like natriuretic peptides, CRP and urinary albumin levels have been extensively studied in high risk patients with cardiovascular disease (particularly heart failure patients) as well as the general population. Of these, the natriuretic peptides Brain Natriuretic Peptide (BNP) and NT-proBNP were found to be the strongest predictors for heart failure, AF, cardiovascular events and mortality.

Natriuretic peptides
The two major natriuretic peptides (NPs) are brain natriuretic peptide (BNP) and atrial natriuretic peptide (ANP). In the setting of wall stress, pre-proBNP is synthesized in the ventricles of the heart. This peptide is cleaved to proBNP and subsequently split to the biologically active BNP and the biologically inactive NT-proBNP. ANP is produced in a similar way in the cardiac atria. NPs circulate through the body and target several organs by interacting with natriuretic peptide receptors. The NPs cause arterial and venous dilatation, natriuresis, diuresis, reduced activity of the renin-angiotensin-aldosterone system and a decreased sympathetic tone. These effects result in a decreased blood pressure, counteracting the hemodynamic stress. In early cardiovascular disease NPs therefore prevent further cardiorenal dysregulation. In advanced heart failure however, sodium and water retention and an increased vascular resistance occur despite sky high NP levels. This may be explained by renal resistance to BNP and a relative deficiency of BNP which have been demonstrated in advanced heart failure animal models.

Natriuretic peptides (NPs) are mainly produced in the heart. NPs are released in the setting of wall stress in the myocardium, induced by volume excess or pressure overload. According to the law of LaPlace, wall stress is a function of interventricular pressure, size of the ventricle and wall thickness. Displayed as: wall stress = (pressure * radius) / (2 * wall thickness). In case of chronic increased pressure overload, the myocardial wall thickness increases (resulting in left ventricular hypertrophy). This can counteract the influence of the increased pressure on wall stress, preventing elevated wall stress. In case of volume overload however, the ventricle will become enlarged (dilated). The myocardium hypertrophies as well, but is does not get thicker (called concentric hyperthrophy, as is the case in chronic pressure overload), but enlarged, due to replication of sarcomeres in series (called eccentric hypertrophy). This leads to increased wall stress and release of larger amounts of NT-proBNP.

The predictive value of BNP and NT-proBNP for cardiovascular events and mortality have been well studied in both high risk patients and the general population. The predictive value of NT-proBNP may be explained by subtle cardiac remodelling and wall
stress induced by (subclinical) cardiovascular disease like hypertension or diastolic dysfunction,\(^{(34)}\) years before the onset of an event or death. The predictive value of this NT-proBNP has never been studied in DTC patients, while these patients are treated with supraphysiological doses of T4 and are therefore at risk for adverse cardiovascular effects. Recently, longstanding iatrogenic thyroid hormone excess was associated with cardiac hypertrophy and cardiac remodelling with increased wall stress in a hamster model.\(^{(37)}\) As wall stress induces the release of BNP and NT-proBNP, these markers in particular may indicate DTC patients with cardiac wall stress and subclinical cardiovascular disease, at risk for cardiovascular events and death.

Both BNP and NT-proBNP are valid biomarkers for the prediction of cardiovascular disease and mortality. However, in a direct comparison NT-proBNP was shown to be the most predictive marker of mortality in a community based cohort.\(^{(38)}\) The circulating half-life of NT-proBNP is 1-2 hours, compared to a half-life of approximately 20 minutes for BNP.\(^{(29)}\) NT-proBNP is therefore more stable in blood and may have a slight preference for usage as a biomarker.

**Aims of the study**

DTC patients on long-term THST are at risk of adverse cardiovascular effects and mortality. However, predictors for cardiovascular events and mortality have never been studied in this patient group. DTC patients are treated with supraphysiological doses of thyroid hormone, which has been associated with increased wall stress. As NT-proBNP is released in response to wall stress in the heart, it may be a marker of subclinical cardiac disease and an excellent predictor for future cardiovascular events and mortality in DTC patients. Primary aim of the study was to evaluate whether NT-proBNP is an independent predictor for cardiovascular events, secondary aim was to study NT-proBNP as an independent predictor of mortality in DTC patients during follow-up. Tertiary aim was to determine the optimal cutoff value of NT-proBNP to discriminate between patients at high or low risk for cardiovascular events and mortality.

**Research questions:**

1. Is NT-proBNP an independent predictor for cardiovascular events in DTC patients in follow-up?
2. Does NT-proBNP independently predict mortality in DTC patients in follow-up?
3. What is the optimal cut-off value for NT-proBNP to discriminate between patients at low and high risk for cardiovascular events and mortality?
2. Methods

Study design and setting
In this retrospective cohort study, the predictive value of NT-proBNP for cardiovascular events and mortality was evaluated in a single center cohort of DTC patients at the University Medical Center Groningen. The cohort consisted of patients in follow-up for DTC, initial DTC treatment was finalized at time of inclusion. Inclusion was between 2004 and 2008, at the moment of first sampling and subsequent freezing of a serum sample. At inclusion patients were at varying lengths of follow-up for DTC. Follow-up ended in October 2012, unless patients died or were lost to follow up prior to that time. All patients diagnosed with DTC between January 1, 1961 and January 1, 2006 with an age of at least 18 years, follow-up data available in the medical record, treatment with total thyroidectomy and radioiodine ablation therapy, follow-up in the UMCG until at least April 1, 2006 and availability of at least 1 frozen serum sample dating from 2004-2008 were included in the study. Patients were excluded in case the NT-proBNP measurement failed. The institutional review board of the UMCG approved the study.

DTC treatment and follow-up
After diagnosis patients underwent standardized DTC treatment and follow-up according to our local protocol,(39,40) and according to the Dutch guidelines implemented in 2007.(3) Treatment consisted of a total thyroidectomy and a central and/or lateral neck dissection in case of lymph node metastases. Postoperatively patients were scored according to the TNM classification.(41) The fifth edition of the TNM classification was used until 2006, thereafter the sixth edition was applied.(42,43) Patients were classified according to the risk of DTC recurrence (later referred to as DTC risk classification) as low (Tx-T2, Nx-N0, Mx-M0), intermediate (any T3 or N1) or high (any T4 or M1 tumor) risk.

Four till 6 weeks post surgery, patients underwent radioiodine ablation therapy to destroy residual normal or malignant thyroid tissue. A whole body scan (WBS) was performed 7 days after administration of the radioiodine dose, to search for remnant radioiodine absorbing tissue. In case of persisting uptake of radioiodine on the WBS, patients were eligible for another radioiodine cycle, until the WBS became negative. In between radioiodine cycles, patients started THST by taking T3, which has a relatively short half-life compared to T4. When T3 was discontinued, hypothyroidism was induced within 2 weeks. The induction of hypothyroidism was combined with a low-iodine diet two weeks prior to diagnostic scanning. Residual thyroid (cancer) tissue is far more likely to take up the radioiodine in a hypothyroid and iodine deficient state, which is necessary to get a reliable diagnostic scan or an effective radioiodine treatment. In case of a negative WBS and absence of elevated Tg levels, follow-up was started and T3 substitution was replaced by T4 therapy.

The TSH target level of THST varied over time and between low, intermediate and high risk patients. A TSH level below the lower limit reference range was pursued in all DTC patients until 2007. From 2007 onwards the TSH target level was <0.1 mU/L in all patients during initial follow-up. After 2 years of follow-up, T4 therapy could be tempered to a TSH level of 1.0 mU/L in low risk patients. The TSH target level remained <0.1 mU/L for intermediate risk patients and was <0.01 mU/L for patients with distant metastatic disease throughout follow-up.
Samples and NT-proBNP measurements
In the period of February 2004 until November 2008, for each patient extra blood was drawn during regular blood testing. The blood was drawn in a coagulation tube and subsequently centrifuged. The serum layer was aspirated and stored at -80°C. This was done because of an abrupt stop of the availability of the then used Tg assay. Therefore, the UMCG laboratory suddenly switched to another assay. Extra blood was stored to retain the possibility to compare old and new blood samples with the same Tg assay. This was important as Tg was used as a sensitive tumor marker during follow-up. However, the frozen samples were not used during the past years and became redundant. After approval of the institutional review board the samples were used for research. The oldest available serum sample per patient was selected and removed from the freezer. Prior to NT-proBNP measurement, the serum samples were thawed at room temperature. The NT-proBNP measurements were performed on the Elecsys 2010, an electrochemiluminescent sandwich immunoassay (Elecsys proBNP, Roche Diagnostics, Mannheim, Germany). Two polyclonal antibodies were used in the assay. The polyclonal antibodies bind at epitopes on residues 1-21 and 39-50 of NT-proBNP, forming a sandwich. The NT-proBNP is subsequently recognized by binding of labeled microparticles to the complex. Intra-assay and inter-assay imprecision are 1.2-1.5% and 4.4-5.0% respectively. The analytical range of the assay is 5-35,000 pg/ml.

Study definitions and outcome
The date at which blood was drawn for later NT-proBNP measurement was defined as baseline and was the start of follow-up for the current study. Survival time was defined as the time from baseline to the date a patient died, was lost to follow-up or the date of last assessment of the medical record in October 2012. Patients were defined as lost to follow-up if they moved and were treated for DTC at another hospital, if treatment was returned to the first line or when the patient did not visit the outpatient clinic for more than 1 year.

Data collection consisted of assessment of the medical record. At baseline the traditional cardiovascular risk factors age, sex, smoking habits, body mass index (BMI), diabetes mellitus (DM), hypertension, hypercholesterolemia and history of cardiovascular disease were assessed. BMI was calculated as weight (kilogram) divided by squared height (meters). DM, hypertension and hypercholesterolemia were defined as the presence of prescribed drugs to treat these conditions. A history of cardiovascular disease was defined as a cardiovascular event prior to the NT-proBNP measurement. Qualifying events were AF, heart failure, cerebrovascular accident (CVA), transient ischemic attack (TIA), myocardial infarction (MI), stable or unstable angina pectoris (AP), percutaneous coronary intervention (PCI), coronary artery bypass graft (CABG), abdominal aortic aneurysm (AAA) or peripheral artery disease. Missing data on baseline patients characteristics were obtained by contacting the patient.

At the end of follow-up medical records were reassessed to score cardiovascular events and death causes. Cardiovascular events were defined as fatal or non-fatal new onset AF, new onset heart failure, CVA, TIA, MI, stable or unstable AP, PCI, CABG, AAA or peripheral artery disease. Heart failure was scored by a heart failure panel. The panel consisted of a cardiologist and an internist specialized in vascular medicine. The panel decided whether or not patients had heart failure, as the diagnostic criteria for heart failure may be ambiguous. In case of disagreement of the panel members a third independent physician could be consulted. Medical records and if available autopsy reports were assessed to obtain death causes. In case of an unknown cause of death the general practitioner was contacted.
Primary endpoint was the predictive value of NT-proBNP for cardiovascular events, secondary endpoint was the predictive value of this biomarker for all-cause mortality in DTC patients. Locating the optimal cutoff point of NT-proBNP for prediction of cardiovascular events and mortality in DTC patients was tertiary outcome.

**Statistics**
Continuous variables with a normal distribution are presented as mean with standard deviation (SD). Median and inter quartile range (IQR) are presented for variables with a skewed distribution. Differences in baseline characteristics between quartiles of NT-proBNP were tested by ANOVA for continuous, normally distributed variables. The Kruskal Wallis test was used for skewed continuous variables. To test differences between proportions, the Chi-square and Fisher exact test (in case of small samples sizes) were used. Differences between NT-proBNP levels for patients with and without an outcome event were assessed by the Mann-Whitney U test.

The Kaplan-Meier method was used to estimate time-to-event curves for both endpoints by NT-proBNP quartiles. The log-rank test was used to test differences between NT-proBNP quartiles and outcome. Cox proportional hazards analyses were used to test the prognostic properties of NT-proBNP for cardiovascular events and all-cause mortality. NT-proBNP was logarithmically transformed and evaluated in the Cox regression models per 1 SD increase. Both a crude analysis and an analysis with adjustment for age and sex (model 1) were performed. Thereafter a stepwise regression procedure was performed by the backward elimination approach (model 2). Initially the model was adjusted for all cardiovascular risk factors and several DTC characteristics; age, sex, DM, BMI, smoking, hypertension, hypercholesterolemia, history of cardiovascular disease, DTC risk classification, tumor histology and median TSH level, which was logarithmically transformed. All TSH measurements in the period of DTC diagnosis until the end of follow-up were used to calculate the median TSH level, except for TSH values dating from before the first radioiodine treatment and periods of thyroid hormone withdrawal. The minus log median TSH was used, as we were interested in the risk for a 10-fold decrease in TSH level rather than a 10-fold increase. During the backward elimination procedure in model 2, variables not contributing to the model (p > 0.1) were deleted, until all variables with a significance level over 0.1 were removed from the model.

The value of NT-proBNP in addition to conventional cardiovascular risk factors for predicting cardiovascular events and mortality was assessed as well. Receiver operating characteristic (ROC) curves were constructed and the area under the curve (AUC) with 95% confidence intervals (CIs) were calculated for both endpoints. First all conventional risk factors were used in the model. Therefore a score was calculated, ranging from 0 to 6. The score 0 indicated that a patient had no risk factors, a score of 6 indicated that all risk factors were present. The following baseline risk factors were scored: BMI >= 27.5, DM, smoking, hypertension, hypercholesterolemia and history of a cardiovascular event. Another curve was drawn for each endpoint after NT-proBNP was added to the conventional risk factors (resulting in a score of 0-7). Again the AUC with 95% CIs were calculated. In these ROC curves, NT-proBNP was defined as a risk factor when it was above the endpoint specific cutoff value. The endpoint specific cutoff value was determined by calculating the sensitivity, specificity and Youden index for each NT-proBNP value. The Youden index was calculated as $J = \text{sensitivity} + \text{specificity} - 1$, and ranges between 0 and 1. If $J = 0$, the particular value of the diagnostic test has no discriminative ability, if $J = 1$ the value of the diagnostic test perfectly predicts the endpoint.$^{45,46}$ The NT-proBNP value with the highest Youden index
for each endpoint was chosen as the optimal cut-off point. The ROC curves and AUCs were repeated after exclusion of all patients with a history of a cardiovascular event at baseline, to eliminate any bias from elevated NT-proBNP levels as a result of previous cardiovascular disease. As the ROC curves only display the value of the addition of NT-proBNP to conventional risk factors, a Cox regression model was used to calculate the difference between both models. First, the conventional risk factors were included in the model, thereafter NT-proBNP was added. The difference of the first model and the model with addition of NT-proBNP was calculated by a chi square test of the difference between the 2-log likelihood values of both models.

Missing BMI values were imputed using the multiple imputation method.\(^{(47)}\) Based on the variables age and sex, SPSS created 5 data sets in addition to the original data set with estimates of the BMI for the missing values. In the Cox regression analysis, all datasets were analyzed and pooled outcome values were created. By using the multiple imputation method, patients with a missing BMI were assigned a BMI value. Therefore all patients could be analyzed in the Cox regression models (model 2), instead of excluding patients with a missing baseline BMI. All Cox regression analyses were repeated without the imputed BMI values.

Results are presented as hazard ratios (HRs) and 95% CIs with corresponding p-values. All tests were two sided, a p-value of <0.05 was considered statistically significant. Software package IBM SPSS for Windows version 20.0 (Armonk, NY) was used for all analyses.
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3. Results

Participants
Figure 2 shows the flow chart of the study. A total of 289 patients met the inclusion criteria, 1 patient was excluded because the NT-proBNP measurement failed. Therefore, 288 DTC patients were analyzed.

Baseline characteristics
NT-proBNP levels showed a right skewed distribution, with a median [IQR] of 69 [40-119] pg/ml (attachment 2). Table 1 shows baseline characteristics according to NT-proBNP quartiles. Mean ± SD age at baseline was 54.8 ± 14.5 years. Age gradually increased with increasing NT-proBNP values, from 45.9 ± 11.0 years in quartile 1 to 65.5 ± 13.9 years in quartile 4, p<0.0001. The total DTC group consisted of 77 men (26.7%) and 211 women (73.3%). The distribution of males and females differed among the quartiles, p=0.006, with more females in the higher NT-proBNP quartiles; median [IQR] NT-proBNP for males was 47 [23 – 109.5] pg/ml, for females 76 [45 – 122] pg/ml. The parameters TNM tumor stage, histology, smoking habits and BMI were not different among NT-proBNP quartiles. Of the total group, 45 patients (15.6%) had an invasive T4 tumor, 102 (35.4%) had regional lymph node metastases (N1) and 21 (7.3%) had distant metastases (M1). Papillary carcinoma was the most frequent DTC type (216 patients, 75.0%), followed by follicular carcinoma (67 patients, 23.3%) and Hurthle cell type carcinoma (5 patients, 1.7%). Forty-nine patients (17.0%) were current smokers, 55 (19.1%) smoked in the past and 184 (63.9%) never smoked. Mean ± SD BMI was 26.9 ± 5.0.

Inclusion criteria:
- DTC diagnosis between January 1 1961 and January 1 2006
- Age at diagnosis at least 18 years
- Follow-up data available in medical record
- Treatment with thyroidectomy and radiiodine
- Follow-up in the UMCG until at least April 1 2006
- Availability of at least 1 serum sample from 2004-2008

289 patients were eligible

1 patient was excluded due to failure of the NT-proBNP measurement

288 patients analyzed

29 patients died

25 cardiovascular events

Figure 2. Flow chart of the study. DTC = differentiated thyroid carcinoma
N-terminal pro-brain natriuretic peptide as a predictor of cardiovascular events and mortality in patients with differentiated thyroid carcinoma

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total</th>
<th>Quartiles of NT-proBNP (pg/ml)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>288</td>
<td>1 (5 – 40)</td>
<td></td>
</tr>
<tr>
<td>Mean age (years) at baseline ± SD</td>
<td>54.8 ± 14.5</td>
<td>45.9 ± 11.0</td>
<td>51.1 ± 12.2</td>
</tr>
<tr>
<td>Sex n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>77 (26.7)</td>
<td>30 (41.7)</td>
<td>18 (25.0)</td>
</tr>
<tr>
<td>Female</td>
<td>211 (73.3)</td>
<td>42 (58.3)</td>
<td>54 (75.0)</td>
</tr>
<tr>
<td>TNM Tumor stage n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tx-T3</td>
<td>243 (84.4)</td>
<td>60 (83.3)</td>
<td>64 (88.9)</td>
</tr>
<tr>
<td>T4</td>
<td>45 (15.6)</td>
<td>12 (16.7)</td>
<td>8 (11.1)</td>
</tr>
<tr>
<td>N stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nx -N0</td>
<td>186 (64.6)</td>
<td>40 (55.6)</td>
<td>45 (62.5)</td>
</tr>
<tr>
<td>N1</td>
<td>102 (35.4)</td>
<td>32 (44.4)</td>
<td>27 (37.5)</td>
</tr>
<tr>
<td>M stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mx -M0</td>
<td>267 (92.7)</td>
<td>71 (98.6)</td>
<td>67 (93.1)</td>
</tr>
<tr>
<td>M1</td>
<td>21 (7.3)</td>
<td>1 (1.4)</td>
<td>5 (6.9)</td>
</tr>
<tr>
<td>Histology n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillary</td>
<td>216 (75.0)</td>
<td>56 (77.8)</td>
<td>55 (76.4)</td>
</tr>
<tr>
<td>Follicular</td>
<td>67 (23.3)</td>
<td>14 (19.4)</td>
<td>17 (23.6)</td>
</tr>
<tr>
<td>Hürthle</td>
<td>5 (1.7)</td>
<td>2 (2.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Smoking n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>49 (17.0)</td>
<td>13 (18.1)</td>
<td>12 (16.7)</td>
</tr>
<tr>
<td>Past</td>
<td>55 (19.1)</td>
<td>11 (15.3)</td>
<td>14 (19.4)</td>
</tr>
<tr>
<td>No</td>
<td>184 (63.9)</td>
<td>48 (66.7)</td>
<td>46 (63.9)</td>
</tr>
<tr>
<td>Body Mass Index*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>26.9 ± 5.0</td>
<td>27.1 ± 4.4</td>
<td>27.1 ± 5.5</td>
</tr>
<tr>
<td>Hypertension n (%)</td>
<td>85 (29.5)</td>
<td>4 (5.6)</td>
<td>20 (27.8)</td>
</tr>
<tr>
<td>Hypercholesterolemia n (%)</td>
<td>34 (11.8)</td>
<td>3 (4.2)</td>
<td>12 (16.7)</td>
</tr>
<tr>
<td>Diabetes Mellitus n (%)</td>
<td>16 (5.6)</td>
<td>0 (0)</td>
<td>6 (8.3)</td>
</tr>
<tr>
<td>History of cardiovascular disease n (%)</td>
<td>22 (7.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Table I. Baseline patient characteristics. DTC = Differentiated Thyroid Carcinoma, SD = standard deviation, * 20 missing values of BMI were imputed
Hypertension was prevalent in 85 patients (29.5%). Prevalence increased from 4 patients (5.6%) in NT-proBNP quartile 1 to 44 patients (61.1%) in quartile 4, p<0.0001. Hypercholesterolemia was present in 34 patients (11.8%) at baseline and the presence of this condition differed among NT-proBNP quartiles, p=0.001. Hypercholesterolemia was most common in quartiles 2 and 4. Sixteen patients (5.6%) were affected by DM, DM was more prevalent in the higher NT-proBNP quartiles, p=0.025. A history of cardiovascular disease was more common in quartile 4 as compared to the other quartiles; 20 of 22 patients with a history of cardiovascular disease were classified in NT-proBNP quartile 4, p<0.0001. BMI was missing for 20 DTC patients, these BMI values were imputed.

Outcome
Table 2 shows outcome data. Median [IQR] follow-up for DTC patients was 6.2 [4.9 – 6.5] years. A total of 32 patients (11.1%) was lost to follow-up, median [IQR] follow-up for this group was 2.4 [1.2 – 3.8] years (not shown). Twenty-five patients (8.7%) had a cardiovascular event during follow-up. Median [IQR] baseline NT-proBNP for this group was 194 [102-403] pg/ml, compared to a value of 63 [37 – 111] pg/ml for patients who had no cardiovascular event.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DTC patients (n= 288)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Follow-up in years</strong></td>
<td></td>
</tr>
<tr>
<td>Median [IQR]</td>
<td>6.2 [4.9 – 6.5]</td>
</tr>
<tr>
<td><strong>Cardiovascular events n (%)</strong></td>
<td>25 (8.7)</td>
</tr>
<tr>
<td>New onset atrial fibrillation</td>
<td>8 (2.8)</td>
</tr>
<tr>
<td>New onset heart failure</td>
<td>3 (1.0)</td>
</tr>
<tr>
<td>Cerebrovascular accident</td>
<td></td>
</tr>
<tr>
<td>CVA</td>
<td>4 (1.4)</td>
</tr>
<tr>
<td>TIA</td>
<td>3 (1.0)</td>
</tr>
<tr>
<td><strong>Cardiac event</strong></td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>2 (0.7)</td>
</tr>
<tr>
<td>MI</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>PCI/CABG</td>
<td>3 (1.0)</td>
</tr>
<tr>
<td>AAA</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Peripheral Artery Disease</td>
<td>0</td>
</tr>
<tr>
<td><strong>Mortality n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>29 (10.1)</td>
</tr>
<tr>
<td>Cardiovascular mortality</td>
<td>6 (2.1)</td>
</tr>
<tr>
<td>Other/unknown †</td>
<td>12 (4.2)</td>
</tr>
<tr>
<td>DTC mortality</td>
<td>11 (3.8)</td>
</tr>
<tr>
<td><strong>Median TSH level</strong></td>
<td></td>
</tr>
<tr>
<td>Median [IQR]</td>
<td>0.1 [0.04 – 0.3]</td>
</tr>
</tbody>
</table>

Table 2. Outcome
DTC = Differentiated Thyroid Carcinoma, IQR = Inter Quartile Range, CVA = cerebro vascular accident, TIA = transient ischemic attack, AP = angina pectoris, MI = myocardial infarction, PCI = percutaneous coronary intervention, CABG = coronary artery bypass graft, AAA = abdominal aortic aneurysm * only the first event per patient after baseline is shown, † 5 unknown causes of death/ 7 due to other causes.
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p<0.0001. New onset AF was the most common first cardiovascular event, with 8 new cases (2.8%), followed by 4 cases of CVA, 3 cases of new onset heart failure, 3 TIA's and 3 PCI/CABGs. Two patients developed AP, 1 had a MI and for 1 patient an AAA was the first cardiovascular event. No patients had peripheral artery disease. Twenty-nine patients died during follow-up (10.1%). A total of 6 patients (2.1%) died from a cardiovascular cause, 11 patients (3.8%) died due to progression or recurrence of DTC, 7 patients (2.4%) died from other causes and for 5 patients (1.7%) cause of death remained unknown. Patients who died during follow-up had a median [IQR] baseline NT-proBNP value of 172 [89 – 508] pg/ml, compared to a value of 64 [38 – 111] pg/ml for patients who were still alive at the end of follow-up, p<0.0001.

Figure 3 shows Kaplan-Meier time-to-event curves for both endpoints by NT-proBNP quartiles. The occurrence of cardiovascular events differed among NT-proBNP quartiles, log rank p<0.0001 (figure 3A). None of the patients classified in NT-proBNP quartile 1 had a cardiovascular event. Two patients of quartile 2 had a cardiovascular event, this applied to 5 patients in quartile 3 and 18 patients in quartile 4. Almost three-quarters of all patients who got a cardiovascular event during follow-up had a baseline NT-proBNP level in the highest quartile.

As can be seen in figure 3B, mortality is dependent upon NT-proBNP quartiles as well, log rank p<0.0001. Especially the patients categorized in the higher NT-proBNP quartiles, quartile 3 and 4, show an increased mortality. Of the patients in quartile 1, 3 died during follow-up. Two patients of quartile 2 died, 7 patients of quartile 3 and 17 patients with a baseline NT-proBNP level in quartile 4.

A. Cardiovascular events by NT-proBNP quartiles

B. All-cause mortality by NT-proBNP quartiles

Figure 3. Kaplan-Meier survival curves

Kaplan-Meier survival curves and log-rank test p-values for A) cardiovascular events and B) mortality by NT-proBNP quartiles (quartile 1: NT-proBNP 5-40 pg/ml (red line), quartile 2: NT-proBNP 40-69 pg/ml (green line), quartile 3: NT-proBNP 69-119 pg/ml (orange line), quartile 4: NT-proBNP 119-6809 pg/ml (blue line). The numbers of patients at risk for both endpoints according to NT-proBNP quartiles are shown below the curves.
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Table 3. Cox regression models for new onset cardiovascular events and all-cause mortality
SD = standard deviation, Log NT-proBNP SD was 0.45. * adjusted for age and sex, ** backward Cox regression model, initially adjusted for age, sex, BMI, DM, smoking, hypertension, hypercholesterolemia, history of cardiovascular disease, DTC risk classification, histology and minus log median TSH level. Remaining variables are shown after the backward selection procedure (threshold criterion p<0.1) for each endpoint.

Cox regression models are shown in table 3. NT-proBNP was a significant predictor for cardiovascular events and mortality, even after adjustment for cardiovascular risk factors, DTC risk classification, histology and TSH level. In the crude model, each SD increase in log NT-proBNP was associated with a HR of 2.97 (95% CI 2.21 – 3.99) for cardiovascular events. Model 1 was adjusted for age and sex, log NT-proBNP was still predictive; HR was 2.55 (95% CI 1.74 – 3.74) per SD increase. In this model male sex was predictive as well, while the predictive value of increasing age was lost. Age was predictive for cardiovascular events while tested separately in a crude model (results not shown). In model 2, initially log NT-proBNP was adjusted for age, sex, BMI, DM, smoking, hypertension, hypercholesterolemia, history of cardiovascular disease, DTC risk classification, histology and minus log median TSH level. After the backward selection procedure, male sex and BMI were the remaining predictive variables for cardiovascular events. After adjustment for these variables, HR for each SD increase in log NT-proBNP was 3.26 (95% CI 2.34 – 4.54).

For mortality the crude HR per SD increase of log NT-proBNP was 3.07 (95% CI 2.20 – 4.28), and 1.87 (95% CI 1.26 – 2.79) in model 1. Increasing age was a predictor as well, while male sex did not predict for mortality. In model 2, the NT-proBNP HR for mortality was 1.93 (95% CI 1.27 – 2.94) per SD increase, other predictors after the backward selection procedure were
increasing age, smoking and the TSH level. For each 10-fold decrease in median TSH, the HR of mortality was 2.49 (95% CI 1.33 – 4.64). All Cox regression analyses were repeated without the imputed BMI data, this did not change the results.

The optimal NT-proBNP cut-off value for cardiovascular events was found to be 138 pg/ml. This NT-proBNP cutoff value had a sensitivity of 68%, a specificity of 84% and a Youden Index of 0.52, table 4. For mortality the optimal cut-off value was 85 pg/ml, this cut-off value had a sensitivity of 79%, a specificity of 62% and a Youden Index of 0.42.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>NT-proBNP cut-off value (pg/ml)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Youden Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular events</td>
<td>138</td>
<td>67.7</td>
<td>84.0</td>
<td>0.517</td>
</tr>
<tr>
<td>Mortality</td>
<td>85</td>
<td>79.3</td>
<td>62.2</td>
<td>0.415</td>
</tr>
</tbody>
</table>

*Table 4. Optimal NT-proBNP cut-off values with corresponding sensitivity, specificity and Youden index.*

Figure 4 illustrates the ROC curves for both endpoints. The AUC for cardiovascular events was 0.73 (95% CI 0.63 to 0.83) for conventional risk factors. When NT-proBNP was added to the model, the AUC was 0.77 (95% CI 0.67 – 0.87). For mortality, the AUC for conventional risk factors was 0.68 (95% CI 0.58 – 0.78), the addition of NT-proBNP resulted in an AUC of 0.74 (0.65 – 0.83). The ROC curves were reproduced after excluding all patients with a history of cardiovascular disease at baseline (fig 4C and 4D). The AUC for cardiovascular events after exclusion of patients with a history of cardiovascular disease was 0.61 (95% CI 0.48 – 0.74) for conventional risk factors and 0.65 (95% CI 0.52 – 0.79) after addition of NT-proBNP. For mortality, the AUC for conventional risk factors was 0.63 (95% CI 0.50 – 0.75) after exclusion of patients with previous cardiovascular disease. When NT-proBNP was added to the model, the AUC was 0.70 (95% CI 0.59 – 0.81). The value of the addition of NT-proBNP to conventional risk factors was assessed by a statistical test. In the Cox regression model, the addition of NT-proBNP to conventional risk factors resulted in a significantly better prediction model for both cardiovascular events (p=0.003) and mortality (p=0.018).
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Figure 4. ROC curves
Receiver operating characteristics (ROC) curves for the predictive value of conventional risk factors (blue line) and the addition of NT-proBNP to the conventional risk factors (green line), for A) cardiovascular events, B) mortality, C) cardiovascular events with exclusion of patients with previous cardiovascular disease and D) mortality with exclusion of patients with previous cardiovascular disease. AUC = area under the curve
4. Discussion

In this study NT-proBNP has shown to be an independent predictor of cardiovascular events and mortality in DTC patients, also NT-proBNP improved risk stratification in this patient group. The optimal NT-proBNP cutoff values to discriminate between patients at high and low risk for cardiovascular events and mortality were 138 pg/ml and 85 pg/ml, respectively.

Interpretation

This is the first study to evaluate the predictive value of NT-proBNP for cardiovascular events and mortality in DTC patients. NT-proBNP has been found to be a predictor of cardiovascular events and mortality in several population based studies.\(^{(25,34,48)}\) In the population based prospective study of Kistorp et al. with 764 participants (50-89 years), the predictive value of baseline NT-proBNP was studied for cardiovascular events and mortality.\(^{(25)}\) Each SD increase in log NT-proBNP was associated with a HR of 1.92 (95% CI 1.42–2.56) for cardiovascular events in the Cox regression model adjusted for cardiovascular risk factors. For mortality an adjusted HR of 1.43 (95% CI 1.10–1.86) was found. Zethelius et al. studied several biomarkers in a community based cohort of 1135 elderly men.\(^{(48)}\) In the Cox regression model adjusted for cardiovascular risk factors, each SD increase in NT-proBNP was associated with a HR of 1.58 (95% CI 1.41–1.76) for all-cause mortality. The HRs found in these studies resemble the HRs found for both endpoints in the present study, indicating a comparable predictive value of NT-proBNP for DTC patients and subjects of the general population.

Overall, the NT-proBNP levels found in DTC patients were relatively high. When compared to a study of a general population sample using the same NT-proBNP assay, levels for DTC patients seemed substantially higher; median [IQR] levels were 68.5 [40 – 119] pg/ml for DTC patients versus 37.7 [16.8 – 73.8] pg/ml for the general population sample.\(^{(34)}\) However, patients at high risk for coronary disease showed even higher NT-proBNP levels.\(^{(49)}\) Increased NT-proBNP levels in patients with an excess of thyroid hormone have been described before.\(^{(50-53)}\) The increased NT-proBNP levels in these patients can be explained by several factors: 1) a direct stimulating effect of thyroid hormone on BNP secretion, 2) secondary cardiovascular effects of thyroid hormone and 3) other factors like age, gender, renal disease and BMI.

Based on the independent association between free T4 and T3 levels and serum (NT-pro)BNP levels in (subclinical) hyperthyroid patients, a direct stimulatory effect of thyroid hormone on serum NT-proBNP levels has been suggested.\(^{(50,51)}\) Schultz et al. did not find an association between resting cardiac output and pulse rate and NT-proBNP levels.\(^{(50)}\) Moreover, an increased expression of BNP mRNA in rat ventricular myocytes in response to thyroid hormone was found\(^{(54)}\) supporting the hypothesis of a direct stimulating effect of thyroid hormone on BNP secretion. More recent studies focus on the role of secondary effects of thyroid hormone on the cardiovascular system as an explanation for elevated BNP and NT-proBNP levels. Heart failure was found to be the major contributing factor for elevated BNP levels in hyperthyroid patients, followed by free T4 levels.\(^{(52)}\) Besides a positive correlation between NT-proBNP and serum thyroid hormone levels, Arikan et al. found a positive correlation between left ventricle end-diastolic parameters and interventricular septum thickness and NT-proBNP.\(^{(53)}\) A negative correlation was found between NT-proBNP and left ventricular ejection fraction, suggesting subclinical cardiac structural changes to be responsible for NT-proBNP elevation in hyperthyroid
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patients, occurring as a secondary effect of thyroid hormone excess. This is conflicting with outcome of Schultz et al., possibly explained by methodological differences i.e. the performance of echocardiographic measurements to assess cardiovascular parameters. The association between cardiovascular effects and NT-proBNP was probably missed by Schultz et al. due to less sensitive measurements. It remains unclear whether a direct effect of thyroid hormone on BNP secretion or a secondary effect of thyroid hormone on the cardiovascular system (or a combination of both) plays the major role in elevation of NT-proBNP levels in (subclinical)hyperthyroid patients. However, we showed that NT-proBNP levels can still adequately predict future cardiovascular events and mortality in DTC patients despite the probably elevated levels in the entire DTC population.

Other factors, like age, gender, renal disease and BMI are able to influence BNP levels as well.\(^{(55-57)}\) Increasing age and female gender have been associated with a significant higher BNP.\(^{(55)}\) In heart failure patients with chronic kidney disease and an estimated glomerular filtration rate below 60 ml/min, plasma BNP levels were increased due to decreased renal BNP clearance.\(^{(56)}\) Furthermore, a BMI over 25 has been associated with lower BNP and NT-proBNP levels in heart failure patients.\(^{(57)}\) Our data show that increasing age and female sex are associated with an increased NT-proBNP level. However, BMI levels were not different among NT-proBNP quartiles. Although chronic kidney disease was not evaluated in the present study, analyses were adjusted for age, sex and BMI if these variables contributed to the Cox regression model (model 2). Therefore we do not expect that these variables caused bias in the analyses. Baseline cardiovascular disease is another factor that may have caused bias in the analyses. However, a history of cardiovascular disease was not predictive of endpoints in the adjusted Cox regression model, while NT-proBNP did independently predict outcome. Therefore, baseline cardiovascular disease alone cannot explain the relation between endpoints and baseline NT-proBNP level.

In this study we aimed to establish the optimal NT-proBNP cutoff values for cardiovascular events and mortality. Cutoff values were 138 pg/ml and 85 pg/ml respectively, based on the optimal sensitivity and specificity. Sensitivity and specificity were best for the cutoff value for cardiovascular events. Therefore NT-proBNP may be most useful for predicting this endpoint. However, these cutoff values cannot be compared to other cutoff values in patients with thyroid hormone excess as to our knowledge no such values exist. When our cutoff values are compared to reference ranges of the general population without cardiovascular disease, they seem to be relatively low.\(^{(58)}\) Therefore, the established cutoff values cannot yet be used in clinical practice.

**Limitations**

The main limitation of the study is the low number of cardiovascular events (n=25) and deaths (n=29) during follow-up. As a result the study did not have enough statistical power for separate analysis for the predictive power of NT-proBNP for AF, heart failure and death causes. Another consequence is that Cox regression models could only be adjusted for a limited number of variables, as the rule of thumb for Cox regression models is that at least 10 events are needed for every predictor variable in the model. The number of relevant predictors was slightly higher than was justified by this rule. However, a more relaxed use of this rule of thumb seems to be acceptable.\(^{(59)}\)
Baseline echocardiographic parameters were not available. This a limitation because these parameters might have been relevant confounders. In daily practice however, echocardiographic measures are usually not available either. Therefore the used models with conventional risk factors represent the current practice of estimating the risk of future cardiovascular events and mortality in DTC patients in follow-up.

Another limitation is that besides the Cox regression analysis, the discriminative ability of NT-proBNP was only assessed by the traditional area under the ROC curve method. Novel performance methods measure the ability of a diagnostic test to correctly reclassify patients in a risk category to improve risk stratification; the net reclassification improvement. Another measure is the net benefit, which measures the clinical usefulness of a diagnostic test taking into account the harm of both false positive and false negative cases. These measures were not used as DTC patients could not be classified in validated risk classification scores like the SCORE table or the Framingham risk score, because of missing baseline variables needed to calculate these scores. Furthermore no data are available on the harms and benefits of treating DTC patients for cardiovascular risk factors, needed to calculate the net benefit.

The samples in which NT-proBNP were measured were obtained at different time points (between 2004 and 2008). Therefore the analysis of NT-proBNP measurements could be biased due to decay of NT-proBNP in the oldest samples. NT-proBNP is however is a stable analyte over time. Hence we do not expect bias of this issue. Moreover, the samples were frozen at minus 80° C, so decay of the analyte is expected to be minimal.

The final limitation is that unlike TSH levels, free T3 and T4 levels were not analyzed in the present study. Several studies found a correlation between NT-proBNP and free T3 and T4 levels. This correlation was not evaluated, as free T3 and T4 levels were not systematically determined during follow-up.

**Clinical Implications**

NT-proBNP was found to be an independent predictor for cardiovascular events and mortality in DTC patients. Also, NT-proBNP was shown to improve risk stratification for cardiovascular events and mortality in this patient group. Adding this biomarker to traditional risk factors in a new model may help to identify patients at risk for cardiovascular events and mortality eligible for active treatment of cardiovascular risk factors, not indicated by conventional risk factors alone. Particularly this group may benefit from the improved risk stratification using NT-proBNP, as an active primary prevention of cardiovascular disease may prevent events and early death. Therefore NT-proBNP may contribute not only to a better survival, but also to a survival with less cardiovascular events and a higher quality of life.

Prior to implementation of this biomarker in daily clinical practice, several issues need to be addressed. First, the NT-proBNP cutoff values need validation in another DTC population. Determination of the optimal cutoff values is important in this population, as cutoff values used in the general population may be too low, causing false positive results. Another question that needs to be addressed is which DTC patients need NT-proBNP testing. We propose to use the NT-proBNP test in DTC patients at risk for cardiovascular disease, like patients with a strict THST. Furthermore, it has never been studied whether an intensified cardiovascular risk
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management benefits DTC patients at risk for cardiovascular events or mortality, as indicated by NT-proBNP. Therefore it is particularly important to study whether treatment according to risk stratification by the new model (traditional risk factors and NT-proBNP combined) improves event-free survival.

A last point that has to be emphasized is that only patients who had undergone treatment for DTC were included in the present study. The results of this study are therefore only applicable for DTC patients in follow-up, not for patients with newly diagnosed DTC. A future perspective may be that NT-proBNP determined prior to start of THST may aid in the determination of the optimal TSH target value prior to start of therapy.

Conclusion
NT-proBNP is a strong independent predictor for cardiovascular events and mortality in DTC patients during follow-up. The optimal NT-proBNP cutoff value was found be to 138 pg/ml for cardiovascular events and 85 pg/ml for mortality. The use of NT-proBNP as a biomarker improves risk stratification for cardiovascular events and mortality in DTC patients during follow-up. Adding NT-proBNP to traditional risk factors in a new model may help to identify patients eligible for a more stringent treatment of cardiovascular risk factors. Further study is needed to establish whether treatment according to risk assessment by the new model can improve event-free survival in these patients.
6. References


6. Jonklaas J, Sarlis NJ, Litofsky D, Ain KB, Bigos ST, Brierley JD, et al. Outcomes of patients with differentiated thyroid carcinoma following initial therapy. Thyroid. 2006;16:1229-42.


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N-terminal pro-brain natriuretic peptide as a predictor of cardiovascular events and mortality in patients with differentiated thyroid carcinoma


7. Attachments

Attachment 1: Abstract

Long-term cardiovascular mortality in patients with differentiated thyroid carcinoma: an observational study

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Abstract

Purpose
Differentiated thyroid carcinoma (DTC) has a favorable survival rate. Therefore, potential adverse long-term effects of TSH suppressive treatment on cardiovascular outcome have gained interest. Primary aim of this study was to evaluate the risk of cardiovascular mortality in DTC patients. Secondary aims were to evaluate all-cause mortality and explore the relation between TSH level and these outcome parameters.

Patients and methods
Subjects from two cohorts were retrospectively compared by Cox regression analyses; 524 DTC patients and 1572 sex- and age-matched control subjects from a large population based study of the same geographic region.

Results
Mean age (±SD) was 49±14 years. Median follow-up [IQR] was 8.5 [4.1–15.9] years for DTC patients and 10.5 (9.9–10.9) years for control subjects. One hundred (19.1%) DTC patients died, 22 (4.2%) of cardiovascular disease, 39 (7.4%) of DTC and 39 (7.4%) of other/unknown causes. Eighty-five (5.4%) control subjects died, 24 (1.5%) of cardiovascular disease and 61 (3.9%) of other/unknown causes. In crude Cox regression analyses DTC patients had an increased risk of cardiovascular mortality and all-cause mortality; hazard ratios (HR) 2.3 (95% CI 1.2–4.6) and 3.2 (2.3–4.4) respectively. After adjustment for age, sex and cardiovascular risk factors, HRs for cardiovascular mortality and for all-cause mortality were 3.3 (1.7–6.7) and 4.4 (3.1–6.1), respectively. Within the DTC group, TSH level was predictive for cardiovascular mortality; crude HR 2.4 (1.2–4.9) for each 10-fold decrease in TSH level and HR 2.7 (1.2–5.9) after
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adjustment for cardiovascular risk factors, DTC risk classification, histology and treatment characteristics.

Conclusion
The risk of cardiovascular and all-cause mortality is increased in DTC patients, independent of age, sex and cardiovascular risk factors. A lower TSH level is associated with increased cardiovascular mortality, supporting the current practice of tempering TSH suppression in patients with low risk of cancer recurrence. Furthermore, DTC patients may benefit from assessment and treatment of cardiovascular risk factors.

Submitted

Attachment 2: Boxplot

Boxplot showing the distribution of NT-proBNPs per quartile. The median, inter quartile range and the lowest and highest NT-proBNP level per quartile of NT-proBNP are shown.
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