Study Protocol

Title: The role of endothelial progenitor and circulating endothelial cells in cardiovascular risk of patients with sub clinical hyperthyroidism

Protocol Number: EPISH 2012

The role of endothelial progenitor and circulating endothelial cells in cardiovascular risk of patients with sub clinical hyperthyroidism

Introduction:

Subclinical hyperthyroidism (SH) is characterised by low serum concentration of thyrotropin (TSH) in the presence of normal serum thyroid hormones and the absence of obvious symptoms of hyperthyroidism. Its prevalence ranges from 0.6 to 16% depending on diagnostic criteria, inclusion of exogenous SH (those on thyroid hormone replacement), the sensitivity of the methods used to measure serum TSH concentrations, lower limit of normal reference range for serum TSH levels, and iodine intake. SH is more common in women than in men (female: male ratio: 1.5: 1) and its incidence increases with advancing age.

The NHANESIII survey found that 1% of people between the ages of 60 and 80 had SH (TSH <0.4 mU/l), rising to 3% of those over 80 (Hollowell et al., 2002). In the recent Cardiovascular Health Study of 3233 American community-dwelling individuals aged 65 years or older, the prevalence of SH (TSH 0.10–0.44 mU/l) was 1.5% (Cappola et al., 2006). The prevalence of thyroid autonomy is inversely correlated with the population‘s iodine intake (Delange et al., 2001). Despite this relationship, even though iodine intake is sufficient, sub clinical hyperthyroidism is common in Singapore.

The implication after diagnosing SH is currently unclear as there is no consensus for ideal management of this condition. Most of the reported studies including the recent ones showed that most of SH remain in this clinical state for longer duration with very few progressing to overt thyrotoxicosis. Sawin et al reported 4.1% rate of progression over 4 yr in 33 patients older than 60 years with SH (TSH less than 0.1 mU/l) (Sawin et al., 1991). Another UK study reported only 4.3% (3 out of 70 subjects older than 60 years) progressed to overt hyperthyroidism in the 10 year follow-up (Parle et al., 2001). In a recent study from Scotland, very few SH patients develop frank hyperthyroidism, whereas a much larger proportion revert to normal, and many remain with SH, when followed up to 7 years after disgnosis; Most SH cases remained as SH at 2 (81.8%), 5 (67.5%), and 7 yr (63.0%) after diagnosis (Vadiveloo et al, 2011). Furthermore, it has been suggested that patients with grade 1 SH (serum TSH between 0.1 and 0.4 mU/l) rarely progress to overt thyrotoxicosis as opposed to grade 2 SH (serum TSH <0.1 mU/l) (Parle et al, 1991). We have done a retrospective evaluation of natural history of

SH at Tan Tock Seng Hospital; Only 6 patients (5.3 %) out of 113 SH patients progressed to overt thyrotoxicosis, while 15 remitted to euthyroid state on follow up (6 to 36 months). In patients with grade 1 SH at diagnosis (60), 37 remained in grade 1 SH, 8 became euthyroid, 13 progressed to grade 2 SH, and 2 progressed to overt thyrotoxicosis, while in grade 2 SH (53), 32 remained at grade 2 SH, 10 became grade 1 SH, 7 remitted to euthyroid state, and 4 progressed to overt thyrotoxicosis (Endo 2011 poster). This is clinically important as most SH remains stable (in SH state) for a long term, they have been denied treatment unless they have overt clinical symptoms or the presence of complications such as atrial fibrillation.

SH and cardiovascular risk:

The cardiovascular risk of subclinical hyperthyroidism is related to both short-term effects (electrophysiological effects of thyroid hormones), and long-term effects resulting from increased left ventricular mass and increased cardiac workload. In most studies, patients with subclinical hyperthyroidism have a higher heart rate and increased prevalence of supraventricular arrhythmias, as assessed by 24-h Holter electrocardiographic monitoring (Reviewed by Biondi and Cooper, 2008). SH is also associated with 2-3 fold increase in the prevalence of atrial fibrillation (Sawin et al, 1994, Auer et al, 2001, Cappola et al, 2006). Other cardiac abnormalities such as reduced heart rate variability and left ventricular hypertrophy have also been reported in SH subjects. Very few studies have assessed other vascular risk factors in SH; A population-based study carried out in Pomerania involving 2086 individuals at least 45 year old with carotid ultrasound and without known thyroid disorders revealed higher carotid intima medial thickness (CIMT) in subjects with decreased serum TSH levels (Volzke et al., 2004). An increase in von Willebrand factor (Coban et al., 2006) and coagulation factor X suggesting a hypercoagulable state (Erem, 2006) have been reported in subclinical hyperthyroid subjects

The association of cardiovascular morbidity and mortality have been assessed in few studies. Few but not all studies have shown increased vascular mortality in SH subjects. Cardiovascular mortality was found to be increased in a community-based review of subjects aged 60 years or older with endogenous hyperthyroidism (with TSH values <0.5 mU/l) monitored for 10 years (Parle et al., 2001). During 9733 person-years of follow-up, 509 of

1191 people died, the expected number of deaths being 496 (standardised mortality ratio [SMR] 1.0, 95% CI 0.9-1.1). Mortality from all causes was significantly increased at 2 (SMR 2.1), 3 (2.1), 4 (1.7), and 5 (1.8) years after first measurement in those with low serum TSH. These increases were largely accounted for by significant increases in mortality due to circulatory diseases (SMR 2.1, 2.2, 1.9, 2.0, at years 2, 3, 4, and 5 respectively). Another population based study (Leiden 85 plus study) followed older subjects (> 85 years) for 4 years; low serum TSH was associated with an increased mortality. Increasing levels of free thyroxine were associated with an increased risk of both cardiovascular (sex-adjusted HR, 1.26; 95% CI, 1.05-1.52) and non cardiovascular (sex-adjusted HR, 1.10; 95% CI, 0.93-1.32) mortality (Gussekloo et al., 2004). However, there were only 17 SH subjects at baseline of the study.

SH and cognition:

Endogenous SH has been shown to be associated with dementia in few studies; Vadiveloo et al reported an increased risk for dementia in SH patients in scotland (adjusted hazard ratio of 1.64 with confidence Interval of 1.20-2.25). One comprehensive recent review evaluated this association of cognitive impairment in SH in both cross sectional and longitudinal population studies; 14 out of 23 studies have shown strong association of SH and cognitive impairment (Gan and Pearce et al, 2012). This association of cognitive changes in SH has not been studied inn local Singapore or Chinese population and also in the younger SH patients.

EPCs and cardiovascular risk:

Endothelial progenitor cells (EPCs) in adult human peripheral blood were originally described, as circulating angioblasts within human peripheral blood which was able to differentiate in-vitro into endothelial cells (Asahara et al., 1997). They reported that human CD34+ cells isolated from peripheral blood, umbilical cord blood and bone marrow could differentiate into endothelial cells in-vitro and in-vivo in mouse models. Postnatal vascularisation or angiogenesis was thought to result exclusively from proliferation, and migration of fully differentiated endothelial cells originated from pre-existing blood vessels. Differentiation of mesodermal cells to angioblasts and subsequent endothelial differentiation was believed to occur only in embryonic development. Hence, this landmark study challenged the traditional

understanding of angiogenesis to suggest that these circulating cells in adult human peripheral blood may also contribute to new vessel formation in post natal life.

Currently the term EPC has been used for different type of cells. Circulating progenitor cells in peripheral blood expressing variable markers identified by flow cytometry (CEPC), and two different types of in-vitro cultured cells using peripheral blood mononuclear cells; EC like cells and Endothelial colony forming cells (ECFC). The quantification of CEPCs using different monoclonal antibodies to various endothelial (VEGFR-2, CD144) and stem cell markers (CD34, CD133) by flow cytometry is the most common method used as it requires very small quantity of peripheral blood and can be done easily compared to in-vitro culture methods.

The role of EPCs on cardiovascular disease progression and outcome had been evaluated in few studies. In one study 120 subjects (43 normal, 44 stable IHD, and 33 acute coronary syndrome) were followed over a median period of 10 months. CD34+/KDR+ EPC levels on flow cytometry were significantly low in patients suffering from cardiovascular events (CV death/ unstable angina/MI/PTCA/CABG/ischaemic stroke). Reduced numbers of EPCs were associated with significantly higher number of cardiovascular events by Kaplan Meier analysis (p=0.0009 (Schmidt-Lucke et al., 2005). In another German study, 519 IHD subjects were followed for 12 months (Werner et al., 2005). An increased number of CD34+/KDR+ EPCs measured at baseline by flow cytometry after adjustment for cardiovascular risk factors were associated with reduced risk of CV death (HR,0.31; 95%CI 0.16-0.63, P=0.001) at 12 months. Similar reduction in other end points (first major cardiovascular event, revascularization and hospitalization) was found in those with higher circulating EPC counts.

Circulating endothelial cells (CEC) and cardiovascular risk:

The measurement of immunologically defined circulating endothelial cells (CECs) in the peripheral blood is gaining ground as an important and novel technique for assessment of endothelial injury. The CECs are part of a family of blood-borne endothelioid cells that include endothelial progenitor cells (EPCs). Endothelial cells line the vascular tree and adhere to a basement membrane. In health, these cells would be expected to remain in this location, with perhaps a very low level of cell loss into the blood, with consequent clearance by the reticuloendothelial system. It would seem logical that pathological processes that cause

damage to the endothelium might also cause endothelial cell detachment, resulting in increased numbers of CECs within the bloodstream.

The precise quantification of CECs is difficult (partly because of the low numbers present in the circulation and because of their differing morphologic appearances), their detection has been improved by cell enrichment techniques and cell labeling using relatively endothelial specific markers. The CECs are counted in whole blood using either immunomagnetic separation or flow cytometry. CD146 molecule has clearly evolved as the most popular marker for the identification of CECs. This adhesion molecule is concentrated at the endothelial junction, where it plays a key role in the control of cell– cell cohesion, permeability, and signalization

CEC and CVD:

There is increasing evidence to support the relationship between CECs and endothelial dysfunction. The CECs have been identified as a useful marker of endothelial damage (Boos et al, 2006). An inverse correlation between CECs and endothelial function measured by brachial artery flow mediated dilatation (a surrogate physiological marker of a disturbed endothelium) has been shown (Rajagopalan et al, 2004 and Chong et al, 2004). In addition, strong correlation between CECs and Von Willebrand factor (plasma marker of endothelial injury) have also been shown in another study (Makin et al, 2004). The CECs are rarely found in normal healthy individuals, in the order of <3 cells/ml. Elevated numbers of CECs have been identified in a wide array of disease processes associated with endothelial injury; pulmonary hypertension (Bull et al, 2003), Behçet's disease (Camoin-Jau et al, 2000), septic shock (Mutunga et al, 2001) and malignancies such as breast cancer (Beerepoot et al 2004).

Acute MI and unstable angina are both associated with significantly increased CEC counts compared with healthy controls (George et al, 1992, Makin et al, 2004, Wang et al, 2005) and CEC counts are either normal or only minimally elevated in patients with stable angina. Other vascular disorders such as peripheral vascular disease, stroke, atrial fibrillation, cardiac failure and diabetes have also been shown to be associated with higher CECs (Boos et al

Rationale for the study:

The above discussion shows there is definite evidence for increased risk of atrial fibrillation, possibly impaired diastolic cardiac function and increased mortality in subjects with subclinical hyperthyroidism. Currently, there is no clear consensus on ideal management of SH. Furthermore, there is no convincing evidence for improvement of clinical abnormalities after treating these subjects with either antithyroid drugs or radioiodine ablation in SH, mainly due to lack of randomised placebo controlled studies. Furthermore, most of SH patients are older with already increased vascular risk due to other concomitant problems such as hyperlipidemia and established cardiac disease. As discussed above, most of SH patients do not progress to overt thyrotoxic state, most of these subjects are not treated and hence, may have increased cardiovascular risk which could persist for a long time.

Reduced circulating EPCs assessed by flow cytometry of peripheral blood using different markers (most commonly CD34+/VEGFR-2+) has been found in subjects with increased cardiovascular risk factors and in established cardiovascular disease. My previous study (during my research at Newcastle University, UK), found lower circulating EPCs in patients with 20 Sub clinical hypothyroidism compared to age matched healthy controls and there was improvement in EPC levels after treatment with thyroxine at 3 months (Abdul Shakoor et al, JCEM 2009). Sub clinical hyperthyroidism occurs in even older population and most of them may have other vascular risk factors compared to sub clinical hypothyroidism and hence we wish to study whether EPC contributes to increased cardio vascular risk in SH. In this present study, we also wish to study other marker of endothelial injury such as circulating endothelial cells (CEC) in patients with SH. It has been suggested that CECs are biomarkers of endothelial damage, and high levels were associated with a poor outcome, where as EPCs are biomarkers of repair with therapeutic potential, and low levels predict poor outcome in vascular disease (Boos et al., 2006).

The association of abnormal number of EPCs or CECs as a newer cardiovascular risk factor in SH will further substantiate the necessity of treating this condition in order to lower the cardiovascular risk, particularly if we prove improvements in both EPC and or CEC levels with achieving euthyroid state in patients with SH

Hypothesis of the study:

Increased cardiovascular risk in sub clinical hyperthyroidism (SH) is contributed by reduction in CEPC and increased circulating endothelial cells.

Primary end points

1. To evaluate whether SH is associated with reduced circulating endothelial progenitor cells (EPC) and increased circulating endothelial cells (CEC) in peripheral blood compared to control group.
2. Furthermore, treatment of SH restoring euthyroid state improves levels of EPC and CECs in peripheral blood.

Secondary endpoints:

Abnormalities in the following parameters in SH compared to controls and improvement after achieving euthyroid state;

1. Anthropometry (BP, weight, and BMI)
2. Endothelial markers such as Hs CRP, ADMA, lipoprotein-associated *phospholipase A2* (*Lp-PLA2*) activity, Neutrophil lymphocyte ratio and monocyte lymphocyte ration
3. Relationship between thyroid hormones, endothelial markers, and EPC/CECs
4. Improvement in cognitive parameters after treatment of SH with carbimazole compared to placebo

II. Methods:

Study subjects (See flow chart and study visit schedule):

This trial will be conducted in compliance with the protocol of the study, and good clinical practice and according to the regulatory requirements by the DSRB. SH patients will be recruited from Endocrine clinic at Tan Tock Seng Hospital after the diagnosis has been confirmed at least on 2 occasions (low serum TSH with normal FT4) and one normal FT3 levels within 3 months prior to the recruitment. For young women, pregnancy will be excluded by pregnancy test at the start of the study. Furthermore, study the participants will be advised

not to use oral contraceptives or estrogen implant but instead use barrier forms of contraceptives during the study period.
40 SH patients will be recruited in total to randomise them to two groups for interventional option; namely anti thyroid drug, carbimazole or placebo.

Inclusion criteria:

- Diagnosis of subclinical hyperthyroidism confirmed at least on 2 occasions (low serum TSH with normal FT4) and one normal FT3 levels within 3 months prior to the recruitment.
- Aged 21-85 years;
- Written informed consent.

Exclusion criteria:

- Sick euthyroid syndrome,
- Recent radioiodine therapy (Within 1 year of screening visit)
- Pregnant or breastfeeding patients.
- Acute medical illnesses such as infections and active cancer.

25 age and sex matched controls will be recruited from friends, staff members, and by means of advertising (posters and local web pages such as Tan Tock Seng Hospital intranet). The same exclusion also applied to the control group. If we include patients with cardiovascular risk factors such as diabetes and IHD, we will match them in the control group.

At the initial visit, all subjects (SH and control group) will have anthropometry, BP, Fasting bloods for Hs CRP, ADMA, LP-LPA 2, Neutrophil/lymphocyte ratio , Monocyte/lymphocyte ratio , EPC and CEC. The cognitive function will be assessed by standardised mini mental state examination (MMSE) and Montreal cognitive assessment (MoCA). Following this visit, SH group will be randomised to 2 sub groups to recieve either carbimazole (5 mg) or placebo. The assessments including MMSE and MoCA will be repeated after 6 months. Carbimazole is an antithyroid drug commonly used for treating thyrotoxicosis. Side effects related to the use of Carbimazole such as agranulocytosis and allergic reactions (rash) are quite rare in clinical practice, but this can not be prevented. If that happens unfortunately to the study subjects, they will be withdrawn from the study.

Randomisation process will be done electronically using randomization codes with the help of medical statistitian at the CRU (TTSH) in blocks of 4 (for carbimazole or placebo pill). Randomization will be done online (intranet access) in which only coordinators (unblinded) will be given access to the system. The research Coordinators will be unblinded and all Investigators and the study subjects will be blinded in the study. The randomization codes will be broken (unblinding process) will only be done at exceptional circumstances.. Unblinding will be done in the event of a life-threatening emergency if it is imperative that precise knowledge of the trial treatment the patient is receiving is required in order to manage the emergency. This will be done online (intranet access) in which only principal investigator and his designate will be given access to the system and unblinding module. The reason for the unblinding as well as the patient allocation number will be entered in the online system before the drug identification is released. Once the code is broken, trial medication will be stopped. The patients will however continue to be followed up in accordance with the trial protocol.

Carbimazole:

This is a 5 mg tablet manufactured by Apex Pharma marketing Pte limited comes in a blister pack. In view of the blinding procedure, the pills will be taken out from the blister pack and research coordinator will repackage in a pill container which will be tainted (or plastic container) and after labeling as a study tablet and instructed to take tablets accordingly. The study patient will be told to return all bottles for drug accountability check.

Placebo:

This is manufactured by Sunward pharmaceutical private limited. The pill is white colored, 7 mm, round with convex single score and made of microcrystalline cellulose, corn starch and PVP. The GMP certificate is available for the placebo manufacturer. As this is not exactly similar to carbimazole tablet (size and color will be same, but it will be convex score) , the placebo pill will be repackaged in a tainted or plastic pill container (as above for carbimazole tablet) and then labeled as study pill and dispensed to the study subjects. The study patient will be told to return all bottles for drug accountability check.

After re-packaging, the study drugs (both carbimazole and placebo) will be stored in a 24hr air-conditioned locked medicinal cooler located at CRU, which its temperature is monitored daily. This is in accordance to advice from Apex pharma's carbimazole's package insert which stated to store below 25 deg C, and protected from light.

Visit 1 (12 weeks):

At 12 weeks after the initial visit, TFT will be repeated and if necessary, dose adjustments will be made to achieve euthyroid state in those on carbimazole. Similar adjustment in the dosage (number of tablets) will be done for those on placebo tablets. We have decided to check thyroid function test at 3 months for the following reason; The follow up arrangements for SH patients vary for each patient (between 4 to 12 weeks). For these SH patients, we generally use small dose of carbimazole (5 mg or less). In some patients, it takes longer than 1 month for achieving euthyroid state, as TSH levels will normalise only after 3 months even in overt thyrotoxic patients on antithyroid drug. This is to avoid making false changes in the treatment plan.

This adjustment of the dosage of carbimazole or placebo pill will be done according to the thyroid function results by the research co-ordinator as they are not blinded. This will be done after discussing the thyroid function test results (depending upon the results of TSH and FT4 as mentioned in the flow chart) with the PI or other study members, who will be blinded as per whether the study subjects are taking carbimazole or placebo. Research co ordinators will not make the decision of change in the dosage on their own. Research Coordinators will then dispense the study drug bottles as per Investigator's prescription order.

Drug accountability and the compliance of drug or placebo will be done at 12 weeks and at the last visit of the study by the research coordinator. This will be done by checking the number of pills in the dispensed bottles. r taking the pill each day.

Discontinuation Criteria:

1. All subjects, who had experienced side effects related to carbimazole or placebo tablet (Any serious side effect such as neutropenia, allergic reactions, feeling unwell, jaundice etc)

2. Voluntary exclusion from the study by the study subjects (any time)

3. Patients who were not compliant with taking the study drugs/placebo

4. Patients who developed any illness which might affect the thyroid status or endothelial progenitor cells after taking part from the study

5. Patients, who need to take medications for other illnesses, which affect the thyroid status or endothelial progenitor cells

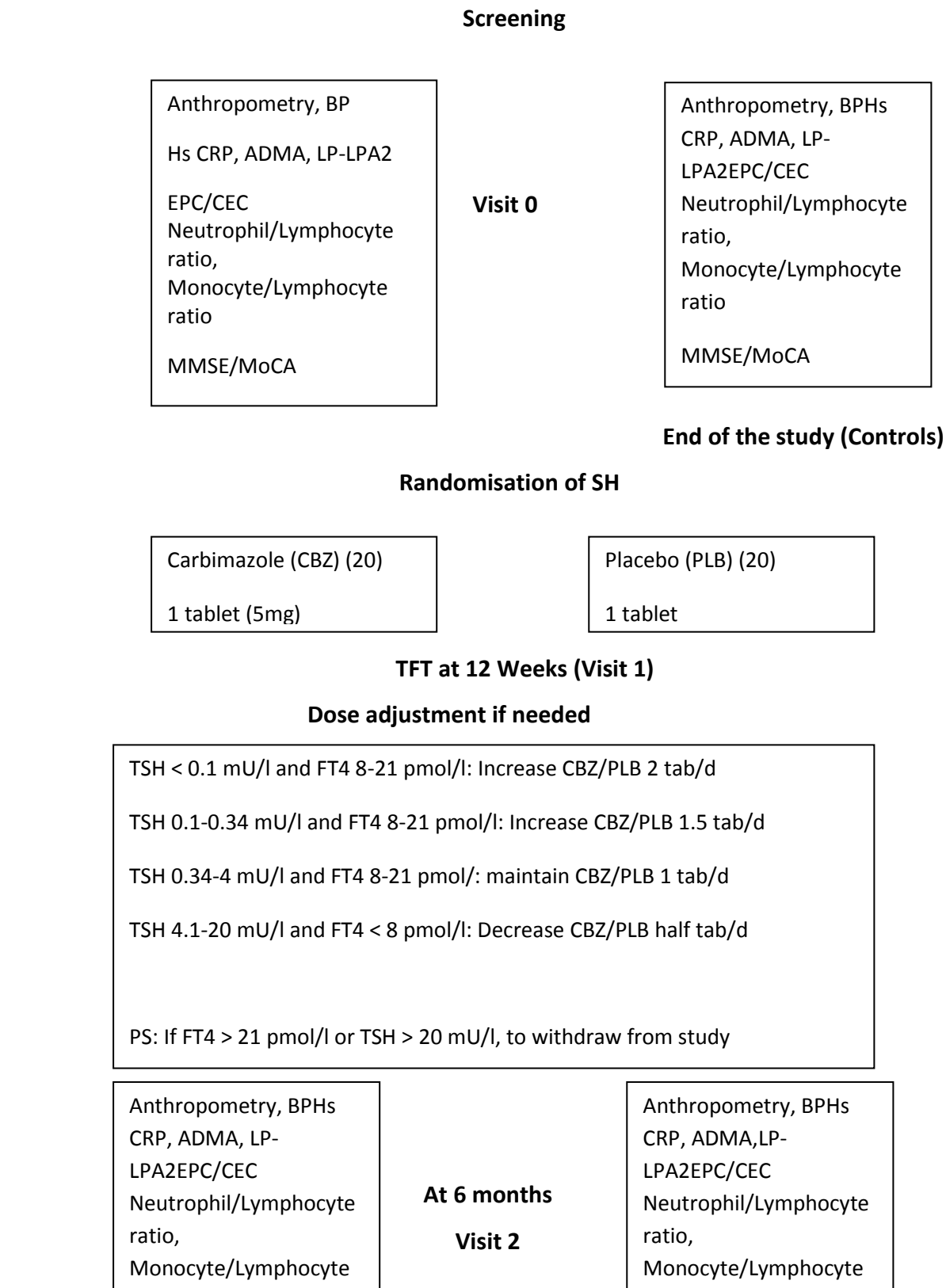
For 4 and 5, these subjects may be included for baseline comparison analysis.

6. Thyroid function test showing FT4 > 21 pmol/L or TSH > 20 mU/L at 12 weeks of the study.

Biochemistry:

Thyroid function tests (Serum TSH, Free T4, free T3), Hs CRP, and Asymmetric di methyl arginine (ADMA) will be studied. For ADMA and LP-LPA 2 quantification, the plasma sample will be stored in the freezer at -20 to 80 degree centigrade and this will be analysed after completion of the study by ELISA method. The other biochemical tests will be done on the same day of collection.

EPISH study flow chart



End of the study

Study visit schedule:

Week	**Screening Visit (Week 0)**	**Visit 1 Week 12**	**Visit 2 Week 24**
nformed Consent	X		
nclusion / Exclusion Criteria	X		
Anthropometry	X		X
Blood pressure	X		X
Fasting bloods for Hs CRP, ADMA, LP-LPA2, EPC and CEC Neutrophil/Lymphocyte ratio, Monocyte/Lymphocyte ratio	X		X
Carbimazole / placebo dosing	X	X	
Adverse event monitoring		X	X
Thyroid function test **Cognitive function (MMSE, MoCA)**	X X	X	X X

EPC analysis:

Circulating endothelial progenitor cells (EPC) and CECs in the peripheral blood will be identified, quantified and characterised by fluorescence activated cell sorter (FACS, Beckton Dickinson, San Jose, CA, USA). This will be done in peripheral blood sample by flow cytometry.

Venous blood will be collected in EDTA tube and analysed in the laboratory within 4 hours. Full blood count analysis will be performed for all subjects before analysing EPC/CEC analysis. Neutrophil/Lymphocyte ratio, Monocyte/Lymphocyte ratio will be anlysed from Full blood count analysis. International Society for Hematotherapy and graft engineering strategy (ISHAGE) with sequential gating protocol will be used for quantification of EPCs (Schmidt-Lucke et al, 2010). Briefly, 1 ml of whole blood will be stained with following fluorescent labelled mono clonal antibodies to CD 45, KDR, and CD 34 after lysis of red blood cells. According to the recommendation, 250,000 events at the initial gating and atleast 100 CD 34+ events will be collected. $CD45^{dim}$ CD 34+/KDR+ cells will be defined as EPCs.

CEC analysis:

This will be done along with quantification of EPCs by flow cytometry using endothelial markerCD144 . CECs will be identified as cells positive for CD144 but negative for CD45.

Cognitive function:

This will be assessed by standard cognitive function tests; standardasised Mini mental state examination (MMSE) and Montreal cognitive assessment (MoCA). These tests are established cognition testing method and are simple to use and does not take longer time to complete (approximately 10-15 minuts each). The Chinese speaking will be using the Chinese version of both these tsets.

Assessment of Efficacy/safety:

Thyroid function test will be done at 12 weeks to make sure that study participants are well clinically . As mentioned in the flow chart, patients with any major deviation in the thyroid function (such as becoming overt thyrotoxic or hypothyroidism) will be discontinued from the study. The compliance of the drug/placebo will also recored in CRF. We will check full blood count (FBC) and liver function tests (LFT) only if clinically suspected eg; (sorethroat or jaundice or feeling unwell).

SAE:

Any adverse effect related to the carbimazole tablet or placebo pill be recorded and will be notified to the DSRB which also include the patients death. The patients who experienced

adverse effect due to the carbimazole such as allergy will be treated by the usual clinical means and monitored accordingly depending on the adverse effect.

Any Unexpected serious adverse effects resulting from using carbimazole (nature or severity of SAE not consistent with that described in carbimazole information leaflet), resulting in significant disability or requiring in patient hospitalisation will be reported to HSA within 15 days and in case of death and life threatening events reported to HSA within 7 days of the occurrence of the event.

Statistics:

We have consulted with medical statistician at our clinical research unit at TTSH. There was no previous study on EPC and CEC levels and effect of treatment on EPC and CEC in SH patients. Hence, we have looked at other studies evaluating circulating EPC and CEC numbers in other vascular disorders. Most of the studies have used between 20 and 40 patients in comparing the level of EPC and CECs; eg. using the difference in mean EPC values and SD, with power of 80% and a significance of < 0.05, in one study evaluating stable sickle cell disease and controls, we need 19 SH patients and 10 control group (Ozdogu et al, 2007). Similarly form the mean CEC values from another study in Type 2 diabetes mellitus, we need 25 SH patients and 13 controls for comparision between SH and controls. Similarly most studies have used 20 or less than 20 subjects comparing different treatment strategy for EPC and CECs in vascular disorders. Hence, we have decided to study 20 subjects in each treatment arms (carbimazole or placebo) in the interventional part of the study. The results will be analysed using the SPSS-16 statistical package (Chicago, IL). Normality of the samples will be assessed by Shapiro-Wilk test. The difference between two groups (SH and controls) and comparision of effect of carbimazole and placebo group in the interventional arm of the study will be analysed either using Students *t* test for normally distributed values or by Mann Whitney test for data which were not normally distributed. Any spurious data will be excluded for the purpose of analysis.

Access to the source data/data handling:

All data pertaining to the study will be stored in a stand alone PC at the Endocrinology department. PI and co investigators only will have access and the data access will be password protected. The entry of the data will be done directly at the CRF at each study visits.

Quality control and assurance:

The internal audit will be carried out by the department of endocrinology and CRU,TTSH. The investigators / institution will permit trial-related monitoring, audits, IRB review and HSA inspections, providing direct access to source data and documents.

Financing and Insurance:

The funding of this study was from NHG-KTPH SIG grant and this study was covered by the NHG clinical trial compensation insurance scheme. The NHG-KTPH SIG is finished now and expired. As the study could not be completed in time, the fund has been transferred to CRU, TTSH. The study has been completed now and hence there wont be any requirement for insurance cover.

Publication:

The study results will be published in the peer reviewed journal and also will be presented as poster presentation in the international mmeting.

Main Contact Person for this study (PI):

Dr. S.K. Abdul Shakoor
Consultant Endocrinologist
Department of Endocrinology
Tan Tock Seng Hospital, Singapore
Tel; 6590675522/65 63577843; Email; Shaikh_shakoor@ttsh.com.sg