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Blood thrombogenicity is independently associated with serum TSH levels in post non ST elevation acute coronary syndrome

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Abstract

**Context:** Higher serum TSH levels, both within the reference range and in those with subclinical hypothyroidism (SCH), have been associated with increased risk of atherosclerosis and cardiovascular (CV) events in a number of cross-sectional and longitudinal studies.

**Objective:** to evaluate blood thrombogenicity (BT) in patients post non-ST elevation acute coronary syndrome (NSTE-ACS) with relation to their thyroid function.

**Design, patients and outcome measure:** Seventy patients one week after troponin positive NSTE-ACS who had been treated with optimal antiplatelet and secondary prevention therapy were studied. Patients with known thyroid disease or on medications affecting thyroid function were excluded. BT was assessed using the ex-vivo Badimon perfusion chamber.

**Results:** Serum TSH was associated with higher thrombus burden ($\beta=0.30; p=0.01$) independent of other well-established CV risk factors. Patients with SCH ($n=12; 17\%$) had a higher thrombus burden than euthyroid individuals as evidenced by the area of the thrombus: mean (SD) [23,608 (10,498) vs 16,661 (10,902) $\mu^2$/mm, $p=0.02$]. However, this association was not evident when the analysis was limited to patients with serum TSH within the reference range. In addition, neither serum free T4 nor free T3 had any significant association with thrombus area.

**Conclusion:** Serum TSH levels, particularly in the subclinical hypothyroid range, are associated with higher thrombus burden despite optimal recommended secondary prevention therapy following NSTE-ACS. This may explain the higher CV risk seen in SCH patients. Future trials to assess the effect of individualised anti-thrombotic as
well as thyroid hormone replacement therapy to reduce atherothrombotic risk in this population are needed.
Introduction

Subclinical hypothyroidism (SCH), a state of mild thyroid failure characterised by a raised serum thyroid stimulating hormone (TSH) level with normal thyroid hormone concentrations, is associated with increased risk of cardiovascular disease (CVD) and CVD-related mortality. Furthermore, higher TSH levels within the reference range are associated with an increased risk of myocardial infarction in patients with established cardiovascular disease. In addition, there is growing evidence that CVD patients with SCH have increased cardiac and all-cause mortality. SCH has been associated with lipid abnormalities, atherosclerotic plaque progression and instability and endothelial dysfunction. However, the association of thyroid dysfunction after non-ST elevation acute coronary syndrome (NSTE-ACS) with blood thrombogenicity (BT) is unknown. We hypothesized that in the setting of NSTE-ACS, the presence of SCH increases blood thrombogenicity.

Materials

Patients: Patients with symptoms of myocardial ischaemia and elevated high sensitive cardiac troponin I levels (>0.1 μmol/L) on at least 2 occasions 12 hours apart and angiographically proven coronary artery disease (>50% stenosis in at least one major vessel) were recruited. In accordance with the current American Heart Association/European Society of Cardiology guidelines on NSTE-ACS management, all patients received 300mg loading and 75 mg daily maintenance dose of both aspirin and clopidogrel along with other three standard secondary prevention medications: hydroxymethyl co-enzyme A reductase inhibitor (HMG-CoA), angiotensin converting enzyme (ACE) inhibitor (or angiotensin receptor blocker) and a beta-blocker (or a calcium channel blocker as clinically indicated). This study
was approved by the Local Research Ethics Committee and all participants gave written informed consent. The study was conducted in accordance with the ethical principles of the declaration of Helsinki.

**Exclusion criteria:** Patients with normal coronary angiogram, current smokers, those on antiplatelet or antithrombotic agents other than aspirin and clopidogrel, individuals with known thyroid dysfunction or on medications known to affect thyroid function such as amiodarone or lithium, coagulation abnormalities, anaemia (Hb<12g/dl), and those with malignancy or being investigated for malignancy were excluded.

**Assessment of platelet dependent thrombus formation:** This was assessed 7 to 10 days after the index event using the validated ex-vivo Badimon perfusion chamber\(^8\). This model consists of three small Plexiglass chambers in series; each chamber was lined with a piece of porcine aorta stripped of the intimal layer to expose the underlying thrombogenic medial layer which simulates atherosclerotic plaque rupture leading to thrombus formation. The first is a low shear chamber unit (inner lumen diameter: 2.0mm; vessel wall shear rate: 212/sec; average blood velocity: 5.3 cm/sec; Reynolds number: 30) that simulates flow in a normal coronary artery and the next two are high shear units (inner lumen diameter: 1.0mm; vessel wall shear rate: 1690/sec; average blood velocity: 21.2 cm/sec; Reynolds number: 60) that simulate flow conditions in a moderately stenosed coronary artery. A peristaltic pump at the distal end drew blood directly from an intravenous catheter over the porcine aorta at a constant rate of 10 ml/minute for 5 min. The aortas with thrombi were fixed in 10% buffered formalin for 72 hours and stained with modified Masson trichrome stain. The stained thrombus was quantified by planimetry using a Leica DM2000 microscope (Leica Microsystems, Weltzler, GmBH) under 10X magnification and the
Image ProPlus 4.0 software (Media cybernetics, MD, USA). Total thrombus area was calculated as mean of individual thrombus area in individual high shear chambers and expressed as $\mu^2$/mm. All the slides were analysed by one independent observer (GV) with intra-observer co-efficient of variation 4.3%.

**Biochemical analysis:** Blood samples were taken immediately after the Badimon perfusion chamber study, 7 to 10 days after the index event. Serum samples were prepared after centrifuging at 1550g for 10 minutes. Samples were stored at -80° Celsius and thawed once prior to analysis. Serum creatinine, lipid levels, troponin I, haemoglobin, fibrinogen, platelet count and platelet volume were analysed by standard techniques. HbA$_1c$ (DCCT aligned) was measured by TOSOH Bioscience G8 analyser (TOSOH biosciences, CA, USA). Serum TSH (reference range 0.4 – 4.0 mU/L), free T4 (reference range 9 – 25 pmol/L), free T3 (reference range 2.5 – 7.5 pmol/L) and thyroid peroxidase (TPO) antibodies (positive if greater than 35 IU/ml) were measured by electrochemiluminescence immunoassay on the Roche cobas e602 (Roche Diagnostics, UK). P selectin and soluble CD40L were measured by ELISA methods (R&D systems, Abington, UK) and TNF alfa was measured by electrochemiluminescence methods (MSD, MD, USA). The within-day and between day co-efficient of variation for all tests were less than 5%.

Patients with serum TSH above the reference range and serum free T4 levels within the reference range were classed as having SCH (n=12) whereas those with both TSH and free T4 within the reference range were categorised as being euthyroid (n=58). In addition, to ensure that the serum TSH abnormality was not transient, individuals with SCH had their levels rechecked a few months after their NSTE-ACS (median 4, range 2 – 14 months). This showed that 9 of 12 individuals were still in the SCH state, one person with the highest TSH of 23 mU/L had been commenced
on levothyroxine, one person had not had their thyroid function rechecked and one participant’s TSH had reverted back to within the reference range. None of the patients had overt hypothyroidism (FT4 < 9 pmol/L), hyperthyroidism (both overt and subclinical), nor had a low free T3 level.

**Statistical analysis:** Patients with SCH were compared to euthyroid individuals using unpaired t-test. Variables that were not normally distributed (serum TSH levels and thrombus area) were log transformed to normality prior to analysis. A multivariate regression analysis was performed with thrombus area as the dependent and established CV risk factors (age, gender, history of prior CVD, diabetes mellitus, hypertension, cerebrovascular disease, body mass index and LDL cholesterol levels) and serum TSH as independent variables. Secondary analyses utilising SCH as a categorical variable or FT4 or FT3 concentrations as a linear variable were also performed. Analyses were performed using SPSS version 21.0 (IBM Corp, NY, USA). Two-tailed significance was set at <0.05.

**Results**

**Baseline Characteristics:**

SCH was identified in 12 (17%) post NSTE-ACS patients. Baseline demographic, clinical, risk factor profile and laboratory characteristics were similar in both the SCH and euthyroid groups (Table 1) except, as expected, serum TSH level was significantly higher, FT4 levels lower and more individuals were positive for antibodies against TPO in the SCH group.
Thrombus area:

NSTE-ACS patients with SCH had higher platelet dependent thrombus formation compared to euthyroid group: mean $\mu^2/mm$ (SD) [23,608 (10,498) vs 16,661 (10,902) $\mu^2/mm$, p=0.02] (Table 1). A representative picture taken from typical patients in each thyroid status group can be seen in Figure 1.

Predictors of thrombus area:

Serum TSH level as a continuous variable was an independent predictor of thrombus area ($\beta=0.30$, p=0.01). Figure 2 (for web only) shows univariate association between serum TSH and thrombus area. Neither FT4 nor FT3 concentrations were found to have any independent association with total thrombus area (results not shown).

In secondary analysis, SCH was an independent predictor of thrombus area ($\beta=0.25$, p=0.027). In addition, history of diabetes mellitus ($\beta=0.47$; p=0.001) and previous cerebrovascular disease ($\beta=0.36$; p=0.002) were also identified as independent predictors of thrombus area. One individual had serum TSH of 23.3 mU/L with normal FT4 level (14.0 pmol/L). We performed all analyses after excluding this individual and found results to be essentially unaltered (results not shown).

Furthermore, analysis was performed after excluding the two individuals who either normalised TSH level subsequently or did not have their thyroid function rechecked. This showed that the results were essentially unchanged for thrombus area, although the strength of the difference was statistically weaker due to smaller number in the SCH group: mean $\mu^2/mm$ (SD) 22,334 (5774) in SCH vs 16,661 (10,902) in euthyroid group; p=0.08.
Discussion

Higher blood thrombogenicity was related to increasing serum TSH levels which was significant in patients with SCH when compared to the euthyroid group despite all patients receiving optimal secondary prevention therapy including aggressive antithrombotic therapy. In addition, SCH was a significant predictor of thrombus burden independent of other well established cardiovascular risk factors such as diabetes mellitus and cerebrovascular disease. This finding may explain the higher cardiovascular risk seen in the SCH population. Moreover, the linear relationship between serum TSH and thrombus area in the post NSTE-ACS setting suggesting that the association may have a causal link although appropriately powered intervention studies alone will prove this. This study also demonstrates that prevalence of SCH in a carefully selected population is high in patients post NSTE-ACS (17%).

SCH has been associated with many CV risk factors such as increased serum lipid levels, diastolic hypertension, impaired endothelial function, increased arterial stiffness, and possibly altered coagulation parameters and elevated C-reactive protein levels. The Rotterdam study suggested that SCH is independently associated with atherosclerosis and with an increased incidence of myocardial infarction in the aging female population. Recent meta-analyses have also confirmed higher CV events and mortality in younger to middle aged adults with SCH. Furthermore, the 12-year mortality follow up in HUNT study demonstrated that high serum TSH levels within the reference range are associated with increased mortality from coronary heart disease in women. L-thyroxine therapy has been shown to positively affect endothelial function, reduce progression of angiographic
coronary artery disease in hypothyroid patients \(^{14}\), improve atherogenic lipid profile\(^{15}\), and is associated with a reduced all-cause mortality as well as fewer CVD events in younger individuals \(^{16}\). Recently, it has been reported that mean platelet volume, a risk factor for CVD, is higher in SCH individuals and was positively correlated with TSH levels \(^{17}\).

As far as we are aware, BT in relation to thyroid function particularly patients with SCH in a post NSTE-ACS state has never been assessed before. The Badimon perfusion chamber system is a validated \textit{ex vivo} model of vessel injury and thrombosis and has previously been used to evaluate the effects of novel antithrombotic agents in human subjects in different disease states \(^{18,19}\). We have used this perfusion system to evaluate the effects of anti-platelet therapy on thrombus formation in various high risk population groups such as patients with established coronary artery disease and type 2 diabetes mellitus. This technique has been well validated in our laboratory \(^{8,20}\). The model enables the measurement of thrombus formation in native (non-anticoagulated) whole blood triggered by exposure to physiologically relevant substrate (collagen in tunica media), and under different rheological conditions mimicking those in narrowed blood vessels. Mechanisms that increase the risk of CV events in patients with established CVD and SCH are unknown. Hence our findings are novel and have the potential for further research in this high CV risk population.

The main limitation of our study is its relatively small sample size particularly in the SCH group. Despite this, the SCH group were found to have a higher thrombus area independent of other traditional risk factors. Furthermore, we found a linear relationship between serum TSH levels and thrombus area that adds to the growing
evidence relating to a link between the two. The other limitation of this study, due to its observational nature, is that causality of thyroid status with higher thrombus burden cannot be assessed. In addition, it is unknown if higher thrombus burden is associated with increased future CV events. Finally, due to the carefully selected nature of participants for this study (exclusion of current smokers, those with anaemia, etc; outlined in detail in Methods), the results of this study may not be generalised to the wider SCH population post NSTE-ACS. For instance, smokers tend to have a lower serum TSH levels but have a higher CVD risk.

In conclusion, this study has shown that post NSTE-ACS individuals with higher serum TSH levels and, in particular SCH, have higher thrombus burden despite optimal secondary prevention therapy which may explain their high CV risk. Future longitudinal studies are required to assess if this increased thrombus burden is indeed associated with adverse CV morbidity and mortality. Eventually, trials that assess the effect of individualised antithrombotic as well as thyroid hormone replacement therapy to reduce the atherothrombotic risk in patients with SCH post NSTE-ACS are required.

Acknowledgments: We would like to thank Mrs Caroline Addison for her help in organising the measurement of thyroid function in these participants.
References:


Cardiology; American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 2002 Guidelines for the Management of Patients With Unstable Angina/Non-ST-Elevation Myocardial Infarction); American College of Emergency Physicians; Society for Cardiovascular Angiography and Interventions; Society of Thoracic Surgeons; American Association of Cardiovascular and Pulmonary Rehabilitation; Society for Academic Emergency Medicine


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Legends to tables and figures:

Table 1: Demographic and biochemical values of patients with SCH and Euthyroidism

Figure 1. Picture of typical thrombus from patients with SCH and Euthyroidism

Figure 2 (for web only). Univariate association between serum TSH levels and ex-vivo thrombus area in post non ST elevation acute coronary syndrome patients.
Table 1: Demographic and biochemical values of patients with SCH and Euthyroidism

<table>
<thead>
<tr>
<th>Characteristics (Mean ± SD or Median (range))</th>
<th>SCH (n=12)</th>
<th>Euthyroid (n=58)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.5 ± 10.0</td>
<td>62.4 ± 12.5</td>
<td>0.78</td>
</tr>
<tr>
<td>Males n (%)</td>
<td>9 (75)</td>
<td>48 (83)</td>
<td>0.53</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>32.5 ± 6.8</td>
<td>31.3 ± 7.3</td>
<td>0.60</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mm Hg)</td>
<td>131.5 ± 16.3</td>
<td>126.9 ± 19.6</td>
<td>0.44</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mm Hg)</td>
<td>74.9 ± 9.9</td>
<td>71.1 ± 9.4</td>
<td>0.21</td>
</tr>
<tr>
<td>Resting Heart rate (beats/minute)</td>
<td>63.9 ± 16.4</td>
<td>62.6 ± 14.2</td>
<td>0.79</td>
</tr>
<tr>
<td>Risk factor profile: n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>8 (66.7)</td>
<td>34 (58.6)</td>
<td>0.60</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>5 (41.7)</td>
<td>27 (46.6)</td>
<td>0.76</td>
</tr>
<tr>
<td>Previous CVD</td>
<td>3 (25)</td>
<td>30 (51.7)</td>
<td>0.09</td>
</tr>
<tr>
<td>Previous CVA</td>
<td>1 (8.3)</td>
<td>7 (12.1)</td>
<td>0.71</td>
</tr>
<tr>
<td>Laboratory data: (Mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>Value 1</td>
<td>Value 2</td>
<td>P value</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>----------</td>
<td>----------</td>
<td>---------</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>13.9 ± 1.0</td>
<td>13.7 ± 1.6</td>
<td>0.66</td>
</tr>
<tr>
<td>Platelets (x1000/mm³)</td>
<td>268 ± 78.4</td>
<td>243 ± 72.6</td>
<td>0.30</td>
</tr>
<tr>
<td>Mean platelet volume (fL)</td>
<td>8.6 ± 1.1</td>
<td>9.0 ± 1.2</td>
<td>0.29</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>4.6 ± 1.3</td>
<td>4.1 ± 1.1</td>
<td>0.48</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>113 ± 42.6</td>
<td>100 ± 21.2</td>
<td>0.13</td>
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<tr>
<td>HbA1c (%)</td>
<td>6.1 ± 1.0</td>
<td>6.5 ± 1.4</td>
<td>0.37</td>
</tr>
<tr>
<td>hs CRP (mg/l)</td>
<td>7.4 ± 7.6</td>
<td>7.5 ± 10.1</td>
<td>0.97</td>
</tr>
<tr>
<td>Troponin I (µg/l)</td>
<td>10.2 ± 14.3</td>
<td>6.16 ± 10.8</td>
<td>0.46</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>3.8 ± 1.0</td>
<td>3.5 ± 0.9</td>
<td>0.42</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>1.7 ± 0.6</td>
<td>1.7 ± 0.7</td>
<td>0.92</td>
</tr>
<tr>
<td>Free T4 (pmol/L)</td>
<td>14.1 ± 1.5</td>
<td>15.6 ± 1.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Free T3 (pmol/L)</td>
<td>4.8 ± 0.78</td>
<td>4.8 ± 0.60</td>
<td>0.79</td>
</tr>
<tr>
<td>TSH (mU/L) median (range)</td>
<td>4.94 (4.32 – 23.3)</td>
<td>1.85 (0.54 – 3.96)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TPO antibody &gt;35 IU/ml n(%)</td>
<td>4 (33.3)</td>
<td>2 (3.4%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P selectin (ng/ml)</td>
<td>61.2 ± 23.3</td>
<td>66.4 ± 26.2</td>
<td>0.51</td>
</tr>
<tr>
<td>sCD40L (ng/ml) median (range)</td>
<td>1504.7 (309.5 – 9368.3)</td>
<td>2532.1 (357.1 – 20727)</td>
<td>0.18</td>
</tr>
<tr>
<td>TNFalfa (ng/ml)</td>
<td>9.4 ± 4.3</td>
<td>8.5 ± 3.4</td>
<td>0.37</td>
</tr>
<tr>
<td>Mean high shear mean thrombus area</td>
<td>23,608 ± 10,498</td>
<td>16,661 ± 10,902</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* P value < 0.05

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; CVA, cerebrovascular accident; hs CRP, high-sensitivity C-reactive protein; LDL, low density lipoprotein; TPO, thyroid peroxidase; sCD40L, soluble CD40 ligand; TNFalfa, tumour necrosis factor alfa.
Figure 1. Representative picture of thrombus from patients with SCH and Euthyroidism
Figure 2 (for web only). Univariate association between serum TSH levels and ex-vivo thrombus area in post non ST elevation acute coronary syndrome patients.

$r=0.41$, $p<0.01$