ARIC Manuscript Proposal #752

PC Reviewed: 11/ 21/ 00	**Status: __A__**	**Priority: __1__**
SC Reviewed: _____	**Status: _____**	**Priority: _____**

1.a. Full Title: Soluble plasma thrombomodulin (sTM), thrombomodulin genotype, and risk of venous thrombosis: The Longitudinal Investigation of Thromboembolism Etiology

b. Abbreviated Title (Length 26 characters): TM and venous thrombosis

2. Writing Group (list individual with lead responsibility first):

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3. Timeline: Winter 2000: analysis and first draft

4. Rationale:

The LITE Study is a prospective study of venous thromboembolism (VTE) in two multicenter, longitudinal, population-based cohort studies: the Atherosclerosis Risk in Communities (ARIC) study and the Cardiovascular Health Study (CHS). The precise etiology of VTE is not fully understood, although it is now generally accepted that activation of the endothelium, in addition to platelets and leukocytes, plays an important role in its development.

Endothelial thrombomodulin (TM) plays an important role in vasoprotection. TM binds thrombin, changes thrombin conformation and allows thrombin to activate Protein C (PC) and TAFI (Thrombin Activatable Fibrinolysis Inhibitor) (1). Activated PC and TAFI inhibit coagulation and fibrinolysis, respectively. TM is thus a link between endogenous control of coagulation and fibrinolysis (2). An impaired TM cofactor function/expression could be an additional risk factor for VTE.

Plasma soluble TM (sTM), cleaved products of cellular TM, also have anti-coagulant and anti-fibrinolytic properties. Plasma sTM levels have been reported to be elevated in TTP and DIC, diabetes mellitus, preeclampsia and SLE. Based on these studies it has been suggested that high sTM values are markers of endothelial damage. On the other hand, Jansson and colleagues' prospective study of patients on long-term oral anticoagulants showed that the concentration of sTM was directly related to the risk of serious hemorrhage (3). It has been shown also that

recombinant human soluble TM has an inhibitory effect on initiation and extension of coagulation through PC activation (4).

A recent report from the ARIC study indicates that the relationship of sTM with thrombosis is more complex than previously considered (5). The study showed that a high plasma sTM level was associated with a lower risk of developing CHD, suggesting that plasma sTM may reflect the level of endothelial expression. The prospective association of sTM with incident CHD differed from its cross-sectional association with carotid atherosclerosis, where plasma sTM reflects not only the basal shedding of TM fragments, but also an increased cleavage of TM molecules due to endothelial injury.

Despite much interest in sTM as a marker of endothelial injury, few studies investigated relationship of sTM concentration and VTE. The findings of the studies are conflicting, partly because of small sample sizes and cross-sectional designs. Yamada N. et al. (6) investigated hemostatic abnormalities in patients with DVT and PE, and reported that sTM levels in both PE and DVT were significantly increased compared to normal volunteers. A study by Trifiletti et al (7) showed no significant difference in plasma level of sTM between newly diagnosed DVT patients and controls. Another study also reported no difference in sTM between controls and DVT patients (8). On the other hand, the inflammatory mediators are also candidate risk factors for venous thrombosis, and it is known that TM is affected by inflammatory factors.

The level of TM expression in healthy subjects is likely to be determined by multiple factors, including both genetic and environmental factors. Several single nucleotide polymorphisms (SNP) or mutations of TM gene in the coding region and the 5'-promoter region have been identified (9-12). Influence of these mutations or polymorphisms on the expression level and/or activity of cellular TM have been reported for Asp468Tyr, G-33A and InsT1689. It is unknown whether any of the mutations affects the plasma level of sTM. The level of plasma TM was inconclusive, ranging from subnormal to increased levels in report on TM gene in 51 patients with VTE (13). A study by Le Flem (14) analyzed the TM proximal promoter region for polymorphisms that could modify TM gene expression in 205 patients with VTE and reported that these genetic variants are not frequent in patients with venous thromboembolism . TM A455V is located in the sixth EGF-like module of TM, a region that is involved in thrombin binding and PC activation. TM A455V was associated with CHD in one study (9), but not with venous thrombosis (15,16). At present it is unknown what impact the Ala-Val substitution has on TM function. The ARIC study recently reported a preliminary analysis of TM A455V in incident CHD, showing that this dimorphism is independent risk factors among African Americans, but not Caucasians (17). Taken together, although few existing data do not support a strong association of TM gene defects with increased thromboembolic risk, there are no existing prospective studies, and additional studies are required.

The prospective LITE study provides an excellent opportunity to increase our understanding of the role of TM in development of VTE. We propose to analyze prospectively the association of sTM levels with VTE. We would like to evaluate if plasma concentration TM is associated with the TMA455V gene polymorphism. We will analyze the association of TM A455V dimorphism with risk of VTE. We will analyze the correlation of sTM and TAFI, PAP, and other fibrinolytic and coagulation factors (FXI, FV) in order to evaluate the significance of any inhibitory effect of sTM on fibrinolysis and its anticoagulant activity on PC.

5. Main Hypothesis/Study Questions:

a. Plasma level of sTM represents the level of endothelial TM expression.
b. Low baseline sTM level, before the onset of disease, is a risk factor for venous thrombosis.
c. Lower sTM will be associated with both incident and recurrent VTE.

d. The TMA455V dimorphism is not associated with plasma level of sTM.
e. The TMA455V dimorphism is not risk factor for VTE.

6. Data (variables, time window, source, inclusions/exclusions):

Sample: Existing LITE nested case-control sample of 323 incident VTE cases and 688 frequency matched controls.

Data: Baseline risk factors measured in ARIC and CHS, including coagulation factors and genotypes measured only on this sample.

Analysis: (1) Examine inter-relations among factors using cross-tabs and correlations.
(2) Logistic regression to test study hypotheses

7.a. Will the data be used for non-CVD analysis in this manuscript? __ √__ Yes ____ No

b. If Yes, is the author aware that the file ICTDER01 must be used to exclude persons with a value RES_OTH = "CVD Research" for non-DNA analysis, and for DNA analysis RES_DNA = "CVD Research" would be used? __ √__ Yes ____ No
(This file ICTDER01 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? __ √__ Yes ____ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER01 must be used to exclude those with value RES_DNA = "No use/storage DNA"? __ √__ Yes ____ No

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