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Derivation and internal validation of a clinical prediction rule to identify patients with low risk of recurrent venous thromboembolism who can discontinue oral anticoagulants after five to seven months of treatment for unprovoked venous thromboembolism.

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Derivation and internal validation of a clinical prediction rule to identify patients with low risk of recurrent venous thromboembolism who can discontinue oral anticoagulants after five to seven months of treatment for unprovoked venous thromboembolism.

By

Marisol Betancourt MD

Thesis submitted to the Faculty of Graduate and Postdoctoral Studies in partial fulfillment for the requirements for the Master of Sciences degree in Epidemiology

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ABSTRACT

Background: Whether to continue or to discontinue oral anticoagulation therapy (OAT) after 6 months of treatment to prevent recurrent or fatal events in unprovoked VTE patients is currently controversial. We sought to develop and internally validate a clinical prediction rule (CPR) to identify patients at low risk of recurrent VTE (at most 3% annual risk) for whom OAT could be safely discontinued.

Methods: Univariate and multivariate analysis techniques were used to identify the best set of predictor variables.

Results and conclusions: We derived and internally validated a CPR for females comprised of D-Dimer over 250 ug/L, post-thrombotic signs present, older age (over 65 years) and obesity (BMI over 30 kg/m²). Women with one or none of the four aforementioned clinical predictors had an annual risk of recurrent VTE of 1.6% and may be able to discontinue OAT. None of the models for males was shown to be safe.

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EXECUTIVE SUMMARY

Statement of the problem

The most menacing and yet preventable complication of a venous thromboembolism (VTE) event is the risk of a recurrent VTE after stopping anticoagulant therapy. Anticoagulant treatment for a period of 3-6 months following a first unprovoked event has been shown to significantly reduce the immediate risk of recurrent VTE while on anticoagulant therapy, however, whether to continue OAT after 6 months of therapy or not is controversial. There is evidence indicating that in some patients the clinical benefit of OAT subsides after the discontinuation of therapy and that prolonged anticoagulation only delays recurrence until OAT is stopped. A method of stratifying patients with low risk of recurrent VTE after a first episode of unprovoked VTE is desirable, as it would help clinicians decide with confidence which patients on OAT for 6 months after an unprovoked VTE event do not need longer anticoagulation therapy and can safely discontinue treatment six months after a first unprovoked VTE, thus reducing the risk of bleeding due to OAT and increasing the patients quality of life. This study sought to develop a clinical prediction rule (CPR) to identify patients at low risk of recurrent VTE (at most 3% annual risk) who could safely discontinue OAT.

Methods

Multi-centre multinational prospective cohort study of patients with a first unprovoked VTE (REVERSE study --Recurrent Venous thromboembolism Risk Stratification Evaluation) in whom potential predictors of recurrent VTE were collected at baseline before stopping OAT after 5 to 7 months of therapy. Recurrent VTE events were investigated in follow-up and

then independently adjudicated. The derivation and internal validation of the CPR was conducted in four phases. Phase I aimed at identifying potential predictor variables ($p < 0.10$) using univariate statistical analyses. Phase II assessed the inter-observer reliability of predictors using a subset of the derivation dataset. Phase III aimed at deriving candidate CPRs by applying logistic regression including variables that were both significant in the univariate analysis ($p < 0.10$) and had good inter-observer reliability (Kappa > 0.6). The logistic models with the highest negative predictive value (risk of recurrent VTE in low risk group $< 3\%$) and specificity were then identified using classification performance analysis. Phase IV selected the final CPR among competitive models and dealt with internal validation of the final CPR using a resampling technique.

Results

The derivation dataset contained 646 participants enrolled over a 4 year period with 91 confirmed episodes of recurrent VTE that occurred in a mean of 18 months of follow-up (range 1-48 months).

In the univariate analysis (Phase I), I found significant differences between genders, with males having 2.4 times (95% CI: 1.49 - 3.85) increased risk of recurrent VTE compared to females. Significant differences by gender were also found for the main dichotomized predictor variables. Therefore, subsequent univariate analyses of predictor variables were carried out for males and for females independently. D-Dimer over 250ug/L, older age > 65 years, lipoprotein (a), history of hypercholesterolemia, statin use in the last year, previous secondary VTE history, body mass index (BMI) > 30 kg/m² and oral contraceptive use in the last year were significant individual predictors for females but not for males. Anticardiolipins

antibodies, hemoglobin over 170g/L, height >188 cm and pulmonary vascular obstruction (PVO) score were significant individual predictors for males but not for females. Post-thrombotic signs (any hyperpigmentation, edema or redness in either leg) and Factor VIII were found to be strong individual predictors of recurrent VTE regardless of gender.

From the inter-observer reliability (Phase II), the combined post-thrombotic sign (PTS) variable, (any hyperpigmentation, edema or redness in either leg) demonstrated good to excellent inter-observer reliability (Kappa =0.73) among all the PTS signs collected, therefore, this variable was selected to be included in the multivariate analyses.

During Phase III and VI, using multiple logistic regression and classification performance analyses, a final CPR for females composed by a D-Dimer > 250 ug/L, PTS signs present, older age (> 65 years) and obesity (BMI > 30 kg/m²) was derived and internally validated. Women with one or none of the four aforementioned clinical predictors had a risk of recurrent VTE of 1.6% per year and included 52% of women. Women with 2 or more of the four clinical predictors had an annual risk of recurrent VTE of 14.1%. Several significant models were identified for the male sub-group. However, the classification performance of these male models showed an unacceptably high annual risk of recurrent VTE in the low risk group (over 3% per year) making any potential CPR for males unsafe.

Conclusion

The final clinical prediction rule derived in this study appears to be a safe and clinically useful tool that can be used for clinicians to segregate female patients treated for 5 to 7 months for unprovoked VTE into a group with an annual risk of recurrent VTE <3% for

whom anticoagulant therapy can be confidently discontinued, and to segregate the remaining patients into a group with an annual risk of recurrent VTE >10% for whom longer term of oral anticoagulant therapy should be strongly recommended. No CPR that can safely segregate male patients at low risk of recurrent VTE was identified in this study because of the high rates of recurrent VTE in the low risk group identified by all the male models. Men are at very high risk of recurrent VTE and longer OAT is recommended.

1.0 INTRODUCTION

1.1 Overall aim

To develop a safe and clinically useful clinical prediction rule (CPR) applicable to patients at six months post-diagnosis of a first episode of unprovoked venous thromboembolism that accurately identifies those patients at low risk of recurrent thrombosis who could safely discontinue oral anticoagulation therapy.

1.2 Statement of the problem

Venous thromboembolism (VTE), manifested as pulmonary embolism or deep vein thrombosis (DVT), is one of the most common life threatening cardiovascular diseases (1;2). The most menacing and yet preventable complication of a VTE event is the risk of a recurrent VTE after stopping anticoagulant medication. The risk of recurrent VTE is much higher for patients with unprovoked VTE (VTE event that occurs in the absence of known risk factors for thrombosis, also known as idiopathic VTE) and a history of previous VTE than in patients with provoked VTE (3;4).

After initial treatment with heparin or low molecular weight heparin, VTE patients are treated with OAT with an international normalized ratio (INR) target of 2-3 for 3 to 6 months (5) to prevent recurrence. It is well recognized that patients with secondary VTE associated with transient risk factors (e.g. surgery, trauma) have a low risk of recurrent VTE and can safely discontinue anticoagulants after three months (6;7), while patients with continuous risk factors (cancer, antiphospholipid antibody syndrome) or a second episode of

“unprovoked” VTE require long-term to indefinite anticoagulant treatment (6;8;9). However, the ideal duration of OAT necessary to prevent recurrent VTE in unprovoked VTE patients has not yet been clearly defined.

Several studies have identified patients with a first episode of unprovoked VTE as being at high risk of recurrence and have recognized that more than 3 months of OAT is necessary to prevent recurrent VTE (3;8;10;11). How much longer these patients should be treated is controversial as the risk of recurrent VTE varies widely across studies. The risk of recurrent VTE in patients with unprovoked VTE after 3 to 6 months of OAT is 5% to 15% in the first year after discontinuation of OAT (12-14). Furthermore, a recurrence as high as 27.4% per year was reported on one arm trial (3 months of OAT), while the arm in continuous OAT showed a 1.3%/year recurrence (3).

Extended OAT has been proven to be very effective at reducing the risk of recurrence during therapy (95% relative risk reduction) in unprovoked VTE patients (3). However, this clinical benefit seems to weaken after discontinuation of OAT (15). Some studies have found that the protective effect of anticoagulants disappears immediately after discontinuation of the medication (10). It appears that extended OAT simply delays recurrence until OAT is stopped, as it has shown to be protective only while on medication. Consequently, some patients with a first episode of unprovoked VTE may need indefinite anticoagulation to effectively prevent recurrences.

Additionally, while extended treatment with OAT is effective in preventing recurrent VTE, it is not risk free. There is a substantial risk of major bleeding associated with prolonged

treatment (16). Major bleeding can be expected in approximately 0.9%-3% of patients with unprovoked VTE per year (4;17;18), and approximately 13% of patients with major bleed will die (19). As such, the trade-off between the risks and the benefits of OAT should be taken into account when deciding whether or not to continue long-term OAT in an individual patient. The frequent blood tests necessary to titrate oral anticoagulant medication is resource-intensive in terms of patients and physician time and technical analysis. OAT greatly affects the patients' quality of life due to limitations in food choices and alcohol consumption, physical activity (contact sports), and interactions with other drugs (10;20).

Thrombosis experts have recommended that the length of OAT for unprovoked VTE patients should be determined based on the patients' individual risk of recurrence, risk of major bleeding and preferences (17). However, the decision to continue OAT in unprovoked VTE patients is currently based on poorly defined risk factors and the discretion of the treating physician (14;16). This results in patients with low risk of recurrent VTE receiving unnecessary extended anticoagulation treatment or in patients with high risk of recurrent VTE being withdrawn from OAT increasing their likelihood of recurrent VTE and a fatal event.

As the majority of unprovoked VTE patients would not have a recurrent event, there is a need for further investigation of individual risk factors for recurrent VTE. Furthermore, there is a need for an instrument to risk stratify unprovoked VTE patients. This need has already been suggested by experts in the field (21-23). Simple effective tools to risk stratify patients would help physicians target patients who are more likely to benefit from extended OAT.

This study is the first to combine known predictor variables in a clinical prediction rule (CPR) that risk stratifies patients with a first unprovoked VTE.

By identifying and combining the predictive power of individual variables of recurrent VTE in patients with history of a first unprovoked VTE, I sought to develop a clinical prediction rule (CPR) that accurately identifies unprovoked VTE patients at low risk of recurrent VTE (at most 3% per year) who can safely discontinue anticoagulants after 5 to 7 months of OAT.

1.3 Description of potential individual predictor variables used in the study

In the last few years, several studies have attempted to identify individual risk factors for recurrent VTE patients (24-28) but none of them have successfully identified risk factors that can safely risk stratify unprovoked VTE patients.

Elevated D-Dimer after discontinuation of OAT, male gender, residual venous obstruction, and elevated Factor VIII have been significantly associated with increased risk of recurrent VTE, and were important variables to consider in the development of this CPR.

D-Dimer is a degradation product of clot dissolution and low levels are used to safely forgo diagnostic image for acute thrombotic events. D-Dimer has also shown high negative predictive value for VTE recurrence when measured after OAT discontinuation on VTE patients (26;29), and has been suggested as the most promising predictor of recurrent VTE in unprovoked VTE patients (30).

Elevated Factor VIII is a known risk factor for VTE (31) and has been suggested to be an important factor in the pathogenesis of recurrent VTE (32). Recently, elevated Factor VIII has been found to be a strong predictor of recurrent VTE in patients with a first unprovoked VTE, and prolonged OAT has been suggested for these patients (25).

DVT patients with residual venous obstruction (persistent abnormalities in ultrasound) off OAT have been found to be at increased risk of recurrent VTE (24;27;28). Although DVT resolves progressively with OAT, approximately 50% of patients still show remaining thrombi in a year (4). Compression ultrasound (CUS) vein images of the leg for patients with DVT of the leg and V/Q scan for patients with pulmonary embolism are used to identify the location of the thrombi and provide comparative images for subsequent suspected recurrent VTE. Residual venous obstruction at CUS has been shown to be a strong predictor of recurrent VTE (27) and was an important predictor variable to include in the development of this CPR. CUS is available in virtually all hospitals, is simple and has been demonstrated to be reproducible (24;33). Prior to this study, there was no prospective assessment of the recurrent VTE prognostic value of follow-up V/Q scan (34).

Studies on VTE patients have consistently shown that males have a higher risk of recurrent VTE than females after stopping OAT (15;35;36). This difference has also been found in unprovoked VTE patients (37). Although there is no clear explanation for this finding, male gender has been identified as a potential predictor of recurrent VTE and was considered an important predictor to study in the development of this CPR.

Recently, an association of post-thrombotic (PTS) signs and recurrent VTE has been suggested (11;15). This association has been confirmed in a study conducted on DVT patients where there was a 2-fold increased risk of recurrent VTE on patients with PTS signs compared with those without (38). PTS is the second most troublesome complication of a VTE event and is manifested by leg pain, skin alterations, occasional to intractable edema and unilateral leg ulcers.

Traditional risk factors for a first VTE are immobilization, surgery, lower extremity fracture, heart failure, stroke, myocardial infarction in the past year, hormone replacement or oral contraceptive therapy either recently or within the last year, family history of VTE, history of previous secondary VTE, pregnancy, varicose veins, age and obesity (increased body mass index). However, these risk factors for first VTE have not previously been examined as risk factors for recurrent VTE.

The following variables have also shown some association with VTE, however the results are not conclusive (4;39): older age, presence of antiphospholipid antibodies such as anticardiolipin antibodies or lupus anticoagulant, elevated levels of homocysteine (15) and some hereditary hypercoagulable disorders (factor V Leiden and the G20210A prothrombin gene mutation heterozygous state)(40). International Normalized Ratio (INR) levels evaluate the quality of OAT and are ideally maintained in therapeutic levels (2.0 to 3.0) to effectively prevent recurrent VTE during OAT. Maintenance of INR therapeutic levels throughout OAT increases the rate of DVT resolution, and INR levels under 2 have been suggested to increase the risk of recurrence (41).

Other novel risk factors that could be associated with recurrent VTE and that were explored in this study were hypercholesterolemia, statin use (42;43), chronic respiratory diseases, and concomitant medication use (NSAIDS).

2.0 METHODOLOGICAL STANDARDS AND RATIONALE FOR THE CPR DERIVATION

2.1 Methodological standards for clinical prediction rules

I derived this CPR for patients with unprovoked VTE using the following methodological standards for clinical prediction rules originally described by Wasson et al (44) and later modified by other authors (45-48).

- 1) **Definition of outcome:** the outcome or diagnosis to be predicted must be clearly defined, clinically important and the assessment of the outcome must be blinded (i.e. the final judge of outcome must have no prior knowledge of potential predictive variables under study).
- 2) **Definition of predictor variables:** the clinical findings to be used as predictive variables must be clearly defined standardized and their assessment must be done without knowledge of the outcome (i.e. blinded).
- 3) **Reliability of predictor variables:** the reproducibility of the clinical findings used as predictive variables must be demonstrated and the reproducibility of the rule must be demonstrated.

- 4) **Selection of subjects:** the patients in the study should be selected without bias and should represent a wide spectrum of clinical and demographic characteristics to increase the generalizability of the study results.
- 5) **Mathematical techniques:** the statistical techniques used to derive the rule must be identified and valid.
- 6) **Accuracy:** the accuracy of the prediction rule in classifying patients with the outcome (i.e. sensitivity) and without the outcome (i.e. specificity) should be demonstrated.
- 7) **Prospective validation:** prospective validation in a second independent set of patients is an essential test of a prediction rule's accuracy and clinical utility (i.e. the effects of clinical use of the rule should be prospectively measured).
- 8) **Sensibility of the decision rule:** clinical prediction rules should be sensible i.e. have a clear purpose, be relevant, demonstrate content validity, be concise, and be easy to use in the intended clinical application. The use of the rule should provide a probability of disease and should imply a course of action.

2.2 Safety and clinical utility of the CPR

A clinical prediction rule that accurately stratifies unprovoked VTE patients as having low risk of recurrent VTE to decide discontinuation of OAT should be:

1) Safe (high sensitivity and negative predictive value): accurately identify low recurrent VTE risk limiting the misclassification of patients at high risk for recurrent VTE. For clinicians to confidently use the rule, it should be near 100% sensitive with very few false negatives and a high negative predictive value. However, a high sensitivity is usually associated with a decreased specificity which may decrease the efficiency of the CPR (48). This trade-off is worth it in order to reduce the possibility that a recurrent VTE may occur on patients at high risk who have been misclassified as being at low risk by the CPR (negative CPR) (45).

2) Clinically useful: exclude a maximum number of patients with low risk of recurrent VTE that can be safely withdrawn from OAT after 6 months of treatment. To be clinically useful and impact positively on patient care, the CPR must identify a significant proportion of patients at low risk. If very few patients are categorised as low risk (e.g. < 5%), it is unlikely that clinicians would use the rule.

This study aimed at developing a CPR with near 100% sensitivity and high negative predictive value that identifies a large sub-group of patients with less than 3% risk of recurrent VTE per year, has good face validity, and is easy to use and remember.

2.3 Rationale for yearly risk thresholds for continuing or discontinuing anticoagulants

The goal of OAT on unprovoked VTE patients is to optimize the preventive action of reducing recurrent VTE and minimizing risk of bleeding. The rationale of this study (identify

unprovoked VTE patients at low risk of recurrent VTE (at most 3% per year)) is based on available information regarding mortality rate of a recurrent VTE event while off OAT and the risk of fatal bleeding due to OAT.

The ratio of case fatality rates from VTE and major bleeding is likely to vary from 1:1 to 1:3, as the case fatality rate of major bleeding in VTE patients with ongoing OAT is approximately 13% (19), and the case fatality rate for VTE patients with recurrent VTE off OAT varies from 5% (49;50) to approximately 13% (50;51).

Yearly event rates (VTE or major bleeding) can be directly compared if they are adjusted by the case fatality rate ratio. The identification of patient sub-groups with an ongoing yearly risk of recurrent VTE off of OAT that is as low as the yearly risk of major bleeding on OAT (i.e. <3%) should safely permit these sub-groups to discontinue anticoagulant therapy.

Conversely, identifying patient sub-groups with an ongoing yearly risk of recurrent VTE off of anticoagulants that is 3 times higher than the yearly risk of major bleeding on OAT (i.e. >9%) should provide clear evidence that these sub-groups should continue anticoagulant therapy (52).

3.0 GOALS AND OBJECTIVES

The objectives of this study were:

- 1) To identify clinical variables that are predictive of recurrent VTE after a first episode of idiopathic VTE subsequent to 5 – 7 months (Phase I - see below).

- 2) To determine the inter-observer reliability of predictive clinical variables at their optimal cut-off points that have not already been validated in previous studies (phase II - see below).
- 3) Using multivariate techniques, to derive clinical prediction rules applicable to patients at 5 to 7 months post-diagnosis of a first episode of idiopathic VTE that will identify patients at low risk of recurrent VTE that can safely discontinue OAT (Phase III – see below).
- 4) To assess the classification performance of the derived clinical prediction rules (Phase III – see below).
- 5) To select a final rule with the best combination of negative predictive value (low risk of recurrent VTE in the low risk group), true negative proportion (high proportion identified as low risk), face validity and ease of use (Phase IV – see below).
- 6) To internally validate the final clinical prediction rule (Phase IV – see below).

4.0 STUDY DESIGN

This study was conducted in four phases: Phase I was aimed at identifying potential predictor variables using the REVERSE derivation dataset (derivation set). Phase II was conducted to determine inter-observer reliability of significant predictors in the univariate analysis ($p < 0.10$) that had not yet been validated. A subset of the derivation dataset was used for this analysis. Phase III was aimed at identifying competing CPRs by applying logistic

regression and classification performance on the derivation dataset. Phase IV was conducted to select the final rule and internally validate the derived CPR using random samples from the derivation set.

4.1 Data collection

The data for this study was collected by the “REcurrent VENous thromboembolism Risk Stratification Evaluation” study (REVERSE) (52). The REVERSE project prospectively collected data in 12 different tertiary care centers in four countries (Canada, Switzerland, France and United States) from October 2001 to March 2006. I have been involved in this study since October 2005. From October 2005 until September 2006 when data collection finished, I continuously surveyed all the REVERSE databases (baseline, follow-up, suspected and adjudicated outcome, death events) to identify extreme observations that required further investigation and corrections from the clinical sites to ensure the accuracy and completeness of the data. During that period I was also responsible for conducting an interim analysis and several other exploratory analyses. From September 2006 to February 2007 I queried, cleaned, linked and locked the REVERSE datasets in order to build a final dataset for analysis, hereafter called the derivation dataset. After the derivation dataset was created, I conducted additional queries to ensure the completeness and accuracy of the derivation dataset. At this stage, I also created new datasets containing information incoming from different clinical sites and updated information (pending laboratory results) in order to link this updated information to the derivation dataset. Finally, to complete the derivation dataset, I created and calculated key variables for the analysis such as age, time to event, index event and body mass index based on the following information: date of birth, date of

enrolment, date of recurrent event, date of death event, last day of follow-up, index DVT signs and symptoms, index PE signs and symptoms, imaging confirmation for PE, imaging confirmation for DVT. Weight (kg) and height (m) were used for BMI calculations (kg/m^2). Percentage of INR therapeutic time was calculated based on 24 INR variables collected since first VTE event to enrolment. Once the derivation dataset was completed, I carried out summary statistics and the complete analyses of the data that led to the derivation of the final CPR (detailed below).

The expert opinion and judgment of researchers and thrombosis experts was used throughout all the stages of the study to ensure that the derived CPR is safe, feasible, relevant, and has good face validity. The REVERSE steering committee played an important role adding content and face validity throughout all the stages of the study. The committee was composed of Susan Kahn (Internists, expert in post-thrombotic syndrome), Richard White (Internists, expert in large administrative database research focused in venous thrombosis), David Anderson (Hematologist, expert in thrombosis and clinical research), Marc Rodger (Hematologist, expert in thrombosis and clinical research), Phil Wells (Hematologist, expert in thrombosis and clinical research), Gregoire Le Gal (Internist, expert on clinical research in thrombosis), Arnaud Perrier (Internist, expert on clinical research in thrombosis), and Marisol Betancourt (Pediatritian and master's student in epidemiology).

Manipulation and linkage of databases as well as the analyses were carried out using SAS V 9.1 software (SAS Institute Inc., Cary, NC, USA).

4.2 Patients and methods

4.2.1 Inclusion criteria and exclusion criteria

Patients were included in the study if they had an objectively proven proximal unprovoked deep vein thrombosis (DVT) or pulmonary embolism (PE) and if they were treated initially with a minimum of five days of heparin or low molecular weight heparin and oral anticoagulants with a target intensity of 2.0 – 3.0 with no recurrence in the subsequent five to six months.

Patients were excluded from the study if they were at high risk of recurrent VTE by virtue of: history of recurrent unprovoked VTE (previous secondary VTE was not an exclusion criterion); known deficiencies of protein S, protein C or antithrombin; persistently positive anticardiolipin antibodies; known persistently positive lupus anticoagulant; combined thrombophilic defects or were unable to give informed consent.

4.2.2 Outcome measures

Oral anticoagulants were withdrawn 5 to 7 months after a first episode of unprovoked VTE and patients were then followed for recurrent VTE after one month, six months and each six months thereafter (mean follow-up = 18 months; range 1 to 48 months) .

Participants were followed for signs/symptoms of recurrent VTE, signs/symptoms of PTS syndrome, changes in VTE risk factors, and changes in their concomitant medications.

All of the suspected recurrent VTE outcomes were independently (investigators from other centers) adjudicated by two adjudicators blinded to the results of the potential predictor variables. The criterion used to document a recurrent VTE event on follow-up differed depending on the suspected diagnosis (DVT, PE or both) as described below.

Deep vein thrombosis

All patients with suspected recurrence (presenting with leg symptoms) had either a compression ultrasound (CUS) or venography and a D-Dimer test. If a CUS was performed, a new area of non-compressibility of a venous segment above the trifurcation of the popliteal vein (compared to baseline ultrasound) was considered diagnostic of a DVT. When the initial ultrasound was not diagnostic it was repeated again in 7 days or the patient underwent a venogram. If a venography was performed, a constant intraluminal filling defect in the deep veins above the trifurcation of the popliteal vein was considered diagnostic of a DVT. Venograms were considered adequate if the entire deep venous system was seen to the level of the common femoral vein.

Pulmonary embolism

All patients with suspected PE had ventilation perfusion (V/Q) scan and a D-Dimer test. If the V/Q scan was normal or unchanged from baseline, PE was considered excluded. If the V/Q scan was non-normal and a new unmatched segmental or greater perfusion defect was documented then PE was diagnosed. If a new matched or sub-segmental perfusion defect was documented a spiral CT scan was performed. If the spiral CT demonstrated an intraluminal filling defect in a segmental or greater vessel in an area of normal perfusion on the baseline V/Q scan then PE was diagnosed. All other patients required pulmonary angiography to diagnose or exclude suspected recurrent or new PE. Pulmonary angiography

demonstrating a constant intraluminal filling defect or a cut-off of a vessel > 2.5 mm in diameter were considered diagnostic for PE. Pulmonary embolism found at autopsy was also considered diagnostic of recurrent VTE.

4.2.3 Sample size

As per accepted methodological criteria for the development of clinical prediction rules, 5-10 patients per each predictor variable studied are required in the smallest outcome category (44). It was estimated *a priori* that approximately 5 recurrent VTE predictor variables that have been mentioned in previous studies would be of primary interest in this study (D-Dimer, elevated factor VIII levels, age, thrombophilia (Factor V Leiden (FVL) or protein gene mutation (PGM) and persistent abnormalities on imaging). The smallest outcome category in this study was expected to be recurrent VTE. As such, it was calculated that the study required a minimum of 25 patients and ideally 50 patients with recurrent events.

4.2.4 Ethics

This study was implemented after approval from each participating institution's research ethics board. Patients participated or refused to participate in the study without interfering with their care. All participants of the study signed informed consent forms that were approved by each institutional ethics review board.

4.2.5 Standardized baseline patient assessment

Once a patient had consented to participate, the study physician completed a standardized patient assessment. This standardized patient assessment was collected prior to

discontinuation of anticoagulants at the six month follow-up visit to mimic actual practice if the clinical prediction rule is adopted. Repeated measures of INR values were collected for the period immediately prior to the commencement of and during anticoagulation therapy. Table 1 describes the 67 predictor variables collected at baseline (five to seven months follow-up visit while the patient was still on oral anticoagulant therapy).

Table 1. Baseline clinical predictor variables.

Predictor variable –definition	Unit of analysis and variable classification
HISTORY	
Demographic Characteristics	
Age	Measured in years (continuous)
Gender	Male or female (dichotomous)
Race	Aboriginal, African Canadian, Asian, Caucasian, Other (nominal)
Socioeconomic status	Household income --<\$25.000, ≥\$25.000 to <\$50.000, ≥\$50.000 to <\$80.000 and ≥\$80.000 (ordinal)
Traditional Risk factors	
Immobilization in the last year	Past year in bed longer than 72 continuous hours over 90% of the time –yes/no (dichotomous)
Surgery in the last year	Yes/no (dichotomous)
Low extremity fracture in the last year	Yes/no (dichotomous)
History of heart failure	Yes/no (dichotomous)
History of stroke	Yes/no (dichotomous)
History of myocardial infarction	Yes/no (dichotomous)
Current or recent (in the last year) hormone replacement therapy.	Yes/no (dichotomous)
Current or recent (in the last year) oral contraceptive therapy	Yes/no (dichotomous)
History of malignancy in the last 5 years	Yes/no (dichotomous)
History of varicose veins	Yes/no (dichotomous)
Trauma in the last year	Yes/no (dichotomous)
Pregnancy in the last year	Yes/no (dichotomous)
Family history of VTE	Yes/no (dichotomous)
History of Heterozygous for FVL or PGM	Present / absent (dichotomous)
Previous secondary VTE	Yes/no (dichotomous)
Novel risk factors	

Current and/or recent (within past year) Statin use	Yes/no (dichotomous)
Current and/or recent (within past year) hypercholesterolemia	Yes/no (dichotomous)
Chronic respiratory diseases	COPD, ILD, Emphysema –yes/no (dichotomous)
Concomitant medication	ASA, clopidogrel, dypiridamol, horse chestnut seed extract, ginko biloba, garlic pills, NSAIDS—yes/no. (dichotomous)
Compression stocking	Yes/no (dichotomous)
PHYSICAL EXAMINATION	
Height	Cm (continuous)
Weight	Kg (continuous)
BMI	Kg/m ² --Calculated from weight and height) (continuous)
Post-thrombotic syndrome monitoring	
Symptoms right leg, left leg	No or minimal, mild, moderate, and severe) (ordinal)
Cramps	
Itching	
Pins and needles	
Leg heaviness	
Pain	
Swelling	
Signs right leg, left leg	No or minimal, mild, moderate, and severe (ordinal)
Pretibial edema	
Skin induration	
Hyperpigmentation	
Venous ectasia	
Redness	
Pain during calf compression	
Warmth	
Dependent cyanosis	
Leg ulcers	Present / absent (dichotomous)
Leg circumference (circumference 10 cm below tibial tuberosity)	Cm (continuous)
LABORATORY TESTS (collected prior to stopping oral anticoagulants)	
Factor V Leiden	Absent / heterozygote (dichotomous)
Prothrombin Gene Mutation (PGM) (Absent or heterozygote)	Yes/no (dichotomous)
Hemoglobin	g/L (continuous)
Platelets	x10 ⁹ /L (continuous)
C-reactive protein	mg/L (continuous)

Homocysteine	Umol/L (continuous)
Factor VIII	U/ml (continuous)
Vidas D-Dimer	Ug /L (continuous)
Lupus anticoagulant (present or absent)	Yes/no (dichotomous)
Anticardiolipin IgG	U/ml (continuous)
Anticardiolipin IgM	U/ml (continuous)
Lipoproteina	(continuous)
INR (multiple tests during OAT)	(continuous)
aPTTSP (prothrombin times)	(continuous)
aPTTFS (prothormbin times)	(continuous)
IMAGING TESTS (prior to stopping OAT at baseline visit- 6 months post diagnosis)	
Ventilation Perfusion Scan (V/Q) (for patients diagnosed with PE or DVT with chest symptoms)	Normal, abnormal, and inadequate (nominal)
Compression ultrasound (for patients diagnosed with DVT or PE with leg symptoms)	Normal, abnormal, and inadequate (nominal)
Abnormal compression ultrasound	Minimal wall thickening, partial resolution, minimal resolution, no resolution, and new thrombus (ordinal)
Pulmonary vascular obstruction (PVO) scores on V/Q scan--Calculated by 1-total perfusion deficit.	(continuous)

5.0 PHASE I – IDENTIFICATION OF UNIVARIATE PREDICTORS

All potential predictor variables for recurrent VTE included in this study were identified in a systematic review conducted prior to the collection of the data and periodically updated throughout the study (52).

In this study, I assessed the prognostic value of predictors of recurrent VTE previously described in the literature such as elevated D-Dimer, gender, residual venous obstruction and elevated Factor VIII. I also evaluated known predictors of first VTE as well as novel variables as potential predictors of recurrent VTE in patients with unprovoked VTE after oral anticoagulation withdrawal (see Table 1).

5.1 Phase I - Objectives

To identify clinical variables that are potential predictors of risk recurrent VTE in patients with a first unprovoked VTE subsequent to 5 to 7 months of oral anticoagulant therapy.

5.2 Phase I - Patients and methods

The potential predictor variables were identified from the derivation set (see data collection in section four).

5.3 Phase I - Data analysis -- Univariate analyses

The individual association between each of the predictor variables and recurrent VTE risk was assessed using the appropriate univariate technique depending on the type of data. For nominal data the Chi-square test with continuity correction or Fisher's exact test (2-tailed) was used. For continuous variables the 2-tailed independent sample t-test was used (pooled-variance t-test for equal variance or Welch-Satterthwaite's t-test adjusted for unequal variances).

All continuous and discrete potential predictor variables with more than two categories were dichotomized using several clinically reasonable cut-off points. The best predictive cut-off point for each variable was then identified by Chi-square or Fisher Exact test. Sensitivity, specificity, and negative predictive values were calculated using 2x2 contingency tables. Dichotomisation of variables facilitates the design of a practical clinical prediction rule that can be easily used and easily remembered.

Although I originally predetermined that potential predictor variables and dichotomised predictor variables with p-values equal or less than 0.20 would be kept as potential predictor variables, I identified a sufficient number of potential dichotomised predictor variables that presented p-values <0.10. Therefore, only predictor variables with a p-value <0.10 were initially kept as potential predictor variables.

5.4 Phase I - Results

5.4.1 Baseline characteristics

From October 2001 to March 2006, 1041 patients were assessed for eligibility; 665 from this sample were enrolled in the study (95 were unable to provide consent and 281 were not eligible). Of the 665 enrolled patients 19 were removed from the study as they were either lost, withdrawn, started anticoagulants for indications other than adjudicated VTE or died prior to first scheduled follow-up. Thus, 646 patients began follow-up and 600 completed follow-up in September 2006. Forty-six patients were censored at the time they were lost to follow-up (n=14), time of withdrawal (n=22) or time of death (n=10).

The 646 patients were followed for a mean of 18 months (range 1-47 months). Of the 646 patients, 306 had a suspected recurrent VTE event and 91 of those were adjudicated as recurrent VTE events (9.4% recurrent VTE per year). None of the deaths during follow-up were adjudicated as due to recurrent VTE.

The mean age of participants was 53 years (range 18-95 years), and 48.6% were females. To examine whether the clinical presentation of VTE at first event (index event) affects the risk of recurrence, the entire population was subdivided in three groups according to their index event (index DVT, index PE, and concomitant Index PE and DVT). Table 2 summarizes the main results of index recurrent VTE diagnoses and their association with recurrent VTE.

Patients with isolated index PE had an annual rate of recurrence of 5%, with isolated index DVT had an annual rate of recurrence of 11.3% and patients with both index DVT and PE had an annual rate of recurrence of 12.6%.

Table 2. Phase I results: VTE diagnosis at index event and recurrent VTE.

Predictor variable**	(n/N) and %	Cut-off point	rVTE incidence (%),	Chi-square	P-value
DVT	(339/646) 52.5	Yes	16.6	3.5	0.06
		No	11.4		
PE	(194/646) 30.0	Yes	7.7	9.3	0.002*
		No	16.8		
DVT & PE	(113/646) 17.5	Yes	17.3	1.5	0.22
		No	13.3		

*p<0.05

** VTE diagnosis at index event (first VTE event).

rVTE= recurrent venous thromboembolism. DVT= Isolated index deep vein thrombosis. PE= Isolated index pulmonary embolism. DVT & PE= includes patients who were diagnosed with both DVT and PE as index event (concomitant DVT and PE).

Patients with isolated DVT at index event were slightly more likely to have a recurrent VTE event compared with patients with isolated PE and concomitant DVT & PE. Patients with isolated PE at index event were significantly less likely to recur compared with patients with isolated DVT and concomitant DVT & PE (Table 2). This positive association of index DVT and recurrent VTE became clear when comparing isolated PE patients with isolated DVT excluding patients with concomitant DVT & PE at index event (see Table 3). Patients with

isolated DVT diagnosed at index event were 2.4 times (95% CI 1.5 – 3.9) more likely to recur than patients with isolated index PE at index event.

Table 3. Phase I results: additional index VTE sub-groups found to be significant predictors of recurrent VTE.

Index VTE sub-groups	(n/N) and % of rvTE	Cut-off point	rVTE incidence (%) (n/N)	Chi-square	P-value
DVT alone Vs. PE alone*	(71/533) 13.3	DVT alone	16.5 (56/339)	8.2	0.004
		PE alone	7.7 (15/194)		
DVT & PE vs. PE alone	(35/307) 11.4	DVT&PE	17.7 (20/113)	7.0	0.008
		PE alone	7.7 (15/194)		

* Sub-group includes patients with DVT alone and PE alone (DVT & PE patients were removed).** Sub-group includes patients with concomitant DVT & PE and PE alone (patients with index DVT alone were removed). rVTE= recurrent venous thromboembolism. DVT= Isolated index deep vein thrombosis. PE= Isolated index pulmonary embolism. DVT & PE= concomitant DVT and PE.

Additionally, when patients with isolated index DVT were removed (see Table 3), patients with concomitant DVT and PE at index event had an increased risk of recurrent VTE (OR=2.6 (95%CI: 1.3-5.2) compared with patients with PE alone at index event.

I also examined the predictive value of a combined PTS signs variable (any hyperpigmentation, edema or redness either leg) and recurrent VTE in different index VTE sub-groups (see Table 4). The PTS signs combined variable (HER) was found to be one of the most significant predictors of recurrent VTE in the univariate analysis (see section 5.4.2 below). The objective of this sub-group analysis was to identify if the predictive capabilities of PTS signs was present only in patients with index DVT, as PTS is known mainly to affect

patients with DVT at index event. However, I found that the PTS signs combined variable (HER) was a strong predictor of recurrent VTE independent of the index VTE sub-groups.

Table 4. Phase I results: predictive value of the post-thrombotic signs in index VTE sub-groups.

Index VTE sub-groups	Predictor variable (n/N)	Cut-off point	rVTE incidence (%) (n/N)	Chi-square	P-value
PE alone	PTS-HER (31/142)	yes	19.4 (6/31)	7.5	0.006
		no	4.5 (5/111)		
DVT alone	PTS-HER (146/305)	yes	21.2 (31/146)	8.3	0.004
		no	9.4 (15/159)		
Concomitant DVT & PE	PTS-HER (38/98)	yes	26.3 (10/38)	4.5	0.03
		no	10.0 (6/60)		
DVT and both (DVT&PE)	PTS-HER (184/403)	yes	22.3 (41/184)	12.4	0.0004
		no	9.6 (21/219)		
DVT & PE vs. PE alone	PTS-HER (69/240)	yes	23.2 (16/69)	13.8	0.0002
		no	6.4 (11/171)		

rVTE= recurrent venous thromboembolism. PE= Isolated index pulmonary embolism. DVT= Isolated index deep vein thrombosis. DVT & PE= concomitant DVT and PE. PTS-HER= Post-thrombotic signs –any hyperpigmentation, edema or redness either leg.

5.4.2 Univariate predictors of recurrent VTE (entire population)

For the entire population, the univariate analysis (t-test) of the continuous variables showed that only four variables (height, weight, leg circumference, and factor VIII) were strong positive predictors of recurrent VTE (Table 5). BMI showed a weak association with recurrent VTE ($p < 0.10$).

Table 5. Phase I results: univariate analysis of the continuous variables of the standardized baseline patient assessment in the entire study population.

Predictor variable	Mean of predictor variable			P-value
	Entire study population, (SD).	rVTE (n=91)	No rVTE (n=555)	
Height (cm)	171.6(10.8)	174.7	171.1	0.004 **
Weight (kg)	87.6(23.5)	93.8	86.5	0.006 **
BMI (kg/m ²)	29.1(7.6)	30.3	28.9	0.08 *
Age (years)	52.5(18.5)	53.6	52.3	0.47
D-Dimer (ug/L)	306.1(402.4)	383.1	293.5	0.27
Hemoglobin (g/L)	142.1(15.0)	143.7	141.9	0.29
Factor VIII (U/ml)	1.7(0.6)	1.8	1.7	0.005 **
Platelets x10 ⁹ /L	256.0(66.4)	251.3	256.8	0.47
C-reactive protein (mg/L)	5.1(6.1)	5.3	5.1	0.75
Lipoprotein a (g/L)	0.13(0.1)	0.16	0.13	0.14
Homocysteine (umol/L)	10.1(7.9)	9.7	10.2	0.4
Anticardiolipin IgM (U/ml)	6.7(10.6)	6.1	6.7	0.45
Anticardiolipin IgG (U/ml)	4.6(4.4)	4.9	4.6	0.59
aPTTSP (Partial thrombin time)	34.7(6.9)	35.1	34.6	0.61
INR percentage of therapeutic time (%)	0.60(0.22)	0.28	0.27	0.82
INR percentage of sub-therapeutic	0.27(0.20)	0.62	0.59	0.46

time (%)				
PVO Score (%)	0.94(0.10)	0.88	0.92	0.12
Circumference 10 cm below tibial tuberosity _Right leg (cm)	39.5(5.1)	40.6	39.3	0.09 *
Circumference 10 cm below tibial tuberosity _ Left leg (cm)	39.7(5.1)	41.1	39.9	0.03 **

****p<0.05, *p≤0.10**

Entire study population (includes males and females). SD= Standard deviation. rVTE = recurrent venous thromboembolism. BMI= Body Mass Index (weight(kg)/height(M)²). INR= International Normalized Ratio. PVO score= Pulmonary vascular obstruction (1-total perfusion deficit)

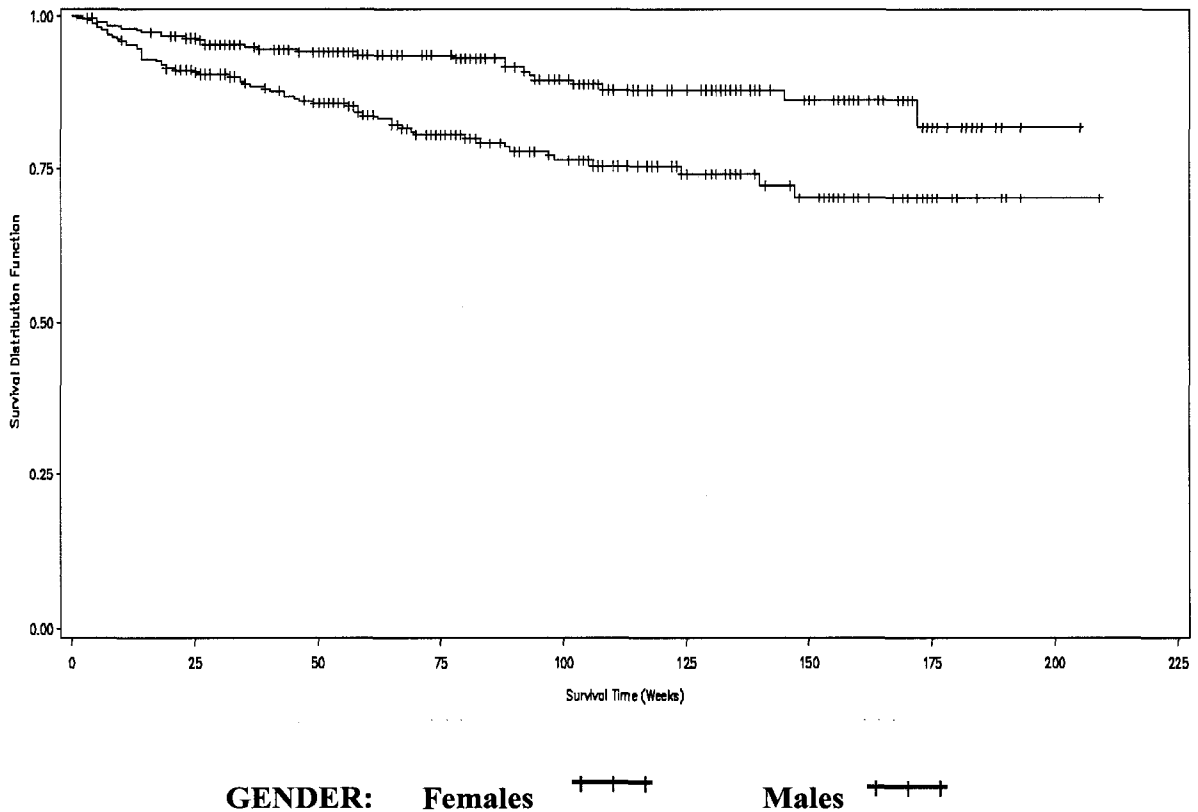
Eighteen continuous predictor variables were categorized by different reasonable clinical cut-off points to identify the optimal cut point for each variable. Discrete variables with more than two categories (19 variables) were re-categorised to different cut-off points with two responses. For example, the PTS signs variables (for the left, right or either leg) all possible cut off points were investigated: none vs. mild or moderate or severe, none or mild vs. moderate and severe, and none or mild or moderate vs. severe. The cut-off point with the best Chi-square was then selected to be brought forward for multivariate analysis.

Additionally, 30 already dichotomous variables were examined. Appendix 1 details the best cut-off points and univariate analysis of the potential predictor variables in the entire population.

From the univariate analysis of the entire study population, gender was the strongest predictor for recurrent VTE. Males were at 2.4 (95%CI: 1.5-3.9) times higher risk of recurrent VTE compared with females ($X^2_{1, 0.05}=13.5$; p 0.0002). This difference in recurrent

VTE incidence rate between men and women can be observed in Figure 1. The log-rank statistic for comparing these curves was highly significant ($X^2_{1,0.05}=15.6$; $p<.0001$).

Figure 1. Kaplan Meier survival curve of unprovoked VTE patients by gender (outcome = recurrent VTE).



Besides gender, strong significant potential positive predictors and optimal cut-off points for recurrent VTE in the entire study population were height over 172 cm, weight over 100 kg, BMI over 48 kg/m², hemoglobin over 170 g/L, factor VIII over 1.5 U/ml, partial thrombin time over 50 second, PVO score < 95%, abnormal V/Q scan and PVO score < 95%, and the combined PTS signs variable (any hyperpigmentation, pretibial edema, or redness either leg) hereafter referred to as HER (Hyperpigmentation/Edema/Redness). All **PTS symptoms** (except daily swelling of the leg for at least a month) did not show any association with

recurrent VTE. Among all the **PTS signs** (pretibial edema, skin induration, hyperpigmentation, venous ectasia, redness, pain during calf compression, warmth, and dependent cyanosis), the signs pretibial edema, skin induration, hyperpigmentation, venous ectasia, and redness were highly significantly associated with recurrent VTE, with pretibial edema being the strongest . 21.9% of patients with HER had recurrent VTE (17.5% annual risk of recurrence), while only 7.9% of patients without HER had recurrent VTE (5.9% annual risk of recurrence). Chronic respiratory diseases (COPD or emphysema) and oral contraceptive therapy within the last year were potential negative predictors for recurrent VTE in the entire study population (see Appendix 1 for chi-squares and P-values).

There were also significant differences by gender for the main predictive variables, like age. Older age (>40) was found to be a significant positive predictor of recurrent VTE for the overall population. Paradoxically however, when I stratified my analysis of age by gender, I found that older women (>65 years of age) had a 2.5-fold (95% CI 1.1-5.5) increase in risk of recurrent VTE while older men (>60 years) showed a 50% reduced in risk (OR 0.5, 95% CI 0.3-0.9).

Therefore, subsequent univariate analyses of predictive variables were carried out for males and for females independently in order to identify a low risk group of men and women separately who could discontinue anticoagulants.

5.4.3 Univariate predictors of recurrent VTE by gender

To identify potential predictor variables by gender, the entire study sample was split into two samples (males and females).

The females study sample contained 314 female patients (48.6% of the entire study sample) and 28 of these patients (8.9%) had an adjudicated recurrent VTE event (5.5% per year) during a mean follow-up of 19 months (range 1-47 months). The mean age was 50.6 years (95% CI: 48.4 – 52.7 years).

The males study sample contained 332 male patients (51.4% of the entire study sample) and 63 patients (19.0%) had an adjudicated recurrent VTE event (13.7% per year) during a mean follow-up of 16 months (range 1-48 months). The mean age was 54.3 years (95% CI: 52.6 – 56.0 years).

5.4.3.1 Univariate analysis for females

Univariate analysis for females-continuous variables

The univariate analysis (t-test) of the continuous variables for females (Table 6) showed that elevated factor VIII and elevated lipoprotein (a) were the strongest variables positively associated with recurrent VTE, followed by BMI, age and anticardiolipin IgM.

Table 6. Phase I results: univariate analysis of the continuous variables of the standardized baseline patient assessment in the female sub-group.

Predictor variable	Mean of predictor variable			P-value
	Population of females mean, (SD).	rVTE (n=28)	No rVTE (n=286)	
Height (cm)	164.2(8.2)	165.3	165.2	0.37
Weight (kg)	79.1(22.5)	86.2	78.4	0.08*
BMI (kg/m ²)	28.8(7.8)	31.9	28.5	0.03**
Age (years)	50.6(19.2)	58.30	49.8	0.03**
D-Dimer (ug/L)	314.2(344.4)	406.9	305.2	0.14
Hemoglobin (g/L)	133.4(13.4)	130.4	133.7	0.22
Factor VIII (U/ml)	1.72(0.6)	2	1.7	0.001**
Platelets x10 ⁹ /L	279.0(70.5)	282.2	278.7	0.80
C-reactive protein (mg/L)	5.4(5.6)	5.8	5.3	0.70
Lipoprotein a (g/L)	0.19(0.2)	0.31	0.18	0.007**
Homocysteine (umol/L)	9.6(9.8)	9.7	9.6	0.93
Anticardiolipin IgM (U/ml)	7.0(8.8)	4.7	7.2	0.01**
Anticardiolipin IgG (U/ml)	4.1(3.2)	3.5	4.2	0.29
aPTTSP (Partial thrombin time)	34.2(7.0)	34.00	34.2	0.90
INR percentage of therapeutic time (%)	0.56(0.22)	0.56	0.56	0.90
INR percentage of	0.30(0.21)	0.33	0.30	0.56

sub-therapeutic time (%)				
PVO Score (%)	0.94(0.13)	0.93	0.94	0.64
Circumference 10 cm below tibial tuberosity _Right leg (cm)	38.8(5.5)	40.9	38.9	0.05*
Circumference 10 cm below tibial tuberosity _ Left leg (cm)	39.1(5.7)	41.8	38.9	0.07*

**** p<0.05, *p≤0.10**

SD= standard deviation. rVTE = recurrent venous thromboembolism. BMI= Body Mass Index (weight(kg)/height(M)²). INR= International Normalized Ratio. PVO= pulmonary vascular obstruction Score on V/Q Scan (1-total perfusion deficit).

Univariate dichotomized potential predictor variables for females

Continuous predictor variables were categorized by different reasonable clinical cut-off points to identify the optimal cut point for each variable. Discrete variables with more than two categories were re-categorised to different cut-off points with two responses. The cut-off point with the best Chi-square and p value <0.10 was then selected for each variable to be used for multivariate analysis for females. The potential significant predictor variables and optimal cut-off points with the highest Chi-square for females were history of hypercholesterolemia in the last year, D-Dimer over 250 ug/L, PTS signs (HER), and Factor VIII over 2U/L, followed by age over 65 years, BMI over 30 kg/m², hemoglobin over 120 g/L, history of previous secondary VTE, lipoprotein (a) over 0.5 and statin use in the last year (Table 7).

Weight over 80 kg was weakly associated with recurrent VTE, and height over 166cm as well as oral contraceptive treatment in the year prior to index VTE were negatively associated with recurrent VTE with p<0.10.

Table 7. Phase I results: univariate analysis of dichotomized potential predictor variables at optimal cut-off points for females.

Dichotomised Potential Predictor Variables at Optimal Cut-points - Females						
Predictor variable (n/N)	Cut-off point	rVTE incidence (%) (n/N)	P-value	Sensitivity (%)	Specificity (%)	NPV (%)
Age (years) (87/314)	=>65	14.9 (13/87)	0.02*	46.4	74.1	93.4
	<65	6.6 (15/227)				
Height (cm) (118/314)	=>166	5.1 (6/118)	0.06*	21.4	60.8	88.8
	<166	11.2 (22/196)				
Weight (kg) (115/313)	=>80	13.0 (15/115)	0.05*	53.6	64.9	93.4
	<80	6.6 (13/198)				
BMI (114/313)	=>30	14.0 (16/114)	0.02*	57.1	65.6	94.0
	<30	6.0 (12/199)				
D-Dimer (ug/L) (121/304)	=>250	14.9 (18/121)	0.003*	66.7	62.8	95.1
	<250	4.9 (9/183)				
Hemoglobin (g/L) (269/311)	=>120	7.1 (19/269)	0.02*	29.6	12.0	81.0
	<120	19.1 (8/42)				
Factor VIII (U/ml) (78/304)	=>2	16.9 (14/83)	0.005*	50.0	75.8	93.9
	<2	6.1 (14/230)				
Factor V Leiden (55/313)	Heterozygote	14.6 (8/55)	0.12	28.6	83.5	92.3
	Absent	7.8 (20/258)				
Homocysteine (umol/L) (24/288)	=>15	12.5 (3/24)	0.48	11.1	92.0	91.0
	<15	9.1 (24/264)				
ACL positive (IgG>4 or IgM>4) or LAC positive) (226/304)	=>4 or LAC+	7.5 (17/226)	0.16	37.0	75.5	92.5
	<4 or LAC-	12.8 (10/78)				
Oral contraceptive use (within last year) (79/253)	Yes	3.8 (7/79)	0.08*	14.3	67.2	90.0
	No	10.3 (18/174)				
Hormone replacement (within last years) (31/248)	Yes	16.1 ((5/31)	0.16	23.8	88.6	92.6
	No	7.4 (16/217)				

Previous secondary VTE (13/314)	Yes	30.8 (4/13)	0.02*	14.3	96.9	92.0
	No	8.0 (24/301)				
Concomitant medication, ASA (10/314)	Yes	20.0 (2/10)	0.22	64.0	66.0	94.5
	No	8.6 (26/304)				
Any edema, hyper-pigmentation and redness, either leg (96/260)	Any	16.7 (16/96)	0.003*	64.0	66.0	94.5
	No	5.5 (9/164)				
Lipoprotein a (8/257)	≥ 0.5	37.5 (3/8)	0.03*	13.0	98.0	92.0
	< 0.5	7.9 (20/249)				
Hypercholesterolemia (62/314)	Yes	19.4 (12/62)	0.001*	52.9	82.5	93.7
	No	6.4 (16/252)				
Statin use (current) (42/314)	Yes	14.3 (6/42)	0.24	21.4	87.4	91.9
	No	8.1 (22/272)				
Statin use (within past year) (7/313)	Yes	42.9 (3/7)	0.02*	10.7	98.6	91.8
	No	8.2 (25/306)				
PVO Score (39/125)	≤ 0.95	12.8 (5/39)	0.14	55.6	70.7	95.4
	> 0.95	4.7 (4/86)				
Abnormal V/Q or PVO $< .95$ (100/144)	Yes	9.0 (9/100)	0.28	90.0	32.1	97.7
	No	2.3 (1/44)				
Abnormal CUS or PVO $\geq .95$ (113/137)	Yes	15 (17/113)	0.20	94.4	19.3	95.8
	No	4.2 (1/24)				

* $P \leq 0.10$

*- Predictor variables shown are ones with 1) strongest association, in the current study, with recurrent VTE and high inter-observer reliability and 2) those previously published that have been suggested to be predictive of recurrent VTE. Predictor variables are dichotomized at the optimal cut-point. NPV= negative predictive value. rVTE= recurrent VTE. BMI= Body Mass Index (weight(kg)/height(M)²). CUS= Compression ultrasound leg vein imaging. COPD= chronic obstructive pulmonary disease. PVO= pulmonary vascular obstruction Score on V/Q Scan (1-total perfusion deficit). V/Q Scan= Ventilation Perfusion Scan.

5.4.3.2 Univariate analysis for males

Univariate analysis for males-continuous variables

The univariate analysis (t-test) of the continuous variables for males (Table 8) showed a weak association of elevated homocysteine and decreasing age. However, no continuous variable was strongly associated with recurrent VTE for males.

Table 8. Phase I results: univariate analysis of the continuous variables of the standardized baseline patient assessment in the male sub-group.

Predictor variable	Mean of predictor variable			P-value
	Population of males, (SD).**	rVTE (n=62)	No rVTE (n=269)	
Height (cm)	178.7(7.8)	179.9	178.4	0.19
Weight (kg)	95.5(21.6)	97.2	95.1	0.49
BMI (kg/m ²)	29.4(6.5)	29.6	29.4	0.78
Age (years)	54.3(15.5)	51.1	55.0	0.07*
D-Dimer (ug/L)	298.3 (452.0)	372.4	280.8	0.42
Hemoglobin (g/L)	150.4(11.2)	149.5	150.6	0.48
Factor VIII (U/ml)	1.65(0.5)	1.7	1.6	0.15
Platelets x10 ⁹ /L	234.4(54.0)	237.8	233.6	0.58
C-reactive protein (mg/L)	4.8(6.6)	5.1	4.8	0.78
Lipoprotein a	0.18(0.1)	0.15	0.18	0.24
Homocysteine (umol/L)	10.6(5.5)	9.7	10.8	0.09*
Anticardiolipin IgM (U/ml)	6.32(12.1)	6.8	6.2	0.62

Anticardiolipin IgG (U/ml)	5.10(5.3)	5.5	5.0	0.53
aPTTSP (Partial thrombin time)	35.2(6.8)	35.6	35.1	0.67
INR percentage of therapeutic time (proportion)	0.63(0.22)	0.65	0.62	0.51
INR percentage of sub-therapeutic time (proportion)	0.24(0.19)	0.26	0.24	0.66
PVO Score (proportion)	0.94(0.09)	0.90	0.94	0.12
Circumference 10 cm below tibial tuberosity _Right leg (cm)	40.1(4.6)	40.5	40.1	0.68
Circumference 10 cm below tibial tuberosity _ Left leg (cm)	40.1(4.5)	40.8	40.0	0.39

* $p < 0.10$

**Population (includes males only).

CI= 95% confidence intervals. rVTE = recurrent venous thromboembolism. BMI= Body Mass Index (weight(kg)/height(M)²). INR= International Normalized Ratio. PVO= pulmonary vascular obstruction Score on V/Q Scan (1-total perfusion deficit).

Univariate dichotomized potential predictor variables for males

As for females, continuous predictor variables were categorized by different reasonable clinical cut-off points to identify the optimal cut point for each variable for males. Discrete variables with more than two categories were re-categorized to different cut-off points with two responses. The cut-off point with the best Chi-square and p value < 0.10 was then selected for each variable to be used in the multivariate analysis for males.

The potential significant predictor variables and optimal cut-off points with the highest Chi-square for males were PTS signs (HER) and PVO score $< 95\%$, followed by age under 65

years, over 40% of sub-therapeutic INR (INR < 2, the combined variable PVO score < 95% or abnormal VQ, and the combined variable anticardiolipin IgM-IgG > 6 U/ml or lupus anticoagulant positive (Table 9). PVO score and V/Q scan results were only available for 28% of the total male sample because PVO score and V/Q scan were done for patients with signs and symptoms of PE at index event and not for patients with index DVT alone. Height ≥ 188 cm, hemoglobin ≥ 170 g/L, and Factor VIII ≥ 1.55 were weakly associated with recurrent VTE ($p < 0.10$).

Table 9. Phase I results: univariate analysis of dichotomized potential predictor variables at optimal cut-off points for males.

Predictor variable (n/N)	Cut-off point	rVTE incidence (%) (n/N)	P-value	Sensitivity (%)	Specificity (%)	NPV (%)
Age (years) (126/332)	≥ 60	12.7 (16/126)	0.02*	25.4	59.1	77.2
	<60	22.8 (47/206)				
Height (cm) (33/331)	≥ 188	30.3 (23/33)	0.07*	16.1	91.5	82.6
	<188	17.5 (52/298)				
Weight (kg) (94/332)	≥ 104	24.5 (23/94)	0.11	36.5	73.6	83.2
	<104	16.8 (40/238)				
BMI (236/331)	≥ 26	16.5 (39/236)	0.10*	62.9	26.8	75.8
	<26	24.2 (23/95)				
D-Dimer (ug/L) (47/314)	≥ 500	14.9 (7/47)	0.46	11.7	84.3	80.2
	<500	19.9 (53/267)				
Hemoglobin (g/L) (9/329)	≥ 170	44.4 (4/9)	0.07*	6.5	98.1	81.9
	<170	18.1 (58/320)				
Factor VIII (U/ml) (168/316)	≥ 1.55	23.2 (39/168)	0.06*	63.9	49.4	85.1
	<1.55	14.9 (22/148)				
Factor V Leiden (45/332)	Heterozygote	24.4 (11/45)	0.31	17.5	87.4	81.9
	Absent	18.1 (52/287)				
Homocysteine (umol/L) (33/301)	≥ 15	9.1 (3/33)	0.19	5.8	88.0	81.7
	< 15	18.3 (49/268)				
ACL(IgG > 6 or IgM > 6 U/ml)	≥ 6 or LAC+	24.7 (39/158)	0.01*	65.0	53.3	86.6

or LAC positive) (158/315)	<6 or LAC-	13.4 (21/157)				
Previous secondary VTE (13/332)	Yes	7.7 (1/13)	0.48	1.6	95.5	80.6
	No	19.5 (62/319)				
Concomitant medication, ASA (26/332)	Yes	7.7 (2/26)	0.19	3.2	91.1	80.1
	No	19.9 (61/306)				
Any edema, hyper-pigmentation and redness, either leg (119/285)	Any	26.1 (31/119)	0.0004 *	64.6	62.9	89.8
	No	10.2 (17/166)				
Lipoprotein a (12/253)	≥ 0.4	25.0 (3/12)	0.42	7.3	95.8	84.2
	< 0.4	15.8 (38/241)				
Hypercholesterolemia (72/332)	Yes	13.9 (10/72)	0.2	15.9	77.0	79.6
	No	20.4 (53/260)				
Statin use (current) (43/332)	Yes	20.9 (9/43)	0.73	14.3	87.4	81.3
	No	18.7 (54/289)				
Statin use (within past year) (11/331)	Yes	18.2 (2/11)	1			
	No	19.1 (61/320)				
PVO Score (33/94)	≤ 0.95	10.6 (10/33)	0.005	66.7	70.9	91.8
	> 0.95	8.2 (5/61)				
Abnormal V/Q or PVO < 0.95 (85/115)	Yes	15.7 (18/85)	0.02*	94.7	69.8	78.8
	No	3.3 (1/30)				
Abnormal CUS or PVO ≤ 0.95 (179/196)	Yes	19.4 (38/179)	1			
	No	17.7 (3/17)				
INR (% of time < 2) (47/200)	≥ 40	29.8 (14/47)	0.04*	64.1	79.5	83.7
	< 40	16.3 (25/153)				

* $P \leq 0.10$

Predictor variables shown are ones with 1) strongest association, in the current study, with recurrent VTE and high inter-observer reliability and 2) those previously published that have been suggested to be predictive of recurrent VTE. Predictor variables are dichotomized at the optimal cut-point. NPV= negative predictive value. rVTE= recurrent VTE. BMI= Body Mass Index (weight(kg)/height(M)²). ACL= Anticardiolipin antibody. LAC= Lupus Anticoagulant. CUS= Compression ultrasound

leg vein imaging. COPD= chronic obstructive pulmonary disease. PVO= pulmonary vascular obstruction Score on V/Q Scan (1-total perfusion deficit). V/Q Scan= Ventilation Perfusion Scan. INR= international normalized ratio.

5.5 Phase I – Discussion

To examine whether the clinical manifestation of VTE at first event (index event) affects the risk of recurrence, the entire population was subdivided in three groups according to their index event (index DVT, index PE, and concomitant index PE and DVT). I observed that patients who presented with DVT alone at index event were slightly more likely to recur compared with patients with index PE alone, and concomitant DVT and PE. This relationship became stronger when patients with concomitant DVT and PE were removed and patients with DVT alone were compared with patients with PE alone (OR=2.4 for patient with DVT compared with PE). Clinical studies have found that patients diagnosed with PE alone at index event are more likely to die for recurrent VTE compared with DVT patients (51;53). However, unlike the present study, no difference has been found on the risk of recurrent VTE for patients with PE alone versus patients with DVT alone. Another noteworthy finding similar to Agnelli et al. was that patients with concomitant DVT and PE at index event were at much higher risk of recurrent VTE (OR=2.6) than patients with index PE alone(10). Therefore, having concomitant DVT and PE at index event appears to be a potential positive predictor of recurrent VTE. Because these findings are not conclusive and because attempts to identify individual predictors of recurrent VTE for each VTE sub-group separately (DVT, PE, and DVT&PE) resulted in small sample sizes that affected the study power and the precision to identified potential predictors of recurrent VTE, it was not possible to identify individual potential predictors of recurrent VTE for each index VTE sub-group. More studies need to be done or a bigger sample need to be collected to confirm these

findings. However, many experts now consider DVT and PE to be a single disease because 90% of patients with PE will have DVT at post-mortem examination and 50% of DVT patients without PE symptoms will have PE if imaged (54). Hence, the majority of patients thought to have PE will have DVT and vice versa, making the relevance of separating VTE population into sub-groups questionable. Highlighting this point, I examined the predictive value of the PTS signs in patients with isolated PE and found that it was as predictive of recurrence in PE patients as DVT patients. PTS has traditionally been thought to be a complication of DVT alone (38;55).

From the univariate analysis of the continuous variables in the entire study population, I observed that only three variables (height, weight, and factor VIII) were significant positive predictors of recurrent VTE. After sub grouping the entire population by gender, Factor VIII remained significant for females but not for males, while height and weight were not significant for either the male or female sub-group. This suggests that the height and weight associations were secondary to confounding by gender. Elevated lipoprotein (a), anticardiolipin IgM, increasing BMI and older age were strong positive predictors of recurrent VTE for females alone ($p < 0.05$). For males, I did not find any variable strongly associated with recurrent VTE ($p < 0.05$) and there were only two variables (elevated homocysteine and decreasing age) that were weakly associated with recurrent VTE ($p > 0.05$ and < 0.10).

Among all the dichotomized predictor variables examined in this study, gender was found to be the most powerful predictor of recurrent VTE with increased risk of recurrent VTE for males compared with females. This result agrees with recent studies that have consistently

shown an increased risk of recurrent VTE in males compared with females (35-37). In addition, older men (>60 years of age) were at lower risk of recurrent VTE than younger men. A gender effect was also observed in most predictor variables analyzed in this study as there were significant differences in the univariate association by gender for the main predictor variables. Based on these findings, the univariate analyses of predictive variables were carried out for males and for females independently in order to hopefully identify a low risk group of men who could discontinue anticoagulants.

The PTS signs (hyperpigmentation, redness, pretibial edema, skin induration, venous ectasia) were strong significant positive predictors of recurrent VTE for the entire study population, and were persistently highly significant for the male and also the female sub-groups. Of all the symptoms of PTS, only reported swelling of the leg showed a significant association with recurrent VTE. Combinations of PTS signs were shown to be more predictive of recurrent VTE than single signs. Of the combined PTS signs, HER was found to be the strongest positive predictor of recurrent VTE independent of gender and was selected for further multivariate analysis because of good inter-observer reliability (see below). In patients with HER, the annual rate of recurrent VTE was 23% in males and 11.9% in females. This strong independent association of PTS signs with increased risk of recurrent VTE has only recently been linked to recurrent VTE by a prospective study conducted on DVT patients (38). It makes biological sense that patients with PTS may be more prone to recurrent VTE than those without it, because PTS is a manifestation of venous damage and residual venous obstruction that should predispose patients to recurrent VTE (55). This is a promising finding, as efforts to prevent PTS in unprovoked VTE patients could also reduce the risk of recurrent VTE.

D-Dimer levels are currently used as an important pointer to forgo diagnostic image for acute thrombotic events (56-59) and have been found to be an independent predictor of recurrent VTE after oral anticoagulation withdrawal in patients with a first event of recurrent VTE (26;29;30). However, no study has been done to determine if D-Dimer is a potential predictor of recurrent VTE on patients still on OAT (5 – 7 month after first VTE event). This is important because of the impracticability and risk, especially to high risk patients, of discontinuing anticoagulants for 3-4 weeks then measuring D-Dimer to decide whether to restart anticoagulants. However, given that anticoagulants reduce D-Dimer levels it was uncertain whether D-Dimer on anticoagulants would be predictive of recurrent VTE. I found that elevated D-Dimer (> 250 ug/L) was a significant positive predictor of recurrent VTE for unprovoked VTE females while still on OAT, but I did not find any significant D-Dimer cut-off points for males. This gender difference has never been reported. D-Dimer >250 ug/L was retained for multivariate analysis for females.

Factor VIII over 1.5 U/ml was a significant positive predictor for recurrent VTE in the entire study population and the cut-off of 2 U/ml was a strong significant predictor in the female sub-group. For the male sub-group, factor VIII over 1.5 U/ml was weakly associated with recurrent VTE. This results agree with previous clinical research that have identified elevated Factor VIII as a risk factor for recurrent VTE (25;32). However, this gender difference has never been reported. Factor VIII was not retained for the multivariate analysis due to concerns with inter-assay and inter-laboratory variability (60-63).

PVO score <95% and the combined variable abnormal V/Q scan or PVO score <95% at baseline as measures of residual venous obstruction in the lungs were significant potential predictors of recurrent VTE in the entire study population and in the male sample but not for females. This is a novel finding, however, these variables were not used in the multivariate analysis due to concerns with cost, difficulties of standardization of these techniques across hospitals and that V/Q is not as widely available and is being replaced by CT for PE diagnosis. Regarding residual venous obstruction of the legs at compression ultrasound (CUS), available research reports are conflicting. Some studies have found that residual venous obstruction at CUS is a strong predictor of recurrent VTE in DVT patients (24;27;28) but other studies (64), like this one, have found no association between residual venous obstruction at CUS and recurrent VTE. Thrombus resolution measured by leg vein compression ultrasound was not a significant potential predictor of recurrent VTE for the entire study population in neither females nor males.

Age >40 years was found to be a significant potential positive predictor of recurrent VTE for the entire study population, and older age over 65 years was found to be a significant potential positive predictor of recurrent VTE for females (2.5-fold increased in risk of recurrent VTE, 95% CI 1.1-5.5 for females > 65 years compared with females <65 years of age). Like this study, older age has been consistently identified for several clinical studies as a positive risk factor for VTE (6;7;14;35;65). However, there is no clear explanation for the increase risk of recurrent VTE found in the present study for males under 60 years of age (50% reduced risk of recurrent VTE for men over 60 years of age compared with men under 60 years of age (OR 0.5, 95% CI 0.3-0.9)). It is possible that menopause, andropause and exogenous hormones (oral contraceptives and hormone replacement therapy) play a role in

this difference. Age over 65 years and age under 60 years were retained for multivariate analyses for women and for men respectively.

Extreme obesity (BMI > 48 kg/m²) was a significant positive predictor of recurrent VTE for the entire study population. Mild obesity persisted as a significant positive predictor of recurrent VTE with a cut-off point > 30 kg/m² for the female sub-group. Surprisingly, BMI over 26 kg/m² was a potential negative predictor of recurrent VTE for the male sub-group. Likewise, weight over 100 kg for the overall population or weight over 80 kg for females and weight over 104 kg for males were significant positive predictors of recurrent VTE. Similar to this study, some clinical studies have found a positive association of obesity and increased risk of VTE (65;66). There is no logical explanation for the protective effect of BMI found in the male group (men with BMI over 25 kg/m² are less likely to recur than men with BMI under 25 kg/m²). BMI > 30 kg/m² for females and BMI < 26 kg/m² for males were retained as potential predictors of recurrent VTE to be considered in the multivariate analysis.

History of hypercholesterolemia in the last year, lipoprotein (a) over 0.5, and statin medication used in the last year were strong potential predictors of recurrent VTE in females but not in males. This is another novel finding, as hypercholesterolemia has not been previously described as a potential predictor of recurrent VTE. Some studies had found a strong correlation between BMI and hypercholesterolemia in DVT patients and had suggested that lack of continuous physical activity, that characterizes obese subjects, may play a role in the association of hypercholesterolemia and increase risk of DVT (67).

However, this is not a likely explanation in this case, as I did not find an association between history of hypercholesterolemia and obesity (BMI > 30 kg/m²). Fibrinogen and plasminogen

activator inhibitor type 1 (PAI-1) have been associated with obesity and hypercholesterolemia in DVT patients, and it has been suggested that hypercholesterolemia may increase the risk of thromboembolism partly mediated through the effects of fibrinogen and PAI-1 on hemostasis (67). Hypercholesterolemia was not retained for the multivariate analysis due to concerns with the accuracy of the self-reported data (68-70). We did not have the appropriate blood samples to test for cholesterol levels (fasting serum samples). Furthermore, given that this was a novel finding the study steering committee had concerns that it lacked the face validity and that this may hamper acceptance of a CPR including the variable history of hypercholesterolemia. Studies with collection of blood samples to appropriately measure hypercholesterolemia in patients similar to this study need to be conducted to confirm this finding.

Height over 172 cm in the entire study population and over 188 cm in the male sub-group were shown to be significant positive predictors of recurrent VTE, results that differed from the female sub-group where height over 166 cm was found to be a significant negative predictor of recurrent VTE. Height has not been previously reported as a predictor for VTE or recurrent VTE. Height may play a role in the increase risk of recurrent VTE in males and may be worth investigating in future studies. However, it is likely that the effect of height on recurrent VTE is explained by gender as there was a 72% Spearman correlation between height and gender. Height over 188 cm was retained for multivariate analysis in the male sub-group.

Oral contraceptive therapy is a known positive risk factor for VTE (71), however I found that history of oral contraceptive use in the last year in the female sub-group was a negative

predictor of recurrent VTE. This protective effect is explained by the common clinical practice of removing oral contraceptive therapy (a known risk factor for VTE) from women after a first episode of VTE to reduce the risk of recurrence, once oral contraceptive therapy was removed, the increase risk of VTE contributed by oral contraceptive therapy appears to have been removed. Consequently, withdrawal from oral contraceptive therapy might have reduced the risk of recurrence. Regarding hormone replacement therapy (HRT), I did not find any association of history of HRT in the last year and recurrent VTE in women. Hormone replacement therapy has been recognized as a risk factor for VTE (72) but no studies have reported an association of HRT and recurrent VTE. Oral contraceptive therapy used in the last year was retained for multivariate analysis in the female sub-group.

The combined variable antiphospholipid antibodies (positive lupus anticoagulant or anticardiolipin antibodies IgM-IgG >6 U/ml) was a significant potential positive predictor variable for men but not for women. This gender difference has not been previously reported. The presence of antiphospholipid antibodies (APLA) is known to be one of the main hypercoagulable states associated with VTE (73) and, as also found in the present study, APLA has been shown to be associated with an increased risk of recurrence in DVT patients (74). This finding is limited by the fact that patients who were persistently positive for antiphospholipid antibodies (APLA) were excluded from the present study, thus the number of patients that were positive for APLA was reduced. Nonetheless, the combined APLA variable was retained for further multivariate analysis for male patients because it was one of the few potential predictor variables found for this sub-group.

In a search for additional risk factors for recurrent VTE that have not been previously studied, hemoglobin over 120 g/l was found to be a significant negative predictor of recurrent VTE for females, while hemoglobin over 170 g/L was found positively associated with recurrent VTE for males. Although the hemoglobin cut-off for females may not be useful to segregate patients at high or low risk of recurrent VTE as the cut-off of 120 g/L is within the normal range for the average female (normal lab ranges for females 115-155 g/l), hemoglobin was used in the multivariate analysis for the female and male sub-groups.

Percentage of INR levels under 2.0 during more than 40% of the time on OAT was a weak positive predictor of recurrent VTE ($p=0.09$) for the entire population and a significant positive predictor for males ($p=0.04$). This finding agrees with previous studies that have reported a 2.7-fold increase in risk of recurrent VTE on patients with INR under 1.5 compared with INR over 1.5 (5). It has been suggested that long periods of sub therapeutic INR might help to identify patients at high risk of recurrent VTE. Sub therapeutic INR was not included in the multivariate analysis due to an important percentage of missing data (40% of the total study population).

There was no association of history of previous secondary VTE and recurrence for the entire population or for the male sub-group, however, there was a significant positive association of secondary VTE and recurrence in the female group, therefore, history of previous secondary VTE was kept for multivariate analysis in the female sub-group.

I found no association between heterozygous carriers of prothrombin gene mutation or Factor V Leiden for the entire population, or for male or female sub-groups separately.

Heterozygous carriers for the inherited defects of coagulation such as carriers of mutations in the prothombin gene (prothrombin gene mutation) and Factor V Leiden mutation are known to be at high risk of VTE and some studies have also linked the presence of these gene mutations with an increase risk of recurrent VTE (75), but other studies like this one have found no association between Factor V Leiden or prothrombin gene mutation heterozygote carriers and recurrent VTE (76).

Chronic respiratory diseases (COPD or emphysema) were found to be significant negative predictors of recurrent VTE for the entire study population. However, there was not a logical explanation for this finding. I did not find an association of chronic respiratory diseases and recurrent VTE for either the male or female sub-group.

To summarize, a total of 13 potential predictor variables for females and 10 variables for males were found significantly associated ($p < 0.10$) with recurrent VTE, however, only 7 variables for females and 6 variables for males were brought forward to multivariate analysis for the reasons explained above. The PTS signs variable "HER" was found to be a strong predictor of recurrent VTE and it was the only variable found to be significantly associated with recurrent VTE for both males and females separately. The significant cut-off points for age, BMI and hemoglobin varied with gender distribution. D-Dimer was a significant predictor of recurrent VTE for females but not for males and the combined variable abnormal lupus anticoagulant and anticardiolipin antibodies was a significant univariate predictor of recurrent VTE for males but not for females.

6. PHASE II - INTER-OBSERVER RELIABILITY OF SIGNIFICANT UNIVARIATE PREDICTORS

To ensure reproducibility of the rule, the reproducibility of the univariate predictors included in the final CPR (phase IV) must first be demonstrated. The REVERSE steering committee decided that only PTS signs and symptoms, baseline compressive ultrasound assessment of residual thrombosis and PVO scoring for determining baseline residual pulmonary embolism required further inter-observer reliability study. All the other variables that were found to be significant in Phase I have been determined to have good inter-observer reliability (age, gender, BMI (77), Vidas D-Dimer (77;78), hemoglobin(79;80). Intra/inter-assay and inter-method variation was a concern for Factor VIII (60-63) and anticardiolipin antibodies (81-83). In the expert opinion of the steering committee elevated levels of Factor VIII is likely to have poor inter-observer reliability and was not brought forward to multivariate analysis (Phase III).

6.1 Phase II - Objectives

To determine the inter-observer reliability of the predictive clinical variables at their optimal cut-off points that have not already been validated in previous studies and that were found to be significant predictors of recurrent VTE in the univariate analysis (Phase I) ($P < 0.10$).

6.2 Phase II - Patients and methods

A sub-sample of 125 patients of the derivation set had duplicated independent examinations of post-thrombotic signs and symptoms. As this was a sub-sample of the REVERSE study,

the same inclusion and exclusion criteria for the main study applied to the inter-observer reliability sample (see item 4.0 for data collection).

6.2.1 Standardized patient assessment

Consecutive, duplicated, independent examinations of PTS signs at baseline for each patient were conducted by two observers (previously trained research nurses). The PTS cut-off points examine for inter-observer reliability were the cut-off points for PTS signs that were found to be significant predictors of recurrent VTE in Phase I.

6.3 Phase II - Data analysis

The inter-observer reliability of each significant PTS cut-off point was determined by calculating a two rater unweighted Kappa statistic. Weighted Kappa statistic was not required as all the significant cut-off points for the PTS signs variables (none, mild, moderate or severe) were dichotomized in Phase I (84). Kappa coefficients and 95% confidence intervals for all reasonable combination of the PTS signs significant in phase I were calculated. Determinants of the magnitude of Kappa (prevalence index, bias index and prevalence-adjusted bias-adjusted kappa (PABAK) were calculated for all the PTS signs cut-off points with Kappa coefficient estimates >0.60.

Kappa (K) indicates the proportion of agreement beyond what is expected by chance if the raters' scores were statistically independent (84). That is:

$$K = \frac{Po - Pc}{1 - Pc}$$

Where P_o is the proportion of observed agreement:

$$P_o = \frac{(a + d)}{n}$$

And P_c is the proportion of agreement expected by chance:

$$P_c = \frac{(a + c)(a + b)/n + (b + d)(c + d)/n}{n}$$

According to Landis and Koch (1977), an almost perfect agreement is considered for Kappa estimates of 1 to 0.81, substantial agreement for Kappa estimates of 0.80 to 0.61, moderate agreement for Kappa estimates of 0.41 to 0.60 and poor agreement for Kappa estimates of 0.40 or less (85). In the present study, the variables with Kappa > 0.60 were considered to have enough agreement to be included in the CPR. Exact binomial 95% confidence intervals were calculated for each Kappa, however, the effects of prevalence and bias on Kappa must be considered when judging the kappa coefficient magnitude (see Table 10 below for an example of a 2X2 table used for kappa calculations)(84).

Determinants of the magnitude of Kappa coefficient estimate.

Prevalence effect exists when the proportion of agreement on the presence of PTS signs differs from that of the absence of PTS signs and it is calculated by the formula:

$$Prevalence_Index = \frac{|a - d|}{n}$$

If prevalence index is high, chance agreement is also high and kappa is reduced accordingly. Bias is the extent to which raters disagree on the proportion of positive PTS signs or negative PTS signs and is calculated by the formula:

$$\text{Bias_Index} = \frac{|b - c|}{n}$$

Kappa was adjusted for high or low prevalence by computing the average of cells (a) and (d) and substituting this value for the actual values in those cells. Kappa was also adjusted for bias by substituting the mean of cells (b) and (c) for the actual cell values. When there is a large bias, kappa is higher than when bias is low or absent. PABAK gives an indication of the likely effects of prevalence and bias (84).

6.4 Phase II - Results

The highest Kappa estimates were identified for the variables “any edema, hyperpigmentation or redness either lower extremity” (Kappa = 0.73), “any moderate or severe edema, hyperpigmentation or redness either lower extremity” (Kappa = 0.74) and “any edema, hyperpigmentation, redness or skin induration either lower extremity” (Kappa = 0.71) which indicate substantial agreement between the two observers (Table 10-12). Most variables for the cut-off point severe PTS signs versus none, mild or moderate PTS signs did not have enough information to compute kappa coefficient (low prevalence of severe PTS signs) while most of the comparisons of moderate or severe PTS signs versus none of mild PTS signs had a Kappa estimate >0.60.

Table 10. 2X2 contingency table for inter-observer reliability of the PTS predictor variable “HER” (any edema, hyperpigmentation or redness either low extremity) evaluated by two observers.

Table “any edema, hyperpigmentation or redness either lower extremity”			
Observer 1	Observer 2		Total
	Yes	No	
Yes	(a) 41	(b) 6	47 (a+b)
No	(c) 10	(d) 68	78 (c+d)
Total	51 (a+c)	74 (b+d)	125 (a+b+c+d)

Calculations of Kappa coefficient for the predictor variable “HER” based on the results of Table 10:

$$P_o = \frac{41 + 68}{125} = 0.87$$

$$P_c = \frac{(51 \times 47) / 125 + (74 \times 78) / 125}{125} = 0.52$$

$$K = \frac{0.87 - 0.52}{1 - 0.52} = 0.73$$

Table 11. Post-thrombotic signs Kappa estimates.

PTS cut-off points	Entire study population (n/N)**	P1%	P2%	Kappa	95% CI
Right leg edema (any)	98/544	16.8	20.0	0.41	0.21-0.62
Left leg edema (any)	104/540	19.2	19.2	0.43	0.23-0.63
Either leg edema (any)	149/545	23.2	27.2	0.39	0.20-0.57
Right leg edema (moderate or worse)	20/544	3.2	2.4	0.85*	0.57-1.0
Left leg edema (moderate or worse)	29/540	3.2	3.2	0.48	0.05-0.91
Either leg edema (moderate or worse)	40/544	4.8	4.0	0.71*	0.41-1.00
Right leg edema (severe)	2/545	0.0	0.0	--	--
Left leg edema (severe)	3/540	0.8	0.0	--	--
Either leg edema (severe)	4/544	0.8	0.0	--	--
Right leg induration (any)	38/545	7.2	8.0	0.37	0.08-0.67
Left leg induration (any)	41/539	9.6	9.6	0.45	0.18-0.71
Either leg induration (any)	58/539	12.8	12.0	0.30	0.06-0.54
Right leg induration (moderate or worse)	9/544	0.8	1.6	0.66*	0.04-1.0
Left leg induration (moderate or worse)	9/539	0.8	0.8	1.0	1.0
Either leg induration (moderate or worse)	12/545	1.6	0.8	0.66*	0.04-1.0
Right leg induration (severe)	3/545	0.0	0.0	--	--
Left leg induration (severe)	4/539	0.0	0.0	--	--
Either leg induration (severe)	5/545	0.0	0.0	--	--
Right leg hyperpigmentation	73/544	11.2	10.4	0.46	0.21-0.71

(any)					
Left leg hyperpigmentation (any)	69/539	11.2	13.6	0.60	0.39-0.81
Either leg hyperpigmentation (any)	93/544	14.4	14.4	0.48	0.26-0.70
Right leg hyperpigmentation (moderate or worse)	20/544	4.0	2.4	0.48	0.05-0.92
Left leg hyperpigmentation (moderate or worse)	19/539	5.6	7.2	0.60	0.31-0.89
Either leg hyperpigmentation (moderate or worse)	28/544	5.6	7.2	0.60	0.31-0.89
Right leg hyperpigmentation (severe)	1/544	0.8	0.8	1.0	1.0
Left leg hyperpigmentation (severe)	3/539	0	0.8	--	--
Either leg hyperpigmentation (severe)	3/544	1.6	0.8	0.66*	0.04-1.0
Right left redness (any)	50/545	14.4	11.2	0.57	0.35-0.77
Left leg redness (any)	52/541	16.8	16.0	0.56	0.37-0.76
Either leg redness (any)	71/545	20.8	16.8	0.56	0.37-0.74
Right leg redness (moderate or worse)	10/545	1.6	0.8	0.66*	0.04-1.0
Left leg redness (moderate or worse)	10/541	0.0	0.8	--	--
Either leg redness (moderate or worse)	13/545	2.4	0.8	0.49	-0.1-1.0
Right leg redness (severe)	2/545	0.0	0.0	--	--
Left leg redness (severe)	3/541	0.0	0.0	--	--
Either leg redness (severe)	3/545	0.0	0.0	--	--
Any edema, hyper pigmentation or redness either leg	215/545	37.6	40.8	0.73*	0.61-0.85
Edema, hyper pigmentation or redness either leg (moderate or worse)	67/545	10.4	10.4	0.74*	0.55-0.94

Edema, hyper pigmentation or redness either leg (severe)	7/544	1.6	1.6	0.49	-0.1-1
Any edema, hyper pigmentation, induration or redness either leg	215/545	42.4	44.8	0.69*	0.56-0.82
Any edema, hyper pigmentation, induration or redness either leg (moderate or worse)	69/545	11.2	10.4	0.71*	0.50-0.91
Edema, hyper pigmentation, induration or redness either leg (severe)	7/544	1.6	1.6	0.49	-0.1-1

***Kappa >.60**

**** n/N= recurrent VTE cases / Entire study population (derivation set) for PTS signs prevalence estimation in the derivation set.**

All Kappa and 95% CI estimates are calculated from the inter-observer reliability set with a total of 125 patients (sub sample of the derivation dataset)

PTS= post-thrombotic signs. P1= Observer #1. P2=observer #2. CI (confidence interval).

To correctly interpret the kappa coefficient results, I calculated the prevalence index, bias index and prevalence-adjusted bias-adjusted kappa (PABAK) for all the PTS signs cut-offs with Kappa > 0.60 (Table 12). Based on these results and also the prevalence of PTS signs on the entire study population the PTS combined variable “any edema, hyperpigmentation or redness either lower extremity” showed to have the highest inter-observer reliability (Kappa = 0.73), smallest prevalence and bias index estimates and the smallest variation between PABAK and Kappa coefficient estimates (0.87 versus 0.73).

Table 12. Determinants of the magnitude of Kappa coefficient for the PTS sign cut-offs with Kappa > 0.60.

	Prevalence Index	Bias Index	Adjusted Kappa (PABAK)	Kappa Coefficient
Right leg edema (moderate or worse)	0.94	0.01	0.95	0.85
Either leg edema (moderate or worse)	0.911	0.01	0.98	0.71
Right leg induration (moderate or worse)	0.98	0.01	0.98	0.66
Either leg induration (moderate or worse)	0.98	0.01	0.98	0.66
Either leg hyperpigmentation (severe)	0.98	0.01	0.98	0.66
Right leg redness (moderate or worse)	0.98	0.01	0.98	0.66
Any edema, hyper pigmentation or redness either leg-HER	0.22	0.03	0.87	0.73
Edema, hyper pigmentation or redness either leg (moderate or worse)	0.79	0.00	0.90	0.74
Any edema, hyper pigmentation, induration or redness either leg	0.13	0.02	0.70	0.69
Any edema, hyper pigmentation, induration or redness either leg (moderate or worse)	0.78	0.01	0.89	0.71

PABAK=prevalence-adjusted bias-adjusted kappa

Prevalence index, Bias index, Adjusted Kappa and Kappa coefficient results shown are only those for the PTS signs cut-offs with Kappa coefficient >0.60 (see Table 8).

Among the individual and combined signs of PTS with Kappa coefficient > 0.60, the PTS combined variable HER showed the smallest prevalence index, small bias index, and the adjusted Kappa for bias and prevalence did not greatly differed (0.73 to 0.87).

6.5 Phase II – Discussion

Most of the individual PTS signs that were significant predictors of recurrent VTE in Phase I (any degree of edema, induration, and hyperpigmentation) had poor inter-rater agreement.

The exception to this was the PTS sign any degree of redness of either leg which showed moderate agreement. Most combinations of PTS signs were found to have a substantial inter-rater reliability. It was also found that moderate post-thrombotic signs have better reproducibility than mild signs, however there was insufficient data to identify inter-rater reliability on severe PTS signs as the prevalence of severe signs was very low in the present study.

The combined variable HER was selected to be included in the multivariate analysis because it showed substantial reproducibility, it is a practical variable (high prevalence of any edema, hyperpigmentation or redness in the entire study population) and it is easy to assess in the clinical setting. In practice a combination of various PTS signs are usually present, as opposed to a single PTS sign, and could be persistent or intermittent (55).

This is the first study evaluating reproducibility of PTS signs in VTE patients and may contribute to enhancing the ability of researchers to compare results of different studies. This may promote further research on one of the most common complications of DVT patients.

7.0 PHASE III - MULTIVARIATE DERIVATION AND CLASSIFICATION PERFORMANCE OF THE CLINICAL PREDICTION RULES

7.1 Phase III - Objectives

The objectives of phase II of the study were:

- 1) Using multivariate techniques, to derive clinical prediction rules (for females and for males respectively) applicable to patients at 5 to 7 months post-diagnosis of a first episode of unprovoked VTE (while still on oral anticoagulant medication) that will identify patients at low risk of recurrent VTE that can safely discontinue OAT.

- 2) To assess the classification performance of the derived clinical prediction rules.

7.2 Phase III - Sample size

As per accepted methodological criteria for the development of clinical prediction rules, 5-10 patients per each predictor variable studied are required in the smallest outcome category (44;45). The smallest outcome category was recurrent VTE. To include approximately five predictors, it was originally calculated that the study required a minimum of 25 patients and ideally 50 patients with recurrent events to avoid over fitting multivariate models. After splitting the overall sample by gender I had 28 recurrent events for females and 63 recurrent events for males that allowed the inclusion of 5 predictor variables for male and female sub-groups.

7.3 Phase III - Variable selection

7.3.1 Variable selection for females

For females, I identified 13 variables that met the selection criteria of association with recurrent venous thromboembolism ($P \leq 0.10$). Within those, lipoprotein (a), history of hypercholesterolemia, statin therapy use in the last year and elevated Factor VIII were not included in the multivariate analysis because of concerns already explained in Phase I. Weight and BMI were highly positively correlated (Pearson correlation = 94%; $p < .001$) as well as weight and height (Pearson correlation = 31.8%; $p < 0.001$). Therefore, I did not include weight over 80 kg and height under 166 cm in the logistic regression analysis.

There were seven potential predictor variables that met the selection criteria of association with recurrent VTE ($P < 0.10$), had good reproducibility (Kappa > 0.60), and were selected by the study steering committee for inclusion in the logistic regression analysis for the female sub-group (Table 13).

Table 13. Dichotomized variables selected for multivariate analysis in the female sub-group (P-value, sensitivity, specificity and NPV).

Predictor variable (n/N)	Cut-off point	rVTE incidence (%) (n/N)	Chi-square	P-value	Sensitivity (%)	Specificity (%)	NPV (%)
Age (years) (87/314)	=>65	14.9 (13/87)	5.4	0.02*	46.4	74.1	93.4
	<65	6.6 (15/227)					
BMI (114/313)	=>30	14.0 (16/114)	5.7	0.02*	57.1	65.6	94.0
	<30	6.0 (12/199)					
D-Dimer (ug/L) (121/304)	>=250	14.9 (18/121)	8.9	0.003*	66.7	62.8	95.1
	<250	4.9 (9/183)					
Hemoglobin (g/L) (269/311)	>=120	7.1 (19/269)	F	0.02*	29.6	12.0	81.0
	<120	19.1 (8/42)					
Oral contraceptive use (within last year) (79/253)	Yes	3.8 (7/79)	3.1	0.08*	14.3	67.2	90.0
	No	10.3 (18/174)					
Previous secondary VTE (13/314)	Yes	30.8 (4/13)	F	0.02*	14.3	96.9	92.0
	No	8.0 (24/301)					
PTS-HER (96/260)	Any	16.7 (16/96)	8.7	0.003*	64.0	66.0	94.5
	No	5.5 (9/164)					

*p>0.10

rVTE = recurrent VTE. PTS-HER = Post-thrombotic signs (either leg any edema, hyper-pigmentation or redness). F = Fishers exact. NPV = negative predictive value.

7.3.2 Variable selection for males

For males I identified 10 variables that met the selection criteria of association with recurrent VTE (P<0.10). Within those, elevated Factor VIII, PVO score under 95% and abnormal V/Q scan were excluded for the male multivariate analysis due to concerns already explained in Phase I.

There were six potential predictor variables that met the selection criteria of association with recurrent VTE ($P < 0.10$), had good reproducibility ($Kappa > 0.60$) and were selected for inclusion in the logistic regression analysis for the male sub-group (Table 14).

Table 14. Dichotomized variables selected for multivariate analysis in the male sub-group (P-value, sensitivity, specificity and NPV).

Predictor variable (n/N)	Cut-off point	rVTE incidence (%) (n/N)	Chi-square	P-value	Sensitivity (%)	Specificity (%)	NPV (%)
Age (years) (126/332)	$\Rightarrow 60$	12.7 (16/126)	5.2	0.02*	25.4	59.1	77.2
	< 60	22.8 (47/206)					
BMI (236/331)	$\Rightarrow 26$	16.5 (39/236)	2.6	0.10	62.9	26.8	75.8
	< 26	24.2 (23/95)					
Height (cm) (33/331)	$\Rightarrow 188$	30.3 (23/33)	3.2	0.07*	16.1	91.5	82.6
	< 188	17.5 (52/298)					
Hemoglobin (g/L) (9/329)	≥ 170	44.4 (4/9)	F	0.07*	6.5	98.1	81.9
	< 170	18.1 (58/320)					
ACL positive (IgG >6 or IgM >6) or LAC positive) (158/315)	≥ 6 or LAC+	24.7 (39/158)	6.5	0.01*	65.0	53.3	86.6
	< 6 or LAC-	13.4 (21/157)					
PTS-HER (119/285)	Any	26.1 (31/119)	12.4	0.0004*	64.6	62.9	89.8
	No	10.2 (17/166)					

* $p > 0.10$

rVTE=recurrent VTE. F=Fishers exact. NPV=negative predictive value. BMI=Body Mass Index (Weight(kg)/Height(M)²). ACL = Anticardiolipin antibody. LAC=Lupus Anticoagulant. LAC+ =LAC present. LAC- =LAC absent. PTS-HE =Post-thrombotic signs (either leg any edema, hyper-pigmentation or redness).

7.4 Phase III - Data analysis

Due to significant gender differences found in the preliminary analysis of potential predictor variables selection under Phase I, analyses for the derivation of clinical prediction rules were carried out independently for males and females. Multivariate analyses were conducted

using logistic regression to identify candidate clinical prediction rules. Classification performance of the rules was then assessed to determine the safety (% risk of recurrent VTE in the low risk group, % risk of recurrent VTE in the high risk group) and utility (excluded proportion of patients at low risk) of the candidate rules.

The REVERSE steering committee (whose names and expertise were previously mentioned in study design, section 4.) played an important role in the final variable and model selection.

7.4.1 Logistic regression

From the derivation subsets for males and females, multiple CPRs were derived using logistic regression analysis. The CPRs were based on those variables that were both significant in the univariate analysis ($p < 0.10$) in Phase I and had good inter-observer reliability ($Kappa > 0.6$) in Phase II as per accepted methodological criteria (44-48). Forward selection was used for model building with recurrent VTE as the dependent variable. The predictor variables were added one at a time creating nested models (with the potential predictor variables entered in the model in order of strength of univariate association), and the reduction in deviance statistic (equivalent to increasing maximum likelihood between nested models) was used with each successive model to test whether each new predictor variable added information and significantly improved the model fit. The smallest models with minimal prediction error were selected as plausible models.

The Hosmer Lemeshow lack of fit test was performed on the final models to assess goodness of fit.

7.4.2 Classification performance

The regression coefficients of each model were used to weight the predictors. Each predictor variable in the model was assigned an integer point score proportional to each variable regression coefficient, and then the best predictive point score cut-off for each model was identified. The variable's weight was simplified by rounding the regression coefficients to the nearest integer to make the CPR easy to use and remember.

Next, the patient's predicted state at the best point score cut-off for each model was compared with the true state by 2X2 contingency tables to determine the risk of recurrent VTE in the low risk group (false negative), risk of recurrent VTE in the high risk group and sensitivity (true positives), rate of low risk excluded proportion $((c+d)/(a+b+c+d))$ as shown in Table 28 below, specificity, and negative predictive value (true negatives). Annual risks of recurrent VTE were calculated for the low and high risk groups by dividing absolute risk of recurrent VTE in the sub-groups by the mean follow-up time in respective sub-groups yielding annual rates of recurrent VTE. Based on these parameters, the classification performance of competing logistic models were assessed in order to identify the best combination of predictor variables to produce a safe and clinically useful prediction rule that accurately classifies patients according to the risk of recurrent VTE.

In selecting the final rule, we attempted to simultaneously optimize several criteria. We wanted the largest number in the low-risk group (highest excluded proportion), the lowest recurrence rate in the low-risk group (at most 3% per year), and the highest recurrence rate in the high risk group (over 10% per year). At the same time, we wanted the rule to have good face validity, and to have a practical number of predictor variables that would be easy to remember and apply. The following section describes our sequence of decisions more precisely.

7.5 Phase III - Results

7.5.1 Model selection for females

Multiple regression analysis

The seven potential predictor variables selected from the univariate analyses (Phase I) for females were entered in the model in order of strength of univariate association as shown in Tables 15-18, and the reduction in deviance statistic was used to test whether each new predictor variable added information and significantly improved the model fit.

To calculate the Deviance statistic, a total of 77 patients from the female dataset were automatically removed from the logistic models due to at least one missing covariate from the model (leaving a total of 228 patients including 25 recurrent VTE events).

An automated forward stepwise logistic regression yielded the following clinical prediction model

$$rVTE = -4.3043 + 1.5851(BMI > 30kg / M^2) + 1.8158(DD > 250ug / L).$$

However, this model did not include the predictor variable post-thrombotic signs HER. This variable was considered an important potential predictor variable by the REVERSE steering committee because it was the strongest independent predictor of recurrent VTE in the univariate analysis.

Female Model 1:

Table 15. Model 1: steps in model building for females using logistic regression with predictor variables entered in order of strength of association.

Model	N	DF	Deviance (-2Log L)	Δ Deviance $X^2_{0.05}$
HER	228	2	122.017	8.80>5.99*
HER+DD>250	228	1	116.233	5.78>3.84*
HER+DD>250+PSVTE	228	1	114.900	1.30<3.84
HER+DD>250+Hb<120	228	1	113.309	2.90<3.84
HER+DD>250+BMI>30	228	1	107.774	8.45>3.84*
HER+DD>250+BMI>30+Age>65	228	1	107.765	0.009<3.84
HER+DD>250+BMI>30+OCP	228	1	106.241	1.524<3.84
HER+DD>250+BMI>30+PSVTE	228	1	106.722	1.052<3.84
HER+DD>250+BMI>30+PSVTE+Age>65	228	1	106.676	0.046<3.84

*Significant likelihood ratio statistic (cut-off p-value 0.05).

DF= Degrees of freedom. HER=post-thrombotic signs (any edema, hyperpigmentation or redness either leg). DD>250=D-Dimer over 250 ug/L. PSVTE=previous secondary venous thromboembolism. Hb<120=hemoglobin over 120 g/L. BMI>30=body mass index over 30 kg/m². Age>65=age over 65 years. OCP=oral contraceptive pill history in the last year.

The addition of the variables history of previous secondary VTE (PSVTE), hemoglobin under 120 g/L, age over 65 years and oral contraceptive therapy in the last year did not significantly improve the model fit. The predictor variables PTS signs (HER), D-Dimer over 250 ug/l and BMI over 30kg/m² substantially improved the model fit and were important independent predictors of recurrent VTE (Table 15). Consequently, the logistic model:

$$rVTE = -3.7800 + 0.7365(HER) + (1.2527(D - \text{dimer} > 250\mu\text{g} / \text{l}) + (1.0830(BMI > 30\text{kg} / \text{m}^2))$$

hereafter referred to as “CPR1” was selected for further classification performance analyses.

Since the Hosmer and Lemeshows test for CPR1 was not significant (p=0.64), there is no reason to suspect lack of fit for this model.

Female Model 2:

Table 16. Model 2: steps in model building for females using logistic regression with predictor variables entered in order of strength of association.

Model	N	DF	Deviance (-2Log L)	Δ Deviance $X^2_{0.05}$
HER	228	2	122.017	8.80>5.99*
HER+DD>250	228	1	116.233	5.78>3.84*
HER+DD>250+BMI>30	228	1	107.774	8.45>3.84*
HER+DD>250+BMI>30+Age>65	228	1	107.765	0.009<3.84

*Significant likelihood ratio statistic (cut-off p-value 0.05).

DF= Degrees of freedom. HER=post-thrombotic signs (any edema, hyperpigmentation or redness either leg). DD>250=D-Dimer over 250 ug/L. BMI>30=body mass index over 30 kg/m².

The predictor variables PTS signs (HER), D-Dimer over 250 ug/l and BMI over 30kg/m² substantially improved the model fit and were important independent predictors of recurrent VTE. Age over 65 years did not significantly improve the model fit (Table 16), however, this variable was included in the model because it was a potential predictor variable of primary interest in this study. Therefore, the logistic model:

$$rVTE = -39717 + 1.2977(BMI > 30\text{kg} / \text{m}^2) + 0.6473(HER) + 0.9155(D - \text{dimer} > 250\mu\text{g} / \text{L}) + 0.8084(\text{Age} > 65\text{years})$$

hereafter referred to as “CPR 2” was selected for further classification performance analysis.

Hosmer and Lemeshows test for CPR 2 was not significant (p=0.45), suggesting reasonable fit for this model.

Female Model 3:

Table 17. Model 3: steps in model building for females using logistic regression with predictor variables entered in order of strength of association.

Model	N	DF	Deviance (-2Log L)	Δ Deviance $X^2_{0.05}$
HER	228	2	122.017	8.80>5.99*
HER+DD>250	228	1	116.233	5.78>3.84*
HER+DD>250+BMI>30	228	1	107.774	8.45>3.84*
HER+DD>250+BMI>30+PSVTE	228	1	106.722	1.052<3.84

*Significant likelihood ratio statistic (cut-off p-value 0.05).

DF= Degrees of freedom. HER=post-thrombotic signs (any edema, hyperpigmentation or redness either leg). DD>250=D-Dimer over 250 ug/L. PSVTE=previous secondary venous thromboembolism.

The predictor variables PTS signs (HER), D-Dimer over 250 ug/l and BMI over 30kg/m² substantially improved the model fit for the CPRs1 and 2, and were important independent predictors of recurrent VTE. Previous secondary VTE did not significantly improve the model fit (Table 17), however, this variable was included in the model because it was a potential predictor variable with good face validity. The logistic model

$$rVTE = -3.8751 + 1.0687(BMI > 30kg / m^2) + 0.7711(HER) + 1.1679(D - dimer > 250ug / L) + 1.6600(Previous_secondaryVTE)$$

hereafter referred to as “CPR 3” was selected for further classification performance analyses.

Hosmer and Lemeshows test for CPR 3 was not significant (p=0.45), again suggesting reasonable model fit.

Female Model 4:

Table 18. Model 4: steps in model building for females using logistic regression with predictors enter in order of strength of association.

Model	N	DF	Deviance (-2Log L)	Δ Deviance $X^2_{,0.05}$
HER	228	2	122.017	8.80>5.99*
HER+DD>250	228	1	116.233	5.78>3.84*
HER+DD>250+BMI>30	228	1	107.774	8.45>3.84*
HER+DD>250+BMI>30+PSVTE	228	1	106.722	1.052<3.84
HER+DD>250+BMI>30+PSVTE+Age>65	228	1	106.676	0.046<3.84

*Significant likelihood ratio statistic (cut-off p-value 0.05).

DF= Degrees of freedom. HER=post-thrombotic signs (any edema, hyperpigmentation or redness either leg). DD>250=D-Dimer over 250 ug/L. PSVTE=previous secondary venous thromboembolism. Hb<120=hemoglobin over 120 g/L. BMI>30=body mass index over 30 kg/m². Age>65=age over 65 years.

As previously seen, the predictor variables BMI>30 kg/m², PTS signs (HER) and D-Dimer>250ug/l were important independent predictors of recurrent VTE, while, age over 65 years and history of previous secondary VTE did not significantly add information to the model (Table 18), however, these two variables were included into model # 4 because they were variables of primary interest in this study and considered important significant potential predictors of recurrent VTE by the steering committee. The logistic model:

$$rVTE = -4.0733 + 1.2945(BMI > 30kg / M^2) + 0.6416(HER) + 0.8455(D - dimer > 250ug / L) + 1.6723(Previous_secondaryVTE) + (0.8326(age > 65))$$

hereafter referred to as “CPR 4” was selected for further classification performance analyses.

Hosmer and Lemeshows test for the CPT 4 was not significant (p=0.94), indicating a good fit for this model.

Female Model 5:

An additional model explored for females was the following:

$$rVTE = -3.5697 + 0.9362(D - \text{dimer}250\mu\text{g}) + 1.0833(\text{BMI} > 30\text{Kg} / \text{M}^2) + 0.7444(\text{age} > 65\text{years})$$

Model 5: steps in model building for females using logistic regression (model without HER).

Model	rVTE/N	DF	Deviance (-2Log L)	Δ Deviance $X^2_{.05}$
DD250	27/303	2	173.221	8.9 > 5.99*
DD250 + BMI30	27/303	1	168.109	5.1 > 3.84*
DD250 + BMI30 + ageover65	27/303	1	165.766	2.3 < 3.84*

*Significant likelihood ratio statistic (cut-off p-value 0.05).

DF= Degrees of freedom. DD>250=D-Dimer over 250 ug/L. BMI>30=body mass index over 30 kg/m². Age>65=age over 65 years.

Classification performance for the five female CPRs derived by multiple logistic regression.

The regression coefficients were used to weight the variables for each of the five female models described above. Each variable within the model was assigned a proportional point score (regression coefficient rounded to the nearest integer) and the best predictive point score (with the smallest recurrent VTE in the low risk group and the highest low risk excluded proportion) for each model was identified (Tables 19). The first four CPRs showed high negative predictive value, less than 3% per year recurrent VTE in the low risk group (safety) and more than 10% per year risk of recurrent VTE in the high risk group. The CPRs 2 and 4 had the highest excluded proportion and the smallest yearly risk of recurrent VTE in the low risk group (Table 19).

Table 19. Classification performance and cut-off points of the CPRs for females.

	SW Forward Selection Model	CPR # 1	CPR #2	CPR # 3	CPR # 4	CPR #5
Cut-off	< 1	<2	<2	<2	<2	<1
# variables	2	3	4	4	5	3
Sensitivity	0.81	0.88	0.88	0.76	0.88	0.89
Specificity	0.40	0.38	0.57	0.65	0.56	0.37
NPV	0.96	0.97	0.98	0.96	0.98	0.97
% rVTE per year (low risk group)	2.4	2.3	1.6	2.9	1.7	1.6%
% rVTE per year (high risk group)	7.8	10.4	14.1	14.8	13.8	7.9%
Low risk excluded proportion	38.3	35.46	52.2	38.7	51.4	34.7%

CPR= clinical prediction rule. NPV=negative predictive value. rVTE= recurrent venous thromboembolism.

7.5.2 Model Selection for males

Multiple regression analysis

The six potential predictor variables selected from univariate analyses (Phase I) for males were entered in the model in order of strength of univariate association as shown in Tables 20-24. The reduction in deviance statistic was used to test whether each new predictor variable added information and significantly improved the model fit. To calculate the Deviance statistic, a total of 63 patients from the male dataset were automatically removed from the logistic models due to at least one missing covariate from the model (leaving a total of 269 patients with 45 recurrent VTE events).

Male Model 1:

An automated forward stepwise logistic regression for the male sub-group yielded the following clinical prediction model

$$rVTE = -2.8391 + 1.0161(HER) + 1.0569(ACL_LPA) + 1.8083(HB > 170g/L).$$

This logistic model was composed of the variables PTS signs HER, anticardiolipin antibodies > 6 U/ml or lupus anticoagulant present (APLA), and hemoglobin over 170 g/L, hereafter referred to as “CPR1 for males” was selected for further classification performance analysis and for the REVERSE steering committee assessment.

Male Model 2:

Table 20. Model 2: steps in model building for males using logistic regression with predictor variables entered in order of strength of association.

Variables	N	DF	Deviance (-2Log L)	Δ Deviance $X^2_{,0.05}$
HER	269	2	232.255	10.68>5.99*
HER+ APLA	269	1	224.455	7.8>3.84*
HER+ APLA +AGE<60	269	1	220.435	4.02>3.84*
HER+ APLA +AGE<60+HB>170	269	1	216.393	4.04>3.84*
HER+ APLA +AGE<60+HB>170 +Height>188	269	1	216.018	0.375<3.84
HER+ APLA +AGE<60+HB>170 +BMI<26	269	1	211.849	4.54>3.84*

***Significant likelihood ratio statistic (cut-off p-value 0.05).**

DF= Degrees of freedom. HER=post-thrombotic signs (any edema, hyperpigmentation or redness either leg). Hb>170=hemoglobin over 170 g/L. Age<60=age under 60 years. APLA= antiphospholipid antibodies (anticardiolipin antibody >6 U/ml or positive lupus anticoagulant).

The Deviance statistics show that the variable height over 188 cm did not significantly improve the model fit. The variables PTS signs (HER), APLA present, age < 60 years,

hemoglobin >170g/L and BMI <26kg/m² substantially improve the model fit and are important independent predictors of recurrent VTE for males (Table 20). Therefore, the logistic model:

$$rVTE = -3.6676 + 1.931(HER) + 1.0253(APLA) + 0.7542(Age < 60) + 1.8141(Hb > 170) + 0.8017(BMI < 26)$$

hereafter referred to as “CPR 2 for males” was selected for further classification performance analyses. The Hosmer and Lemeshows test for CPR2 for males was not significant (p=0.80), indicating a good fit for this model.

Male Model 3:

Table 21. Model 3: steps in model building for males using logistic regression with predictor variables entered in order of strength of association.

Variables	N	DF	Deviance (-2Log L)	Δ Deviance X ² _{,0.05}
HER	269	2	232.255	10.68>5.99*
HER+APLA	269	1	224.455	7.8>3.84*
HER+APLA+AGE<60	269	1	220.435	4.02>3.84*
HER+ APLA+AGE<60+BMI<26	269	1	216.387	4.05>3.84*

*Significant likelihood ratio statistic (cut-off p-value 0.05).

DF= Degrees of freedom. HER=post-thrombotic signs (any edema, hyperpigmentation or redness either leg). APLA= antiphospholipid antibodies (anticardiolipin antibody >6 U/ml or positive lupus anticoagulant). BMI<26= body mass index under 26 kg/m².

The variables PTS signs (HER), APLA present, age < 60 years, and BMI <26kg/m² substantially improve the model fit and are important independent predictors of recurrent VTE for males (Table 21). Therefore, the logistic model:

$$rVTE = -3.5861 + 1.2967(HER) + 0.8449(APLA) + 0.9011(Age < 60) + 0.7271(BMI < 26)$$

hereafter referred to as “CPR 3 for males” was selected for further classification performance analyses. The Hosmer and Lemeshows test for CPR 3 for males was not significant (p=0.97), suggesting reasonable fit for this model.

Male Model 4:

Table 22. Model 4: steps in model building for males using logistic regression with predictors entered in order of strength of association.

Variables	N	DF	Deviance (-2Log L)	Δ Deviance $X^2_{,0.05}$
HER	269	2	232.255	10.68>5.99*
HER+APLA	269	1	224.455	7.8>3.84*
HER+APLA+AGE<60	269	1	220.435	4.02>3.84*
HER+APLA+AGE<60+HB>170	269	1	216.393	4.04>3.84*

*Significant likelihood ratio statistic (cut-off p-value 0.05).

DF= Degrees of freedom. HER=post-thrombotic signs (any edema, hyperpigmentation or redness either leg). APLA= antiphospholipid antibodies (anticardiolipin antibody >6 U/ml or positive lupus anticoagulant). Age<60=age under 60 years. HB>170=hemoglobin over 170g/L.

The variables PTS signs (HER), APLA present, age over 60 years and hemoglobin over 170 g/L substantially improve the model fit and are important independent predictors of recurrent VTE for males (Table 22). Therefore, the logistic model:

$$rVTE = -3.2899 + 1.1205(HER) + 1.0361(ALPA) + 0.6448(Age < 60) + 1.6440(HB > 170)$$

hereafter referred to as “CPR 4 for males” was selected for further classification performance analyses. The Hosmer and Lemeshows test for CPR 4 for males was not significant (p=0.86) suggesting reasonable fit for this model.

Male Model 5:

Table 23. Model 5: steps in model building for males using logistic regression with predictor variables entered in order of strength of association.

Variables	N	DF	Deviance (-2Log L)	Δ Deviance $X^2_{,0.05}$
HER	269	2	232.255	10.68>5.99*
HER+ALPA	269	1	224.455	7.8>3.84*
HER+ALPA+AGE<60	269	1	220.435	4.02>3.84*

*Significant likelihood ratio statistic (cut-off p-value 0.05).

DF= Degrees of freedom. HER=post-thrombotic signs (any edema, hyperpigmentation or redness either leg). ALPA= antiphospholipid antibodies (anticardiolipin antibody >6 U/ml or positive lupus anticoagulant). Age<60=age under 60 years.

The variables PTS signs (HER), LPA present and age < 60 years substantially improve the model fit and are important independent predictors of recurrent VTE for males (Table 23).

Therefore, the logistic model

$$rVTE = -3.2459 + 1.2179(HER) + 0.8646(LPA) + 0.7952(Age < 60)$$

hereafter referred to as “CPR 5 for males” was selected for further classification performance analyses. The Hosmer and Lemeshows test for CPR 5 for males was not significant (p=0.89), indicating a good model fit.

Male Model 6:

Table 24. Model 6: steps in model building for males using logistic regression with predictor variables entered in order of strength of association.

Variables	N	DF	Deviance (-2Log L)	Δ Deviance $X^2_{,0.05}$
HER	269	2	232.255	10.68>5.99*
HER+ALPA	269	1	224.455	7.8>3.84*

*Significant likelihood ratio statistic (cut-off p-value 0.05).

DF= Degrees of freedom. HER=post-thrombotic signs (any edema, hyperpigmentation or redness either leg). ALPA= antiphospholipid antibodies (anticardiolipin antibody >6 U/ml or positive lupus anticoagulant).

The variables PTS signs (HER) and ALPA positive substantially improve the model fit and are important independent predictors of recurrent VTE for males (Table 24). Therefore, the logistic model:

$$rVTE = -2.6680 + 1.0782(HER) + 0.8866(ALPA)$$

hereafter referred to as “CPR 6 for males” was selected for further classification performance analyses. The Hosmer and Lemeshows test for CPR 6 for males was not significant (p=0.99), suggesting reasonable model fit.

Male Model 7:

As with the previous male models and in the univariate analysis in Phase I, the variable PTS signs (HER) was the strongest univariate predictors of recurrent VTE for males. Therefore, the following model including the variable PTS signs (HER) alone, hereafter referred to as “CPR 7” for males, was selected for further classification performance analysis.

$$rVTE = -2.1707 + 1.1274(HER)$$

Classification performance for the seven male CPRs derived by multiple logistic regression

Similarly to the derivation of female CPRs, the regression coefficients were used to weight the variables for each of the seven models for males described above. Each variable was assigned a proportional point score (regression coefficient rounded to the nearest integer) and the best predictive point score (with the smallest recurrent VTE in the low risk group and the highest low risk excluded proportion) for each model was identified (Table 25). The automated forward stepwise logistic model (CPR 1) was shown to be unsafe (unacceptably low NPV, more than 3% risk of recurrent VTE in the low risk group) and not very clinically useful (only 29% of patients will approximately be removed from anticoagulant therapy). CPR 2 had the smallest risk of recurrent VTE in the low risk group and a 35% excluded proportion. Consequently, CPRs 1 and 2 for males were selected for assessment by the REVERSE steering committee. All other models for males (CPR 3 to CPR 7) were shown to be unsafe (very high risk of recurrent VTE in the low risk group) and thus unlikely to validate. CPRs 3 to 7 were not used for further analyses.

Table 25. Classification performance and cut-off points of the CPRs for males.

	CPR 1*	CPR 2*	CPR 3	CPR 4	CPR 5	CPR 6	CPR 7
Cut-off	<1	<2	<2	<2	<1	<1	<1
# variables	3	5	4	4	3	2	1
Sensitivity	64.6	0.91	0.89	0.82	0.96	0.89	0.65
Specificity	62.9	0.42	0.41	0.53	0.11	0.33	0.63
NPV	0.89	0.96	0.95	0.94	0.93	0.93	0.90
% rVTE per year (low risk group)	3.7	3.4	4.1	5.0	6.5	4.5	7.7
% rVTE per year (high risk group)	18.8	19.9	20.1	22.3	14.9	18.9	23.1
Low risk excluded proportion	29.0	35.0	35.8	47.2	10.0	29.5	58.3

*Male models selected for assessment by the study steering committee.

CPR= clinical prediction rule. NPV=negative predictive value. rVTE= recurrent venous thromboembolism.

7.6 Phase III – Discussion

Multivariate analysis was conducted separately for females and for males. Seven potential predictor variables for females and six potential predictor variables for males met the selection criteria of association with recurrent VTE ($P < 0.10$), had good reproducibility ($\text{Kappa} > 0.60$), and were considered by the REVERSE steering committee important predictor variables for inclusion in the multivariate logistic regression analysis.

The PTS signs (HER) variable was found to be one of the most important independent predictors of recurrent VTE for males and for females in univariate and multivariate analysis.

From multivariate analysis, competing logistic models for males and for females were identified separately. Classification performance analysis was then performed for each model to identify the best predictive model.

For females, automatic forward stepwise logistic regression yielded a model that was not selected for further analyses because it did not include potential predictors that were strong predictors in univariate analysis (i.e. PTS signs-HER). From multivariate regression analysis, four good predictive models (including variables that significantly improved the model fit and had good face validity) were identified. These four models were selected because they all had high NPV (over 96%), less than 3% annual risk of recurrent VTE in the low risk group, and more than 10% annual risk of recurrent VTE in the high risk group. CPRs 2 and 4 had the highest low risk excluded proportion (the CPRs identified more than 50% of patients at low risk of recurrent VTE).

For males, seven logistic regression models composed of variables that significantly improved the model fit were identified. Among the seven logistic models, CPR 1 and 2 were the models with the best classification performance. All other CPRs (CPR 3 to CPR 7) were showed to be unsafe (had a very high risk of recurrent VTE in the low risk group) and thus unlikely to validate.

CPRs 1 to 4 for females and CPR 1 and 2 for males were chosen for further examination by the study steering committee.

8. PHASE IV- SELECTION OF THE FINAL CLINICAL PREDICTION RULE AND INTERNAL VALIDATION.

In this phase, the CPR with the best classification performance and that would be easiest to apply in the clinical setting was identified. Once the final CPR was chosen, internal validation analysis for the final CPR using a resampling technique was conducted to

investigate how well the prediction model might perform if it is to be applied in a new dataset and to exclude models that appeared over-fit. The REVERSE steering committee also played an important role in the selection of the final CPR.

8.1 Phase IV - Goals and Objectives

The objectives of phase IV were to:

- 1) Select a final rule with the best classification performance, good face validity, and ease of use.
- 2) Internally validate the final clinical prediction rule.

8.2 Phase IV - Selection of the final CPRs

Competing logistic models identified in Phase III (CPR 1-4 for females and CPR 1 and 2 for males) were assessed in consensus with the REVERSE steering committee members to select the best predictive model for females and hopefully for males. The clinical prediction rule to be selected for males and for females separately was the CPR with the highest low risk excluded proportion, the smallest yearly risk of recurrent VTE in the low risk group (at most 3% per year), the highest yearly risk of recurrent VTE in the high risk group (>10% per year), negative predictive value closer to 100%, and easy to remember and apply in the clinical setting. Often, when comparing potential rules, these criteria are competing. Expert opinion is required to weigh the importance of these trade-offs e.g. will clinicians adopt a very safe rule (low risk group < 1% annual risk) that only suggests discontinuing anticoagulants in 10% of patients versus another rule that is safe (low risk group <3% annual risk) but suggests discontinuing anticoagulants in 30% of patients.

8.2.1 Selection of the final CPR for females

The selection of the final rule was based on logistic multivariate analysis, classification performance results, and consensus of the REVERSE steering committee.

After assessment of the four final female rules (Phase III), CPR 2--composed of the predictor variables BMI over 30 kg/m², PTS signs (HER), D-Dimer over 250 ug/l and age over 65 years—was selected as the final CPR for females. All these variables and cut-off points were considered to have good face validity (make clinical sense) and are easy to remember.

The final CPR for females was obtained from the logistic regression model described in Table 26. To weight each variable of the final model, the regression coefficients of the logistic model were rounded to the nearest integer and then added to create a point score variable.

Table 26. Regression coefficients and weights attributed to each variable of the final female model.

Variable	Regression Coefficient	Odds Ratio	Odds Ratio (95% LCI)	Odds Ratio (95% UCI)	Model Point Scores *
BMI >30	1.2977	3.661	1.424	9.409	1.0
PTS signs(HER)	0.6473	1.910	0.757	4.821	1.0
D-Dimer>250	0.9155	2.498	0.850	7.344	1.0
Age > 65 years	0.8084	2.244	0.794	6.346	1.0

* Point scores are the model regression coefficients rounded to the nearest integer. LCI= lower confidence interval. UCI= upper confidence interval. BMI>30=body mass index over 30 kg/m². HER=post-thrombotic signs (any edema, hyperpigmentation or redness either leg). DD>250=D-Dimer over 250 ug/L. Age>65=age over 65 years.

The score variable (range 0 to 4) was dichotomized to several cut-off points and the best cut-off point was chosen (Tables 27-28). The score cut-off point less than 2 was found to have the smallest yearly risk of recurrent VTE in the low risk group (1.6%), the highest NPV (98%), the highest low risk excluded proportion (52%), a high yearly risk of recurrent VTE in the high risk group (14.1%), and it was considered to have a practical number of predictor variables that would be easy to remember and apply by doctors in the clinical setting.

In addition, the likelihood ratio for a negative result (low risk of recurrent VTE) predicted by the final CPR was 0.21, which indicates that the final CPR for females has a moderate value in sorting out persons without recurrent VTE (small probability of recurrent VTE for patients with a negative CPR). The negative likelihood ratio estimates the probability for the CPR results (negative) for a person that had recurrent VTE versus the probability of a CPR result (negative) for a person that did not have a recurrent VTE event, in which sensitivity and specificity are expressed as proportions rather than percentages (45).

Table 27. Classification performance of the final CPR for females at each score cut-off point.

	Cut-off <1	Cut-off < 2*	Cut-off < 3	Cut-off < 4
Sensitivity	0.96	0.88	0.52	0.12
Specificity	0.31	0.57	0.81	0.97
NPV	0.99	0.98	0.94	0.91
LR-	0.12	0.21	0.59	0.91
% VTE per year (low risk group)	1.0	1.6	4.4	6.7
% VTE per year (high risk group)	10	14.1	19.7	26.9
Low risk excluded proportion	28.3	52.2	22.3	4

*Two or more points= continuation of oral anticoagulant treatment.

One or less = discontinuation of oral anticoagulant treatment

NPV=negative predictive value. VTE= venous thromboembolism. LR-= negative likelihood ratios.

Table 28. 2X2 table and classification performance of the final CPR for females—score cut-off < 2 points.

	Recurrent VTE	No Recurrent VTE
Two or more logistic regression model points (high risk—Continue anticoagulants)	(a) 22	(b) 98
One or none logistic regression model point (low risk—Discontinue anticoagulants)	(c) 3	(d) 128

Sensitivity= $(22/25) \times 100 = 88\%$ (75.3%-100%)

Specificity= $(128/226) \times 100 = 57\%$ (50.2%-63.1%)

Negative predictive value= $(128/131) \times 100 = 98\%$ (95.2%-99.5%)

Excluded proportion = 52.2%

Negative LR= $(1-0.88)/0.57 = 0.21$ (0.07-0.62)

Positive LR= $(0.88 / (1-0.57)) = 2.0$ (1.6-2.5)

Pre-test probability= $25/251=0.1$

Pre-test odds= $0.1 / (1-0.1) = 0.11$

Post-test odds= $0.11 \times 2.0 = 0.22$

Post-test probability of disease= $0.22 / (1+0.22) = 0.28$

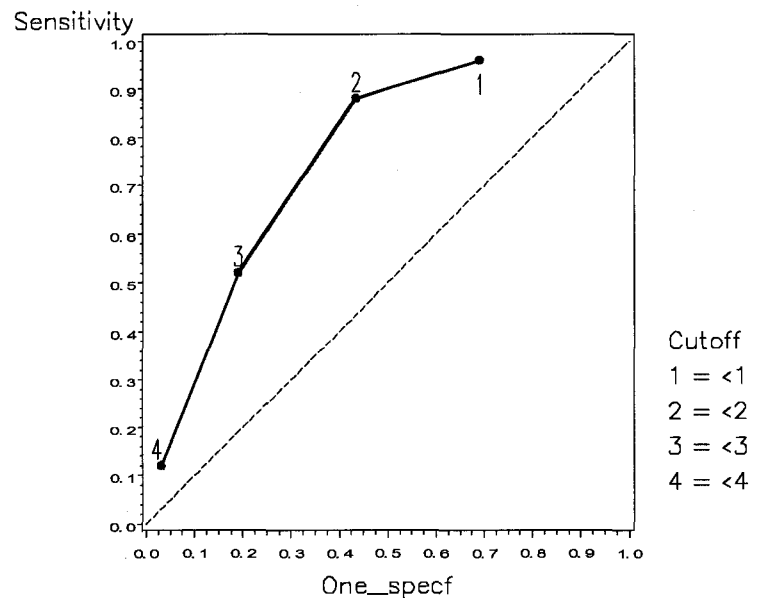
Thus, women with 2 or more of the following variables after 5 to 7 months of oral anticoagulation should continue oral anticoagulants: 1) Any hyperpigmentation, pretibial edema or redness of either lower extremity, 2) Vidas D-Dimer >250 ug/l, 3) obesity – BMI > 30 kg/m² and 4) older age >65 years. Women with less than two of these variables can safely discontinue oral anticoagulation therapy.

Receiver Operating Characteristic curve for the final female CPR

The Receiver Operating Characteristic (ROC) curve (Figure 2) is a graphical representation of the trade off between false negative and false positive rates for the 4 cut-off points of the CPR. The plot shows the false positive rate (1-specificity) on the X axis and the true positive rate (sensitivity or 1-false negatives) on the Y axis using the different cut points of the CPR. The CPR cut-off point < 2 gives the CPR the highest true positive rate and the lowest false positive rate, demonstrating the accuracy of the CPR for identifying patients that presented

recurrent VTE in this study. The good accuracy of the CPR to correctly classify cases with recurrent VTE and without recurrent VTE can be also inferred by the area under the ROC curve (0.76).

Figure 2. ROC curve for the final female CPR (accuracy of the model).



AUC = 0.76

8.2.2 Selection of the final CPR for males

CPR 1 and 2 for males (from Phase III) were assessed to identify a CPR with good predictive ability for males. CPR2 (including the predictor variables BMI<26kg/m² and age <60 years) lacked face validity since it seems questionable to conclude that young and thin males are at high risk of recurrent VTE. CPR 1 had a very low NPV (89%) and both CPR 1 and 2 had more than 3% risk of recurrent VTE in the low risk group. A low NPV and an annual risk of recurrent VTE over 3% in the low risk group makes the CPR unsafe to apply as there would

be an unacceptable number of patients at high risk of recurrent VTE mistakenly classified as having a low risk of recurrent VTE.

We were unable to produce a safe and clinically useful CPR to identify patients a low risk of recurrent VTE in the male population of this study because they showed to be at high risk of recurrent VTE independently of the presence or absence of the predictor variables assessed in this study. Consequently, we concluded that the male patients in this study were generally at high risk of recurrent VTE, and all should be considered for long term anticoagulation therapy (continuation of OAT after 5 to 7 months of treatment).

8.3 Phase IV – Internal validation

Prior to widespread implementation of a clinical prediction rule, a CPR should be validated in a separate sample population (and ideally in different populations) with data for the validation group gathered prospectively. A successful prospective validation of a CPR ensures clinicians that patients can be properly classified by the rule (86).

Internal validation using random samples of the derivation population can be used as an approximation of an external validation to determine how good the prediction model would perform if the CPR is to be applied in a new dataset. Internal validation helps to identify rules that aren't likely to validate well, but it does not ensure that the rule will validate well with prospective independent data.

To estimate what the misclassification rate would be if the CPR for females is to be applied in a new clinical setting, the final CPR for females was tested 500 hundred times in 50% random samples of the same data used for model building (a 50% resampling validation technique).

8.3.1 Internal validation using a 50% resampling technique – Patients and methods

For the random 50% validation procedure (50% resampling validation technique), I re-sampled 500 hundred random samples of 150 female patients, which was approximately 50% of the female derivation set. Each observation in the dataset had an equal chance of being selected for each sample, and once selected, it could not be chosen again (a simple random sample without replacement method). Then, the derived final rule for females was applied systematically to each sub-sample of the 500 random samples of 150 patients to determine the female CPR classification performance for each sub-sample. This resampling validation technique uses the variability of the original female derivation dataset to simulate the performance of the clinical prediction rule in a new population.

8.3.2 Internal validation – Data analysis.

The 50% resampling validation technique was used to generate a better estimate of the parametric variance than the observed sample variance (in the derivation set) by applying the female final predictive model to each of the 500 hundred pseudo-replicate samples. Then, measures of classification performance of the CPR, including sensitivity, specificity, negative predictive value, yearly risk of recurrent VTE in the low risk group, yearly risk of

recurrent VTE in the high risk group, and low risk excluded proportion were calculated for each pseudo-replicate sample.

8.3.3 Internal validation – Results

The variability of the classification performance on the 500 hundred random sub-samples of the female derivation dataset is graphically represented in Figures 3-5.

Figure 3. Annual rate of recurrent VTE in the low risk group (500 samples).

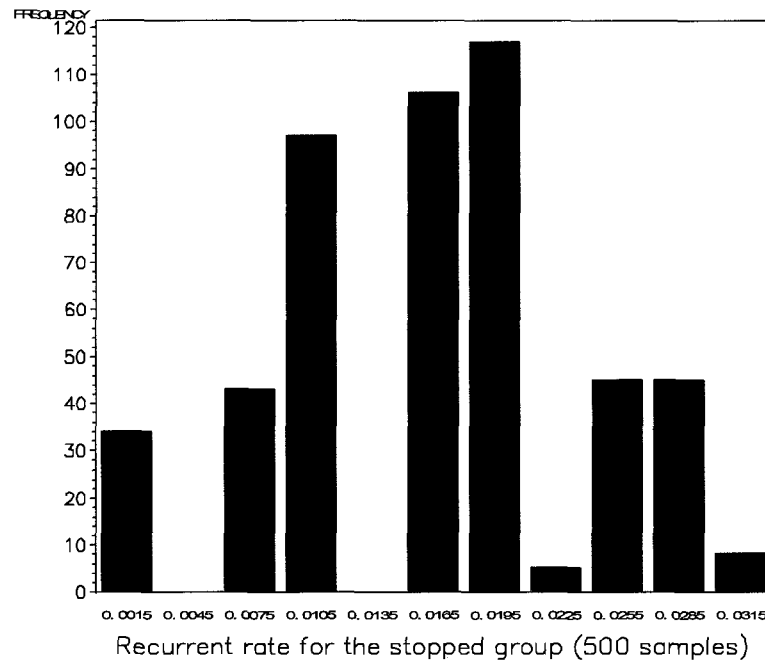


Figure 3 shows number of random samples (y axis), and indicated VTE annual rate of recurrence in the low risk group (x axis).

The grand mean for the annual rate of recurrence of the low risk group between the 500 hundred random sub-samples was 1.6% (the same mean of the derivation set) with a standard deviation of 0.8%, a minimum value of 0%, and a maximum value of 3.2%.

Figure 4. Annual rate of recurrent VTE in the high risk group (500 samples).

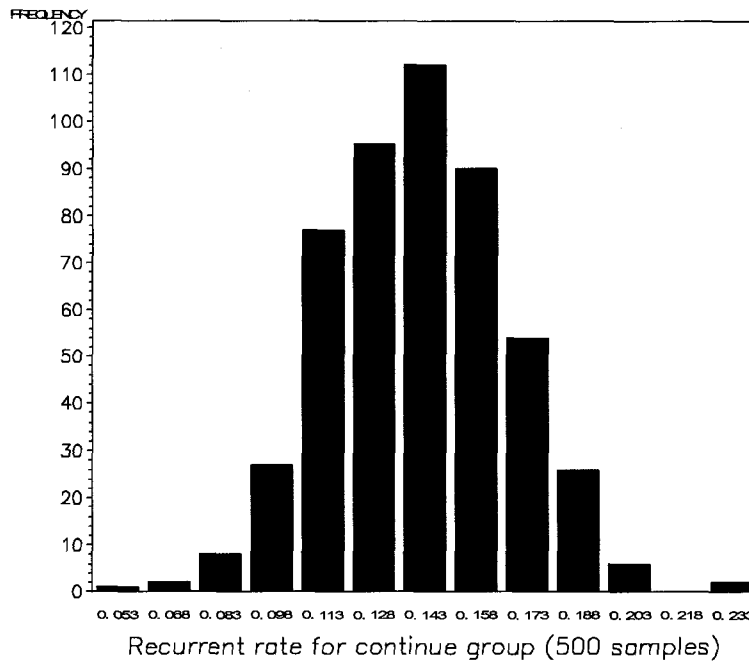


Figure 4 shows number of random samples (y axis), and indicated VTE annual rate of recurrence in the high risk group (x axis).

The grand mean for the annual rate of recurrence of the high risk group between the 500 hundred random sub-samples was 14.1% with a standard deviation of 0.3%, a minimum value of 0.5%, and a maximum value of 23.5%.

Figure 5. Rate of low risk excluded proportion.

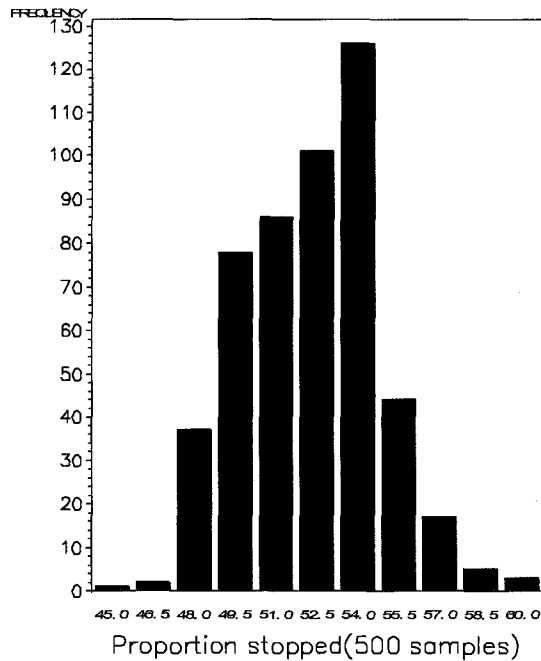


Figure 5 shows number of random samples (y axis), and indicated proportion of females classified as low risk(x axis).

The grand mean for the risk excluded proportion between the 500 hundred random sub-samples was 52.2% with a standard deviation of 2.4%, a minimum value of 44.7%, and a maximum value of 60.0%.

The grand mean for the negative predictive value between the 500 hundred random sub-samples was 0.98 (the same mean of the derivation set) with a standard deviation of 0.01, a minimum value of 0.96, and a maximum value of 1.

The grand mean for sensitivity between the 500 hundred random sub-samples was 0.88 (the same mean of the derivation set) with a standard deviation of 0.06, a minimum value of 0.67, and a maximum value of 1.

The grand mean for specificity between the 500 hundred random sub-samples was 0.57 (the same mean of the derivation set) with a standard deviation of 0.03, a minimum value of 0.49, and a maximum value of 0.64.

8.4 Phase IV – Discussion

The final CPR was selected after simultaneous comparison of several competing logistic regression models for males and for females obtained in Phase III. The selection was based on classification performance of the CPRs (the highest low risk excluded proportion, the smallest risk of recurrent VTE in the low risk group and the highest risk of recurrent VTE in the high risk group), and easy to apply in the clinical setting and to remember.

The final CPR for females includes four variables that have been previously studied as useful individual predictor variables of recurrent VTE. D-Dimer has been recognized to predict low risk of recurrent VTE after discontinuation of OAT in patients with a first episode of VTE or first unprovoked VTE. Obesity (65;66) and older age (7;35;65;87) have consistently been shown to be associated with increase risk of VTE and recurrent VTE. PTS signs (HER) is a novel finding that have recently been associated to increased risk of recurrent VTE in DVT patients (15;38).

To simulate the performance of the final CPR for females if the CPR were to be applied in a new clinical setting, the final CPR was tested in sub samples of the same study population from which the rule was derived (female derivation set). The classification performance of

the final CPR showed small variability among the 500 sub-samples of the derivation set. The annual risk of recurrent VTE in the group classified by the rule as being at low risk of recurrence vary from 0% to 3.2%. These results suggest that the final CPR for females may validate well in a new population, and ultimately, should be safe for identifying patients at low risk of recurrent VTE.

Although the small variability of the classification performance results among the 500 hundred random sub-samples indicate that the final CPR for females is likely to validate in prospective independent datasets, a prospective validation of the final CPR on independent samples would be necessary for the rule to be accepted and used. Deriving the best rule for a given dataset usually results in a rule that performs better on the derivation dataset than it would on an independent dataset, and might result in a poor rule for practical use. A good rule, however, will also perform well on independent datasets.

No CPR was selected for males, because the two selected logistic models in Phase III showed a high risk of recurrent VTE in the low risk group, had a low percentage of low risk excluded proportion (not very useful in the clinical setting) and some of the individual predictors were considered to lack face validity (i.e. age under 60 years and weight under 26 kg/m²).

Based on the results of Phase IV the REVERSE steering committee concluded that unprovoked VTE male patients are at high risk of recurrent VTE (pre test probability = 13.7% / year and post test probability of 14.9 to 23.1% /year, see Table 25) and should be considered for long term anticoagulation, while unprovoked VTE female patients who have

one or none of the four CPR clinical predictors had a low risk of recurrent VTE (1.6% /year) and may be able to safely discontinue anticoagulants. Women with 2 or more of the four CPR clinical predictors have a high risk of recurrent VTE (14.1%/year) and should be considered for long term anticoagulation.

For the final CPR to be easy to teach and remember, the REVERSE steering committee developed a mnemonic memory aid formula for the CPR: “**Men continue and HER DOO2**”, which stands for any Hyperpigmentation, Edema or Redness (**HER**) of either lower extremity, Vidas **D-Dimer** >250ug/ml, **Obesity** –BMI>30 kg/m², **Older age** – age >65 years. The presence of **2** or more of the four predictor variables will suggest continuation of OAT.

I believe the final CPR is a plausible approximation to reality, a good model to predict low risk of recurrent VTE in patients with unprovoked VTE after 5 to 7 months of OAT, and easy to use by physicians of varying levels of experience in a variety of clinical settings.

9.0 DISCUSSION

In this study I have derived and internally validated a clinical prediction rule that provides clinicians and patients with a clear guidance on whether to continue or discontinue anticoagulants after 5 to 7 months of treatment for unprovoked venous thromboembolism. This rule would allow the discontinuation of oral anticoagulants in 52.2% of unprovoked VTE patients on anticoagulant treatment for 5 to 7 months with a reasonably low risk of recurrent VTE (1.6%/year), and would segregate the remaining patients into a group with an annual risk of recurrent VTE >10% for whom longer term oral anticoagulant therapy should be strongly recommended.

The final CPR was obtained after a thorough examination and statistical analysis (Phase I to Phase IV) of a total of 67 known and novel potential predictor variables that have been previously individually linked to increase risk of VTE or recurrent VTE. Several single variables such as gender (15;35;36), D-Dimer levels after discontinuation of oral anticoagulants (26;29;30;64), residual venous obstruction (24;27;28;64), and elevated Factor VIII (25;32) have been previously studied in an effort to risk stratify VTE patients into low and high risk of recurrent VTE. These studies found that the above variables were significantly associated with recurrent VTE, however, their results have not been helpful to risk stratify patients with unprovoked VTE because these studies were conducted on patients with a heterogeneous risk of recurrence (provoked and unprovoked VTE patients (28;88), distal and proximal DVT (29;30;64) or variable duration of OAT (25;28-30;35;88)), and because the predictive power of single variables was too low to accurately and safely stratify VTE patients by risk of recurrence.

The present study used a multivariate approach to discriminate risk strata. The result is the first CPR that provides clinicians and patients with a safe and clinically useful tool to decide whether or not to continue anticoagulants after 5 to 7 months of treatment for unprovoked venous thromboembolism.

Among all the dichotomized potential predictor variables examined, gender was found to be the most powerful predictor of recurrent VTE with males having 2.4 times the risk of recurrent VTE of females. This result agrees with other studies that have consistently found an increased risk of recurrent VTE in males compared with females (35-37). Although, there

was a significant difference in the means of age between men (54.3 years) and women (50.6), age alone does not seem to explain this differences. Significant differences by gender were also found for most of the individual potential predictor variables examined, as a result, univariate analyses were carried out for males and females independently. It has been suggested that men show a higher risk of recurrence because of a higher incidence of hypercoagulability in those men who develop VTE (37).

There were 13 potential predictor variables for females and 10 variables for males that met the selection criteria of association with recurrent VTE ($P < 0.10$) and had good reproducibility ($Kappa > 0.60$). The most significant findings were:

1) History of hypercholesterolemia was the strongest univariate predictor of recurrent VTE for females but it did not show any association with recurrent VTE for males. Although, the association of hypercholesterolemia and unprovoked VTE has been previously mentioned (67), this is the first study to describe hypercholesterolemia as a potential predictor of recurrent VTE. History of hypercholesterolemia was excluded from multivariate analysis due to concerns with the accuracy of the self-reported data and concerns with lack of face validity and that this may hamper acceptance of a CPR including the variable history of hypercholesterolemia.

2) Post- thrombotic signs HER was found to be the most powerful individual predictor for increased risk of recurrent VTE for males and was also a significant predictor for females (male patients with HER had 3.1 times the risk of recurrent VTE compared with males without HER, and female patients with HER had 3.4 times the risk of recurrent VTE

compared with females without HER). PTS signs is a novel finding that has been only recently linked to increased risk of recurrent VTE in a study conducted on DVT patients (38).

3) Factor VIII was a highly significant predictor of recurrent VTE for females and a significant predictor for males. Elevated Factor VIII is a known thrombophilic factor that predisposes to VTE and has recently been found to be positively associated with an increased risk of recurrent VTE after secondary coagulation is stopped (25;32). Factor VIII was not included in the multivariate analysis due to concerns with inter-assay and inter-laboratory variability (60-63). There have been no previously published inter-observer reliability studies of elevated factor VIII levels. However, in the expert opinion of the steering committee elevated levels of Factor VIII are likely to have poor inter-observer reliability. Factor VIII levels varies with blood group (89), age groups (90) and can increase with physical activity (91) and exogenous hormones (92). There are multiple commercial assays and machines and reference ranges for each assay/ machine combination for this indication that would have to be developed (60-63).

4) D-Dimer over 250 ug/L was a significant predictor of recurrent VTE for females but not for males. Elevated D-Dimer levels after discontinuation of OAT has been identified as a predictor of increase risk of recurrent VTE (26;29;30;64), however, no study has reported the differences found between men and women regarding the predictive value of D-Dimer.

5) Pulmonary vascular obstruction (PVO) and the combined variable abnormal V/Q scan or PVO score <95% at baseline as measures of residual venous obstruction were significant

predictors of recurrent VTE for males but not for females. However, I did not find any association of residual venous obstruction on leg vein compression ultrasound and recurrent VTE. These variables were not used in the multivariate analysis due to concerns with cost, difficulties of standardization of these techniques across hospitals and that V/Q is not widely available and is being replaced by CT for PE diagnosis.

6) Older age (>65 years of age) and obesity (BMI>30 kg/m²) were found to be significant predictors of recurrent VTE for females but not for males. Similar to the current study, older age has been consistently identified for several clinical studies as a positive risk factor for VTE (6;7;14;35;65) as well as obesity (65;66). I have no logical explanation for the protective effect of increased BMI and older age found in this study for males, however.

Only 7 predictor variables for females and 6 predictor variables for males were included in the multivariate analysis as they were considered by the REVERSE steering committee to have good face validity (that is, the predictor variable makes good clinical sense) and to be feasible to apply in different clinical settings.

The variables that were retained for multivariate logistic analysis for females were age over 65 years, BMI over 30 kg/m², D-Dimer over 250 ug/ml, hemoglobin under 120 g/L, PTS signs (HER), absence of history of oral contraceptive used in the past year, and history of previous secondary VTE. The variables that were retained for multivariate analysis for males were age under 60 years, height over 188 cm, BMI under 25 kg/m², hemoglobin over 170 g/L, PTS signs (HER) and the combined variable abnormal lupus anticoagulant or elevated anticardiolipin antibodies over 6 U/ml.

After classification performance analysis of competing logistic models for males and females, we (the REVERSE steering committee) selected the rule with the best classification performance (safe and clinically useful), that has good face validity and is easy and feasible to apply in the clinical setting. We were unable to produce a safe and clinically useful CPR to identify patients a low risk of recurrent VTE for males because the two competing logistic models for males had an unacceptably high risk of recurrent VTE in the group classified by the rule as being a low risk (high percentage of patients at high risk that would discontinue OAT when they should continue.).

Among the four logistic models for females, the logistic model with the variables elevated D-Dimer, older age, obesity and post-thrombotic signs of the leg (HER) was the model with the best classification performance (safe and clinically useful) and was considered by the REVERSE steering committee to have good face validity and to be easy to apply in the clinical setting. The four predictor variables included in the final CPR have previously been reported by clinical and epidemiological studies as individual predictors of VTE recurrence, which adds content validity to the rule. We concluded that unprovoked VTE male patients are at high risk of recurrent VTE (13.7% / year) and should be considered for long term anticoagulation, while unprovoked VTE female patients who have one or none of the four CPR clinical predictors had a low risk of recurrent VTE (1.6% /year) and may be able to safely discontinue anticoagulants.

This study adhered to the methodological guidelines for clinical prediction rule development (44;45;47;48;93) before prospective validation, including:

- 1) The primary outcome (recurrent VTE versus no recurrent VTE) was clearly defined (objective biological diagnosis), clinically important, and was independently and blindly assessed.
- 2) The array of clinical variables used as predictor variables were clearly defined, standardized and prospectively collected, and their assessment was done without knowledge of the outcome recurrent VTE.
- 3) To ensure reproducibility of the CPR, only variables known to have good reproducibility were included in the final rule (age, BMI (77), Vidas D-Dimer (78)). The combined PTS variable (any hyperpigmentation, edema or redness, either leg) was found to have good to excellent reproducibility as per the results of the inter-observer reliability analysis for PTS conducted in this study (Kappa = 0.73 (95%CI: 0.61-0.85)).
- 4) The study population has been well defined: a sample of patients from 12 different tertiary care centers in four countries (Canada, Switzerland, France and United States) representing a wide spectrum of clinical and demographic characteristics was consecutively selected without bias, increasing the generalizability of the study results. Only patients older than 18 years old were selected for the study.
- 5) The statistical techniques used to derive and internally validate the CPR were identified and valid. “Logistic regression is a commonly used statistical approach to determine the most parsimonious set of predictor variables in a multivariate CPR that maximizes the accuracy of

diagnosing the condition of interest”(45), as well as classification performance and internal validation.

6) The accuracy of the CPR in classifying the patients with the outcome recurrent VTE (i.e. sensitivity) and without the outcome (i.e. specificity, NPV, negative LR) was demonstrated.

7) The CPR is sensible as it has a clear purpose (to safely identify patients at low risk of recurrent VTE in patients with unprovoked VTE), is relevant (currently there is uncertainty surrounding the clinical decision for continuing or discontinuing anticoagulants in patients with unprovoked VTE), has content validity (there is a recognized correspondence between the predictive variables included in the CPR and risk of recurrent VTE in patients with unprovoked VTE), is concise (there are only four variables), is easy to use in the clinical setting (the CPR is simple, easy to interpret and is applicable at the point in time at which the decision to continue or withdraw OAT is required) and suggests a course of action (continuation or discontinuation of oral anticoagulants).

The final female CPR identified a high proportion of excluded patients (>50%) stratified as having low risk of recurrent VTE (<3% per year), and it showed a high NPV (98%) and a high yearly risk of recurrent VTE in the high risk group (14.1%). Over 50% of excluded proportion represents a significant number of patients that could safely be withdrawn from oral anticoagulant medication reducing the intrinsic risk of bleeding due to OAT and increasing the quality of life of these patients.

Limitations of this study

Not all of the potentially significant variables ($P < 0.20$) were included in the multivariate analysis to avoid over-fitting the data as the power of this study was reduced by the need to split the derivation dataset by gender and by the existing missing data. The final model was missing 24% of the data due to at least one missing covariate for the model. The PTS signs variable was the predictor variable with the most missing data, because collection of PTS signs started after the first 107 patients were entered into the study (16.4% of PTS signs values were missing). The inclusion of significant potential predictors that were excluded from the multivariate analysis may have yielded a better prediction rule, but this would have only been possible with a larger derivation set. Additionally, important variables such as hypercholesterolemia in women that were demonstrated to be important predictors of recurrent VTE in this study warrant further investigation, as it may be a useful variable to include in the CPR.

The D-Dimer variable and optimal cut-off point of over 250 ug/L was identified using one reagent on one instrument (Vidas D-Dimer on a Vidas instrument) using mostly one laboratory (London Health Science Center). Other D-Dimer tests will require separate studies prior to the CPR to be adopted to identify the optimal cut-off point and performance within the rule.

The final CPR was found to have good internal validity, which indicates that the rule is likely to validate in independent samples. However, the effect of biases in selecting patients or collecting data can not be eliminated by internal validation, because all testing is carried out on patients from the derivation set population. This means that the error rate of any CPR is

likely to be higher when that CPR is used prospectively in an independent group of patients (44). To ensure that similar results are replicated in a different population of patients or in a different care setting, three additional important methodological criteria must be met prior to widespread implementation of the rule in clinical practice (45;48;94):

- 1) The safety of the rule must be prospectively validated in a different population of patients.
- 2) The inter-observer agreement of the rule as a whole must be prospectively determined.
- 3) The clinical impact of use of the rule must be prospectively evaluated.

When the safety and reproducibility of this clinical prediction rule has been validated in an independent prospective population, dissemination of the clinical decision rule should provide physicians with important information to allow them to more confidently identify unprovoked VTE patients at low risk of VTE recurrence who may not need to continue OAT.

From this study we have also identified the need to update the methodological guidelines for derivation and validation of clinical decision rules originally described in 1985 by Wasson JH et al (44) and later modified by other authors (46;48) including the most recent update published by Stiell IG et al in 1999. It is apparent that new steps should be added in order to ensure that CDRs are accepted in clinical practice. We recommend the following additions to the CDR methodological guidelines: 1) a systematic review should be conducted to identify the potential clinical predictor variables to be included in the study; the search

strategy should be designed and revised by an expert panel in the clinical topic and all the variables considered should be listed in an appendix of the article, 2) a check list of information to be included for CDR publication should be developed to ensure that the reader receives all the information needed to identify if the CDR applies to their patients (data should be presented to the reader, inclusion and exclusion criteria should be clearly stated, and the authors should comment about the external validity of their study population to facilitate physicians to identify if their patients are similar to those patients used in deriving and validating the rule).

10.0 CONCLUSION

The final CPR is simple (only four parameters), makes sense, is easy to understand and easy to apply in the clinical setting.

The final clinical prediction rule derived in this study is as follows:

“All males should not discontinue anticoagulant treatment, and all women with the absence of more than two of the four following clinical predictors can safely discontinue oral anticoagulants”:

- Presence of PTS signs (any hyperpigmentation or edema or redness on either leg).
- D-Dimer over 250 ug/L.
- Obesity – BMI over 30 kg/m².
- Older age – over 65 years of age.

This is a rule which is likely to be a safe and clinically useful tool, and it suggests a reasonable course of action to follow after six months of anticoagulant treatment in patients with history of a first episode of unprovoked VTE: discontinuation of anticoagulant treatment or long-term anticoagulation. This CPR should help clinicians decide with more confidence which patients do not need extended OAT and should result in an increase in quality of life in this common group of patients.

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Appendix 1. Phase I—Univariate analysis of baseline patient assessment at optimal cut-off points for the entire study population.

APPENDICES

Appendix 1. Phase I—Univariate analysis of baseline patient assessment at optimal cut-points for the entire study population.

Demographic variables							
Predictor variable (n/N)	Cut-off point	rVTE incidence (%)	Chi-square	P-value	Sensitivity (%)	Specificity (%)	NPV (%)
Age (years) (488/646)	=>40	15.8	4.7	0.03**	85	26	91.1
	<40	8.9					
Height (cm) (322/645)	=>172	18.0	8.8	0.003**	64.4	52.4	90.1
	<172	9.9					
Weight (kg) (166/645)	=>100	21.1	9.1	0.003**	38.5	76.4	88.3
	<100	11.7					
BMI (16/644)	=>48	31.3	4.1	0.04**	5.6	98.0	86.5
	<48	13.5					
Gender (332/646)	Males	19.0	13.5	0.0002*	30.8	48.5	81.0
	Females	8.9					
Income (\$) (258/538)	>50,000	16.3	0.3	0.6	50.6	52.5	85.4
	<=50,000	14.6					
Ethnicity (597/646)	Caucasian	14.6	1.5	0.22	95.6	8.1	91.8
	No Caucs.	8.2					
Traditional Risk factors for VTE							
Immobilization (57/646) (past year)	Yes	17.5	0.6	0.43	11	91.5	86.3
	No	13.8					
Surgery (past year) (50/646)	Yes	10.0	0.7	0.39	5.5	91.9	85.6
	No	14.4					
Hormone replacement (within past 2 months) (2/614)	Yes	50	Fisher's Exact	0.26	1.2	99.8	86
	No	14.1					
Hormone replacement (within last years) (32/515)	Yes	15.6	Fisher's Exact	0.57	8.2	94.1	88.4
	No	11.6					
Oral contraceptive use (within past 2 months) (7/573)	Yes	14.3	Fisher's Exact	1			
	No	15.6					

Oral contraceptive use (within last year) (79/585)	Yes	3.8	8.3	0.004**	3.6	84.8	84.0
	No	16.0					
Malignancy (past year) (15/646)	Yes	6.7	Fisher's Exact	0.7	1.1	97.5	85.7
	No	14.3					
Varicose veins (191/645)	Yes	14.1	0.0	1			
	No	14.1					
Trauma (past year) (27/646)	Yes	14.8	Fisher's Exact	0.8	4.4	95.9	86
	No	14.1					
Family history of VTE (153/643)	Yes	11.8	0.9	0.33	19.8	75.5	85.1
	No	14.9					
Heterozygous for FVL or PGM (123/645)	Yes	15.5	0.22	0.6	20.9	81.2	86.2
	No	13.8					
Previous secondary VTE (26/646)	Yes	19.2	Fisher's Exact	0.39	5.5	96.2	86.1
	No	13.9					
Novel risk factors							
Statin use (current) (85/646)	Yes	17.7	1.0	0.31	16.5	87.4	86.5
	No	13.6					
Statin use (within past year) (18/644)	Yes	27.8	Fisher's	0.15*	5.5	97.7	86.3
	No	13.7					
Known hypercholesterolemia (current) (118/646)	Yes	16.1	0.5	0.49	20.9	82.2	86.4
	No	13.6					
Known hypercholesterolemia (within past year) (32/646)	Yes	18.8	Fisher's	0.43	6.6	95.3	86.2
	No	13.8					
Chronic respiratory diseases (COPD or Emphysema)	Yes	2.5	4.7	0.03**	1.1	93.0	85.2
	No	14.9					

(40/646)							
Compression stocking (215/646)	Yes	15.8	0.79	0.37	37.4	67.4	86.8
	No	13.2					
Concomitant medication							
ASA (36/646)	Yes	11.1	0.3	0.6			
	No	14.3					
Garlic (11/644)	Yes	18.2	Fisher's Exact	0.65			
	No	13.9					
NSAIDS (44/645)	Yes	18.2	Fisher's Exact	0.42			
	No	13.8					
Laboratory Results							
Factor V Leiden (100/645)	Heterozygote	19	2.3	0.13	20.9	85.4	86.8
	Absent	13.2					
Prothrombin Gene Mutation (37/646)	Heterozygote	5.4	2.4	0.12	2.2	93.7	85.4
	Absent	14.7					
Hemoglobin (g/L) (9/640)	≥ 170	44.4	7.1	0.008**	4.5	99.1	86.5
	< 170	13.5					
C-reactive protein (mg/L) (81/537)	≥ 8	13.6	0.04	0.83	14.3	84.8	85.5
	< 8	14.5					
Homocysteine (umol/L) (88/589)	≥ 13	12.5	0.07	0.79	13.9	84.9	86.4
	< 13	13.6					
Factor VIII (335/621)	≥ 1.50	16.9	4.7	0.03**	69.7	42.5	89.3
	< 1.50	10.7					
D-Dimer (ug/L) (227/618)	≥ 250	17.6	3.7	0.05*	46.0	64.9	88.0
	< 250	12.0					
Lupus anticoagulant (3/616)	Present	33.3	0.94	0.3	1.2	99.6	86.1
	Absent	13.9					
Anticardiolipin IgM(U/ml) (482/604)	≥ 2	15.2	2.0	0.16	84.9	21.8	89.8
	< 2	10.2					
Anticardiolipin IgG(U/ml) (576/609)	≥ 1	13.6	2.1	0.15	90.7	5.4	77.8
	< 1	22.2					
Lipoprotein a (12/510)	≥ 0.5	33.3	Fisher's	0.05*	6.3	98.2	88.0
	< 0.5	12.1					

			Exact				
INR (% of time <2) (105/391)	>=40%	19.1	2.9	0.09*	36.4	74.7	87.8
	<40%	12.2					
aPTTSP (Partial thrombin time) (21/616)	>=50 s	33.3	Fisher's Exact	0.02**	8.1	97.4	86.7
	<50 s	13.3					
Imaging Results							
Ventilation Perfusion Scan(V/Q) (127/309)	Abnormal	14.3	1.6	0.20	68.4	42.4	90.6
	Normal	9.5					
Compression ultrasound (CUS) (231/452)	Abnormal	19.5	2.0	0.16	58.4	50.4	85.5
	Normal	14.5					
CUS (153/384)	Worse than minimal wall thickening	15.0	0.00	0.93	40.4	60.2	85.3
	Minimal wall thickening	14.7					
CUS (75/384)	Worse than partial resolution	18.7	Fisher's	0.28	24.6	81.4	86.1
	Partial resolution	13.9					
CUS (23/384)	Worse than minimal resolution	21.7	Fisher's	0.36	8.8	94.5	85.6
	Minimal resolution	14.4					
CUS (4/384)	Worse than no resolution	50.0	Fisher's	0.11	3.5	99.4	85.5
	No resolution	14.5					
PVO scores (72/219)	<0.95	20.8	10.7	0.001**	37.5	29.8	79.2
	=>0.95	6.1					
Abnormal V/Q or PVO	Yes	14.6	7.5	0.006**	93.1	31.3	97.3
	No	2.7					

score<0.95 (185/259)							
Abnormal CUS or PVO<0.95 (292/333)	Yes	18.8	2.03	0.15	93.2	13.5	90.2
	No	9.8					
Clot Resolution (228/443)	Yes	19.7	3.1	0.08*	60.8	50.4	86.5
	No	13.5					
Post-thrombotic syndrome monitoring							
Symptoms either leg (right leg or left leg)							
Cramps (183/544)	Any, either leg	15.3	0.8	0.36	38.4	67.1	87.5
	No	12.5					
Itching (99/544)	Any, either leg	17.2	1.5	0.23	23.3	82.6	87.4
	No	12.6					
Pins and needles (115/544)	Any, either leg	14.8	0.2	0.6	23.3	79.2	87.0
	No	13.1					
Leg heaviness (134/545)	Any, either leg	13.4	0.00	1			
	No	13.4					
Pain (161/545)	Any, either leg	13.7	0.01	0.9			
	No	13.3					
Swelling (223/542)	Any, either leg	17.5	5.3	0.02**	53.4	60.8	89.3
	No	10.7					
Signs either leg (right leg or left leg)							
Pretibial edema (149/545)	Any, either leg	26.2	28.9	<.0001* *	53.4	76.7	91.4
	No	8.6					
Skin induration (58/539)	Any, either leg	22.4	4.6	0.03**	18.1	90.4	87.7
	No	12.3					
Hyperpigmenta tion (93/544)	Any, either leg	21.5	6.3	0.01**	27.4	84.5	88.3
	No	11.8					
Venous ectasia (219/535)	Any either leg	17.4	4.8	0.03**	52.8	60.9	89.2
	No	19.8					
Redness (71/545)	Any, either leg	21.1	4.2	0.04**	20.6	88.1	87.8
	No	12.2					
Any edema,	Any	21.9	21.9	<.0001*	64.4	64.4	92.1

hyper-pigmentation and redness, either leg (215/545)	No	7.9		*			
Pain during calf compression (49/543)	Any, either leg	14.3	0.03	0.86	9.6	91.1	86.6
	No	13.4					
Warmth (17/541)	Any, either leg	5.9	Fisher's	0.71	1.4	96.6	86.3
	No	13.7					
Dependent cyanosis (16/541)	Any, either leg	18.8	Fisher's	0.46	4.1	97.2	86.7
	No	13.3					
Leg ulcers (15/536)	Yes	14.3	0.00	1			
	No	13.5					
Leg circumference (15/536)	=>50 cm	33.3	Fisher's	0.04**	6.9	97.8	87.1
	<50cm	12.9					
Leg difference between Right and Left leg (385/536)	=>0.5 cm	15.6	5.4	0.02**	83.3	30	92.1
	<0.5 cm	8.0					

** p=<0.05, *p=<=0.10

Predictor variables shown are the most important predictor variable for the entire population previously published that have been suggested to be predictive of recurrent VTE (with or without significant univariate association with recurrent VTE. Only the optimal cut-off point per each potential predictor variable is showed. VTE= Venous thromboembolism. NPV=negative predictive value. BMI= Body Mass Index (weight(kg)/height(M)²). HRT= Hormone Replacement Therapy. OCP= Oral Contraceptive. CUS= Compression Ultrasound Leg Vein Imaging. COPD= Chronic Obstructive Pulmonary Disease. PVO= Pulmonary Vascular Obstruction Score on V/Q Scan. V/Q Scan= Ventilation Perfusion Scan. FVIII= Factor VIII levels. INR= international normalized ratios.